

SPECIAL FEATURE

NEW PERSPECTIVES IN WHOLE-PLANT SENESCENCE

Longitudinal analysis in *Plantago*: strength of selection and reverse age analysis reveal age-indeterminate senescence

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Summary

1. Senescence is usually viewed as increased age-specific mortality or decreased age-specific fecundity due to the declining ability of natural selection to remove deleterious age-specific mutations with age. In herbaceous perennial plants, trends in age-specific mortality are often confounded by size. Age-indeterminate senescence, where accumulated physiological damage varies strongly with environment, may be a better model of senescence in these species.

2. We analysed trends in size and fertility in *Plantago lanceolata*, using a long-term demographic census involving > 10 years and > 8000 individuals in four cohorts. We used elasticity and pairwise invasion analysis of life-history function parameterized age × stage matrices to assess whether the force of natural selection declined with age. Then, we used reverse age analysis of size and fertility to assess whether age-indeterminate senescence occurred. Reverse age analysis uses longitudinal data for individuals that have died to look at trait patterns as a function of both age and remaining time to death. We hypothesized that (i) the strength of natural selection would decline strongly with age, and (ii) physiological condition would deteriorate for several years prior to death.

3. Both elasticity and invasion analyses suggested that the strength of natural selection through mortality declined strongly with age once size was accounted for. Further, reverse age analyses showed that individuals shrank for 3 years prior to death, suggesting physiological decline. Inflorescence production declined with age, and also declined in the 3 years prior to death regardless of overall age.

4. *Synthesis.* The hypothesis that plants escape senescence generally assumes that plants can continue to grow larger and increase reproduction as they get older. The results here show that size and reproduction decline with age and the rates of these declines towards death are life span- and age dependent. Further research is needed to delineate the importance of age-determinate vs. age-indeterminate factors in senescence patterns across species.

Key-words: age at death, ageing, life history, physiological decline, plant development and life-history traits, senescence, size, strength of selection

Introduction

Senescence is thought to have evolved because the force of natural selection to remove age-specific mutations with negative consequences generally declines with the age at which the mutation is expressed (Hamilton 1966). Although the physiological mechanisms involved in senescence are diverse

and vary from individual to individual, senescence is most generally observed as increased age-specific mortality or decreased age-specific fecundity with age beginning at or at some point beyond the age at maturity (i.e. actuarial senescence; Hamilton 1966; Bell 1984; Roach 1993). The mathematical theory behind the evolution of senescence is solid enough that the evolution of senescence is seen by many as a universal property of life (Rose *et al.* 2007), although models have been developed that highlight the circumstances when an escape from senescence may be expected (Vaupel *et al.*

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2004; Baudisch 2008), and some species with negligible senescence have been identified (Finch 1998).

Senescence is most easily documented in organisms in which mortality is strongly determined by age. Senescence in such organisms may occur due to the deleterious effects of mutations or pleiotropic genes with age-specific effects (Medawar 1952; Williams 1957). This is in contrast to the observation that in many organisms in which senescence has not been observed, strong size determination in mortality and fertility has been found (Finch 1990; Silvertown 1991). Herbaceous perennial plants are modular, growing new modules and replacing old modules with age (Solbrig 1980). As they grow older, such plants generally grow bigger, and plant mortality is strongly negatively related to size (Bierzychudek 1982). Because plants lack an exact or approximate age at which growth and reproduction must necessarily stop or slow down, it could be hypothesized that many plant species escape senescence. However, this hypothesis may imply that plants generally die at a large or even peak size.

Do plants senesce? Hamilton suggested that senescence in some plants may be negligible, meaning that it may be difficult if not nearly impossible to observe because of its slow rate (Hamilton 1966). An alternative theory to Hamilton's predicts negative senescence, in which the physiological quality and health of an organism improves with age (Vaupel *et al.* 2004). Under this theory, reallocating small amounts of energy and resources away from current reproduction to growth can set the stage for negative senescence provided that the growth achieved in place of current reproduction results in dramatic increases to future reproduction (Sparkman, Arnold & Bronikowski 2007; Baudisch 2008). Thus, in organisms with size-determined life histories, the forces of natural selection may decrease only mildly with age, if at all, due to inherent life-history trade-offs (Vaupel *et al.* 2004).

