# Age-Specific Demography in *Plantago*: Uncovering Age-Dependent Mortality in a Natural Population

**Deborah A. Roach**[1,*] **and Jutta Gampe**[2,†]

1. Department of Biology, University of Virginia, Charlottesville, Virginia 22904-4328;
2. Max Planck Institute for Demographic Research, Rostock, Germany 18057

ABSTRACT: Accurate measures of age-dependent mortality are critical to life-history analysis and measures of fitness, yet these measures are difficult to obtain in natural populations. Age-dependent mortality patterns can be obscured not only by seasonal variation in environmental conditions and reproduction but also by changes in the heterogeneity among individuals in the population over time due to selection. This study of *Plantago lanceolata* uses longitudinal data from a field study with a large number of individuals to develop a model to estimate the shape of the baseline hazard function that represents the age-dependent risk of mortality. The model developed here uses both constant (genetics, spatial location) and time-varying (temperature, rainfall, reproduction, size) covariates not only to estimate the underlying mortality pattern but also to demonstrate that the risk of mortality associated with fitness components can change with time/age. Moreover, this analysis suggests that increasing size after reproductive maturity may allow this plant species to escape from demographic senescence.

*Keywords:* age-dependent mortality, *Plantago lanceolata*, natural population, hazard regression, plant senescence.

Age-dependent mortality is a critical variable in our calculations of fitness and thus to our understanding of natural selection, yet accurately quantifying this variable in natural populations is very difficult. In order to understand how natural selection has acted to shape mortality patterns, it is important to conduct experiments under the

* Corresponding author; e-mail: droach@virginia.edu.

† E-mail: gampe@demogr.mpg.de.

same environmental conditions that the population has experienced for many generations of selection; yet in these environments, high levels of age-independent mortality can decrease population size, and the external factors influencing mortality may not be constant. Demographic studies in laboratory populations, designed to study senescence, have shown that postmaturation mortality is initially a monotonically increasing function (cf. Vaupel et al. 1998; Promislow et al. 1999). This is in contrast to studies in natural populations that have shown that seasonal variation in environmental conditions and reproduction can cause variation in rates of mortality (Vavrek et al. 1997; Roach 2003). Acceleration of mortality rates at older ages has been documented in natural populations (cf. Promislow 1991; Loison et al. 1999), but it is not clear whether this effect is the result of increases in external or internal causes of death (Zwaan 1999). In order to determine whether a species shows demographic aging, the age-independent shifts in mortality caused by changes in external forces must be distinguished from age-dependent changes in mortality. The objective of this study is to develop a model to estimate the shape of the baseline hazard function that represents the age-dependent risk of mortality. Abiotic factors, from weather to fine-scale spatial variation, and biotic factors such as genetics, size, and reproduction are used as covariates in the model to reveal the underlying shape of the age-dependent mortality function in an experimental field population.

In practice, our estimates of fitness are usually calculated using information on reproductive components of fitness from individuals, yet our estimates of mortality are calculated at either the population level or some subpopulation grouping of individuals, for example, the mortality for different families within a population or environment. Mortality is then quantified as the number of deaths within a census interval divided by the number of individuals alive at the beginning of the interval. These population-level measures of mortality may be biased due to phenotypic mortality selection (Endler 1986). Within a population, even within an inbred strain of individuals in a laboratory setting (Curtsinger et al. 1992), individuals are

heterogeneous with respect to their risk of mortality, and this can result in a compositional change in a population over time. This change can then lead to an erroneous estimation of age-specific mortality because later-age mortality patterns will be calculated from a nonrandom subset of individuals (Vaupel and Yashin 1985). One approach to minimize the influence of heterogeneity is to do separate analyses on relatively homogeneous groups of individuals within the population. "Groups" in this case can be defined by some measure of quality, for example, time of reproduction (cf. McDonald et al. 1996). The disadvantage to this approach is that it may lead to many groups that are too small for a given difference in mortality to be detected. Moreover, it is often difficult to define homogeneous groups and the "quality" of an individual (Cam and Monnat 2000). An alternative approach to a population-level measure of mortality is to model an individual hazard function. Individual risk models have been developed and used for human populations and captive animals, but except for modified capture-recapture models (cf. White and Burnham 1999; Conroy et al. 2002), they have rarely been used in ecology and evolution studies (Zens and Peart 2003). The advantage of individual hazard models is not only that they can demonstrate the impact of cohort selection in the deviation between population- and individual-level survival estimates (e.g., Cam et al. 2002) but also that they can help to reduce the bias of our estimates of mortality. The hazard regression model developed in this study uses both constant and time-varying covariates to improve our estimates of age-dependent mortality.

Even when mortality is quantified using an individual hazard function, there are several other experimental design issues that must be overcome to obtain an accurate description of age-dependent mortality. First, it requires that large numbers of individuals be tracked longitudinally for a long period of time. A review of the literature on life table data from natural populations has shown that survivorship curves are often based on small sample sizes and that thus the resulting mortality estimates are not very precise (Finch 1990). Large sample sizes are particularly important in experimental situations where potentially high levels of extrinsic mortality may result in a very small proportion of the population living to late ages. From our current knowledge we assume that individuals that become the oldest old do so because they are a nonrandom sample of their cohort, but we need longitudinal data linking mortality, fertility, and growth across life stages from more model organisms to determine what kinds of individuals, in demographic terms, survive to become the oldest old (Partridge and Mangel 1999).

