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Experimental Gerontology 36 (2001) 687–694

Experimental Gerontology

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# Environmental effects on age-dependent mortality: a test with a perennial plant species under natural and protected conditions

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**Abstract**

Most experimental studies of senescence have been done with short-lived organisms under controlled laboratory conditions and it is not clear whether the insights gained from these studies can be broadly generalized. This study was designed to detect senescence in a natural population and to compare the patterns of mortality for a single species in natural and protected conditions. It was done with *Plantago lanceolata*, a perennial plant for which the demography of a large population of individuals in their natural environment is relatively straightforward. An initial cohort of 10,000 individuals was established in the natural field environment. In order to separate the effects of environment- and age-dependent factors on mortality, an additional cohort was planted in the field one year later. To study the demography of mortality under protected conditions, a population of 1000 individuals was established in the greenhouse. The results of the comparative analysis of two different-aged cohorts in the field and of the field and greenhouse populations show that senescence patterns can be very plastic. The results show that senescence in the natural environment is caused by an increased vulnerability of older individuals to environmental stress. Under the protected environmental conditions of the greenhouse senescence was negligible. 

*Keywords*: Mortality rate; Age-dependent mortality; Age-independent mortality; Intrinsic senescence; Different-aged cohorts; Natural populations; Protected populations; Plants

## 1. Introduction

Our current understanding of mortality patterns is limited by the experimental conditions under which demographic aging has been studied. To date, most of our understanding of the process of aging has been derived from short-lived organisms that have been studied in an

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0531-5565/01/$ - see front matter 
PII: S0531-5565(00)00235-7

environment less harsh than that in which their life history evolved (Charlesworth and Partridge, 1997). Humans live under the protected conditions of civilization, and all of the other species commonly used in aging studies, *Drosophila*, medflies, nematodes, and mice have been studied under the protected conditions of the laboratory. Can the insights gained about the demography and genetics of aging from limited species, under protected conditions, be broadly generalized to all species under all experimental conditions? Fifty years ago, Medawar (1952) suggested that senescence, defined as an increased rate of dying with increased age, was characteristic of populations in 'captive' protected environments, for which the level of random mortality had been reduced. His general hypothesis was that life in the wild is short, and hard, and that genes governing late-life processes in the wild do not matter because few individuals survive to old age. He thus predicted that only in populations sheltered from high extrinsic mortality factors, which thus had an increasing proportion of individuals living to late ages, could senescence patterns be observed. Medawar's illustrative examples included man and *Drosophila*, both of which under historically improved or protected laboratory conditions have an increased proportion of individuals surviving the early-life stages, and both of which manifest an increasing rate of mortality late in life. There are two questions raised by Medawar's hypothesis. First, can an experiment be designed to detect senescence in a natural population despite a high level of extrinsic, age-independent mortality? Secondly, how do the patterns of mortality for a single species compare in natural versus protected conditions, and is senescence most apparent under protected conditions? This study is designed to address these two questions.

There has been no direct test of Medawar's hypothesis because comparative studies of populations in wild versus captive environments are difficult to undertake, particularly with animals. Not only do we not know very much about the comparative patterns of mortality under controlled and natural conditions but our understanding about mortality in natural populations alone is very limited. Demographic studies in natural populations are often based on small sample sizes, thus the resulting mortality estimates of late-age are not very precise (Finch, 1990). Field studies with animals often have the additional experimental and statistical problems inherent to mark-recapture data (Lebreton et al., 1992), which adds further imprecision to the mortality estimates. In order to avoid these problems, this study used a plant, *Plantago lanceolata*, to study patterns of mortality under both natural and controlled conditions. Plants offer unique opportunities for natural field studies on senescence (Roach, 1993). The demography of plants is relatively easy compared to most animals, and a large population of individuals of known age can be marked in the wild and followed for their entire life span. Plants can be studied in their natural environments with minimal experimental disturbance, and growing plants under greenhouse conditions can easily create controlled, protected populations.

