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LONG-TERM COST OF REPRODUCTION WITH AND WITHOUT ACCELERATED SENESCENCE IN *CALLOSOBRUCHUS MACULATUS*: ANALYSIS OF AGE-SPECIFIC MORTALITY

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Abstract.—Age-specific mortality is measured to characterize the costs of reproduction in the beetle *Callosobruchus maculatus*, providing explicit details of the timing, duration, magnitude, and acceleration of mortality. We experimentally manipulated reproductive effort in four cohorts of 200 individually housed females by controlling exposure to males and to an artificial oviposition substrate. We demonstrate that (1) early reproduction produces long-term increases in age-specific mortality; (2) egg-laying effort affects the onset of age-specific mortality but not its shape or rate of change; and (3) mating with subsequent reproduction increases the rate of change in age-specific mortality relative to virgins. Accelerated senescence is defined demographically as an increase in the rate of change of age-specific mortality. Our results challenge the hypothesis that reproductive effort accelerates senescence but provides evidence that mating itself may have this effect.

Key words:—Age-specific mortality, Bruchidae, cost of reproduction, force of mortality, senescence, survival analysis.

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An adequate demographic description of mortality that accounts for the trade-off between reproduction and life expectancy is crucial to an understanding of the evolution of senescence. Genetic variation and physiological mechanisms associated with reproduction that influence life span can best be understood in terms of their explicit effects on mortality risk as a function of age. Although it is well documented that reproduction decreases life expectancy (Stearns 1976; Dunlap-Pianka et al. 1977; Partridge and Harvey 1985; Bell and Koufopanou 1986), tests of evolutionary theories of life history and of aging typically consider only differences in survivorship or mean longevity (e.g., Partridge and Farquhar 1981; Rose and Charlesworth 1981; Browne 1982; Partridge et al. 1986; Partridge et al. 1987; Roitberg 1989; Fowler and Partridge 1990; Ernsting and Isaaks 1991). Our purpose is to apply rigorous analysis of age-specific mortality as a measure of cost of reproduction. Age-specific mortality, to varying degrees, has been examined in studies of reproduction in freshwater triclads (Calow and Woollhead 1977) and red deer hinds (Clutton-Brock et al. 1983), in genetic analyses of *Drosophila* and nematodes (Rose 1984; Johnson 1990), and in a comparative study of mammals (Promislow 1991).

The effect of reproduction on the timing and pattern of mortality and on the demographic rate of senescence (see Finch 1990) can be estimated by considering age-specific mortality, the probability of death at a given age conditional upon survival to that age (Chaing 1984). In contrast, survivorship and life expectancy are cumulative functions of age-specific mortality, which integrate episodes of mortality, obscuring details of timing, magnitude, and pattern (Gavrilov and Gavrilova 1991). In the context of cost of reproduction, Partridge and Farquhar (1981) and Partridge (1987) introduced these distinctions by noting that reproduction may decrease life expectancy because it temporarily increases mortality risk or because it accelerates senescence. However, an adequate evaluation of these ideas has been hampered by small sample sizes that permit testing only for differences in overall survivorship between treatments (e.g., the log-rank test; Elandt-Johnson and Johnson 1980). Understanding the cost of reproduction in terms of the pattern of age-specific mortality will permit a more complete description and provide a better basis for testing theories of the mechanism and evolution of senescence.

Three current issues in the study of life-history evolution may benefit from this approach. First, a debate concerns the validity of comparing the mortality cost of reproduction as determined by physiological manipulation versus genetic analysis (Reznick 1985, 1992; Partridge 1992). If age-

specific mortality is explicitly characterized, more information will be available to distinguish between the effects of each technique. For instance, Reznick (1992) suggested that the longevity of selected lines be compared when reproductive effort is manipulated. Comparison of age-specific mortality would strengthen the design because the genetic and physiological treatments may qualitatively affect the patterns of age-specific mortality in different ways even while they have the same quantitative effect on life expectancy (Pollard 1982; Vaupel 1986).

Second, age-specific mortality is the most appropriate demographic metric for evaluating evolutionary theories of senescence. Senescence is defined in the evolutionary literature as a decrease in the components of fitness, reproduction, and life expectancy, with advancing age (Charlesworth 1980; but see Abrams 1991). Implicitly, senescence entails a progressive increase in the age-specific mortality rate (Kirkwood 1987). Thus, to characterize senescence in demographic experiments, it is necessary to explicitly measure age-specific mortality and to estimate its pattern of change. As well, one goal of evolutionary senescence theory is to account for positive and accelerating mortality rates as seen in the efforts of Medawar (1955, 1981), Hamilton (1966), and Kirkwood (1990; Kirkwood and Rose 1991), although Abrams (1993) has recently demonstrated that nonincreasing mortality trajectories may be expected under various conditions of population dynamics and genetic mechanisms.

