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Evolution of late-life fecundity in *Drosophila melanogaster*

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late life;
mortality;
mortality-rate plateau;
viability.

Abstract

Late-life fecundity has been shown to plateau at late ages in *Drosophila* analogously to late-life mortality rates. In this study, we test an evolutionary theory of late life based on the declining force of natural selection that can explain the occurrence of these late-life plateaus in *Drosophila*. We also examine the viability of eggs laid by late-age females and test a population genetic mechanism that may be involved in the evolution of late-life fecundity: antagonistic pleiotropy. Together these experiments demonstrate that (i) fecundity plateaus at late ages, (ii) plateaus evolve according to the age at which the force of natural selection acting on fecundity reaches zero, (iii) eggs laid by females in late life are viable and (iv) antagonistic pleiotropy is involved in the evolution of late-life fecundity. This study further supports the evolutionary theory of late life based on the age-specific force of natural selection.

Introduction

Late-life mortality-rate plateaus

Mortality rates have been shown to decelerate and 'plateau' late in life in a number of organisms, including *Drosophila*, medflies, wasps, yeast, nematodes and humans (Greenwood & Irwin, 1939; Comfort, 1964; Carey *et al.*, 1992; Curtsinger *et al.*, 1992; Fukui *et al.*, 1993; Brooks *et al.*, 1994; Curtsinger *et al.*, 1995; Charlesworth & Partridge, 1997; Vaupel *et al.*, 1998; Drapeau *et al.*, 2000; Rose *et al.*, 2002; Miyo & Charlesworth, 2004). When mortality-rate plateaus were first definitively demonstrated (Carey *et al.*, 1992; Curtsinger *et al.*, 1992), they challenged theories of aging that predicted an exponential increase in age-specific mortality rates (Finch, 1990). The plateauing of cohort mortality rates late in life suggested that, in an infinite population, some organisms could theoretically live forever. Although there is currently no widely accepted explanation for mortality-rate plateaus, many theories have been proposed (Abrams & Ludwig, 1995; Mueller & Rose, 1996; Charlesworth & Partridge, 1997; Pletcher & Curtsinger, 1998; Wachter, 1999; Demetrius, 2001; Gavrilov & Gavrilova, 2001; Weitz & Fraser, 2001). Among these theories, two types have received the most attention: evolutionary and demographic.

The demographic theories of late-life mortality suggest that mortality rates plateau because of lifelong differences in individual robustness (Vaupel *et al.*, 1979; Vaupel, 1988,1990; Pletcher & Curtsinger, 2000). This effect only results in mortality-rate plateaus when heterogeneity in robustness is extreme and sustained throughout life (Service, 2000a). However, heterogeneity this extreme and this consistent has yet to be found experimentally (Curtsinger *et al.*, 1992; Fukui *et al.*, 1996; Brooks *et al.*, 1994; Vaupel *et al.*, 1994; Khazaeli *et al.*, 1998; Drapeau *et al.*, 2000; but see Service, 2000b; Mueller *et al.*, 2000).

One evolutionary theory of late-life mortality is based on the plateau, at or near zero, in the age-specific force of natural selection after the end of reproduction (Mueller & Rose, 1996; Rose & Mueller, 2000; Charlesworth, 2001; see Pletcher & Curtsinger, 1998 for caveats). This theory has been experimentally corroborated by the result that the onset of mortality-rate plateaus evolutionarily fluctuates with the age at which reproduction ends (Rose *et al.*, 2002).

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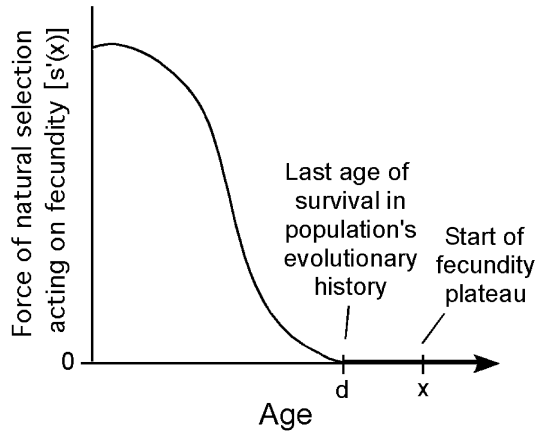

Fig. 1 The force of natural selection acting on age-specific fecundity. '*d*' indicates the last age of survival in the population's evolutionary history. After '*d*' the force of natural selection plateaus and remains at zero. '*x*' indicates the start of the fecundity plateau.