Determining the age-dependent patterns of mortality for species in their natural environment is critical for us to begin to gain an understanding of the breadth of senescence processes. A second objective of this study is to expand the breadth of our understanding of senescence to plants. A wealth of information from the fields of plant demography and plant population biology suggests that plants may offer unique opportunities for studies on senescence. Senescent declines may be found in both annual and perennial species, but most of the data are based on short-term, relatively small experiments (for reviews, see Watkinson 1992; Roach 1993; Pedersen 1999). Silvertown et al. (2001) reviewed published life tables from iteroparous perennial plants and found that over one-half of the species showed higher mortality at later ages, but their interpretation of these results was confounded by both small sample sizes and the possibility that this increase in mortality could have been environmentally induced. The demography of plants is comparatively easy because plants can be studied in their natural environments with minimal experimental disturbance. It is possible to mark and follow individuals for their entire life span; thus it is relatively easy to follow large numbers of individuals in a population, and there are no problems with recapturing individuals. The results of the study presented here suggest that growth after reproductive maturity may facilitate an escape from senescence.

## Material and Methods

### Field Experiment

*Plantago lanceolata* (ribwort plantain) is a short-lived perennial species with a basal rosette. It germinates in the fall and spring and flowers in midsummer. It remains green all year; thus individuals can be easily followed, and an accurate assessment of mortality can be made in every month. The "natural" habitat for this species is roadsides and mown fields, and it is found in these habitats throughout the world (Van der Aart and Aulto 1992).

The field site for this study was located in Durham, North Carolina, on a long-term research site, which had been maintained with one or two mowings per year for over 50 yr. Seeds, from which the experimental plants were derived, were collected from naturally established plants in the same field where the experiment was conducted. *Plantago lanceolata* is a wind-pollinated species; thus the seeds collected from a maternal plant were most likely related as half-sibs. For the study described here, 30 half-sib families, with approximately 150 (range 103–171) individuals per family, were used for the analysis. This study was part of a large, multicohort demographic study, and the details of the variation in demography across cohorts are described elsewhere (Roach 2003). The analysis presented here is restricted to a subset of cohort 1 plants (only blocks 1–3 and the 30 largest families) for which we

had complete data for all of the covariates that were included in the regression model.

A total of 4,476 individuals were planted into the field in April. The seedlings were planted in a randomized block design with complete replication of all families across three blocks within a 15 × 24-m$^2$ area. The details about how the seedlings were raised before establishment in the field are given elsewhere (Roach 2003). After transplanting, individuals were censused every 4 wk for mortality for 4.5 yr, until only 7% of the population remained alive. Monthly mortality was calculated both at the population level as $q(x)$, the number of deaths between age $x$ and $x + 1$ divided by the number of individuals alive at age $x$, and as an individual hazard rate (see "Model Development"). Size of the experimental individuals was quantified every 6 mo as the total number of leaves. Leaf number is highly correlated with aboveground biomass ($r = 0.70$, $P < .0001$, $n = 80$). Individuals began reproducing in their second year, and seeds were collected continuously during the reproductive season. Reproduction was quantified as the number of inflorescence spikes produced by an individual plant.

Meteorological data, including daily minimum, maximum, and average temperatures, and precipitation were obtained from the State Climate Office of North Carolina at North Carolina State University in Raleigh. The West Durham weather station is located approximately 3 miles from the field site.

## Model Development

The objective of this modeling analysis was to determine the shape of the age-dependent influence on mortality after all other covariates had been incorporated into the model. There were several factors that were critical to the development of the model. First, information on the age at death $T_i$ of an individual $i$ was limited to the monthly census intervals and thus was discrete. Because many individuals died at the same age, that is, the same census interval $t$, the model had to accommodate a large number of ties in death times for many census dates. Furthermore, some of the covariates in the model, like weather, size, and reproduction, changed from one census interval to the next. We thus needed to allow for a time-varying component for these effects. Finally, because we were interested in determining the age-dependent influence on mortality, we needed a model that would allow us to explicitly estimate the baseline hazard. The most common model in survival analysis, the Cox proportional hazards model (Cox 1972), circumvents the estimation of this baseline hazard by focusing on the effect of the other covariates, apart from time (age). Additionally, the Cox model assumes observations in continuous time, and estimates in

the presence of tied events have to be corrected for by formulas that can become laborious if the number of ties is large. We therefore chose a discrete-time survival model to analyze our data (Efron 1988; Fahrmeir and Tutz 1997).

The discrete hazard $h_i(t)$ of individual $i$ at time $t$ is defined as the conditional probability that the individual dies at age $t$ given that it has survived up to age $t$, that is, $h_i(t) = P(T_i = t | T_i \geq t)$, which is the discrete-time analog to a continuous hazard rate. In discrete-time survival models an individual's contribution to the likelihood function is broken down into a sequence of discrete time units that can be treated as distinct observations with no need to correct for pseudoreplication (Allison 1982; Fahrmeir and Tutz 1997). For example, consider an individual dying in some census interval $t^*$: its contribution to the likelihood is $P(T_i = t^*)$ and can be written as

$$P(T_i = t^*) = P(T_i = t^* | T_i \geq t^*)P(T_i \geq t^*)$$

$$= h_i(t^*) \prod_{s=1}^{t^*-1} [1 - h_i(s)].$$

Censored individuals contribute the product

$$P(T_i > t^*) = \prod_{s=1}^{t^*} [1 - h_i(s)].$$

If we denote by $t_i$ the census interval in which individual $i$ either died or was censored, and we denote by $\delta_i$ a censoring indicator that takes the value 1 if the individual died and 0 if censored, then the log-likelihood for the whole sample of $n$ individuals is