Third, in life-history theory, reproduction and survival are considered competing processes because of the need for resource allocation (Sibly and Calow 1986). This trade-off is often assumed to be temporary and concurrent (Charlesworth 1990a). If long-term effects of oviposition effort on age-specific mortality are found and described in terms of duration, magnitude, and functional form, the conclusions derived form this assumption must be reevaluated. For instance, in some quantitative genetics models (Charlesworth 1990b), trade-offs across ages can give rise to unexpected changes in the signs of genetic correlations.

Our objective is to describe age-specific mortality for large female cohorts of the bruchid beetle *Callosobruchus maculatus* in response to experimental manipulation of reproductive effort and mating experience. *Callosobruchus maculatus* is a stored-grain pest that oviposits on dried legumes (Johnson and Kistler 1987). Larvae develop completely within the seed and eclose after several weeks. Adults are facultatively aphagous and nutritionally self-contained, capable of living without food for at least 30 d and laying up to 120 eggs (Mitchell 1990). Mean life span of *C. maculatus* females increases when reproductive effort is reduced by limiting oviposition opportunities through crowding or through withholding hosts or males (Wightman 1978; Smith and Lessells 1985; Møller et al. 1989). To understand better the nature of this cost of reproduction, we ask (1) when and for how long is age-specific mortality elevated in response to differences in reproductive effort and; (2) is the change in the pattern of age-specific mortality caused by a change in the timing of the onset of senescence or by a change in the rate of senescence?

Materials and Methods

Analysis of age-specific mortality requires initial cohorts large enough to provide reasonable estimates over the whole study period. We formed treatments each of 200 females housed individually in 35 × 10-mm plastic petri dishes containing a disk of bench top skid net, without food or water. To maintain independence of post-treatment experience among individuals (Hurlbert 1984), dishes were interspersed and rotated daily throughout a single incubator. Mortality was recorded daily. *Callosobruchus maculatus* was obtained from a bean warehouse (San Francisco, California; Fox 1993a) and maintained on azuki beans (*Vigna angularis*) under the experimental conditions (25°C, constant light, rh < 25%) and at a density of four to six eggs per bean for five generations before our study.

To produce treatment cohorts, azuki beans with one to two eggs per bean were held individually in tissue culture cells. Over a 5-d period, virgin females were collected (entered age-class 0 d) and assigned to one of four treatments; three mated and one unmated. Mated females were individually exposed to single males for 24-h posteclosion. Unmated females were placed in dishes at this age but received no males (treatment 1). Reproductive effort was manipulated among the mated treatments by the presence or absence of five 6-mm diam. solid glass beads (Baxter Scientific Products), water, and acetone washed. These beads elicited similar egg laying as those treated by the methods of Credland and Wright (1988) (M. Tatar unpubl. data). Fresh glass beads

TABLE 1. Mass, mean, and SE at emergence of predesignated subcohort of 50 females per treatment and relationship to individual life span. Probabilities are for the F-test of the Pearson product-moment correlation.

Treatment	Mass (SE) (in mg)	Coefficient of determination (r^2) between mass & life span
Unmated (treatment 1)	4.7 (0.11)	+ 0.08 $P = 0.05$
Beads-age-0 (treatment 2)	5.1 (0.09)	+ 0.01 $P = 0.53$
Beads-age-0 & 1 (treatment 3)	4.7 (0.11)	+ 0.11 $P = 0.02$
Beads-all-ages (treatment 4)	5.0 (0.10)	+ 0.01 $P = 0.55$

were present through age 0 d (treatment 2), present through ages 0 and 1 d (treatment 3), and present at all ages (treatment 4).

To investigate whether beads caused increased mortality independently of their influence on beetle reproduction, an additional group of 58 unmated females were exposed to beads as in treatment 4. We observed a difference in overall mortality between this control group and that of treatment 1 ($\chi^2 = 3.4$, $P = 0.07$, log-rank test, BMDP 1L; Dixon 1990) but in the opposite direction than expected if the presence of beads increased mortality (median life span: with beads = 20 d, no beads = 18 d). No differences in age-specific mortality were observed until age 16 d.