Senescence may not be directly linked to age *per se*, but the accumulated effects of the wear and tear of life may inevitably show up in all organisms. Common trends in age-specific mortality measured in wild vs. captive bird species, for example, suggest that senescence is inherently age determined, and thus probably related to the negative age-specific effects of particular genes (Ricklefs 2000). However, an alternative to this age-determined model of senescence suggests that the physiological decay that occurs with age is more strongly linked to environmental variability than to age-specific gene action (Ricklefs 2000). This 'damage accumulation' hypothesis suggests that the age of onset and rate of senescence should vary with environmental quality between individuals, while the classic model of senescence predicts little or no such variability (McNamara *et al.* 2009; Martin & Festa-Bianchet 2011). Under the 'damage accumulation' hypothesis, trends in ageing may be best observed via analyses of health and vitality relative to the time remaining prior to death in longitudinal data from organisms that have already lived and died (Martin & Festa-Bianchet 2011; Hammers *et al.* 2012). At some point prior to death, physiological condition should decline, and the point at which this decline occurs should vary with environment (Martin & Festa-

Bianchet 2011). In plants, this should be observed as reduced size prior to death, and potentially also as reduced fertility.

We investigated the demography of a large, experimental population of the ribwort plantain, *Plantago lanceolata* L., growing in a wild meadow. First, we developed age \times stage matrix models with function-based life-history models and used them together with elasticity analysis and invasion analysis, to ask whether the forces of natural selection strongly decline, mildly decline, or do not decline at all with age. Then, we used reverse age analysis to ask whether plants generally showed evidence of deteriorating health and vitality prior to death, or if they died generally at peak health. In reverse age analysis, the physiological condition of individuals that have been tracked across all life stages is analysed with respect to both age and the remaining time to death and may also be analysed with respect to previous size. We hypothesized that (i) the force of natural selection would be observed to decline strongly with age once size had been accounted for, and (ii) plants would not simply keep growing prior to death, but shrink and reduce reproduction for 1 or more years before death due to increasing sensitivity to extrinsic stresses via senescence.

Materials and methods

STUDY SPECIES AND SITE

We studied the ribwort plantain, *P. lanceolata* L. (hereafter *Plantago*). This plant is a wind-pollinated, short-lived perennial with a Holarctic distribution and is commonly found in disturbed habitats. It produces a basal rosette that persists year-round in Virginia, USA, where the study population was located. Size is very plastic, and plants may increase or decrease in size dramatically from year to year (Roach, Ridley & Dudycha 2009). In central Virginia, flowering may occur from April to September, although May to August is most common, and inflorescences may be formed by the same individual multiple times per breeding season. Although reproduction may occur in many years throughout the lifetime of the plant, a high proportion of individuals reproduce only once at this field site (Shefferson & Roach 2010). Seed dormancy has been noted (van Groenendael & Slim 1988); however, in another study in our field site, we found no evidence for dormancy in this population (D.A. Roach, unpublished data).

This study took place in an experimental field plot at the Shadwell Preserve of the Jefferson Monticello Foundation in Virginia, USA. Monthly, temperatures at this site vary from approximately -5 °C in the winter to 22 °C in the summer, and there is spatial heterogeneity in soil moisture and light levels across the plot (D. Roach, unpublished data). A large natural population of *Plantago* at this site was used as the source of the parental plants used in this experiment. We used a modified North Carolina II breeding design with a total of 20 sire genotypes, ten dam genotypes and 40 dam-sire combinations (Lynch & Walsh 1998). This design was repeated for each cohort. Seeds were planted in the greenhouse, raised, planted in the field and marked as described elsewhere (Roach 2003). Seedlings, approximately 6-week old, were planted 15 cm apart in rows, which were 20 cm apart. This spacing was sufficient to avoid competition between study individuals and is within the natural range of densities exhibited by *Plantago* at the study site. Seedlings were planted in the field in a randomized block design with replication of genotypes and

cohorts within 1×14 m sections, which were separated by 1 m to allow researchers to measure plants while causing minimum disturbance. Four adjacent sections constituted a block, and here, we use data on the 12 main blocks that contained all cohorts. The total planting area was approximately 75×45 m. These plantings occurred within, and with minimal disturbance to, the natural plant community, to keep ecological interactions as they would be in a wild population. This plant community is an old field dominated by annual grasses. The different cohorts were planted as follows: cohort 1, October 2000; cohort 2, October 2001; cohort 3, April 2002; and cohort 4, October 2002. The survival of plants was assessed weekly, whilst size was measured in November and May. We thus used November to November as our census interval in the analysis. This study includes data from the time of planting until August 2011. Although the total number of individuals planted approaches 30,000, only those individuals for which size was consistently measured across all life stages were analysed here (cohort 1, 3058 plants; cohort 2, 2803 plants; cohort 3, 1073 plants; cohort 4, 528 plants).

We also conducted further experiments to assess both the number of seeds produced by individual plants, as well as field germination and field survival of seedlings. These were generally performed on an annual basis to allow exploration of temporal variation in demographic patterns, and these methods are described elsewhere (Shefferson & Roach 2012).