$$\log L = \sum_{i=1}^{n} \delta_i \log \left[ \frac{h_i(t_i)}{1 - h_i(t_i)} \right] + \sum_{i=1}^{n} \sum_{s=1}^{t_i} \log [1 - h_i(s)],$$

which is equivalent to

$$\log L = \sum_{i=1}^{n} \sum_{s=1}^{t_i} y_{is} \log \left[ \frac{h_i(s)}{1 - h_i(s)} \right] + \sum_{i=1}^{n} \sum_{s=1}^{t_i} \log [1 - h_i(s)],$$

where $y_{is}$ denotes a binary indicator whether individual $i$ experienced death in interval $s$ ($y_{is} = 1$) or not ($y_{is} = 0$). This is exactly equal to the log-likelihood function of regression models for binary responses (Allison 1982).

To model the dependence of the discrete individual hazard $h_i(t)$ on covariates $x_{it}$, the hazard is linked to an appropriately defined regression function $\eta_{it} = f(x_{it})$ of $x_{it}$. The subscript indicates that the covariates not only may depend on the individual's characteristics but also may vary with time. Standard choices for the link functions are the logistic function $h_i(t) = \exp(\eta_{it})/[1 + \exp(\eta_{it})]$, leading

to a usual binary logit model, or the inverse complementary log-log link $h_i(t) = 1 - \exp[-\exp(\eta_{it})]$, which is a discrete analog to the Cox model but allows for immediate estimation of the baseline hazard. Both link specifications lead to rather similar results, which was also true for our data. We chose the logit link, which is the more popular of the two, because of its easier interpretation of the regression parameters. For logistic regression models the basic risk parameter is the odds of an event, in our case the odds of the hazard $h_i(t)/[1 - h_i(t)]$. If the regression function $\eta_{it} = f(x_{it})$ is linear in the covariates, that is, $\eta_{it} = \beta_0 + \beta_1 x_{1it} + \cdots + \beta_p x_{pit}$, then $\exp(\beta_j)$ gives the factor by which the odds of an event change if the respective covariate $x_j$ is increased by one unit.

Due to their similarity to binary regression models, any software that allows one to fit logistic regression or generalized linear models (GLM) can estimate discrete survival models. We used the GLM function of S-Plus (2000; equivalent to the same function in the freely available software R [R-Project 2004]).

The actual modeling objective was to find the appropriate form of the predictor $\eta_{it} = f(x_{it})$ as a function of the individual covariates. From the design of the field experiment, the covariates used to model the individual hazards $h_i(t)$ were as follows:

*Weather.* Two external time-varying covariates were used to describe the weather conditions, and their effect was specified to occur during the precensus intervals. To characterize the external climate conditions within a precensus interval, we calculated the average of the daily maximum temperatures during the time interval between the current and the previous census and the cumulative precipitation in the last 40 d before a census. All plants experienced the same values of these variables at the same age. To allow for a flexible treatment of these two quasi-continuous variables, they both were coded as factors after selecting appropriate breakpoints based on quantile information on the different variables. As temperature showed a pattern that was piecewise linear, we included this variable via a piecewise linear spline in the final model with breakpoints at 15°, 20°, and 25°C, respectively. Precipitation was coded into four categories: 0–5, 5–10, 10–15, and 15–40 cm. The breakpoints here and elsewhere were always included as right endpoints in the lower interval. The last broad category is the result of two merged categories that showed almost the same effect in the analysis.

*Spatial location.* This is an external covariate that is constant. The data used for this model were from three spatial blocks; thus this was modeled as a three-level factor.

*Genetic family.* This time-constant covariate was included as one level for each of the 30 families.

*Reproduction.* This covariate, given by the number of inflorescences, is the result of an endogenous process and is thus time varying. As an endogenous variable, reproduction was given a lagged effect such that all reproduction 3 wk before a census was allowed to show an impact on mortality. During the nonreproductive season, reproduction was equal to 0. To capture potential nonlinear influences of this continuous variable, it was coded as a factor with the following categories: 0 (no reproduction), 0–5, 5–10, 10–20, 20–35, and 35 to the maximum number of inflorescences, which was 99.

*Size.* The number of leaves per plant was measured in the field every 6 mo (June and November) and is a time-varying covariate. Given the difference in the census interval (monthly) and the size measurement interval, we made the simplifying assumption that size was a constant function between the measurement times. In other words, we carried forward the most recently observed value until the next interval. Size was grouped into the following intervals, again to allow for nonlinearities if necessary: 0–5, 5–15, 15–30, 30–50, 50–75, 75–105, and 105 to the maximum number of leaves, which was 197.

Given that the primary question was how mortality changes with age itself after adjusting for the influence of the other covariates, no rigid parametric assumptions about the baseline function could be made. To avoid interference with the monthly varying weather conditions, we defined age to be a five-level factor implying a year-specific constant baseline that was allowed to adjust freely between years. Given that the experiment started in April and was stopped 4.5 yr later at the end of October, the last factor level refers only to a half-year period. In order to confirm that this last half-year interval did not bias our results, we also ran the model backward in time such that the final yearly interval was a year ending in October and the first year was truncated. This "backward" analysis yielded the same results for the year-specific baseline; thus only the forward analysis is presented here. All factors included in the models were coded as treatment contrasts, that is, by dummy variables with the first category being the reference group.

