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Authors

Mueller, Laurence D
Rauser, Casandra L
Rose, Michael R

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An evolutionary heterogeneity model of late-life fecundity in *Drosophila*

Laurence D. Mueller · Casandra L. Rauser ·
Michael R. Rose

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Abstract There is now a significant body of research that establishes the deceleration of mortality rates in late life and their ultimate leveling off on a late-life plateau. Natural selection has been offered as one mechanism responsible for these plateaus. The force of natural selection should also exert such effects on female fecundity. We have already developed a model of female fecundity in late life that incorporates the general predictions of the evolutionary model. The original evolutionary model predicts a decline in fecundity from a peak in early life, followed by a plateau with non-zero fecundity in late life. However, in *Drosophila* there is also a well-defined decline in fecundity among dying flies, here called the “death spiral”. This effect produces heterogeneity between dying and non-dying flies. Here a hybrid evolutionary heterogeneity model is developed to accommodate both the evolutionary plateau prediction and the death spiral. It is shown that this evolutionary heterogeneity model gives a much better fit to late-life fecundity data.

Keywords Aging · *Drosophila* · Fecundity plateau · Late life · Natural selection

Introduction

It has been established that a variety of organisms, including humans, show a deceleration in mortality rates at advanced ages (Carey et al. 1992; Curtsinger et al. 1992; Tatar et al. 1993; Vaupel et al. 1998; Carey 2003). This deceleration results in a rough plateau with high mortality rates late in adult life. In view of the unusual features of late life relative to aging, we have proposed that this period be considered a third phase of life (Rauser et al. 2006a). Such a distinctive, and previously unanticipated, phenomenon calls for the development of appropriate theory to explain it.

There have been two general classes of explanations offered for these plateaus: natural selection and lifelong heterogeneity. Several different theories of natural selection have been developed (Abrams and Ludwig 1995; Mueller and Rose 1996; Charlesworth 2001), but the type of selective theory which has received the most attention is that based on the asymptotic plateau in the forces of natural selection acting on both age-specific survival and age-specific fecundity (Rauser et al. 2006a). The chief alternative theory is that lifelong heterogeneity between sub-cohorts is the cause of these late-life plateaus (Beard 1959; Vaupel et al. 1979; Service 2000). There have been a variety of published criticisms of evolutionary theories of late life (Pletcher and

L. D. Mueller (✉) · C. L. Rauser · M. R. Rose
Department of Ecology and Evolutionary Biology,
University of California, Irvine
CA 92697-2525, USA
e-mail: ldmuelle@uci.edu

Curtsinger 1998; Wachter 1999) and lifelong heterogeneity theories of late life (Mueller et al. 2003).

The evolutionary theory of late life that is based on Hamilton's (1966) age-specific forces of natural selection has been tested several times and the results have generally been consistent with that theory (Rose et al. 2002; Rauser et al. 2006a, b). The few empirical tests of the heterogeneity theory have been either ambiguous or negative in their results (Khazaeli et al. 1998; Drapeau et al. 2000; Mueller et al. 2003; Steinsaltz 2005). An interesting aspect of the Hamiltonian evolutionary theory of late life is that it also makes formal predictions about late-life fecundity (Rauser et al. 2006a), specifically that in late life there can also be a more or less constant fecundity, in other words a late-life plateau. There is already experimental support for this prediction (Rauser et al. 2003, 2005a, b, 2006b).

A two-stage Gompertz model has been used for empirical curve-fitting in tests of theories for mortality plateaus, in an hypothesis-free manner. This simple formula provides an excellent description of age-specific mortality for organisms during the periods of aging and late life (e.g. Rose et al. 2002).

No such standard descriptive model for female age-specific fecundity exists, making the quantitative analysis of fecundity data problematic. Furthermore, while mortality data roughly seem to fit a Gompertzian pattern of exponential increase followed by a plateau, our late-life fecundity data often seem to conform more to a progressive exponential decline in age-specific fecundity (e.g. Rauser et al. 2005a), at least visually, even though a two-stage model of age-specific fecundity incorporating a late-life plateau fits the data better than a model with linear or exponential declines in fecundity (Rauser et al. 2005a).

A further motivating complication is our detection of later-life (but not *lifelong*) heterogeneity for age-specific fecundity (Rauser et al. 2005a). Female flies that are about to die exhibit plummeting fecundity. We call this phenomenon a "death spiral."

Thus we have two anomalies in the late-life fecundity data: (1) an apparent, though not genuine (Rauser et al. 2005a), fit to statistical models

with continuing exponential decline in fecundity; and (2) the presence of a pre-death heterogeneity within cohorts (Rauser et al. 2005a). The question we address in this article is whether the second anomaly explains the first. That is, can we explain the observed "weakening" or "crumbling" of the late-life fecundity plateau by the death spiral?

In this paper, we use both the general predictions from the evolutionary theory of late life and our own observations of the fecundity of females near death to develop a general statistical model of late-life fecundity in *Drosophila*. This model may have more general use than our applications to *Drosophila*. However, since the focus of our research has been experimental data from *Drosophila*, we have chosen to focus on this organism in our use of this more complex analysis.

In addition, with the development of this more detailed model of female fecundity, we can determine if the less detailed statistical methodology that we used in the past yields substantially different predictions when applied to the same experimental data.

Finally, there are several different experimental techniques which can be used to estimate the parameters of this new model. We consider each of these methods and discuss their strengths and weaknesses. Ultimately, the analysis that we develop here provides an objective basis upon which anyone may design further experimental tests of theories of late life using fecundity, in any organism.

Materials and methods

Experimental populations

We used replicated laboratory-selected populations of *D. melanogaster*, derived from the South Amherst, Massachusetts, IVES population (IV) (Ives 1970), and collected from the wild in 1975 (Rose 1984). The IV population was the ancestral population of the five replicate O populations (having subscripts 1–5) in 1980, which were cultured using females of increasingly greater ages until females had to attain 70 days of age from egg (Rose 1984).

The five CO populations used in this study were derived from five corresponding O populations in 1989 (Rose et al. 1992), and in recent years the CO populations have been cultured using females that are 28 days of age (Rose et al. 2004). These populations are grown up in vials until age 14 days from egg, when they are placed in population cages until they are 28 days of age, from the egg stage. At that age, eggs are collected to propagate the next generation. The five replicate CO populations are maintained separately and had been under this selection regime for at least 150 generations at the time of these experiments. (Because our experimental design used staggered block replication, the number of generations of an experimental population prior to each assay varies.)