We measured the effect of bead schedule on egg laying by recording for each mated treatment the fecundity of 50 females, randomly designated at age 0 d. These females were also weighed to the nearest 0.1 mg at emergence (table 1). From age 0 to 4 d, females in treatment 4 laid approximately 1.5 times more eggs than females in treatments 2 and 3 (fig. 1). All groups exhibited similar, low levels of oviposition after age 4 d; eggs laid in the absence of glass beads were deposited on the petri dish. We designate ages 0 to 4 d as the early period with age 5 d onward as the late period, and treatment 4 as high reproductive effort with treatments 2 and 3 as low reproductive effort.

Unmated *C. maculatus* lay some unfertilized eggs (Wilson and Hill 1989). Such ovipositions were rarely observed and not recorded in the present study, but we subsequently observed 1850 unmated females and found that 66% laid no eggs, whereas the remainder laid an average of

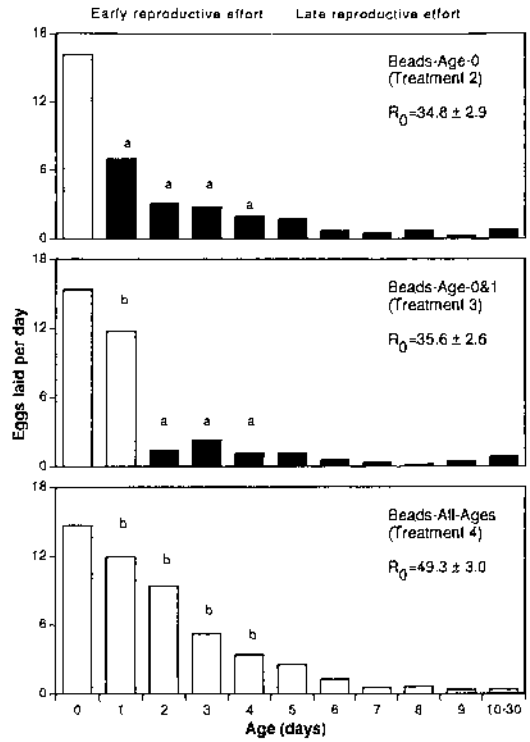

FIG. 1. Average daily eggs laid per surviving female. Open bars represent days with beads. Closed bars represent days without beads. Each day, bars with different letters report significant differences in means ($b > a$), Scheffe's procedure ($P < 0.05$). R_0 is mean life-time reproductive effort ± standard error. Mean eggs laid per surviving female (SE) in the early period was the following: treatment 4 = 44.62 (2.82) > treatment 2 = 29.41 (2.45), and treatment 3 = 31.86 (2.39) (Scheffe's procedure, $F = 10.1$, $P < 0.001$). Mean eggs laid per surviving female (SE) in the late period is not distinguishably different across treatments ($F = 2.02$, $P = 0.13$): treatment 4 = 4.78 (0.94), treatment 2 = 5.39 (0.92), treatment 3 = 3.1 (0.64).

5.13 ± 0.20 eggs (SE) (M. Tatar unpubl. data). In the present study, we observed egg-maturation dynamics of unmated females by dissecting an independent sample of 25 newly emerged females and comparing the number of mature eggs in their abdomens to the number found in the carcasses of a predesignated subcohort of 50 from the unmated treatment. Within 24 h of emergence, females had 14.7 ± 1.0 (SE) mature eggs compared to 6.4 ± 1.2 mature plus partial eggs at death. Consistent with observations of Wilson and Hill (1989), egg maturation begins before emergence from the bean. Wilson and Hill reported that maturation ceases once the ovaries are full; unmated females asymptote at about 30

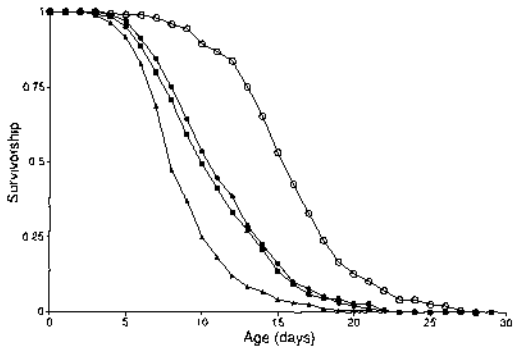

FIG. 2. Survivorship. Treatment 1, unmated (open circle); treatment 2, beads-age-0 (filled circle); treatment 3, beads-age-0 & 1 (filled square); treatment 4, beads-all-ages (filled triangle).