## Results

### Field Measurements: Population-Level Analysis

The population-level mortality dynamics showed seasonal and yearly fluctuations (fig. 1). The yearly mortality, $q(x)$, was 0.44, 0.72, 0.19, 0.15, and 0.13, respectively, for the 5 yr of the study. This pattern of mortality was influenced by many covariates, including spatial location, reproduction, size, and genetics, each of which is discussed individually below. These factors were included as covariates in the regression model. Environmental effects were also included in the model, and details of their impact on mor-
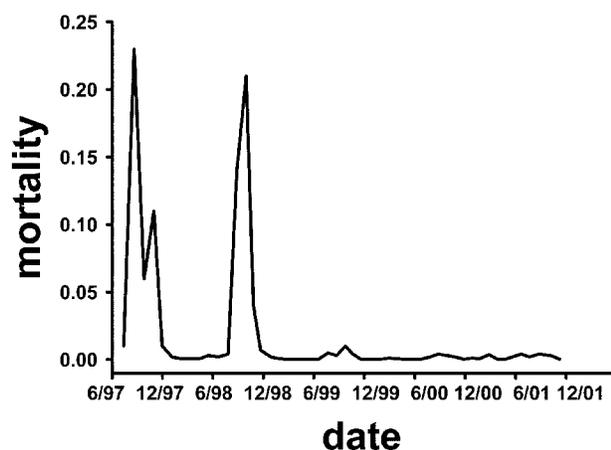
Figure 1: Monthly field mortality by date. The data shown here are a subset of the data reported in Roach (2003) and are limited to cohort 1 in blocks 1–3.

tality in this population have been described elsewhere (Roach 2003).

*Spatial location.* Within the randomized block design, blocks showed differences in mortality across years (Proc Lifetest, log-rank $\chi^2 = 434.40$, df = 2, $P < .001$). A significantly higher percentage of individuals survived in block 2 over the 5-yr study (5%, 19%, and 4%, respectively, for blocks 1, 2, and 3; $\chi^2 = 262.51$, df = 2, $P < .001$), and most of this advantage was derived from a lower rate of mortality at this location during the first 2 yr of life.

*Reproduction.* Individuals in the field flowered for the first time in May of year 2. The risk of mortality varied across levels of reproduction and across years (fig. 2; see also Roach 2003). In the first year of reproduction, individuals with the largest number of inflorescences had the highest mortality. Individuals with minimal or no reproduction had lower mortality. In later years, this hierarchy was reversed such that the class of individuals with the largest level of reproduction had the lowest mortality.

*Size.* There was a large size-dependent component to mortality. Table 1 shows the size distribution of individuals in June of each year and, within each year, those individuals who survived or died before the next June (year 5 is not included here because size was not measured in June of that year). First, looking across years, the mean size (number of leaves) of individuals in the population is increasing with age. Second, except for year 2, the size of the survivors is higher than those who died. Year 2 was the first year of reproduction for this cohort, and the cost of reproduction in that year was higher than in later years.

*Genetic effects.* Across the 30 half-sib families, there were significant differences in the survival patterns over the study period (Proc Lifetest, log-rank $\chi^2 = 50.41$, df =

29, $P < .008$). At the end of the study, the percentage of individuals alive within each family ranged from 4.03% to 12.26%. Reproduction and size also varied across families. In an ANOVA of total lifetime reproduction, including only individuals who survived to reproduce at least once, spatial block and family were both highly significant ($P < .001$). Size at every measurement interval also showed significant block variation at all measurement times ($P < .001$) and a significant family component to variation through June of year 4 ($P < .001$). There were no significant differences in size among families after that date.

### Statistical Model: Hazard Regression Analysis

Sequential inclusion of all variables listed in "Model Development" were performed, and except for genetic family, the addition of each variable led to significant improvements in model fit; thus family was not included in the final model due to the lack of improvement in fit. The two final, most complete models included covariates for spatial block, temperature, and precipitation as fixed effects across years. These two models differed, however, in their treatment of size and reproduction. The population-level analysis had shown that the influence of both size and reproduction on mortality varied across years; we thus contrasted a restricted model in which size and reproduction were forced to act identically for all years with an interaction model in which the impact of size and reproduction was allowed to change from year to year (i.e., size and reproduction were nested within year). Moving from the restricted model with a uniform shape of size and reproduction for all years to the interaction model led to a decrease in residual deviance of $-388.48$ (with an increase of 36 df). This corresponds to an Akaike Information Criterion of 19,641.81 (with 25 df) for the restricted model versus 19,325.33 (with 61 df) for the interaction model. In other words, there was a distinct improvement in the fit with the interaction model despite the increased number of parameters. Thus, our final model was stated as follows:

$$\text{logit } h_i(t|x_{it}) = \beta_0 + f_{\text{block}}(\text{block}_i) + f_{\text{temp}}(\text{temperature}_t)$$
$$+ f_{\text{precip}}(\text{precipitation}_t) + f_{\text{year}}(\text{year}_t) + f_{\text{size}}(\text{size}_{it}) \times \text{year}$$
$$+ f_{\text{reproduction}}(\text{reproduction}_{it}) \times \text{year},$$