In 1991, the five ACO populations used in this study were derived from the corresponding five CO populations, and cultured using females that are 8–10 days of age (Chippindale et al. 1997). These populations are grown up in vials until they are 8–10 days of age, when they are placed in population cages for 1 day to collect eggs to propagate the next generation. The five replicate ACO populations are also maintained separately and had been under this accelerated-development selection regime for at least 360 generations at the time of these experiments. All these populations have been maintained at effective population sizes of at least 1,000 individuals and so are not heavily inbred (Chippindale et al. 2004). While there is some evidence that the ACO flies suffer from a miniaturization syndrome resulting from selection for faster development (vid. Chippindale et al. 2003, 2004), this possible complication does not affect the chiefly methodological purpose of the present article.

The difference in age of reproduction between the ACO and CO populations resulted in late-life mortality-rate plateaus that started at a significantly greater age in the CO populations, relative to the ACO populations (Rose et al. 2002), as was predicted by the evolutionary theory for late life. The difference in the age of reproduction between these populations is positively correlated with the age of last survival because of the way these populations are maintained. This difference corresponds to the ages at which the force of

natural selection acting on fecundity declines to zero and plateaus (earlier in the ACO populations, relative to the CO populations). Together, these 10 populations have been used as a system in which to test the evolutionary theory of late-life, based on the force of natural selection, as it applies to fecundity (vid. Rauser et al. 2006b).

To explore the utility of the models developed here we study a number of different data types that are commonly encountered in life history studies. (1) Data in which we have fecundity and survival records for individual females, (2) data which consist of fecundity of groups of females and survival of a separate cohort, and (3) fecundity on groups of females and no survival data.

Fecundity assays

All flies used in the fecundity assays described here were raised as larvae in 5 ml of standard banana-molasses food at densities of between 60 and 80 eggs per 8-dram vial for two generations. During this controlled density rearing, the ACO_i and CO_i populations were reared in parallel using a 2-week generation time in incubators at 25°C and under constant illumination.

During each assay, adults were kept in 5 ml food vials containing charcoal-colored medium, so that eggs could easily be seen and counted using a dissecting microscope, and 5 mg of yeast so that nutrition was not a limiting factor for fecundity (vid. Chippindale et al. 1993). At the beginning of each assay, four females and four males, age 12 days, were placed in each vial and transferred to fresh vials daily so that eggs could be counted. Eggs were counted daily from 100 randomly selected vials from each replicate population. As mortality occurred, flies from different vials were combined daily to forestall any age-dependent density effects (cf. Nusbaum et al. 1993; Carey et al. 1993; Graves and Mueller 1993, 1995; Curtsinger 1995a, b; Khazaeli et al. 1995, 1996). When the number of vials fell below 100, eggs were counted in all remaining vials until the end of the assay. All assays started with 3,200 females per replicate population, and as many males. Each fecundity assay continued until all flies were dead. Age is measured in days from the egg stage unless indicated otherwise.

ACO and CO survival

Large numbers of CO flies adult flies were lightly anesthetized with carbon dioxide and separated into new 8-dram food vials, in groups of 12 males and 12 females per vial. Survival was determined every 2–4 days. Living flies were transferred to new vials with fresh food and number of dead flies per sex was recorded. When necessary, flies were recombined in order to maintain a density of approximately 24 flies/vial, to rule out any possible density effects on both early and late-life mortality rates (Graves and Mueller 1993, 1995; Curtsinger 1995a, b; Khazaeli et al 1995, 1996). Survival assays were continued until all flies were dead. The average sample size for the CO mortality assay was 2,168 females and 2,352 males. We purposely used large sample sizes in order to reduce sampling variance in our estimations of mortality rates (Promislow et al. 1999).

We did not do a separate survival assay for the ACO populations. However, to determine if there were differences in the average break day for our new model required estimates of the survival of the ACO flies used in these fecundity experiments. We obtained rough estimates of these parameters by counting the number of survivors in the fecundity experiments. These numbers can only be accurately estimated once 2,800 of the original 3,200 flies had died. At that point we followed every vial of four females in the experiment and so have good estimates of the numbers that died every day. However, these ACO results are much less detailed than the CO survival data and thus are utilized for only the comparison of the ACO and CO fecundity break days.

Experiments on individual females

In one experiment, a large cohort of flies from the CO₁ replicate population was used in each of three assays (Rauser et al. 2005a). We refer to the populations created for each of these three assays as CO₁₋₁, CO₁₋₂, and CO₁₋₃. For each replicate assay, individual females were housed with two males in vials containing charcoal-colored medium and 5 mg of yeast. Fecundity was first measured at age 12 days from egg (all ages reported are in days from egg). Assays one and two started

with 1,111 females and twice as many males, to insure that all females were mated, while assay three started with 606 females and twice as many males. The three replicate assays were temporally staggered to reduce the large amount of work required in measuring daily fecundity for such a large number of females. Over all three cohorts, we collected lifetime daily fecundity data for 2,828 females, with 3,169,101 eggs counted in total.

During the assays, we transferred flies to fresh yeasted vials daily and counted the number of eggs laid for each female until she died. Male flies were recombined between vials as they died, to ensure a supply of mates for females. We wanted to measure lifetime individual female fecundity for all females in each cohort and compare the age-specific fecundity of females that died before the onset of the late-life fecundity plateau with those females that live to lay eggs at very late ages.

Results

Predictions from the evolutionary theory of late life

The force of natural selection acting on fecundity should decline with age until the last age of survival in the environment in which a population evolves (Hamilton 1966). The force of natural selection acting on age-specific fecundity scales according to $s'(x) = e^{-rx}l_x$, where x is the age of a genetic effect on fecundity, r is the Malthusian parameter for the population, and l_x is survivorship to age x . After the last age at which individuals survive in the population's evolutionary history (say d , which is not necessarily the last age of cohort survival under protected conditions) $s'(x)$ converges on and remains at zero thereafter. The results of a simulation model of such fecundity plateau evolution are given in Rauser et al. (2006a)

According to this evolutionary theory for late life, fecundity should mimic the age-specific force of natural selection. That is, fecundity should decline in mid-life and plateau at very late ages, in a fashion analogous to mortality rates. However, as with mortality, it may not be possible to detect these plateaus in female fecundity unless very large cohorts are examined. If we examine age-

specific fecundity in a variety of organisms, there are some general patterns which emerge (Finch 1990 chapter 3; Carey 2003 chapter 4; Rauser et al. 2006b).