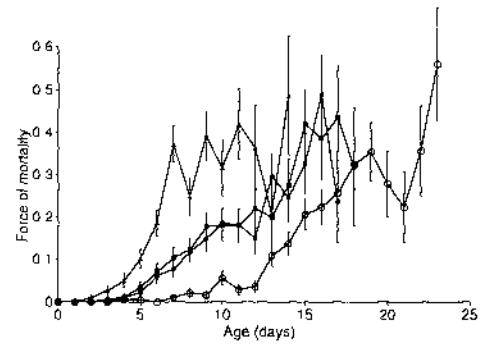

FIG. 3. Force of mortality, μ_x with ± SE, for ages where number surviving, n_x, is greater than 10. Treatment 1, unmated (open circle); treatment 2, beads-age-0 (filled circle); treatment 3, beads-age-0 & 1 (filled square); treatment 4, beads-all-ages (filled triangle).

mature eggs by age 5 d. Based on our observations of older, dead females, we infer that unlaid mature eggs are resorbed. Unmated females exhibit some ovarian activity, but of a different nature than that in mated females.

RESULTS

The mortality of the treatment cohorts is analyzed in several ways, including nonparametric comparisons of survival curves; nonparametric comparison of the force of mortality; estimation and comparison of Gompertz parameters with linear regression; and estimation and comparison of parametric mortality distributions by maximum likelihood. Each approach provides different insight and is applied in turn.

Survival Curves

To determine whether two or more samples could have arisen from identical survivor functions, nonparametric tests can be used to summarize differences in the estimated survivorship curves over the entire study period (e.g., log-rank test; Elandt-Johnson and Johnson 1980). We illustrate this approach for our treatments because it has a precedent in evolutionary studies. Overall survivorship is the lowest in the high reproductive-effort treatment (treatment 4), it is similar and intermediary for the low reproductive-effort treatments (treatments 2, 3), and it is greatest in the unmated treatment (treatment 1) (fig. 2; $\chi^2 = 267.3$, $P < 0.0001$, Mantel Cox trend test, BMDP 1L; Dixon 1990).

Force of Mortality

Differences in survivorship curves do not describe the timing, duration, and magnitude of mortality differences between groups. Direct consideration of age-specific mortality, q_x, or of the force of mortality, μ_x, for each treatment provides insight into the nature of survivorship differences. The force of mortality represents the limiting value of age-specific mortality as the age interval becomes infinitesimally small; thus, it is independent of the census interval and not bounded by 1.0. When the census interval is 1 d

$$\mu_x \approx -\ln(1 - q_x), \text{ and } \operatorname{se}(\mu_x) \approx \frac{q_x}{n_x(1 - q_x)},$$

where x = age, $q_x = d_x/n_x$, d_x = number of individuals dying in the interval, and n_x = number of individuals entering the interval (Elandt-Johnson and Johnson 1980, p. 143).

In figure 3, the force of mortality increases rapidly to age 7 d in the high reproductive-effort treatment (treatment 4). At that age, a mortality change point is evident (Muller and Wang 1990; Nguyen et al. 1984), a sudden shift from an accelerating to a horizontal trajectory. The low reproductive-effort treatments (treatments 2, 3) are considered together. Their age-specific mortality is less than that of treatment 4 up to approximately age 13 d. The effect of reproduction on mortality after this point is obscured because the variance and stochasticity of μ_x increases rapidly because of small remaining n_x and because the trajectories may be converging. Unmated females (treatment 1) exhibit a low level of continuously increasing age-specific mortality relative to all mated treatments up to approximately age 18 d.

Differences in reproductive effort in the early period (ages 0–4 d) produce a long-lasting effect

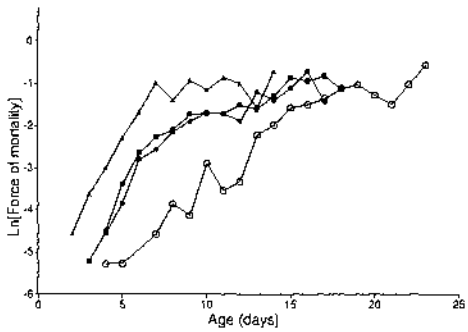

FIG. 4. Natural log of force of mortality, ln μ_x, for ages where number surviving, n_x, is greater than 10. Treatment 1, unmated (open circle); treatment 2, beads-age-0 (filled circle); treatment 3, beads-age-0 & 1 (filled square); treatment 4, beads-all-ages (filled triangle).

on mortality; the difference in age-specific mortality between the high and low reproductive treatments is evident at age 3 d and continues for at least 10 d. Comparison of mated with unmated females indicates that a long-term effect on mortality, first evident at age 5 d, is associated with mating combined with reproduction.