where the different functions $f(.)$ denote the respective factor or spline expression, the subscripts denote variables that change between individuals and/or census intervals, and multiplication crosses denote interactions. A complete list of estimates from this final model is given in the ap-
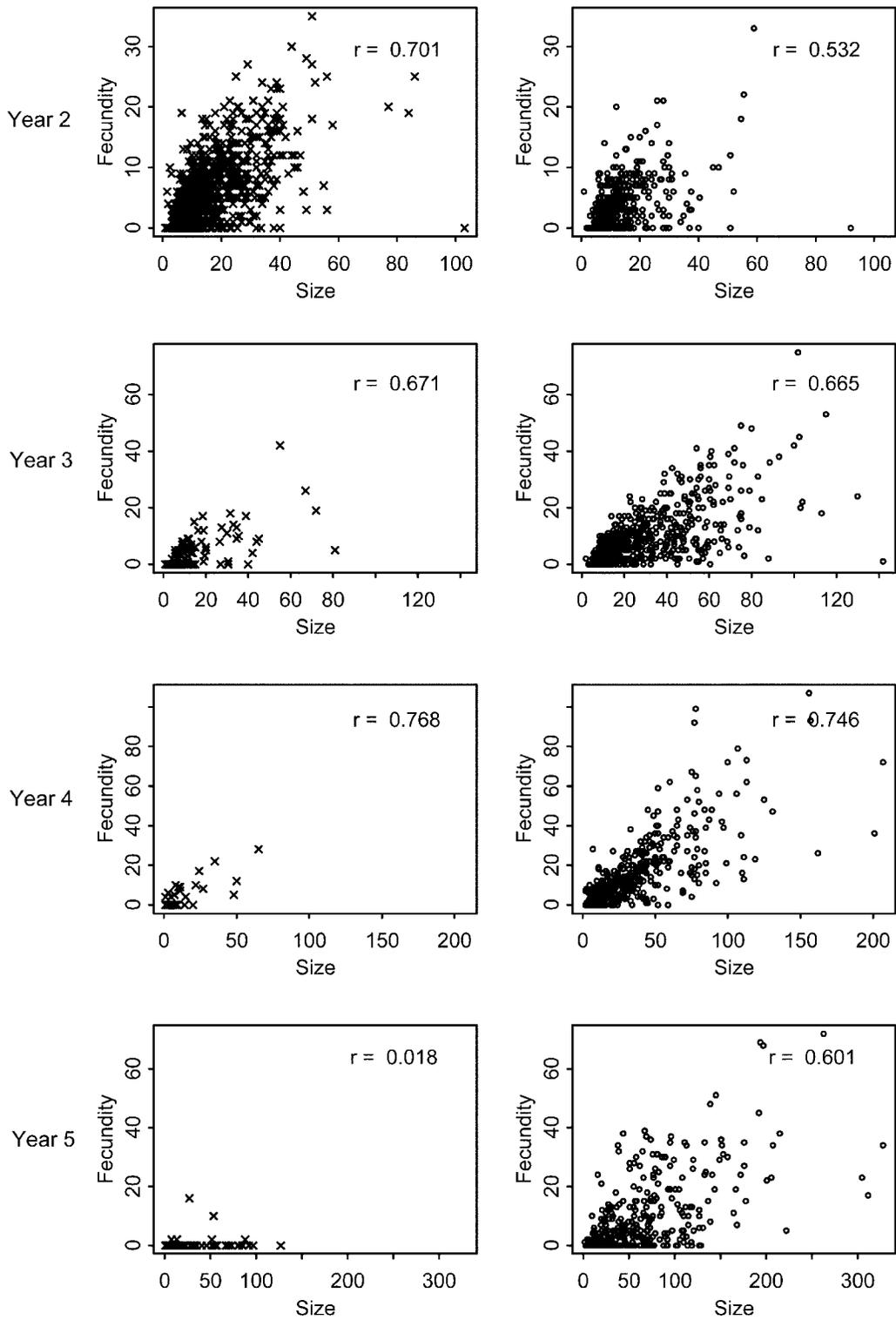
**Figure 2:** Size-dependent fecundity by year for nonsurviving (*left*) and surviving (*right*) individuals in the field population. Survival was measured through the reproductive season from May to October. Pearson's correlation coefficients are given within the individual figures. All correlations were significant (*P* < .00001) except for dying individuals in year 5.

**Table 1:** Size and mortality by year

| Year | Size of survivors + SE | N | Size of dying + SE | N |
|------|-----------------------|------|-------------------|-------|
| 1 | 5.74 + .03 | 2,498 | 4.93 + .03 | 1,932 |
| 2 | 11.06 + .33 | 692 | 11.33 + .22 | 1,786 |
| 3 | 28.57 + .95 | 563 | 14.21 + 1.35 | 129 |
| 4 | 34.29 + 1.34 | 452 | 19.44 + 2.86 | 82 |

Note: For each year, size was measured in June as the number of leaves, and survivorship was calculated through the following June. The size of individuals that would die within the year was significantly smaller than size of the survivors for every year except year 2 ($P < .0001$). $N$ = sample sizes.

pendix available in the online edition of the *American Naturalist* (table A1).

The parameter estimates from the model for block, temperature, and precipitation were consistent with the population-level results. Plants in block 2 experienced a much lower risk than those in the almost equally performing blocks 1 and 3. An increase in temperature led to a decrease in hazard of death up to 20°C and a much steeper increase in mortality risk with temperature above 20°C. The risk of death fell with increasing precipitation except at the highest levels of rain (>15 cm/40-d interval) when the risk of mortality is similar to the driest intervals.