Many species show an increase in fecundity following sexual maturity. After a peak in fecundity is reached, some time in early or mid-life, there is a decline in fecundity at later ages. Very generally, we expect that many organisms undergoing a laboratory fecundity assay will show a unimodal age-specific fecundity curve which may either decline steadily to a low value or show some type of plateau at late life. Our simulations (Rauser et al. 2006a) predict the evolution of fecundity toward a general pattern of decline from a peak in early life to a plateau at later age.

While it is possible to ignore early fecundity and only model fecundity in mid and late-life with our evolutionary model, there are additional phenomena that affect the fecundity of individual females just before they die that we must incorporate.

A stochastic model of female fecundity

Recently we found in a study of individual females that their fecundity declines more rapidly just before death compared to similarly aged females that are not dying (Rauser et al. 2005a). We call this pathological deterioration of fecundity the “death spiral.” A similar phenomenon has been observed in medflies (Müller et al. 2001).

We develop a statistical model of late life fecundity by distinguishing between the egg-laying of females before and during their death spiral. The basic pattern of female age-specific fecundity before the death spiral according to our evolutionary theory is as follows. In mid to late life, fecundity shows a roughly linear decline until the fecundity break day (fbd), after which fecundity remains constant (Fig. 1). These assumptions lead to the following relationship between age (t) and fecundity ($f(t)$),

$$f(t) = \begin{cases} c_1 + c_2t, & \text{if } t \leq fbd \\ c_1 + c_2fbd, & \text{if } t > fbd \end{cases} \quad (1)$$

Just before death, during the death spiral, we have found that the fecundity of individual flies

declines at a more rapid rate (Fig. 1). If the duration of the death spiral is w days and a particular female dies at age d , then her fecundity for w days prior to death, $\tilde{f}(t)$, is given by,

$$\tilde{f}(t) = f(d - w) + f(d - w)c_3(w + t - d). \quad (2)$$

This formulation of the death spiral assumes that the slope of the decline is proportional to the average fecundity of females at the age the death spiral begins. Both $f(t)$ and $\tilde{f}(t)$ are constrained to have non-negative values. Accordingly, the complete four parameter model for age-specific fecundity with parameters, $\theta = (c_1, c_2, c_3, fbd)$, is,

$$F(t, d, \theta) = \begin{cases} f(t) & \text{if } t < d - w \\ \tilde{f}(t) & \text{otherwise} \end{cases} \quad (3)$$

To make predictions from Eq. 3, we need to know the age at death of every female in the cohort. With this information, it is possible to determine *both* which females are in a death

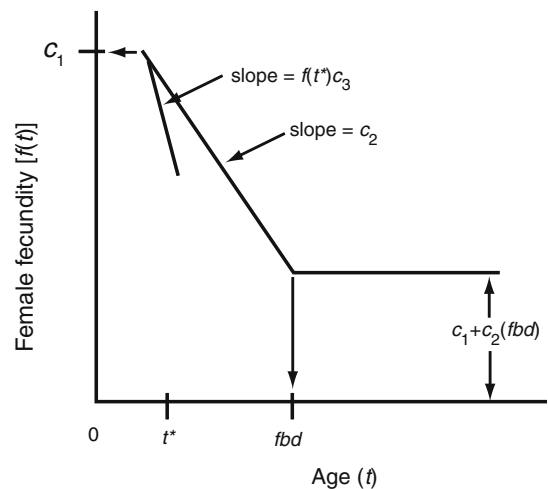


Fig. 1 A model of female fecundity. During middle ages the decline in female fecundity at age t is described by the line $f(t) = c_1 + c_2t$. At age fbd , called the fecundity break day, female fecundity reaches a plateau of $c_1 + c_2fbd$ eggs per day. Females about to die enter a death spiral or steep decline in fecundity. If a female begins this death spiral at age t^* , then fecundity declines linearly from that age until death with a slope of $c_3f(t^*)$. This slope may be the same for all flies or may vary for pre- and post plateau females. The duration of the death spiral is assumed to be a fixed length. It may be estimated independently from data or via regression from the population fecundity data

spiral, and hence have their fecundity best predicted by Eq. 2, and which females are not about to die and thus have fecundities predicted by Eq. 1. To explore the patterns of female fecundity predicted by Eq. 3, we used the two-stage Gompertz, to generate a sample of longevities for populations of different sizes. With the two-stage Gompertz model, the instantaneous mortality rate at age t -days is,

$$\begin{cases} A \exp(\alpha t) & \text{if } t \leq mbd \\ \bar{A} & \text{if } t > mbd \end{cases} \quad (4)$$

where mbd is the mortality break day.

One prediction from this model is that the length of the fecundity plateau will be greater in larger cohorts, as shown in the numerical simulations presented in Fig. 2A. This occurs because larger cohorts are more likely to have greater numbers of females surviving to extreme ages. In this model, stochasticity is due to random variation in ages of death. The survival of one cohort is a single realization of this process. As we look at larger number of realizations, the mean trends, especially at advanced ages, become more apparent (Fig. 2B).

An important parameter of this stochastic fecundity model is the duration of the death spiral, w . While the value of this parameter could be estimated from a regression analysis, we first examined individual fecundity patterns to see what an empirically estimated value of w might be. To accomplish this, we analyzed the individual fecundity data collected for the CO₁₋₁ population in Rauser et al. (2005a).