Gompertz Analysis

The rate of change of age-specific mortality is an index of demographic senescence (Finch et al. 1990) and is often quantified by estimating the Gompertz parameters (e.g., Finch et al. 1990; Johnson 1990; Promislow 1991). Gompertz (see Finch 1990) postulated that adult mortality increases exponentially at a constant rate b starting from some initial level a such that $\mu_x = ae^{bx}$. Plots of ln μ_x against age x are linear with slope b and intercept ln a and least-squares linear regression are used to estimate these parameters. Parallel, noncoincidental plots have similar rates of change in age-specific mortality (b) but differ in initial mortality rate (a). Plots that diverge in slope have different rates of change in age-specific mortality. The coefficient for the rate of change in age-specific mortality, b, is an index of the rate of demographic senescence (Finch et al. 1990), and changes in this value are a way to quantify experimental effects on the rate of senescence.

We analyze our data with the Gompertz model because of its simplicity and precedence, although its application presents two difficulties. First, although it is common to use least-squares to estimate and test the regression parameters, the assumption that observations are independent is violated because q_x at successive ages includes repeated observations on the same individuals. One solution is to apply maximum likelihood (see below). Second, in the present data, the complete trajectories of ln μ_x are not linear as assumed by the Gompertz model (fig. 4); age-specific mortality does not accelerate at a constant rate. However, the plots appear to be linear initially, particularly in treatment 4 up to age 7 d. For the present, we assume that treatment 4 is linear through age 7 and use this cut point to truncate the curves of treatments 2, 3, and 4. Based on visual inspection of the curve for treatment 1, we assume linearity below age 19 d.

Graphically (fig. 4), the initial segments of the mated treatments appear parallel. The ln μ_x plot of the high reproductive-effort treatment (treatment 4) differs from the low effort treatments (treatments 2, 3) solely by a shift up or to the left. The slope of the unmated females (treatment 1) rises less rapidly than that of the mated treatments. We verified these observations by estimating and comparing the Gompertz parameters for the initial linear segments with least-squares regression and a single-model test of coincidence (Kleinbaum et al. 1988), weighted by $(n_x)^{1/2}$ to correct for the dependence of the variance on sample size (table 2).

The rate of demographic senescence is not changed by the early manipulation of reproductive effort among mated females. The mortality rate doubling times (MRDT = ln $2/b$) among mated females is 0.85–0.94 d. Rather, a four- to sixfold increase in the initial mortality rate (a) is associated with high early egg-laying effort (table 2). In contrast, the demographic rate of senescence for mated females is initially less than half that of unmated females (MRDT = 2.31 days).

Parametric Maximum-Likelihood Estimates (MLE)

Maximum-likelihood analysis of parametric mortality models does not require independence among mortality rates at each age or linearity of ln μ_x (Kalbfleisch and Prentice 1980; Nelson 1981). The models are forms of specific distributions of time-to-death that have associated force of mortality functions such as the Weibull, exponential, lognormal, and gamma. Maximum-likelihood estimates the distribution parameters using individual survival times within a group, and analytical methods are applied to test for differences among the parameters of the treatments.

The deceleration of age-specific mortality ob-

TABLE 2. Least-squares regression, weighted by $(n_x)^{1/2}$, of ln(force of mortality) for initial linear segments. Single-model comparison of two straight lines, pariwise against treatment 2, with the backward testing procedure and the model $\ln \mu_x = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 XZ + E$, where X is age and $Z = 0$ for treatment 2 and $Z = 1$ for treatment 1, 3, or 4 (Kleinbaum et al. 1988, p. 275). Partial F-tests are presented. Do not reject H_0: treatments 3 and 2 are coincidental. Reject H_0: treatments 4 and 2 are coincidental but do not reject H_0: treatments 4 and 2 are parallel. Reject H_0: treatments 1 and 2 are coincidental and H_0: treatments 1 and 2 are parallel but do not reject H_0: treatments 1 and 2 have similar intercepts. For comparison with Finch (1990): mortality rate doubling time MRDT = ln 2/b, initial mortality rate IMR = a.