The results show that the shape of the influence of size and reproduction is not consistent across years (fig. 3). A change in size had the most significant influence on the risk of mortality in year 1. One approach to comparing the relative impact of a change in size on the risk of mortality is to consider the odds ratio, which specifies the multiplicative change in the odds of mortality associated with an increase in size. This analysis showed that an increase in size in year 1 from the smallest class (0–5 leaves) to the next larger class (5–10 leaves) decreases the odds of dying (all other factors being equal) by a factor of 0.44 ($\exp [-0.832]$). There is an even more dramatic decrease in the risk of dying for the next size class where an increase in size from the smallest size class to 10–15 leaves decreases the odds of dying by a factor of 0.03 ($\exp [-3.445]$). Size continues to be beneficial at the largest size classes in year 1. The standard errors for the parameter estimates for the largest size classes were very large; thus the exact patterns of change in the risk of mortality are not known.

The size-specific risk of mortality in year 2 is very different from that found for year 1. At the smallest size classes in year 2, an increase in size decreases the risk of mortality, but this decrease is at a slower rate than in year 1. However, for the largest individuals in year 2, the risk of mortality increases. This increase is most likely due to a cost of reproduction. For years 3 and 4, the largest individuals have the lowest risk of mortality. Even with large confidence bands, it is clear that an increase in size significantly reduces the risk of dying in these years. In year

5, size was measured in the previous December and not in June at the early stages of reproduction; thus whereas the values for this year show relatively little change in the risk of mortality across size classes, it is difficult to compare these values with the trends from the other years.

The reproduction-specific risk of mortality also changes across years. Year 2, the first year of reproduction, shows a high risk of mortality for all reproductive classes. Relative to being nonreproductive, the odds of dying for the first reproductive category increase nearly twofold ($1.94 = \exp [0.661]$) and more than threefold for the largest reproductive classes. For years 3 and 4 there is no evidence for a change in the risk of mortality across reproductive classes, although in year 4 individuals with a low level of reproduction do seem to have some mortality advantage over those who are not reproducing. The pattern for year 5 is very different and suggests that the most fecund individuals have the lowest risk of mortality. It should be noted, however, that this is also the year that was missing the June size measures; thus this dramatic shift in the risk of mortality may reflect the absence of a true covariate for size. We also tested the significance of an interaction of size-by-reproduction nested within year, but this was not significant ($P > .40$).

Our objective in this modeling analysis was to estimate the shape of that part of the baseline hazard function that represents the age-dependent risk of mortality. Because we used only one cohort for this study, age and year are synonymous here. The year-specific hazard is given in figure 3. The results show that the risk of mortality increases in years 2 and 3 relative to year 1 but that there is no evidence for a continued increase through years 4 and 5. In the last 2 yr, the baseline hazard is relatively constant, with no evidence for an age-dependent increase in mortality, in other words, no aging.

## Discussion

The results of this study show that there are many factors that influence mortality and that the shape of age-dependent mortality is difficult to discern for populations studied in their natural environment. At the population-level, separate analyses of individuals across spatial blocks, or size, or reproductive class all demonstrated that each of these factors could have a significant influence on differential mortality. In this experiment the results from the mortality calculations from the population-level analysis showed roughly the same general trend as the results from the covariate analysis; yet, given the large impact of both extrinsic and intrinsic factors on mortality, it would be difficult to extrapolate any conclusions concerning the age dependence of these mortality patterns without a covariate analysis. Moreover, in most cases it is unlikely that these
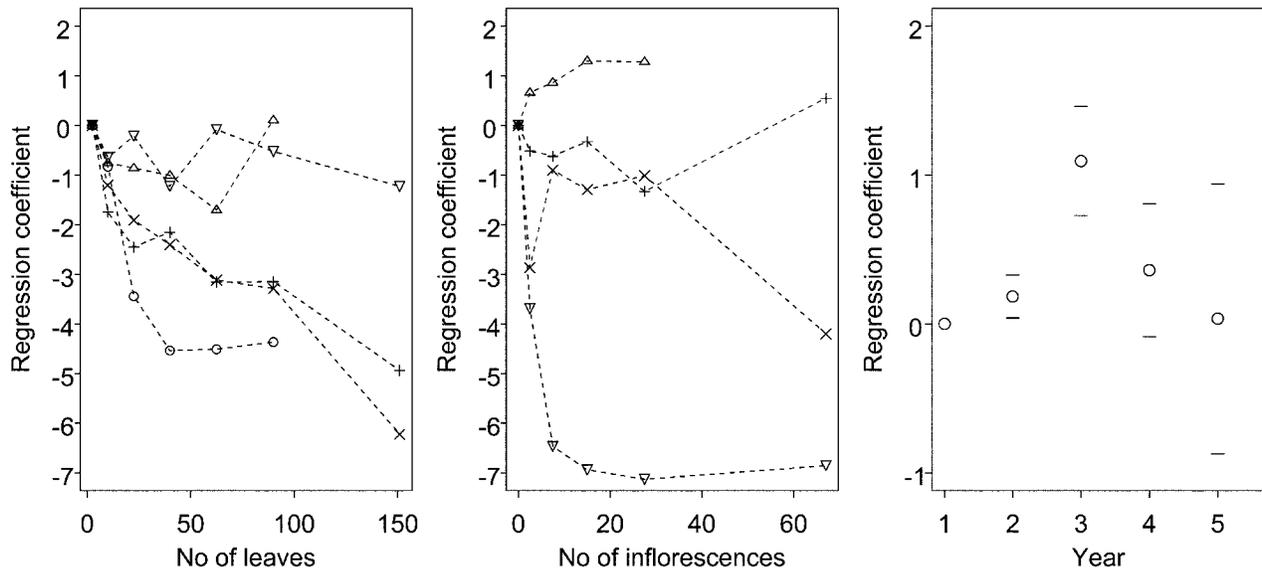
**Figure 3:** Year-specific effects of size (*left*) and reproduction (*middle*) on the risk of mortality and the baseline year-dependent mortality (*right*). Parameter values are plotted over the midpoints of the respective size- or reproductive-classes. *Circle* = year 1, *triangle* = year 2; *plus sign* = year 3; *multiplication cross* = year 4; *upside-down triangle* = year 5. Parameter estimates for year-dependent mortality are given ±2 SE.