We separated all females into two groups, those dying before the break day (fbd) and those dying after fbd . The age of these flies has then been rescaled to days before death, rather than absolute age. From these data we then estimated the slope of female fecundity on days before death using different numbers of observations, varying the duration of the death spiral. Our expectation was that as we added observations further back in time from the day of death, the fecundity pattern should return to the average cohort fecundity, causing the magnitude of the slope of the fecundity decline to fall

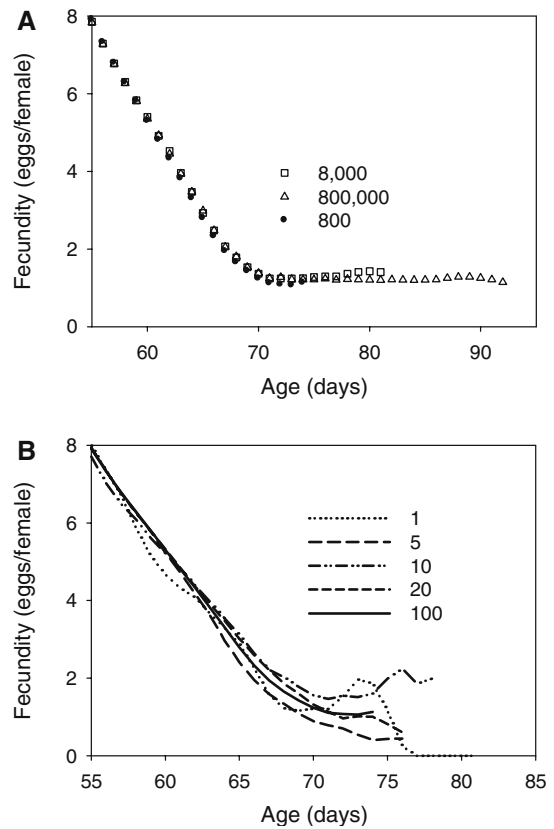


Fig. 2 Deaths for cohorts of 800, 8,000, or 800,000 females were simulated from the two-stage Gompertz (Eq. 4). The average fecundity at each age was then predicted from Eq. 3. The parameter values were taken from the final estimates for the CO₁ population and were: $A = 0.00452$, $\alpha = 0.076$, $\bar{A} = 0.3679$, $mbd = 71$, $c_1 = 75.0$, $c_2 = -1.53$, $fbd = 54.8$, $c_3 = -0.243$. In **A** the results for 100 replicate samples of each cohort size are shown. In **B** the results for a single cohort size (800) are shown using five different sample sizes

relative to its otherwise-expected value, when only the few days before death are used to estimate this slope. This analysis showed that the slope remains unchanged for non-plateau females until 16 days before death, suggesting a death spiral duration of 15 days (Fig. 3). In plateau females, the change occurs at day 7 suggesting a death window of 6 days. Based on these results, we used a death spiral duration of 10 days in models which treat w as a fixed constant.

Our basic model of female fecundity (Eq. 3) has four parameters, $\theta = (c_1, c_2, c_3, fbd)$. We also examined three variants of this model. First var-

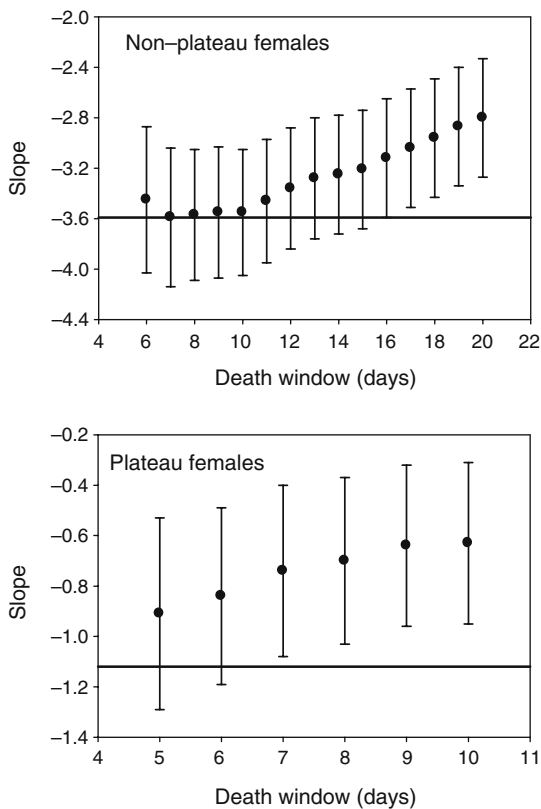


Fig. 3 The slope of the death spiral as a function of the death window for non-plateau and plateau females. The horizontal line shows the base slope of the death spiral using only five (non-plateau females) or four (plateau females) days of fecundity observations before death. Each point represents the slope with additional observations added. The error bars can be used to determine when these slopes are significantly different from the base slope. The error bars are twice the square root of the variance of the sum of the two estimated slopes (the base slope and the current slope)

iant: a five-parameter model which assumes that the slope of the death spiral, c_3 , may be different for pre-plateau and post-plateau females. Second variant: we generalized this five-parameter model to a six-parameter model by making the duration of the death spiral a model parameter. Third variant: this model was the same as the six parameter model except that the duration of the death spiral was allowed to differ for flies dying before the plateau and after the plateau. To provide a perspective on these models we have also fit these data to the simple two-stage linear model (Eq. 1).

Statistical inference of model parameters

To estimate the parameters of these stochastic fecundity models requires information on both age-specific fecundity and mortality. Without the mortality data, we can not directly infer the timing of female death spirals. We have analyzed three classes of experimental data using these models.

1. Experiments that have measured fecundity on individual females and have also recorded the age at death of these females. These are the best data and permit direct estimates of model parameters. These data were collected but not analyzed with Eq. 3 in Rauser et al. (2005a)
2. Experiments where the number of deaths of a cohort of females is recorded at regular time intervals, but fecundity is observed on groups of females, not individuals. None of our previous work analyzed data like this. We present the analysis of these types of data for the first time here.
3. Experiments where fecundity has been observed on groups of females but no survival data has been recorded. Most of our previous work consisted of data with these characteristics (Rauser et al. 2003, 2005b, 2006b). We discuss the results and methods of analysis for each of these three groups of experiments next.

1. *Individual fecundity and survival records.* Let the observed number of eggs laid by female- i at age- x be, f_{ix} , where $i = 1, \dots, N$ and $x = t_b \dots t_d$. Thus, t_b is the age from egg at which female reproduction begins and $t_d + 1$ is the greatest age at death of the N females. For each of the N females let the observed age at death be d_i . With these observations we can compute the average fecundity at each age by,

$$f_x = \frac{1}{n_x} \sum_{i \text{ such that } d_i > x} f_{ix} \tag{5}$$

based on records of n_x females still alive at age- x .