Treatment	Parameter estimates		Comparison to treatment 2			Mortality rate constants	
	Slope, b (SE)	Intercept, ln a (SE)	Coincidence $F(XZ, Z \mid X)$	Parallel $F(XZ \mid X, Z)$	Intercept $F(Z \mid X)$	MRDT (days)	IMR day^{-1} ($\times 10^{-4}$)
Beads-age-0 (treatment 2)	0.734 (0.081)	−8.95 (0.546)				0.94	1.28
Beads-age-0 & 1 (treatment 3)	0.815 (0.057)	−9.31 (0.332)	2.07 NS			0.85	0.09
Beads-all-ages (treatment 4)	0.753 (0.037)	−7.52 (0.183)	171.95 $P < 0.001$	0.008 n.s.		0.92	5.42
Unmated (treatment 1)	0.310 (0.023)	−7.22 (0.202)	55.72 $P < 0.001$	7.38 $P < 0.05$	3.71 NS	2.31	7.34

served in figures 3 and 4 suggests fitting the lognormal mortality distribution (see Kalbfleisch and Prentice 1980, pp. 24–25). To evaluate the goodness-of-fit, we test the linearity of the normal probability plot when age is log transformed (Nelson 1981, p. 113). Each treatment fits the expectation (table 3). An alternative model that can also exhibit a decelerating rate of change in age-specific mortality is the Weibull (Kalbfleisch and Prentice 1980, p. 23). We discriminate between the Weibull and the lognormal distributions by comparing the log likelihoods of each model (Kalbfleisch and Prentice 1980, p. 65). The lognormal model provides the best fit among the mated treatments, whereas the Weibull is superior for the unmated females (table 3). We choose the lognormal for the present analysis.

The force of mortality of the lognormal can have a logisticlike shape, increasing to some maximum followed by a nearly level or decreasing trend with the trajectory depending on two parameters, σ and α (notation of regression formulation; Kalbfleisch and Prentice 1980). The shape parameter σ (referred to as "scale" in BMDP 2L; Dixon 1990) determines the overall pattern of the force of mortality; it is the variance of the mortality distribution. Small values of σ give shallower and flatter patterns, indicating slower rates of change in μ_x. The location parameter α (referred to as "constant" in BMDP) determines the placement of the μ_x on the age axis; it is the mean of the mortality distribution. Parameter values with asymptotic variances are estimated by BMDP 2L (table 3).

For each parameter, we test for similarity among the treatments by calculating maximum-likelihood simultaneous limits (Nelson 1981, p. 531). For the shape parameter σ, the three mated treatments form a group of indistinguishable values that are greater than that of the unmated females (table 3). Among the mated treatments, the location parameter α varies significantly and each differs in this respect from the unmated group.

It is evident from this analysis that the level of reproductive effort early in adult life, given mating, changes only the placement of the age-specific mortality trajectory on the age axis and not its rate of change. Mating combined with egg laying, however, produces a fundamental change in the pattern of age-specific mortality. Mated and unmated females differ in the shape of their force of mortality as described by the lognormal model and in the form of mortality model that best fits the distribution of deaths.

DISCUSSION

A mortality cost of reproduction is evident in females of *Callosobruchus maculatus* when early reproductive effort is manipulated phenotypically. The nature of this cost is evident from estimation of age-specific mortality; risk is elevated during the period of differential reproduction and continues for at least 10 d (treatments

TABLE 3. Lognormal mortality model parameters estimated by maximum-likelihood estimate (MLE) with BMDP 2L (Dixon 1990); location and shape parameters (asymptotic variance). Model parameters compared by maximum-likelihood simultaneous limits; superscript letters denote sets of parameters with significant differences, $P < 0.01$. Goodness-of-fit estimates correlation (Pearson product moment) for quantile-quantile plot of natural-log longevity. The log-likelihood difference compares the maximized-log likelihood for the lognormal against the Weibull model. A value greater than positive 1.92 [$P(\chi^2_{(1)} > 1.92) = 0.025$] indicates that the lognormal distribution is more appropriate than the Weibull based on the standard maximized log-likelihood of the generalized F-model (Kalbfleisch and Prentice 1980, p. 65).