two approaches will yield similar results. The results of this study have demonstrated not only that there is no age-dependent increase in mortality at late ages but also that in order to uncover the age-dependent component to mortality the interactions between fitness components and age must be clearly understood.

The influence of size and reproduction on the risk of mortality can change with age. Over years 1, 3, and 4, small increases in size lead to large decreases in mortality risk. Other plant ecological studies have shown size-dependent trends in mortality (cf. Cook 1980; Solbrig 1981; also in *Plantago lanceolata*: Antonovics and Primack 1982), and other recent studies have demonstrated stochastic variation in demographic rates with size (cf. Rees et al. 1999; Rose et al. 2002). The results reported here show that size-dependent mortality can change over time and not necessarily in a linear manner. In year 2, the influence of size on mortality is minimal, yet the cost of increasing reproduction is highest in this year. During this first year of reproduction, the largest-sized individuals had a higher probability of reproducing, and mortality associated with reproduction in this year thus influenced the size-dependent component of mortality. This suggests that not only is there evidence for significant interactions of reproduction- and size-by-year but also there is an important relationship between size and reproduction that can influence mortality patterns, despite the fact that the size-by-reproduction interaction was not significant. The importance of the relationship between size and repro-

duction is underscored by our results from year 5. In that year, our size measures were not taken in June, just before reproduction, and the regression coefficients associated with reproduction had confidence bands too large to make any conclusions concerning the shape of the reproduction risk to mortality. In other words, in the absence of information on size, our understanding of the patterns of reproduction was less clear. Size of an individual plant is very plastic, and at any one particular time individuals within this cohort, who were all the same age, showed large variation in their number of leaves. If we are interested in identifying the age-dependent component of the demography, then empirical evidence for variation in these correlations, and their interactions, is critical (Rees et al. 1999; Rose et al. 2002).

One interaction that may have influenced the results but that was not included in the model was age-by-environment. Because we included only one cohort, there is the possibility that what we are interpreting as age-dependent mortality may be strictly due to environmental effects. If, for example, we had found an increase in mortality with age, then with this design we could not have dismissed the possibility that this increase was not caused by an unmeasured deterioration of the environment. An alternative, experimental approach to avoid this correlation would be to include data from another cohort within the same field. Individuals of different ages would then be experiencing the same environment, and the effects of age and environment could be more clearly separated. In the

experiment reported here, there were other cohorts (Roach 2003), but size, the most important covariate, was only measured on cohort 1; thus the other cohorts could not be included in this modeling analysis. The contrasting population-level demographic patterns found across cohorts within this field (Roach 2003) underscore the need for multiple-cohort studies to unravel the age-dependent component to mortality in the field.

This study has demonstrated that there are many factors, biotic and abiotic, that can have major impacts on mortality patterns in natural populations, and once the age-dependent risk is uncovered, change in the age-specific hazard is negligible after the third year. The value of this approach was that we did not have to make any assumption about the shape of the mortality function. The first step in most studies designed to test for patterns of senescence is to select an appropriate model to fit the mortality distribution; but this need to specify a function may be restrictive. If, as in our case, there is no a priori expectation of a particular shape to the mortality function, then the approach used here may have broader appeal. Using a demographic definition of senescence, in other words, an increase in mortality after reproductive maturity (Medawar 1952; Hamilton 1966), and with the assumption that there was no age-by-environment interaction, these results suggest that there is no evidence for senescence in this plant species at the ages studied here. An alternative manifestation of senescence as a physiological decline in function with age cannot be ruled out by this study. Unfortunately, this later measure has not generally been applied to nonhuman population-level evolutionary studies of senescence.

Most of our information on demographic aging is currently based on studies with insects under laboratory conditions. Clearly more information is needed on a wider range of species in their natural habitats if we are to understand the breadth of senescence processes. However, determining the age-dependent component to the observed demographic patterns can be difficult. The covariate analysis presented here provides an alternative approach to classifying individuals into homogeneous groups in order to minimize heterogeneity and understand demographic dynamics. Defining individual quality, particularly when quality itself may be a dynamic state, can be difficult (cf. McNamara and Houston 1992, 1996; Cam and Monnat 2000). Moreover, in order to access "hidden" age-related demographic parameters, we first need more data from the longitudinal monitoring of large numbers of individuals of known age, and then we can apply the tools of hazard regression models to more clearly understand the age-dependent patterns of mortality under natural environmental conditions.

## Literature Cited

Allison, P. D. 1982. Discrete-time methods for the analysis of event histories. Sociological Methodology 13:61–98.

Antonovics, J., and R. B. Primack. 1982. Experimental ecological genetics in *Plantago*. VI. The demography of seedling transplants of *Plantago lanceolata*. Journal of Ecology 70:55–75.