The predicted average fecundity $(F_x(\hat{\theta}))$ at age- x for the set of parameter values $\hat{\theta}$ is calculated as,

$$\frac{1}{n_x} \sum_{i \text{ such that } d_i > x} F(x, d_i, \hat{\theta}), \quad (6)$$

where $F(x, d_i, \hat{\theta})$ is one of the fecundity models such as Eq. 3. The model parameters, $\hat{\theta}$, are then chosen to minimize,

$$\frac{1}{(t_d - t_b + 1)} \sum_{x=t_b}^{t_d} [f_x - F_x(\hat{\theta})]^2. \quad (7)$$

Since there are so many more females at the young ages, we have chosen a least-squares statistic that treats each age as an equivalent sampling unit. However, since there are fewer observations at the older ages, we expect the fecundity predictions at late ages to be less reliable. This uncertainty will be reflected in the size of the confidence intervals we compute with these regression predictions.

To evaluate the uncertainty in the predicted values of female fecundity we utilized bootstrap resampling of our data. A bootstrap sample, \tilde{f}_{ix} , was generated by taking a sample of N females with replacement from the original set of N females. This sampling also produced N bootstrap ages at death, \tilde{d}_i . With this bootstrap sample, we utilized the methods summarized in Eqs. 5, 6, 7 to obtain a least squares estimate, $\tilde{\theta}$. The parameter $\tilde{\theta}$ was then used to predict the mean fecundity at each age,

$$\tilde{F}_x(\tilde{\theta}) = \frac{1}{\tilde{n}_x} \sum_{i \text{ such that } \tilde{d}_i > x} F(x, \tilde{d}_i, \tilde{\theta}),$$

where \tilde{n}_x is the number of females alive at age- x in the bootstrap sample. One hundred bootstrap samples were generated and 96% confidence bands on the average value of the 100 $\tilde{F}_x(\tilde{\theta})$ were derived from the second smallest and 99th largest value of $\tilde{F}_x(\tilde{\theta})$.

2. *Individual survival records and group fecundity records.* To estimate the basic model (Eq. 3) parameters, and to provide confidence intervals about the estimated parameter values, we compared observed fecundity data with those

derived from simulations. The simulations generated ages at death from the two-stage Gompertz mortality model. The parameter estimates for the two-stage Gompertz model were obtained in independent mortality experiments.

Our experimental data for this model consisted of an initial cohort of 3,200 females, reared as described above. Unlike the previously described experiment with individual fecundity records, the females in this experiment were maintained in vials with four females per vial. At each age, if there were more than 400 surviving females, a sample of 100 vials was chosen to estimate fecundity. Once the number of surviving females dipped below 400, all vials were used to estimate fecundity. Thus, the per-capita fecundity of females in vial- i at age- x is given by $f_i(x)$, $i = 1, 2, \dots, n_x$, where n_x is the number of vials used to estimate fecundity at age- x . Age-specific fecundity estimates started at age t_b , which was 30 days from egg for all five populations, and ended at day t_d , the last day there were four live females, which varied among populations.

In our numerical analysis, the bootstrap fecundity sample at age- x was generated by taking n_x samples with replacement from $f_i(x)$. This bootstrap sample is represented as, $\tilde{f}_i(x)$, $i = 1, 2, \dots, n_x$.

The independent mortality data were used to estimate the simulation parameters of the two-stage Gompertz. The distribution function of the two-stage Gompertz, $G(x)$ is,

$$\begin{cases} \exp\left\{\frac{A[1-\exp(-\alpha x)]}{\alpha}\right\} & \text{if } x \leq mbd \\ \exp\left\{\frac{A[1-\exp(-\alpha x)]}{\alpha}\right\} \exp[\tilde{A}(mbd - x)] & \text{if } x > mbd \end{cases}$$

The age at death, d , for 3,200 females in the bootstrap sample was simulated by the inverse-transform algorithm as $d = G^{-1}(U)$, where U is a uniform random number on the interval (0,1) (Fishman 1996). At each age we took data from a sample of 400 females, or if there were fewer than 400 survivors, all females were used. Let the number of females used at each age be \tilde{n}_x . With the simulated age at death for these females and an estimate of the model parameter θ_0 , we estimated the predicted fecundity of each female as

$F(x, d_i, \theta_0)$ (Eq. 3), for $i = 1, \dots, 3,200$. The bootstrap estimate of fecundity at age- x , for parameter θ_0 , was then estimated from the average, $\tilde{F}_x(\theta_0) = 1/\tilde{n}_t \sum_{i=1}^{i=\tilde{n}_t} F(x, d_i, \theta_0)$. The least-squares estimates were found by minimizing the sum, $\sum_{j=t_b}^{j=t_d} \sum_{i=1}^{i=n_j} [\tilde{F}_j(\theta_0) - \tilde{f}_i(j)]^2$. From this first bootstrap sample, one bootstrap estimate of the parameter vector, θ_i was obtained. One hundred bootstrap samples were then generated and their mean was used as the final parameter estimate, $\hat{\theta}$. These least squares estimates treat the vials as the units of observation. Since the number of vials used was limited at the early ages, these regressions do not weight the very early ages excessively, although the very late ages contribute less to minimizing the squared deviations due to the small number of survivors.

3. *Group fecundity records only.* When only fecundity data from groups of females exist, it is not possible to estimate all the parameters in Eq. 3. However, using the fecundity data alone we can get estimates of the parameters for Eq. 1 using standard nonlinear regression techniques. From these we can use the estimated break days to make important evolutionary inferences. For this procedure to be valid it is important to assume a correspondence between the estimated value of the break day utilizing only Eq. 1 versus the value for the break day derived from the full model (Eq. 3). We explore this problem later.

Application of the stochastic fecundity model to *Drosophila*

1. *Individual fecundity and survival records.* The four parameter stochastic fecundity model (Eq. 3) was fit to individual data as well as the five, six and seven parameter variants of Eq. 3. The success of the four models was then compared using the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) and a cross validation index. We looked for the model that consistently had the smallest value of AIC, BIC and the cross validation index. To compute the cross validation index, we divided the raw data into halves. One half was used to estimate the model parameters. We then computed the mean prediction sum of squares with the second half of the data set. This process was repeated 100 times with different random partitions of the raw data and the average values of the cross validation index are reported in Table 1.