Treatment	Lognormal: MLE		Goodness of fit***	Log-likelihood difference
	Location α	Shape σ	r	
Unmated (treatment 1)	2.860[a] (0.018)	0.251[a] (0.013)	0.978	−4.27
Beads-age-0 (treatment 2)	2.557[b] (0.021)	0.294[b,c] (0.015)	0.998	8.44
Beads-age-0 & 1 (treatment 3)	2.523[c] (0.023)	0.310[b] (0.016)	0.998	18.67
Beads-all-ages (treatment 4)	2.262[d] (0.022)	0.300[c] (0.015)	0.996	6.66

*** $P < 0.001$ for all treatments.

2, 3 vs. treatment 4). Based on the Gompertz and maximum-likelihood estimates (MLE) parametric analyses, this difference is caused by a change in the initial mortality rate or in the timing of mortality and not to a difference in the rate of change of age-specific mortality. We conclude that early reproductive effort produces a consistent, long-term increase in mortality that is not associated with accelerated senescence.

Mating with subsequent reproduction also affects mortality (treatments 2, 3, 4 vs. treatment 1). Mated females have a qualitatively different pattern of age-specific mortality from virgins. Considering treatments of mated females as a group, age-specific mortality increases faster than for the unmated females. This difference is not observed among the reproductive effort treatments of mated females. The *shape* of the age-specific mortality pattern changes only in response to mating status; among the mated treatments that differ by reproductive effort, the shape is the same. We suggest that a physiological event associated with mating itself has triggered a change in mortality pattern, one characterized by accelerated demographic senescence.

Because *C. maculatus* is facultatively aphagous, the patterns of mortality may reflect death by nutrient exhaustion rather than progressive loss of physiological homeostasis, that is, biological senescence. As in other insects (Dunlap-Pianka et al. 1977; Leather 1984; Kaitala 1991), longevity is increased in *C. maculatus* when adults are fed (Møller et al. 1989; Fox 1993b). *Callosobruchus,* however, are physiologically suited for adult aphagy. Females emerge with adequate energy to develop and lay most of their potential eggs (Fox 1993b). Among bruchid beetles, adults emerge with a high proportion of their wet weight as lipids (Nwanze et al. 1976; Wightman 1978; Sharma and Sharma 1979). In *C. analis,* lipids provide about 95% of adult metabolic energy and water (Wightman 1978). Lack of adult food is not a contrived condition that produces an artificial mortality pattern. However, nutrient exhaustion still may be a leading factor in the distribution of deaths if variation in lipid storage at emergence affects individual life span. In this case, a correlation between initial body mass and longevity is expected among nonreproductive individuals. Such correlations are small in the present data (table 1). We conclude that variation in nutrient reserves and hence their time of exhaustion does not account for the patterns of mortality.

Each of the four treatments demonstrates a slowing of the rate of change in age-specific mortality as confirmed by the adequacy of the lognormal mortality model to fit the distribution of the observed deaths. This deceleration may be explained in two ways. It may be an artifact of compositional change in the cohort, resulting from heterogeneity in mortality patterns within the population of genotypes and phenotypes (Vaupel and Yashin 1985). Alternatively, the trend may reflect a physiological change within aging individuals such as a slowing or reversal of biological senescence. An important conclusion is that the Gompertz mortality model, which assumes continuous acceleration of adult mortality, is not universal. The strength and generality of this conclusion is demonstrated unambiguously by the decelerating age-specific mortality trajectory of 1.2 million medflies (Carey et al. 1992) and the smaller life tables of several other animals (Calow and Woollhead 1977; Economos 1982; Curtsinger et al. 1992).

Our approach to measuring cost of reproduction is similar to that of Gustafsson and Part (1990) in that we estimate the effect of early reproductive effort on later age-specific fitness

components. Gustafsson and Part evaluated age-specific clutch size; we are concerned with age-specific mortality. A major difference between these studies is that, unlike Gustaffson and Part, we reject the idea that early reproduction accelerates senescence. This difference is because of our explicit definition of senescence as a progressive change in age-specific fitness (mortality) and accelerated senescence as an increase in the rate of change. In contrast, Gustafsson and Part assumed that merely a persistent decrease in age-specific fitness equals accelerated senescence. They overlooked the importance of rate of change that is implicit in their definition of senescence (Gustafsson and Part 1990, p. 279): "decline of fitness components . . . with *advancing* age." By our criteria, Gustafsson and Part have demonstrated a long-term cost of reproduction but have not established an effect on the rate of senescence.