ELASTICITY ANALYSES

We tested hypothesis 1, that the force of natural selection would strongly decline with age once size had been accounted for, via elasticity analysis of age \times stage matrix models using function-based models of life-history parameters derived from the demographic data set. Elasticity analysis assesses the relative ability of proportional changes in population projection matrix elements to impact λ , the deterministic population growth rate (Caswell 2001). As Hamilton's theory for the evolution of senescence proposes that the force of selection is scaled by age, and does not require that the strength of selection on any particular trait be known, we suggest that Hamilton's selection scaling coefficient is analogous to the elasticity of λ with respect to the summed mortality-related transitions associated with each age. This assumes only that λ is a good proxy for fitness (Caswell 2001; Metcalf & Pavard 2007) and allows us to test how strongly selection is expected to modify age-specific traits such as age-specific mortality. This use is supported by theoretical work showing that sensitivities, which are used to estimate elasticities, are mathematically equivalent to selection gradients under specific assumptions (Caswell 2001).

We began by parameterizing general linear models (GLMs) with the data corresponding to the probability of survival from time t to time $t + 1$ (binomial), the probability of transition from size x at time t to size y at time $t + 1$ (binomial), and the number of inflorescences produced in time t (Poisson). Model building began with the most parameterized model, which included cohort and year as nominal factors, and size at time t as a discrete variable that could not be < 1 , and all interactions between these three variables. We then removed interaction terms and factors to produce all possible models until the three demographic factors were constants. The model with the lowest AIC was considered the most parsimonious, with the caveat that only models in which all linear coefficients were estimable were compared.

We used these GLMs to produce matrices for deterministic age \times stage matrix projection analysis. These methods allow age to

be incorporated in stage-structured systems, and so allow age-specific trends to be explored even in systems in which size is the strongest determinant of demography (Caswell 2001). Age \times stage matrices were created for each cohort. First, we size-stratified the model according to one-leaf increments from one to 100 leaves, as few plants exceeded 100 basal rosette leaves in size. We then parameterized the survival-growth elements of each cohort's projection matrix as the product of the probability of survival from time t to $t + 1$ and the probability of growth from size x at time t to size y at time $t + 1$ conditional on survival, per the GLM functions previously described. Each matrix included a seedling stage, and fertility was estimated as the number of expected inflorescences, produced in each stage in each year per cohort via the previous GLM function, multiplied by several quantities, including the mean weight of inflorescences in that cohort in that year; the slope of the seed vs. weight regression developed in a separate study (Shefferson & Roach 2012); and the mean germination rate (0.69). As seed dormancy did not occur in this system, we did not include a dormant seed stage. Additionally, the probability of transition of seedlings to each size of adult was estimated as the mean probability of seedling survival over one winter (0.141) multiplied by the probability of a one-leaved adult individual achieving the transition to each size of adult plant conditional upon survival.

In these matrices, the top row of the matrix includes all fertilities, the next 100 rows of the first column included transitions to the seedling stage, and a 100×100 matrix for each age is interspersed in the supermatrix below the seedling stage such that each 100×100 stage-based matrix from one age leads directly to the next. To allow plants to live on past the number of years monitored, we included a final 100×100 matrix developed as the element-by-element mean matrix of all years used in the study. The final supermatrices were of dimensions 1001×1001 (cohort 1), 901×901 (cohort 2) and 801×801 (cohorts 3 and 4).

Elasticity analysis was conducted in relation to the projected deterministic population growth rate (λ) of each of the cohort matrices (Caswell 2001), which was our proxy for fitness in this analysis (Metcalf & Pavard 2007). As elasticities are orthogonal, we summed appropriate elasticities in each matrix to assess the relative abilities of altered demographic rates at different ages to impact fitness. We reasoned that declining summed elasticities associated with each age would be consistent with the declining efficacy of natural selection with increasing age under Hamiltonian senescence, while initially declining summed elasticities followed by increases in later life would be consistent with negative senescence. Matrix building and elasticity analyses were conducted using the *popbio* package in *R* 2.15.2 (Stubben & Milligan 2007; R Core Team 2012).

We also tested for Hamilton's declining forces of natural selection using invasion analysis. As the results are essentially the same as in the elasticity analysis, the methods and results from that analysis are provided in Appendix S1 in Supporting Information.