Late-life fecundity

As with mortality, 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 (Fig. 1). 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.

According to this evolutionary theory, 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, after the last age of survival. We have already experimentally demonstrated that population fecundity indeed plateaus at late ages in three large cohorts of *Drosophila melanogaster* (Rauser *et al.*, 2003) and that the occurrence of late-life fecundity plateaus is not affected by nutrition or mate age (Rauser *et al.*, 2005).

This evolutionary theory not only implies that population fecundity should plateau at late ages, but that these plateaus should evolve according to the last age of survival in the population's evolutionary history. In this study, we test this prediction by comparing the onset of population fecundity plateaus in populations that have long had different last ages of survival. If fecundity plateaus in late life and evolves according to the evolutionary theory, then our results will support the evolutionary theory.

Late-life offspring viability

Offspring viability generally declines with parental age in *Drosophila* (Price & Hansen, 1998; Hercus & Hoffmann, 2000), except in later reproducing lines (Kern *et al.*, 2001). Thus, it may be important to include reproductive factors, such as viability, in the evolutionary theory of aging because it is conceivable that the same genetic mechanisms that affect age-specific mortality and fecundity rates could similarly affect offspring viability.

In this study, we determined egg-to-adult viability for offspring from mid-and late-life parental ages in early and late-reproducing populations. Our objective was to test the effects of parental age on the evolution of offspring viability, and to determine whether eggs laid in late life are viable and how that viability compares to eggs laid in mid life.

Antagonistic pleiotropy and fecundity

Antagonistic pleiotropy occurs when genes that are beneficial early in life are deleterious later in life (Williams, 1957; Rose, 1985; Charlesworth, 1994). Late-acting deleterious genes that cause reproductive senescence late in life can persist in a population because these same genes enhance reproduction, or other fitness characters, at earlier ages when the force of natural selection is much stronger.

Because the start of late-life fecundity plateaus depends on the timing in the drop in the force of natural selection, we predict that switching to a selection regime with an earlier last age of reproduction should lead to an earlier age for the onset of fecundity plateaus if antagonistic pleiotropy is a genetic mechanism underlying late-life fecundity patterns. In this study we subject late-reproducing populations to an evolutionary reversion to earlier ages of reproduction (cf. Rose *et al.*, 2002, 2004) and statistically test whether these newly derived early reproducing populations have an earlier plateau onset compared to the late-reproducing populations from which they were derived. Antagonistic pleiotropy is distinguished from mutation accumulation and genetic drift in this experiment by selecting for an earlier age of reproduction for only 24 generations, which is too little evolutionary time for mutation accumulation or drift to have a significant effect at the population sizes we employ.

In this paper, we experimentally test the predictions of the evolutionary theory for late life based on the declining force of natural selection, using *D. melanogaster* populations having different last ages of survival. We predict that all of these populations should show plateauing in late-life population fecundity, and have corresponding differences in the age at which fecundity stops declining and plateaus. Viability of eggs laid in mid- and late-life is also analysed. Lastly, we test a population

genetic mechanism that may shape the evolution of late-life fecundity: antagonistic pleiotropy.

Materials and methods

Experimental populations

We used replicated laboratory-selected populations of *D. melanogaster*, derived from the South Amherst, MA 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 the five corresponding O populations in 1989, and in recent years cultured using females that are 28 days of age (Rose *et al.*, 1992). These populations are grown up in vials until age 14 days from egg when they are placed in population cages until age 28 days when 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, also 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 8–10 days of age when they are placed in population cages for one 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 1000 individuals and so are not inbred (Chippindale *et al.*, 2004).

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. 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 provide a platform with which to test the evolutionary theory of late life, based on the force of natural selection, as it applies to fecundity.

Fecundity assays

All flies used in the fecundity assays 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 coloured 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 (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 2003; Graves & 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 3200 females per replicate population, and as many males. Each fecundity assay continued until all flies were dead.