One of the major problems associated with studying populations in their natural field environments is that these populations are often subject to harsh biotic and abiotic factors, which can result in a high level of age-independent mortality. Variation in these extrinsic mortality factors over time can mask any age-dependent mortality. Experimental designs of field studies need to be able to separate age-dependent from age-independent variation in mortality patterns. This separation was

achieved in this study through a comparative analysis of the mortality patterns of two cohorts grown concurrently in the field. These two cohorts shared the same environment but differed in age. It was thus possible to separate the effects of time- and age-dependent variation on the rates of mortality.

## 2. Materials and methods

*P. lanceolata* (ribwort plantain) is a plant with a nearly cosmopolitan distribution (Van der Aart and Vulto, 1992). It is a short-lived herbaceous perennial with a basal rosette which remains green all year. Reproduction is by wind pollination and individuals are self-incompatible. The natural habitat for this species is a mown field. The field site for this study was located in Durham, NC, on a long-term research site that has been maintained as a mown field for over 50 years. Seeds, from which the experimental plants were derived, were collected from this same field.

For the initial experiment, a cohort of 10,000 six-week-old seedlings was planted into the field. The seedlings had been raised in sterilized field soil, which had been collected from the same field where the seed had been collected, and they were grown in the Duke University Phytotron, a controlled growth facility. The seedlings for this experiment were thus of uniform age, yet sufficient size and precondition to maximize survival during transplantation.

Individual seedlings were planted and marked with an identification label, and except for the 3 cm hole created for each seedling, the surrounding plant community was left undisturbed. The seedlings were planted in a randomized block design within a $23 \times 24$ m$^2$ area. Within each block, plants were located 15 cm apart in 10 staggered rows each 10 cm apart. Individuals were censused every four weeks for mortality.

This initial cohort was planted into the field in April 1997. To separate the effects of age and environment, an additional cohort of 10,000 seedlings was planted in April 1998 into adjacent blocks in the field. The same protocol was used to collect seed and to raise the seedlings prior to planting. The census of both cohorts was done concurrently in order to make a direct comparison of the fluctuations in mortality in the different populations. The mortality patterns of the different-aged cohorts were then compared to evaluate age- versus environment-dependent mortality patterns.

To evaluate the demographic patterns of mortality under protected conditions, a parallel experiment was set up in the greenhouse in April 1997. The greenhouse population initially consisted of 1000 individuals. Each individual was grown in its own pot, and the plants were closely monitored to maintain ideal conditions including water, fertilizer, weeding, and spraying for pests as needed. In order to ensure that the soil environment of these plants did not deteriorate over time and continued to remain as favorable as possible, the plants were repotted every six months in fresh soil. Similar to the field experiment, the plants in the greenhouse were censused once a month.

Mortality rates were calculated, as $q_x$, the number of deaths between age $x$ and $x + 1$ divided by the number of individuals alive at age $x$. These monthly mortality rates were standardized to 31-day intervals to reflect minor variation in the number of days included in the census interval.

## 3. Results

Mortality in the natural field environment is very high (Fig. 1). Of the original 10,000 individuals in cohort #1, 48% died within the first year, and by the third year of the study less than 10% of the original individuals were still alive (Fig. 1). Field mortality is not constant, rather it shows both yearly and seasonal variation. Across years, mortality was highest in the second year, and lowest in the third and fourth years. The yearly mortality rates, after reproductive maturity, were 0.78, 0.20, and 0.09, for years 2, 3, and 4, respectively. The relatively high mortality in the second year may be partially attributed to extremely dry summer weather ('El Nino'). In addition to variation among years there is also within-year variation in mortality. Mortality consistently peaks during the summer months and is separated by a period of substantially lower, stable mortality during the winter and early spring (Fig. 1).