Our four parameter model (Eq. 3) was the most frequent best model over all three indices used for assessing model fit. The simple two-stage linear model (Eq. 1) has the fewest parameters but has the highest AIC and BIC values of any model tested (Table 1). Clearly, taking into account the effects of the death spiral provides a dramatic improvement over Eq. 1. These results combined with the general preference for the most simple model suggests that Eq. 3 is the best

Table 1 The model fitting results for three different data sets and four different models

Model	Criteria	CO ₁₋₁	CO ₁₋₂	CO ₁₋₃
Eq. 1	AIC	7.28	9.24	10.5
	BIC	8.02	10.1	11.5
4-par	AIC	4.14	4.55	5.17
	BIC	4.67	5.12	5.67
5-par	Cross-validation	3.93	6.33	9.92
	AIC	4.32	4.28	5.05
	BIC	4.98	4.99	5.68
6-par	Cross-validation	5.01	5.20	11.48
	AIC	4.34	4.37	4.83
	BIC	5.14	5.22	5.58
7-par	Cross-validation	5.12	6.63	10.57
	AIC	4.29	4.95	4.33
	BIC	5.23	5.94	5.21
	Cross-validation	4.59	6.10	10.23

The lowest (best) value for each criteria are bold

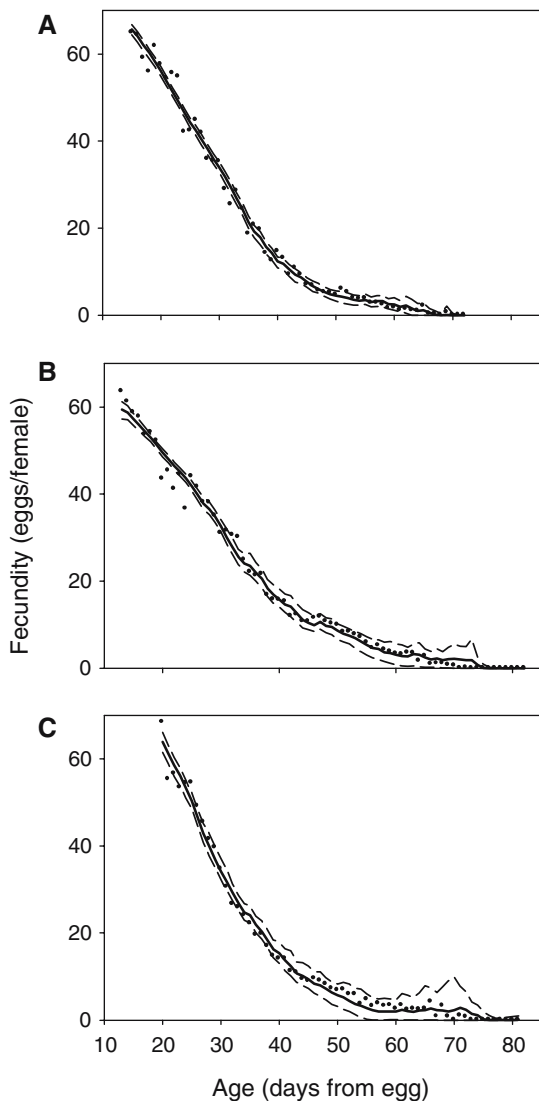


Fig. 4 The four parameter fecundity model (*dark solid line*) and 96% confidence interval (*dashed black lines*) for populations CO₁₋₁ (**A**), CO₁₋₂ (**B**), and CO₁₋₃ (**C**). The circles are the mean observed fecundity at each age

description of age-specific female fecundity. We have used this model to compare the average predicted fecundity from the model with the observed average fecundity in the three populations, CO₁₋₁, CO₁₋₂, and CO₁₋₃ (Fig. 4).

Although the fit of the four parameter model is very good we do not consider goodness of fit alone to be the only important criterion for assessing the utility of this type of model. This model is our hypothesis about the evolutionary

forces molding age-specific fecundity, incorporating the heterogeneity arising from individual physiological declines prior to death. It is thus both of a priori interest and phenomenologically credible, in terms of fitting actual data.

2. *Individual survival records and group fecundity records.* Except for one case out of the ten examined, the four-parameter model (Eq. 3) had the smallest values of both AIC and BIC, as shown in Table 2. Accordingly, we have focused on this model in the detailed analysis of the CO data presented here.

Fecundity and mortality rates were measured for each of the five replicate CO populations. Figure 5 shows the data for both age-specific fecundity and female mortality, along with their respective fitted models for all five populations. Although the fecundity model is composed entirely of linear functions, the fact that the population is composed of two types of females, the normal and the dying, produces predicted fecundities that decline in a nonlinear fashion with age (Fig. 5). The age of onset of the late-life fecundity and mortality-rate plateaus for a population, and their respective break days, were estimated from either the stochastic fecundity model (see Table 3) or the two-stage Gompertz model (see Table 4), respectively. Though these estimated ages for the start of late life differ between age-specific fecundity and mortality, with pleiotropic effects that affect fecundity and mortality differentially, the break days do not need to correspond, at least not on the basis of the evolutionary theory of late life, when pleiotropy is allowed.

Table 2 Summary of the stochastic fecundity model statistics

Model	Parameter	CO ₁	CO ₂	CO ₃	CO ₄	CO ₅
4-par	AIC	100.1	80.55	76.19	63.72	97.10
	BIC	100.8	81.16	76.64	64.20	97.85
5-par	AIC	100.6	81.20	82.49	63.64	102.23
	BIC	101.5	81.96	83.21	64.25	103.17
6-par	AIC	100.1	81.02	76.78	63.49	98.38
	BIC	101.2	82.10	77.65	64.22	99.50
7-par	AIC	101.1	80.76	76.54	63.97	98.84
	BIC	102.4	81.83	77.55	64.82	100.15

The four (4-par) and five parameter (5-par) models use fixed widths of 10 days. The lowest values of AIC and BIC are shown in bold face for each population