Statistical tests for the existence and evolution of late-life fecundity plateaus

We tested whether fecundity plateaus at late ages by statistically testing the fit of a model with a late-life plateau to mid- and late-life fecundity data in all 10 populations. Average population fecundity in *Drosophila* increases at early adult ages until it reaches a peak, and then starts to decline. Therefore, we defined mid-life as those ages where average population fecundity starts to decline, and late-life as those ages where the decline in average population fecundity stops or slows. The model we fit to the data was a 3-parameter two-stage linear model, having a second stage slope of zero, analogous to the two-stage models fit previously to our mortality (Drapeau *et al.*, 2000) and fecundity data (Rauser *et al.*, 2003). Under the two-stage model the fecundity at aged t-days is

$$\begin{cases} \varphi_1 + \varphi_2 t & \text{if } t \le \varphi_3 \\ \varphi_1 + \varphi_2 \varphi_3 & \text{if } t > \varphi_3 \end{cases} \quad (1)$$

We chose the two-stage linear model *a priori* because the evolutionary theory of late life predicts a mid- life decline and a late-life plateau in fecundity. We are not attempting to model-fit our fecundity data, but to use the 2-stage model as a way to test the predictions made by the evolutionary theory. Our data does not necessarily have to fit this model, and if it generally did not,

then the evolutionary theory of late-life would be falsified.

The model was fitted using all of the fecundity data at each age (100 observations), starting at an age in mid-life where the average fecundity for that population started to decline (age 30 days for all CO populations except CO_3, where fecundity did not start to decline until age 46 days, and age 26 days for all ACO populations). Each population was fitted to the model independently. This model was fit to the data using a non-linear least-squares function in the R-project for statistical computing (http://www.R-project.org). We wrote a self-starting R-function for the two-stage linear model that provided initial estimates for the parameter values as well as the predicted fecundity from eqn 1.

We tested whether fecundity plateaus evolve according to the last age of survival using the ACO and CO *Drosophila* populations described above. The replicate ACO populations have an earlier age of reproduction and shorter life spans compared to the CO populations. However, these average life span patterns and ages of reproduction by themselves do not indicate the timing or nature of fecundity plateaus for these populations. A pair-wise comparison between the ACO and CO populations allows us to properly test whether the onset of fecundity plateaus, or the break day, would occur later in the CO populations relative to the ACO populations.

This experimental design resulted in one ACO_i and one CO_i population that were matched by a common index being tested at one time. The common index indicates that the two populations had a common population of origin (O_i). Thus, the pairs of populations form blocks that have a common evolutionary origin and a common set of experimental conditions. Each population also has its own unique history of genetic change due to random genetic drift. Thus, there are three sources of random variation in this experiment: populations, blocks and individual measurement errors.

In this formulation, we will let the index i indicate one of the 10 populations, j be one of the five blocks or population pairs and k a vial of four individuals, which is the smallest unit of observation within a population. If each population has a total of n_i individuals, then the number of eggs per female in population-i, block-j, individual-k is y_{ijk}. The basic non-linear model is given by

$$y_{ijk} = f(\varphi_{ijk},\ v_{ijk}) + \varepsilon_{ijk},$$

where φ_{ijk} is the vector of parameters, v_{ijk}, is the covariate vector and ε_{ijk} is the within population variation. The covariate vector contains the age of individual ijk, t_{ijk} and the population code, δ_i, which is zero if the population is ACO (e.g. $i = 1, 2, 3, 4$ or 5) and one if the population is CO (e.g. $i = 6, 7, 8, 9$ or 10).

For the two-stage linear model the functional relationship is

$$f(\varphi_{ijk}) = \begin{cases} \varphi_{1ij} + \varphi_{2ij} t_{ijk} & \text{if } t_{ijk} \le \varphi_{3ij} \\ \varphi_{1ij} + \varphi_{2ij}\varphi_{3ij} & \text{if } t_{ijk} > \varphi_{3ij} \end{cases}$$

We assume that the values of the model parameters are affected by both fixed and random effects. The fixed effects can be examined to determine if the selection treatment has a significant effect. The parameters are also assumed to vary randomly between populations due to founder and drift types of effects and between blocks. The between block variation may be due to different experimental conditions or due to founder effects. These two sources of variation cannot be separated. These assumptions translate into the system of equations,

$$\begin{aligned} \varphi_{1ij} &= \beta_1 + \gamma_1\delta_i + b_{1i} + c_{1j} \\ \varphi_{2ij} &= \beta_2 + \gamma_2\delta_i + b_{2i} + c_{2j} \\ \varphi_{3ij} &= \beta_3 + \gamma_3\delta_i + b_{3i} + c_{3j} \end{aligned} \qquad (2a - c)$$

where the γ_k ($k = 1$–3) are the fixed effects due to selection, the b_{ki} are the random population effects and the c_{kj} are the random block effects. An important statistical test will be to determine if the γ_k are significantly different from zero. If so, this will indicate that the selection treatment has a statistically significant effect on the regression model parameter.