REVERSE AGE ANALYSIS

We tested hypothesis 2 that plant vitality would decline prior to death on a schedule determined by the time of death rather than absolute age, via reverse age analysis of size and fertility (Martin & Festa-Bianchet 2011; Hammers *et al.* 2012). First, we stratified the data set within each cohort by the timing of death, with all plants dying between December of 1 year and November of the next grouped together. Utilizing all plants living for specified time periods from three years until December 2010 (maximum life span for cohort 1: 10 years, cohort 2: 9 years, cohorts 3 and 4: 8 years), we then

analysed the size ($size_t$, given as the number of leaves in the basal rosette in year t) and fertility ($inflo_t$, given as the number of inflorescences in year t) of each plant as a function of the number of years until death (time to death, or ttd) and current age as fixed effects, as well as their interaction. To assess changed rates of growth and shrinkage with age and time to death, we also included quadratic age (age^2) and quadratic time to death (ttd^2), and the interaction between the quadratic terms (Hammers *et al.* 2012). We also included cohort (coh) and previous year's size ($size_{t-1}$) as fixed effects, and all second- and third-order interactions between fixed-effects terms. We corrected for the effects of individual history by including individual identity as a random effect. We analysed these mixed models using function *glmer* in package *lme4* for R 2.15.2 (Bates, Maechler & Bolker 2012; R Core Team 2012), assuming a Poisson distribution for both size and fertility. As inflorescence number could be 0, we added 1 to this variable prior to analysis to correct for zero inflation. We then developed all biologically relevant models simplified from this most parameterized model, leaving individual identity as the sole random effect in all models, and identified the most parsimonious model via comparison of model AIC. We anticipated that our hypothesis would be supported by increasing size and fertility with increasing time to death, while negative senescence would yield decreasing size and fertility with increasing time to death.

Results

The elasticity of λ to survival-transitions and fertilities in our cohort-specific age \times stage projection supermatrices decreased consistently with age, with the biggest decreases occurring within the first 3 years of life (Fig. 1). These elasticities never increased in later years, even incrementally. Further, elasticities summed to 1 for all four cohorts. Therefore, we conclude that the force of natural selection declines with age in this system once size is accounted for.

While size was negatively correlated with mortality in this study, plants generally experienced their largest sizes in the earlier or middle years of life. Across all cohorts, mortality peaked at 0.60–0.70 in individuals with only one leaf, and dropped sharply as size increased to approximately 30 leaves (Fig. S1). Beyond that the slope of the decline in mortality progressively became flatter, but never reached 0 (Fig. S1). Further, across all cohorts, plants started small in their first year of life, grew to large size either in the second year (cohort 1, Fig. 2a) or thereaf-

ter (cohorts 2 and 4) and shrank thereafter (Fig. S2). Plants in cohort 3 generally grew to their largest size in their first year, and then usually shrank (Fig. S2).

Annual fertility increased linearly with size (Fig. S3). Inflorescence production generally exhibited an early peak, followed by years of low production, and finally, another peak production year at the end of life in the longest-lived individuals (Fig. S4). In cohort 1, this latter peak was small relative to the early-life peak (Fig. 2b), but it was approximately equal, or even greater in size in all other cohorts (Fig. S4). This increase in late-life reproduction was during the same year in all cohorts (2009), which suggests an environment-dependent cause.

Plant size decreased over a roughly 3-year period prior to death (Figs. 3, S5–S7), consistent with our hypothesis that senescence is strongly influenced by environmental variability. Our best-fit mixed model of plant size incorporated a nonsignificant but positive effect of time to death, suggesting that individuals were larger in the middle of life than at the end. Furthermore, time to death and previous size formed a significantly positive interaction term determining current size, suggesting that larger plants experience greater declines in size as they get close to death (Table 1). The significantly negative effect of quadratic time to death suggests that plant shrinkage accelerates as time to death decreases (Table 1), and time to death interacted significantly with cohort, as well, suggesting that senescence is subject to cohort effects (Table 1). Size also significantly decreased with age, although the largest individuals sometimes increased in size prior to death (Fig. 3d), and a significantly positive quadratic age term suggests that the slope of this change increased with age (Table 1). Age also displayed significant interactions with cohort, suggesting demographic cohort effects (Table 1). Both age \times time to death and quadratic age \times quadratic time to death significantly influenced size (Table 1), suggesting that the effects of age and time to death vary with life span. Indeed, although size declined as death approached, this decline generally started from either a larger or a smaller size depending on age and varied across cohorts (Fig. 3, S5–S7). Previous size also significantly positively determined current size, suggesting an important influence of individual history (Table 1).

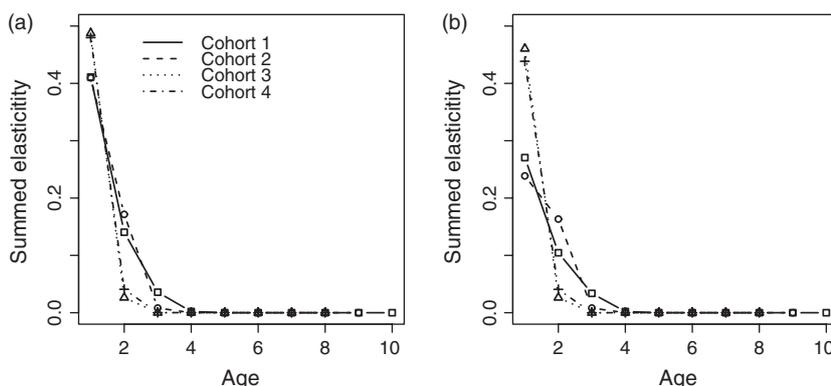


Fig. 1. The strength of natural selection via mortality and fertility in *Plantago lanceolata* declines with age. Summed elasticities of deterministic λ in response to (a) survival-growth transitions and (b) fertility. Elasticities were estimated from age \times stage supermatrices from each of four cohorts planted at the field site in Shadwell, Virginia, USA, across 3 years (November 2001–2003) and tracked until August 2011.