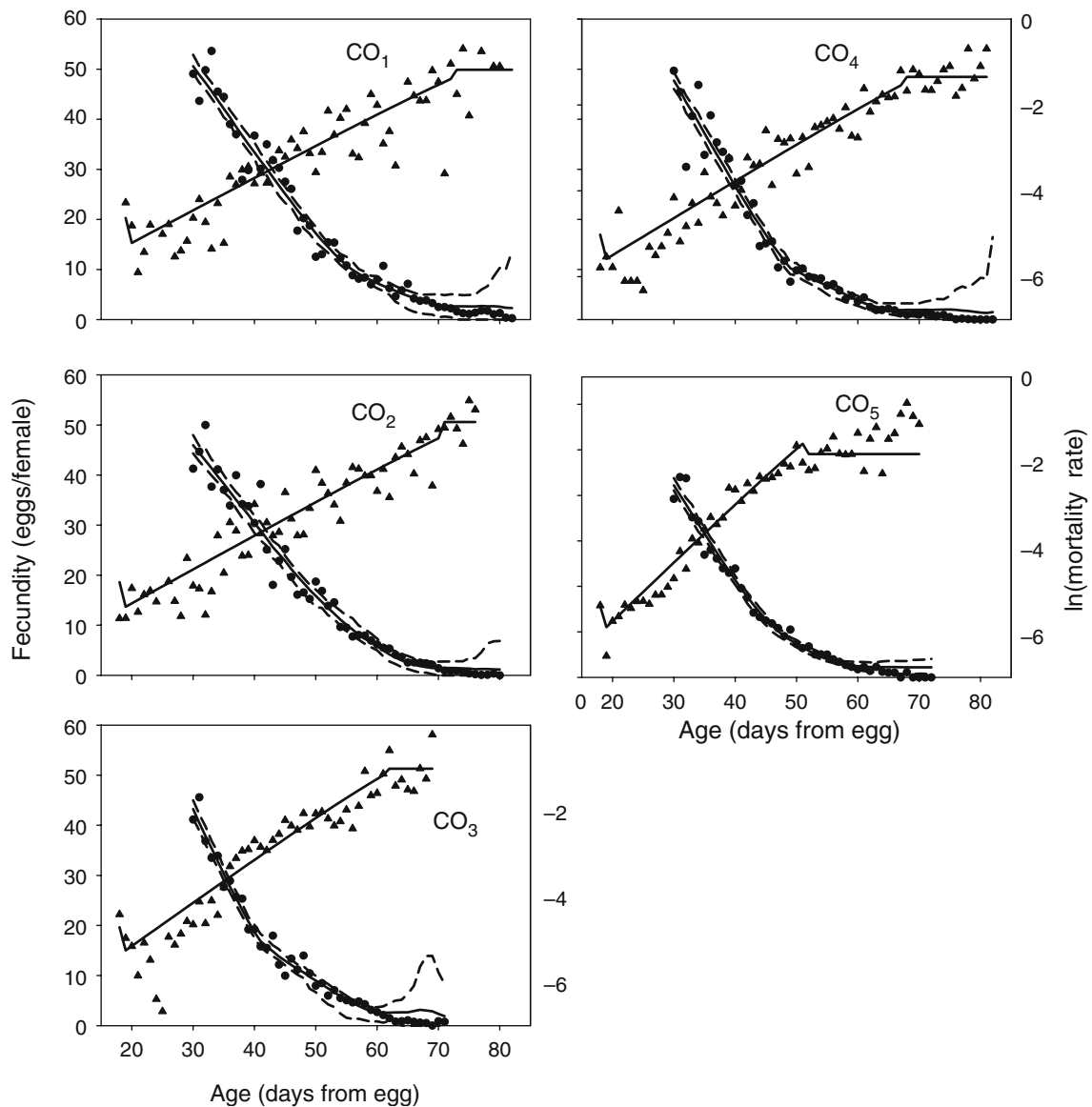


Fig. 5 Age-specific female mortality and fecundity data along with the respective model predictions for all five CO populations. The *circles* are the observed mean fecundity and the *triangles* are the observed mortality rates. A two-stage Gompertz model was fit to the mortality data and the four-parameter stochastic fecundity model was fit to the fecundity data to determine the break days, or the onset of

the late life plateaus, for both mortality and fecundity. The *dashed lines* are the upper and lower 96% confidence interval for the fecundity predictions. Fecundity plateaued earlier than mortality in all five populations. The average pairwise difference between the onset of the two types of plateaus was 12.7 days

This is not a post hoc invocation of pleiotropy. As shown in Rose et al. (2002) and Rauser et al. (2006b), we have independent evidence for the occurrence of pleiotropy affecting late-life.

An important question for this new model is whether there is a significant difference between

the break day in the CO populations and the ACO populations. Using the survival data from the ACO fecundity experiment and the techniques outlined in this section the average fecundity break day in the ACO populations was estimated as 14.6 days of adult life. This is

Table 3 The stochastic fecundity model (Eq. 3) least squares estimates and 96% bootstrap confidence intervals

Population	c_1	c_2	fbd	c_3
CO1	75.0 (72.1, 78.4)	-1.53 (-1.80, -1.20)	54.8 (40.4, 58)	-0.243 (-2.51, -0.00965)
CO2	69.1 (65.9, 73.0)	-1.36 (-1.65, -1.00)	54.7 (47, 61.0)	-0.735 (-2.71, -0.000803)
CO3	82.2 (75.4, 88.4)	-2.37 (-2.74, -1.96)	40.6 (37.5, 41.9)	-0.189 (-1.97, -0.116)
CO4	79.6 (75.3, 82.5)	-1.88 (-2.13, -1.65)	48.9 (47.6, 50)	-0.411 (-1.76, -0.00118)
CO5	70.8 (67.6, 73.7)	-1.77 (-2.08, -1.51)	53.7 (48, 59.3)	-0.104 (-0.135, -0.0690)

The parameter fbd is the fecundity break day measured as days from egg

Table 4 Parameter estimates from the two-stage Gompertz model that was fit to male and female age-specific survival data

Population	Females				Males			
	α	A	mbd	\bar{A}	α	A	mbd	\bar{A}
CO ₁	0.0760	0.00452	71.0	0.3679	0.0745	0.00452	68.0	0.3012
CO ₂	0.0794	0.00370	69.0	0.4066	0.0797	0.00335	71.0	0.4066
CO ₃	0.1013	0.00409	60.0	0.4493	0.1271	0.00222	51.7	0.2069
CO ₄	0.0866	0.00303	66.0	0.3012	0.0854	0.00335	60.0	0.2466
CO ₅	0.1371	0.00209	50.0	0.1821	0.1262	0.00286	48.4	0.1706