The second cohort of field plants was established in order to differentiate environmental- and age-dependent variation in mortality. Both cohorts were growing adjacent to each other, and thus experienced the same environmental conditions, yet the second cohort was exactly one year younger than the first. Any significant differences in the mortality rates between the two cohorts can thus be attributed to age. The first year of growth for cohort #2 was in 1998, during the extreme 'El Nino' weather conditions. Juvenile mortality was high and 95% of the seedlings from cohort #2 died during this first summer. To test patterns of senescence, the most important comparative analysis is an evaluation of the two cohorts following maturation of both cohorts. In the third and fourth years of this study (1999 and 2000), there was a consistent difference in the relative

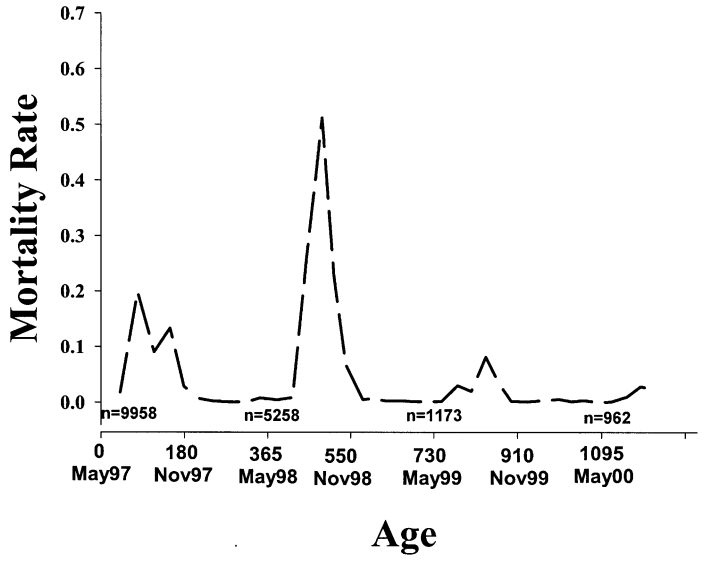


Fig. 1. Mortality rate of *P. lanceolata* individuals in a natural field population. The *x*-axis includes both the calendar date and the age of individuals in the population in days. The numbers along the base of the curve give the number of surviving individuals at yearly intervals.

mortality patterns of the two cohorts. During the summer and early fall, cohort #1 showed a significantly higher peak in mortality than cohort #2 in both years (contingency test, $p < 0.001$ for both 1999 and 2000, Fig. 2). It should be noted that the total number of individuals alive in each cohort was still relatively large during this period (Fig. 2), thus these results reflect true age-specific variation in mortality. During the winter months there was minimal mortality in both cohorts and there were no differences between the two cohorts (Fig. 2).

Mortality under the protected conditions of the greenhouse was negligible and aseasonal (Fig. 3). After four years of study, 94% of the individuals in the greenhouse were still alive whereas only 9% of the individuals in cohort #1 in the field were still alive after this same period.

## 4. Discussion

The results of this study demonstrate that age-dependent patterns of mortality can be masked by high rates of mortality caused by age-independent environmental factors. In the natural field study, there was evidence for large seasonal variation in mortality, which was primarily caused by the extreme environmental conditions of hot, dry, summers. Among years, there was variation in these environmental conditions that resulted in interannual variation in yearly mortality rates. For cohort #1, if juvenile mortality in the first year is ignored, there was an overall decline in the yearly rate of mortality as the plants progressed through their second, third and fourth years. Without a comparative analysis of a second cohort, it would not be possible to determine whether this yearly decline in mortality was due to an absence of aging and a decrease in age-specific mortality, or to yearly variation in external environmental factors. The comparative analysis of the two cohorts