Fig. 2. Mean plant size and inflorescence production of *Plantago lanceolata* individuals living specific numbers of years in cohort 1. (a) Plant size, given as the number of leaves in the basal rosette in November of that year. (b) Inflorescence production. The terminal point in each line is the final size measure prior to death, and only data for individuals dying within the study period are shown.

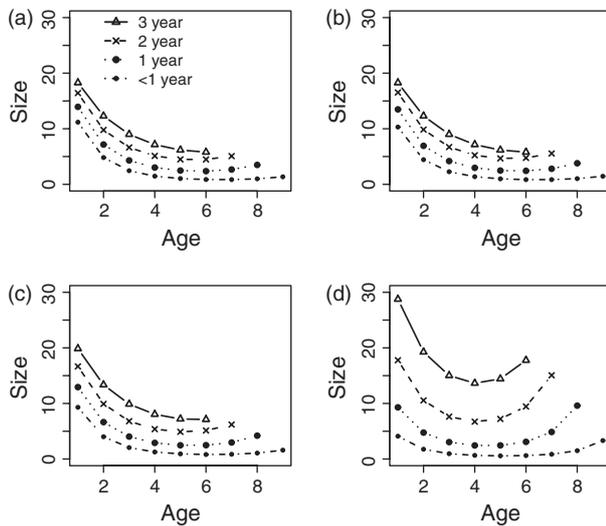
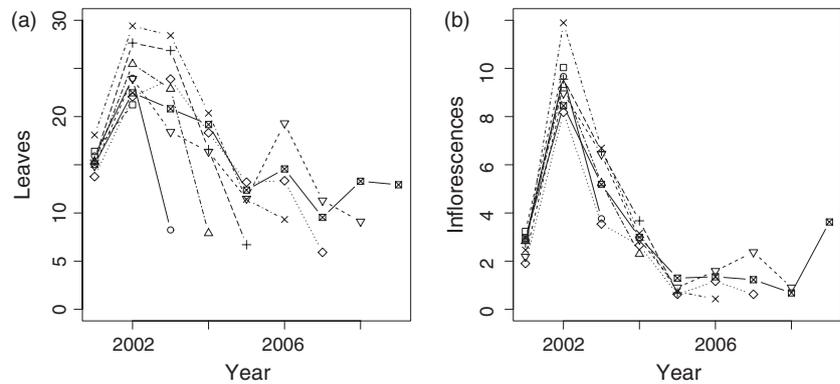


Fig. 3. Predicted size of *Plantago lanceolata* in a long-term field study in Shadwell, Virginia, USA, as a function of age, time to death and previous size in cohort 1. On the y-axis, size is given as the number of leaves in the basal rosette for the associated age on the x-axis. We include model predictions for 3, 2, 1 and < 1 years prior to death, and these predictions are estimated from the best-fit mixed model (Table 1). Panels a–d indicate trends of plants with 1, 5, 10 and 50 leaves in the previous year, respectively.

Mixed modelling of inflorescence production also supported the damage accumulation hypothesis for reproductive senescence in *Plantago*. Time to death and age had significantly negative and positive main effects on inflorescence production, respectively, suggesting that older plants flowered less and flowering decreased even more as death drew near (Table 1). Quadratic age and quadratic time to death also had significant impacts on inflorescence production (Table 1), supporting variation in senescence rate with life span (Figs. 4, S8–S10). Previous size also had a significantly positive effect on current inflorescence production, and this effect became more negative with increasing age and more positive with decreasing time to death (Table 1). Age had a significantly negative effect on inflorescence production, and this effect varied with cohort (Table 1). Cohort also affected inflorescence production via interactions with size and time to death, further supporting strong cohort effects on demography in this species (Table 1).