α is an age-dependent parameter, A is an age-independent parameter, mbd is the mortality break day measured as days from egg, or the age that mortality rates plateau, and \bar{A} is the late-life mortality rate, or the mortality rate after the onset of the plateau

Table 5 Parameter estimates for the two-stage fecundity model (Eq. 1)

Model parameters							
Population	c_1		c_2		fbd		
	Eq. 3	Eq. 1	Eq. 3	Eq. 1	Eq. 3	Eq. 1	
CO ₁₋₁	75.4	76.5	-1.72	-1.56	35.8	34.2	
96% c.i.	(73.6,76.8)		(-1.96,-1.63)		(33.1,46.4)		
CO ₁₋₂	64.7	81.0	-1.28	-1.62	37.8	33.1	
96% c.i.	(62.3,66.7)		(-1.46,-1.06)		(31.4,57.8)		
CO ₁₋₃	85.0	74.3	-1.97	-1.48	39.0	34.3	
96% c.i.	(80.2,89.5)		(-2.39,-1.58)		(31.0,50.0)		
CO ₁	75.0	98.3	-1.53	-1.61	37.8	40.5	
96% c.i.	(72.1,78.4)		(-1.80,-1.20)		(33.4,41.0)		
CO ₂	69.1	86.3	-1.36	-1.39	38.7	44.1	
96% c.i.	(65.9,73.0)		(-1.65,-1.00)		(31.0,45.0)		
CO ₃	82.2	102.3	-2.37	-2.04	24.6	30.7	
96% c.i.	(75.4,88.4)		(-2.74,-1.96)		(21.5,25.9)		
CO ₄	79.6	103.5	-1.89	-1.90	32.9	36.3	
96% c.i.	(75.3,82.5)		(-2.13,-1.65)		(31.6,34.0)		
CO ₅	70.8	99.2	-1.77	-1.93	37.7	32.6	
96% c.i.	(67.6,73.7)		(-2.08,-1.51)		(32.0,43.3)		

The column labeled Eq. 1 has derived these estimates solely from fecundity data. The column labeled Eq. 3 has utilized fecundity and survival data in combination with Eq. 3 to estimate the model parameters. 96% bootstrap confidence intervals are provided for the estimates obtained from Eq. 3. The break day (fbd) is measured in days of adult life

19.7 days earlier than the average CO fecundity break day and the difference is statistically significant (t test, $P = 0.003$). This result is consistent with our earlier result using only group fecundity records (Rausser et al. 2006b)

3. *Group fecundity records only.* For eight different experimental data sets we have estimated the parameters of Eq. 1 from the fecundity data only. Three of these data sets, referred to as CO₁₋₁, CO₁₋₂, and CO₁₋₃, are replicate

experiments on individual females from the CO₁ population (Rauser et al 2005a). For these three populations, the parameters of Eq. 1 were also estimated from the stochastic fecundity model (Eq. 3) using the techniques described previously for individual fecundity and survival records. The remaining five populations are the entire set of five CO populations (CO_{1–5}). However, for these data, fecundity was recorded on groups of females and survival was observed on a separate group of females. Accordingly, the parameters of the stochastic fecundity model were estimated by the techniques described previously for individual survival records and group fecundity records. These analyses were done using an adult age time-scale. Thus, time zero is the start of adult life.

The results (Table 5) suggest that the best-fit values for the parameters c_1 and c_2 may vary depending on the technique used. This is not surprising since the decline in female fecundity with age is described by two parameters in the stochastic fecundity model (Eq. 3), c_2 and c_3 , while the simple model (Eq. 1) summarizes this decline with just one parameter, c_2 . The numerical estimates of the break day (*fbd*) are more similar (Table 5). In fact, of the eight estimated values, six estimated by Eq. 1 are within the confidence interval of the estimates obtained from the stochastic fecundity model (Eq. 3). Given these findings, we suggest that reasonable estimates of the break day for the stochastic fecundity model can be obtained when survival data is absent by simply fitting the two-stage fecundity model.

Discussion

A long term goal of this research is to understand the forces that shape late-life survival and fecundity. Studying this problem experimentally requires statistical models of the patterns of age-specific survival and fecundity, regardless of the hypothesis under test. There already exist several statistical models of age-specific mortality that provide a good fit to the data found for many different organisms. There has been less work on providing a general statistical model of age-specific fecundity in later life. In this paper we have attempted to fill that void.

The statistical models that we have developed and tested here may be used both for quantitative prediction and to gain understanding of basic biological processes. Ultimately however, making precise predictions is not our most important goal. We are more interested in using these statistical models as a means of generating testable predictions about the action of natural selection. One useful prediction has been the age of onset of a fecundity plateau. An explanation of late life based on natural selection would predict that this onset of a fecundity plateau should occur at younger ages in those populations where early reproduction is at a premium.

The statistical analyses presented here demonstrate that, even in circumstances where the longevity of females has not been estimated, fecundity data alone can be used to get reasonably good estimates of the start of a fecundity plateau. This is true even though the simpler model with a strict plateau at late life may not appear to accurately predict the observed per-capita fecundity at later ages. Our conclusion is that the findings of previous papers on late-life fecundity (Rauser et al. 2003, 2005a, b, 2006b) that were based on estimates of the fecundity break day from Eq. 3 remain scientifically valid. That is, if more detailed data on female survival had also been available, we expect that our previous conclusions about differences in fecundity break days would be largely unchanged.

While our discussion and data analysis have focused on the model organism *Drosophila melanogaster*, our novel statistical model could be used with other organisms as well. It has been noted previously that Mediterranean fruit flies also show a similar decline in fecundity prior to death (Müller et al. 2001). In addition, Carey (2003) has noted that males exhibit a characteristic supine behavior prior to death. Current male calling behavior in medflies may also be used to predict remaining lifespan (Zhang et al. 2006). These observations suggest a physiological decline prior to death that may exhibit its effects in a variety of characters, not just female fecundity. Carey and his colleagues have used this concept in their attempt to predict the longevity of individuals (reviewed in Carey 2003), as opposed to

predictions of individual female fecundity as is the case in the present paper.

The ability to examine other organisms for the presence of fecundity plateaus is currently limited by the small number of studies that have sampled sufficient numbers of females. However, just as the examination of the mortality rates of very large numbers of individuals in experimental cohorts has become more common over the last 15 years, we expect the same will happen with experimental work on age-specific female fecundity.

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