Our results advance the analyses of the cost of reproduction that are based on survivorship curves. Such analyses are incomplete because the timing, duration, and magnitude of mortality costs are often ambiguous. For instance, Partridge et al. (1986, p. 927) concluded that exposure to males places female *Drosophila melanogaster* at temporary rather than permanent risk. They based this conclusion on a failure to distinguish statistically the survivorship of treatment females (males removed day 10) from control females (no males) at age 36 d onward. However, this inference appears to be based on incorrectly applied statistics. First, Partridge et al. used the log-rank test, which combines the contribution of each age to overall differences in survivorship. Conceivably, overall survivorship could be found to differ if the test was begun at some age *subsequent* to 36 d. Second, a log-rank test conducted on the tail end of survivorship curves is not likely to reject the null hypothesis of no difference because the remaining sample size is small, reducing the power of the test (Elandt-Johnson and Johnson 1980). Last the inference is based on an a posteriori specification of the null hypothesis, because sequential ages were tested until no difference was detected. Nonparametric survival analysis preclassified by age may be able to determine when mortality is affected, but the power is limited because of the small initial cohort sizes.

The estimates of age-specific mortality in *C. maculatus* show that mating per se can have a permanent, rather than temporary, effect on female mortality. Mating leads to a change in the pattern of age-specific mortality in the direction of increased rate of change. However, the conclusion is tentative, and a more direct test is desirable. Potentially, the change in pattern could be due to differences in reproductive effort between unmated and mated females. The untested assumption is that variation in reproductive effort in the range of 0–5 eggs (unmated) to 30–50 eggs (mated) affects only the onset (rather than pattern) of mortality acceleration as does the variation in the range of 30 eggs (treatments 2, 3) to 50 eggs (treatment 4). We define an increase in the rate of change of age-specific mortality as accelerated demographic senescence. To our knowledge, the effect of mating on mortality in *C. maculatus* is the first demonstration of accelerated senescence in response to reproductive activity. Whereas Johnson (1990) reported a decrease in the Gompertz parameter b in the age-1 strain of *Caenorhabditis elegans*, the reduced reproduction of age-1 has been found to be independent of the life-span mutation itself (Johnson and Lithgow 1992).

The effect of reproductive effort itself on mortality is obtained by manipulating oviposition opportunities while controlling male exposure. Based on such a design, Partridge et al. (1987) found that egg laying decreases overall survivorship in *D. melanogaster*, but the details of the relationship between reproduction and mortality are unclear. Among groups that immediately exhibit a 5 to 10-fold difference in egg laying (Partridge et al. 1987; pp. 746–747), statistically significant differences in survivorship are not detected until 47 d. It is unclear whether this difference is due to a temporary increase in age-specific mortality in the high-effort treatment during the sixth w or to a permanent increase in mortality in this group. Ernsting and Isaacks (1991) varied early egg production in the beetle *Notiophilus biguttatus* by manipulating prey availability and temperature. At later ages when *conditions* were equalized, they report an overall difference in mortality as measured by survivorship curves. However, it is not clear if *egg production* was actually equal in the late periods.

Details of the effect of reproductive effort on mortality are evident in *C. maculatus* when age-specific mortality is explicitly considered. Differences in early egg-laying effort produce a long-term increase in mortality. This change is not due to accelerated demographic senescence. Rather, the age of onset of senescence is de-

creased, or the initial level of mortality is increased. Sacher (1977) described similar changes in age-specific mortality caused by irradiation in rodents. He suggested that it represents either a change in the onset of senescence by promotion of the development of preexisting disease ($\ln \mu_x$ plots shift horizontally) or the addition of an increment of permanent injury to cell and tissue function, increasing vulnerability to a level characteristic of later ages ($\ln \mu_x$ plots shift vertically). Although distinguishing between these hypotheses is not possible when the $\ln \mu_x$ plots are linear, the nonlinear patterns of *C. maculatus* may be diagnostic. If the plateau of mortality at advanced ages can be shown to occur at similar levels among reproductive-effort treatments, the interpretation is a left shift of the plots rather than a vertical displacement, implying the senescence onset model. Such a left shift is our interpretation of the difference between the high effort and low effort curves in figure 4. Arking (1987; Arking and Wells 1990) and Rose (1984) also suggested that increases in longevity associated with reproduction result from changes in the onset of senescence. These authors worked with selected lines of *Drosophila* and applied different analytical approaches. Strikingly, none of these genetic studies or the present physiological manipulation of *C. maculatus* shows an effect on the rate of senescence associated with reproductive effort.

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