Discussion

Strongly declining age-specific elasticities of λ and declining size prior to death both support the existence of senescence, rather than negative senescence, in *Plantago*. Strong drops in elasticity in particular suggest that natural selection may be relatively ineffective at eliminating age-specific deleterious genes over much of the adult life of the species. However, senescence patterns here are more consistent with the ‘damage accumulation’ hypothesis, in which the onset of senescence varies with environment (Martin & Festa-Bianchet 2011). Indeed, declining size at the tail end of life suggests increasing sensitivity to extrinsic sources of mortality (Fig. 3). Such age-dependent shifts in sensitivity to the environment may be caused by age-related decline in plasticity. Organisms are at their most plastic in their earliest ages, when developmental pathways adjust to environmental context (Leimar, Hammerstein & Van Dooren 2006). Thus, senescence in this and other size-determined species may be a process in which observed mortality is strongly mediated by a combination of trends in the environment, and the individual’s changing ability to respond to those changes given individual history, genetic background and previously experienced environmental contexts. Alternative models of the demographic impacts of late-life mutations may also yield insights into this system, as late-life mutations may fundamentally impact not just age-specific mortality and fertility, as Hamilton framed his theory, but rates of instantaneous intrinsic and extrinsic mortality and other parameters with potentially nonintuitive impacts on the evolution of senescence (Baudisch 2005).

The primary plant trait that fine-tunes organismal response to environment is likely to be size. Size in herbaceous plants is highly plastic, and plant size was the subject of some of the first experiments on phenotypic plasticity in nature (Clausen, Keck & Hiesey 1947). Altered plant size is likely to be a response to differing levels of stress and resource in the environment, with even shrinkage adaptive at times (Salguero-Gómez & Casper 2010). Declining plant size over time may also indicate a strong influence of competition on plant size and density, although we argue that this does not explain trends in size here because competition should yield a strong influence of year (i.e. age) but not time to death in a data set in which many life spans have been recorded.

Table 1. Estimates of effects on size and fertility of *Plantago lanceolata* in a long-term demographic study at an experimental population in Shadwell, Virginia. The response variables were the number of leaves in the basal rosette (Size_{*t*}) and 1 + the number of inflorescences (Inflo_{*t*}, or fertility). Independent variables included size in the previous year (Size_{*t-1*}), age (Age_{*t*}), remaining number of years to death (Ttd_{*t*}, or time to death), and cohort (Coh). Best-fit models are presented. Mixed model analysis was conducted using function *glmer* in package *lme4* in R 12.15.2 (Bates, Maechler & Bolker 2012; R Core Team 2012)