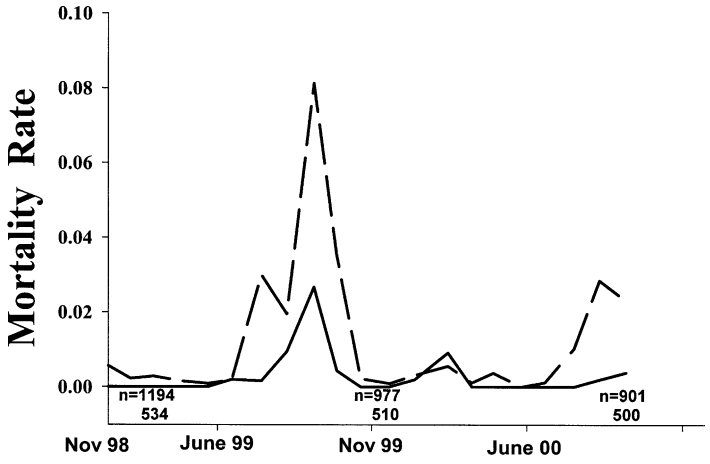


Fig. 2. Post-maturation mortality rates of cohort #1 (dashed line) and cohort #2 (solid line) from November 1998–September 2000. The numbers along the base of the curve give the number of surviving individuals for cohort #1 (top) and cohort #2 (bottom).

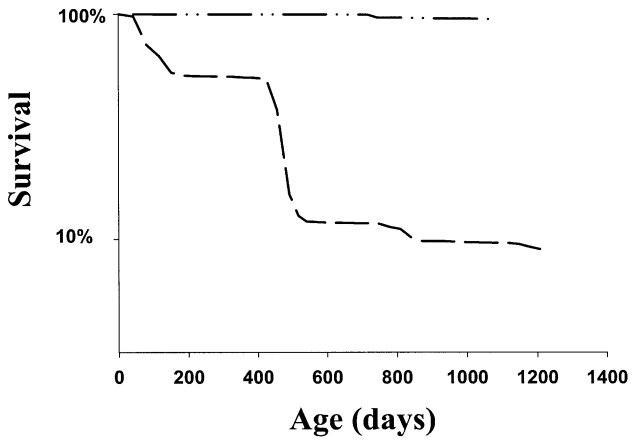


Fig. 3. Survival curves (log %survival) of *P. lanceolata* in the field (cohort #1, dashed line) and in the greenhouse (dotted–dashed line).

demonstrates that despite an overall yearly decline in mortality, there is evidence, within years, for an age-dependent increase in mortality rates.

Age-dependent variation in mortality can be caused by an increase in vulnerability of older individuals to the external environment, or to an increase in intrinsic physiological decline. The results of this study suggest that the higher mortality rate of the older-aged cohort of *P. lanceolata* is due an age-dependent increase in vulnerability to the external environment. After maturation of both cohorts, when cohort #1 was in its third and fourth years and cohort #2 was in its second and third years, respectively, the younger cohort consistently showed a lower rate of mortality than the older cohort during the summer months. It is interesting that it was only during the summer months, when the external environmental conditions were most extreme, that these differences between the cohorts were apparent, but there were no differences in the mortality rates of the two cohorts during the winter months. This seasonal variation in the differences between the two cohorts makes it possible to distinguish between senescence caused by an age-dependent increase in vulnerability to the external environment, and senescence caused by an intrinsic physiological decline. In this study, underlying a large age-independent mortality there is an age-dependent increase in mortality caused by an increased vulnerability to the extreme environmental conditions during the summer. The absence of any difference between the two cohorts during the winter suggests an absence of any intrinsic physiological difference between the cohorts, and thus there is no evidence for senescence independent of an age-dependent interaction with environmental stresses.