Cam, E., and J.-Y. Monnat. 2000. Stratification based on reproductive state reveals contrasting patterns of age-related variation in demographic parameters in the kittiwake. Oikos 90:560–574.

Cam, E., W. A. Link, E. G. Cooch, J.-Y. Monnat, and E. Danchin. 2002. Individual covariation in life-history traits: seeing the trees despite the forest. American Naturalist 159:96–105.

Conroy, M. J., J. C. Senar, and J. Domènech. 2002. Analysis of individual- and time-specific covariate effects on survival of *Serinus seninus* in north-eastern Spain. Journal of Applied Statistics 29:125–142.

Cook, R. E. 1980. Germination and size dependent mortality in *Viola blanda*. Oecologia (Berlin) 47:115–117.

Cox, D. R. 1972. Regression models and life tables (with discussion). Journal of the Royal Statistical Society B 74:187–220.

Curtsinger, J. W., H. H. Fukui, D. R. Townsend, and J. W. Vaupel. 1992. Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*. Science 258:461–464.

Efron, B. 1988. Logistic regression, survival analysis, and the Kaplan-Meier curve. Journal of the American Statistical Association 83:414–425.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, N.J.

Fahrmeir, L., and G. Tutz. 1997. Multivariate statistical modeling based on generalized linear models. Springer, New York.

Finch, C. E. 1990. Longevity, senescence, and the genome. University of Chicago Press, Chicago.

Hamilton, W. D. 1966. The moulding of senescence by

natural selection. Journal of Theoretical Biology 12:12–45.

Loison, A., M. Festa-Bianchet, J. M. Gaillard, J. T. Jorgenson, and J. M. Jullien. 1999. Age-specific survival in five populations of ungulates: evidence of senescence. Ecology 80:2539–2554.

McDonald, D. B., J. W. Fitzpatrick, and G. E. Woolfenden. 1996. Actuarial senescence and demographic heterogeneity in the Florida scrub jay. Ecology 77:2373–2381.

McNamara, J. M., and A. I. Houston. 1992. State-dependent life-history theory and its implications for optimal clutch size. Evolutionary Ecology 6:170–185.

———. 1996. State-dependent life histories. Nature 380: 215–221.

Medawar, P. B. 1952. An unsolved problem in biology. H. K. Lewis, London.

Partridge, L., and M. Mangel. 1999. Messages from mortality: the evolution of death rates in the old. Trends in Ecology & Evolution 14:438–442.

Pedersen, B. 1999. Senescence in plants. Pages 239–274 *in* T. O. Vuorisalo and P. K. Mutikainen, eds. Life history evolution in plants. Kluwer, Dordrecht.

Promislow, D. E. L. 1991. Senescence in natural populations of mammals: a comparative study. Evolution 45: 1869–1887.

Promislow, D. E. L., M. Tatar, S. Pletcher, and J. R. Carey. 1999. Below-threshold mortality: implications for studies in evolution, ecology and demography. Journal of Evolutionary Biology 12:314–328.

Rees, M., A. Sheppard, D. Briese, and M. Mangel. 1999. Evolution of size-dependent flowering in *Onopordum illyricum*: a quantitative assessment of the role of stochastic selection pressures. American Naturalist 154: 628–651.

Roach, D. A. 1993. Evolutionary senescence in plants. Genetica 91:53–64.

———. 2003. Age-specific demography in *Plantago*: variation among cohorts in a natural population. Ecology 84:749–756.

Rose, K. E., M. Rees, and P. J. Grubb. 2002. Evolution in the real world: stochastic variation and the determinants of fitness in *Carlina vulgaris*. Evolution 56:1416–1430.

R-Project. 2004. http://www.r-project.org/.

Silvertown, J., M. Franco, and R. Perez-Ishiwara. 2001. Evolution of senescence in iteroparous plants. Evolutionary Ecology Research 3:393–412.

Solbrig, O. T. 1981. Studies on the population biology of the genus *Viola*. II. The effect of plant size on fitness in *Viola sororia*. Evolution 35:1080–1093.

S-Plus. 2000. User's guide. Data analysis products division, MathSoft, Seattle.

Van der Aart, P. J. M., and J. C. Aulto. 1992. Biogeography and human effects. Pages 5–6 *in* P. J. C. Kuiper and M. Bos, eds. *Plantago*: a multidisciplinary study. Springer, Berlin.

Vaupel, J. W., and A. I. Yashin. 1985. Heterogeneity's ruses: some surprising effects of selection on population dynamics. American Statistician 39:176–185.

Vaupel, J. W., J. R. Carey, K. Christensen, T. E. Johnson, A. I. Yashin, N. V. Holm, I. A. Iachine, et al. 1998. Biodemographic trajectories of longevity. Science 280: 855–860.

Vavrek, M. C., J. B. McGraw, and H. S. Yang. 1997. Within-population variation in demography of *Taraxacum officinale*: season- and size-dependent survival, growth and reproduction. Journal of Ecology 85:277–287.

Watkinson, A. R. 1992. Plant senescence. Trends in Ecology & Evolution 7:417–420.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46(suppl.):120–139.

Zens, M. S., and D. R. Peart. 2003. Dealing with death data: individual hazards, mortality and bias. Trends in Ecology & Evolution 18:366–373.

Zwaan, B. J. 1999. The evolutionary genetics of ageing and longevity. Heredity 82:589–597.