Size model				Fertility model			
Effects	Estimate	SE	<i>P</i> ≤	Effects	Estimate	SE	<i>P</i> ≤
Intercept	3.440	0.079	0.0001	Intercept	2.580	0.097	0.0001
Coh ₂	-1.547	0.095	0.0001	Coh ₂	-0.954	0.046	0.0001
Coh ₃	-2.445	0.141	0.0001	Coh ₃	-1.350	0.087	0.0001
Coh ₄	-1.914	0.143	0.0001	Coh ₄	-1.250	0.092	0.0001
Size _{<i>t-1</i>}	-0.019	0.002	0.0001	Size _{<i>t-1</i>}	0.021	0.001	0.0001
Age _{<i>t</i>}	-1.087	0.034	0.0001	Age _{<i>t</i>}	-1.201	0.048	0.0001
Ttd _{<i>t</i>}	0.046	0.029	0.118	Ttd _{<i>t</i>}	0.087	0.037	0.018
Age _{<i>t</i>} ²	0.082	0.003	0.0001	Age _{<i>t</i>} ²	0.121	0.004	0.0001
Ttd _{<i>t</i>} ²	-0.023	0.002	0.0001	Ttd _{<i>t</i>} ²	-0.012	0.003	0.0001
Size _{<i>t-1</i>} × Age _{<i>t</i>}	-0.002	0.0005	0.0001	Size _{<i>t-1</i>} × Age _{<i>t</i>}	-0.002	0.0002	0.0001
Size _{<i>t-1</i>} × Ttd _{<i>t</i>}	0.013	0.001	0.0001	Size _{<i>t-1</i>} × Ttd _{<i>t</i>}	0.001	0.0002	0.0001
Age _{<i>t</i>} × Ttd _{<i>t</i>}	0.190	0.009	0.0001	Age _{<i>t</i>} × Ttd _{<i>t</i>}	0.039	0.015	0.007
Age _{<i>t</i>} ² × Ttd _{<i>t</i>} ²	-0.005	0.0002	0.0001	Age _{<i>t</i>} ² × Ttd _{<i>t</i>} ²	-0.0004	0.0004	0.241
Coh ₂ × Age _{<i>t</i>}	0.414	0.036	0.0001	Coh ₂ × Age _{<i>t</i>}	0.208	0.029	0.0001
Coh ₃ × Age _{<i>t</i>}	0.799	0.052	0.0001	Coh ₃ × Age _{<i>t</i>}	0.165	0.046	0.0003
Coh ₄ × Age _{<i>t</i>}	0.725	0.056	0.0001	Coh ₄ × Age _{<i>t</i>}	0.186	0.047	0.0001
Coh ₂ × Ttd _{<i>t</i>}	0.095	0.043	0.025	Coh ₂ × Ttd _{<i>t</i>}	0.001	0.008	0.878
Coh ₃ × Ttd _{<i>t</i>}	0.261	0.062	0.0001	Coh ₃ × Ttd _{<i>t</i>}	0.023	0.015	0.116
Coh ₄ × Ttd _{<i>t</i>}	0.090	0.063	0.149	Coh ₄ × Ttd _{<i>t</i>}	0.033	0.015	0.028
Coh ₂ × Size _{<i>t-1</i>}	0.021	0.003	0.0001	Coh ₂ × Size _{<i>t-1</i>}	0.009	0.001	0.0001
Coh ₃ × Size _{<i>t-1</i>}	0.051	0.003	0.0001	Coh ₃ × Size _{<i>t-1</i>}	0.004	0.001	0.0001
Coh ₄ × Size _{<i>t-1</i>}	0.043	0.004	0.0001	Coh ₄ × Size _{<i>t-1</i>}	-0.001	0.001	0.328
Coh ₂ × Age _{<i>t</i>} ²	-0.012	0.004	0.002	Coh ₂ × Age _{<i>t</i>} ²	-0.002	0.004	0.569
Coh ₃ × Age _{<i>t</i>} ²	-0.046	0.006	0.0001	Coh ₃ × Age _{<i>t</i>} ²	0.039	0.007	0.0001
Coh ₄ × Age _{<i>t</i>} ²	-0.047	0.006	0.0001	Coh ₄ × Age _{<i>t</i>} ²	0.032	0.007	0.0001
Coh ₂ × Ttd _{<i>t</i>} ²	0.001	0.004	0.890				
Coh ₃ × Ttd _{<i>t</i>} ²	-0.006	0.006	0.377				
Coh ₄ × Ttd _{<i>t</i>} ²	-0.005	0.006	0.396				
Coh ₂ × Size _{<i>t-1</i>} × Age _{<i>t</i>}	-0.007	0.001	0.0001				
Coh ₃ × Size _{<i>t-1</i>} × Age _{<i>t</i>}	-0.004	0.001	0.0003				
Coh ₄ × Size _{<i>t-1</i>} × Age _{<i>t</i>}	-0.006	0.001	0.0001				
Coh ₂ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>}	0.005	0.002	0.004				
Coh ₃ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>}	-0.010	0.001	0.0001				
Coh ₄ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>}	-0.006	0.002	0.001				
Coh ₂ × Age _{<i>t</i>} × Ttd _{<i>t</i>}	-0.025	0.005	0.0001				
Coh ₃ × Age _{<i>t</i>} × Ttd _{<i>t</i>}	-0.052	0.008	0.0001				
Coh ₄ × Age _{<i>t</i>} × Ttd _{<i>t</i>}	-0.032	0.009	0.0002				
Coh ₁ × Size _{<i>t-1</i>} × Age _{<i>t</i>} ²	0.0007	0.0001	0.0001				
Coh ₂ × Size _{<i>t-1</i>} × Age _{<i>t</i>} ²	0.001	0.0001	0.0001				
Coh ₃ × Size _{<i>t-1</i>} × Age _{<i>t</i>} ²	0.0004	0.0001	0.005				
Coh ₄ × Size _{<i>t-1</i>} × Age _{<i>t</i>} ²	0.0009	0.0002	0.0001				
Coh ₁ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>} ²	-0.001	0.0001	0.0001				
Coh ₂ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>} ²	-0.002	0.0002	0.0001				
Coh ₃ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>} ²	-0.0007	0.0002	0.0001				
Coh ₄ × Size _{<i>t-1</i>} × Ttd _{<i>t</i>} ²	-0.001	0.0002	0.0001				
	SD						
Plant identity (random)	0.559			Plant identity (random)	0.598		

However, plasticity is likely to be greater and perhaps more responsive to environment when the plant is young and developing, if only because developmental plasticity should be greatest in early life and should set the stage for the kinds of plasticity of which organisms are capable of later in life (Diggle 2002; Leimar, Hammerstein & Van Dooren 2006). In later ages, adaptively plastic shifts may be slower

or not as great. Thus, adaptive shifts in size may become less likely with increasing age, and this decline in ability to adapt at the within-individual level may be spurred by extrinsic stressors. In other herbaceous species, body architecture may also be plastic and as important as size, but our system does not offer sufficient architectural plasticity to test this hypothesis.