The absence of any intrinsic physiological decline in *P. lanceolata* is also evident from the comparative analysis of the field and greenhouse populations. Medawar (1952) had hypothesized that mortality in populations protected from extreme environmental conditions, should reflect intrinsic senescence. The results of this study suggest that senescence in *P. lanceolata* is negligible under greenhouse conditions, and there is no apparent age-specific pattern to the mortality. The extreme longevity of individuals in the greenhouse

does not rule out the possibility that there may be a slow increase in age-specific mortality. It is apparent, from the results of this study, however, that age-specific variation in mortality under the environmental conditions of the greenhouse does not occur at the same ages as it does under the environmental conditions of the field. The maximum age of *P. lanceolata* reported from field data is seven years (Mook et al., 1992). With the current negligible mortality, it is clear that individuals in the greenhouse population will live beyond this reported maximum. It may be that at older ages, beyond four years, individuals in the field and the greenhouse populations will begin to show an increasing rate senescence independent of external environmental conditions.

The comparative analysis of the populations of *P. lanceolata* in the field and in the greenhouse demonstrates the plasticity of mortality patterns. In the field environmental plasticity is evident both within years with seasonal variation in mortality and between years with variation in overall rates of mortality. The plasticity of mortality is most apparent in the comparison of field and greenhouse populations. After four years, just over 9% of the field population remained alive, yet in the greenhouse 94% of the population was still alive. Other studies have also demonstrated that environmental factors are a major determinate of both the overall rate of mortality and of life span. Studies with bristlecone pine for example have demonstrated a three-fold difference in life span between plants grown at low and high elevations (Lanner, 1999). Similarly, ferox trout, found in deep waters have longer life span than populations found in shallower waters (Greer, 1995; Mangel, 1996). For both of these species, specific environmental conditions created negligible rates of senescence.

There have only been two other studies of this kind comparing the mortality rates of field and protected populations. In one other study with a plant species, *Rumex hastatulus*, Roach (2001) found a high rate of age-independent mortality in the field and a late-age increase in mortality in the greenhouse. The late-age increase in mortality was only apparent long after all individuals in the field had died, suggesting that the increase in mortality may have been due to intrinsic senescence at extremely late ages. In the study with *R. hastatulus*, it was not possible to distinguish time-dependent environmental changes from aging because only one cohort was used. Thus the increase in late-age mortality may have been caused by some unmeasured environmental change (Roach, 2001). In a comparative study of natural and zoo populations of 28 species of birds, Ricklefs (2000) found no differences in aging between populations in the two types of environments. For birds, these results suggest that age-related mortality is associated with intrinsic causes of death that is independent of the external environment.

Mortality rate is a composite measure of deaths due to age-independent and age-dependent factors. Additionally, an age-dependent increase in mortality may be caused by two mechanisms. First, senescence may be due to an increase with age in intrinsic causes of death caused by a physiological decline. This first mechanism of senescence is independent of the external environment. Secondly, senescence may be caused by an interaction between age and environment that is manifest as an increasing susceptibility of older individuals to extrinsic factors (e.g. adverse weather). In humans, and in birds, aging is associated primarily with increases in intrinsic causes of death (Hayflick, 1994; Ricklefs, 2000). The results of this study with a plant species show that these results cannot be generalized to all organisms expected to show senescence. In the absence of

environmental stress, in other words during the winter months in the field and in the greenhouse, senescence is negligible. Yet, with the proper experimental design, senescence can be detected in a natural population despite high levels of extrinsic mortality. Senescence in the field was caused by an increased susceptibility of the older-aged individuals to environmental stress. The dogma about aging in natural populations has been that there is a relatively high age-independent rate of mortality that can obscure age-dependent mortality. Senescence is thus difficult to detect because few individuals survive to ages when biological senescence is expressed. In this, the largest demographic study in a natural population, senescence was demonstrated despite a high rate of age-independent mortality. Without two large concurrently grown cohorts, these age-dependent mortality patterns would be masked by extreme age-independent mortality. Large demographic studies in natural populations are not always feasible, but the results of this study demonstrate that in order to understand the variation in patterns of senescence, we need to increase the diversity of the types of species studied and the conditions under which they are studied.

## Acknowledgements

This work was supported by NIH Grant P01 AG08761. I would like to thank Jeff Dudycha for his comments on the manuscript.

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