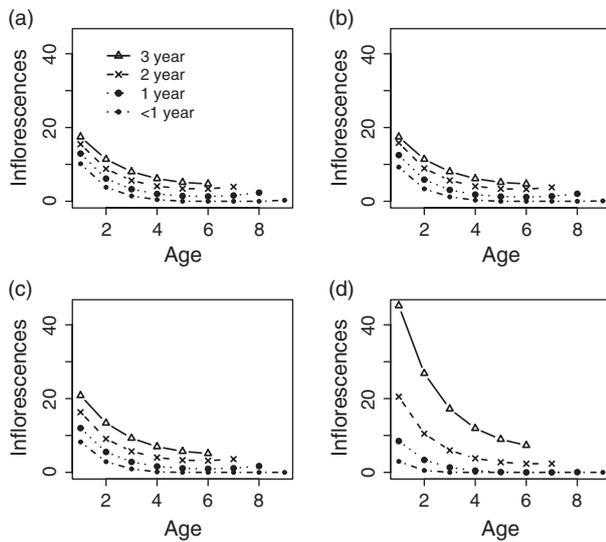


Fig 4. Predicted inflorescence production of *Plantago lanceolata* in a long-term field study in Shadwell, Virginia, as a function of age, time to death and previous size in cohort 1. On the y-axis, inflorescence number is for the associated age on the x-axis. We include model predictions for 3, 2, 1 and < 1 years prior to death, and these predictions are estimated from the best-fit mixed model (Table 1). Panels a–d indicate trends of plants with 1, 5, 10 and 50 leaves in the previous year, respectively.

Fertility patterns were consistent with the damage accumulation hypothesis as well. The closer the individual was to death, the fewer the inflorescences produced (Fig. 4). Our data contradict some more contemporary models of senescence, such as the disposable soma, which postulates that reproduction should increase in old age as the organism shifts resources away from survival and growth to reproduction (Kirkwood 1977). While such terminal investment may be common in age-structured species such as many birds (Ricklefs 2000), perennial plants may not express such patterns because of the lesser influence of age on demographic patterns.

The theory of negative senescence suggests that plants and other organisms must keep growing to maintain some critical size above which mortality is minimal and fertility is very high (Vaupel *et al.* 2004). Size is often a proxy for vitality in studies of plant fitness. In animals, increased size results in increased chance of successful reproduction as well as decreased mortality (Wilbur & Collins 1973; Doak, Kareiva & Klepetka 1994). However, in plants, growth may not be constantly positive, and consistently positive growth may not necessarily be adaptive. Herbaceous perennial plants may even reach a maximal size dictated by the senescence of older rooting tissue, as in the case of some orchids (Kull 1999). In trees, the loss of branches or death of vascular tissue may cause sudden and dramatic shrinkage. For example, living bristlecone pines (*Pinus aristata*) have some of the greatest longevities ever recorded, but may be alive only as thin strips of vascular tissue on otherwise dead tree trunks (Finch 1998). Shrinkage may be adaptive under some circumstances, particularly as a means of dealing with a temporally variable environment (Salguero-Gómez & Casper 2010), and high growth

can increase mortality if the environment then shifts to a more stressful state (Shefferson & Roach 2010). Thus, some analyses of plant demography may make unrealistic assumptions about growth, and these assumptions may translate into unrealistic predictions of life span.

We have shown that a system without evidence of actuarial senescence may still exhibit evidence of physiological senescence suggestive of the accumulation of damage with age. Actuarial senescence has been noted in a number of iteroparous herbaceous perennial species (Silvertown, Franco & Perez-Ishiwara 2001), but patterns in *Plantago* have been more complex to expose (Roach 2012), requiring further cohorts and periods of stress to identify (Roach & Gampe 2004; Roach, Ridley & Dudycha 2009). We encourage further research on age-determinate vs. age-indeterminate factors at work in senescence, particularly in plants and other organisms in which they have not been well documented. Studies of plasticity mechanisms may be particularly important, given that age-indeterminate mechanisms of senescence may be linked to the breakdown of sensitivity or responsiveness to environmental change.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Invasion analysis methods and results.

Figure S1. Annual mortality decreases with increasing size in *Plantago lanceolata*.

Figure S2. Mean size of *Plantago lanceolata* individuals per year.

Figure S3. Annual fertility as a function of size in *Plantago lanceolata*.

Figure S4. Mean inflorescence production of *Plantago lanceolata* individuals per year.

Figure S5. Predicted size of *Plantago lanceolata* as a function of age, time to death and previous size in cohort 2.

Figure S6. Predicted size of *Plantago lanceolata* as a function of age, time to death and previous size in cohort 3.

Figure S7. Predicted size of *Plantago lanceolata* as a function of age, time to death and previous size in cohort 4.

Figure S8. Predicted inflorescence production of *Plantago lanceolata* as a function of age, time to death and previous size in cohort 2.

Figure S9. Predicted inflorescence production of *Plantago lanceolata* as a function of age, time to death and previous size in cohort 3.

Figure S10. Predicted inflorescence production of *Plantago lanceolata* as a function of age, time to death, and previous size in cohort 4.