Fecundity decreases substantially with age in these populations, which suggests that we should model within population variance as a function of mean fecundity. The general formulation is

$$\mathrm{Var}(\varepsilon_{ijk}) \cong \sigma^2 g^2(\hat{u}_{ijk}, v_{ijk}, \delta)$$

where $\hat{u}_{ijk} = E\left(y_{ijk}\middle|\mathbf{b}_i, \mathbf{c}_j\right)$. In this analysis, we used $g(.) = |y_{ikj}|^{\delta}$, where δ is estimated from the data. The $\mathbf{b}_i$ were assumed to be distributed as

$$\mathbf{b}_i \sim N\left(0, \begin{bmatrix} \Psi & 0 & 0 \\ 0 & \Psi_{22} & 0 \\ 0 & 0 & \Psi_{33} \end{bmatrix}\right)$$

The $\mathbf{c}_j$ are assumed to be distributed as

$$\mathbf{c}_j \sim N\left(0, \begin{bmatrix} Z_{11} & 0 & 0 \\ 0 & Z_{22} & 0 \\ 0 & 0 & Z_{33} \end{bmatrix}\right)$$

The maximum likelihood techniques used to estimate the model parameters and test their significance are reviewed in Pinheiro & Bates (2000), chapter 7). These techniques were implemented with the non-linear mixed effects package in R (Version 1.6).

Lastly, using the parameter estimates from the model, the height of the late-life fecundity plateau is

$$\hat{\varphi}_4 = \hat{\varphi}_1 + \hat{\varphi}_2\hat{\varphi}_3 \qquad (3)$$

Since $\hat{\varphi}_4$ is a non-linear function of the three estimated parameters, its variance was estimated using the delta method (Mueller & Joshi, 2000, p. 83). The variance in plateau height is then

$$\begin{aligned}\mathrm{Var}(\varphi_4) = {} & \mathrm{Var}(\varphi_1) + \varphi_3^2\mathrm{Var}(\varphi_2) \\ & + \varphi_2^2\mathrm{Var}(\varphi_3) + 2\varphi_3\mathrm{Cov}(\varphi_1\varphi_2) \\ & + 2\varphi_2\mathrm{Cov}(\varphi_1\varphi_3) + 2\varphi_2\varphi_3\mathrm{Cov}(\varphi_2\varphi_3) \quad (4)\end{aligned}$$

Asymptotic 95% confidence intervals on the plateau height, $\hat{\phi}_4$, are estimated as $\hat{\varphi}_4 \pm 1.96\sqrt{\mathrm{Var}(\hat{\varphi}_4)}$. The variances and covariances in equation (4) are estimated from the non-linear least squares procedures.

Testing late life viability

Offspring viability was determined in the ACO_{3-5} and CO_{3-5} populations, using a portion of the same cohort used in the pair-wise fecundity assays. Therefore, flies from the ACO_i and CO_i populations were reared as described above. At the beginning of each pair-wise viability assay, approximately 2000 individuals from each cohort were placed in a population cage and fed banana food medium. Eggs were collected from each population at weekly intervals, over a 2- to 8-hour interval using yeasted charcoal-food plates, until the corresponding fecundity assay ended. Each week, exactly 60 eggs were transferred from the charcoal-food plate to each of 10 8-dram vials containing 5 mL of standard banana-molasses food for both populations and incubated at 25 °C with constant light. When the number of eggs laid by the population decreased at later ages to numbers too low to set-up 10 vials, we completed as many vials as possible, always keeping the density of eggs per vial the same. The number of adults that emerged from each vial was counted starting at the day of first emergence, and thereafter for the next seven days. Viability was determined for four parental age classes in the ACO populations (ages 22–25, 29–32, 36–39 and 43–45 days from egg) and seven age classes in the CO populations (ages 22–25, 29–32, 36–39, 43–46, 50–53, 57–60 and 64–66).

We defined egg-to-adult viability as the proportion of eggs that resulted in eclosed adults. To test whether offspring viability declined with parental age over all age classes, including late life and whether viability differs between selection regimes, we used a linear regression model fitted to the angular-transformed [$\arcsin(\sqrt{x})$] proportions (Sokal & Rohlf, 1995, pp. 419–422) in R (http://www.R-project.org). The viability of the offspring at each parental age was considered the response variable and parental age and selection regime were the covariates used, separately or together, within the model. Offspring viability at each parental age class from the replicate populations within each selection treatment was pooled.

We compared the viability before the onset of the plateau with the viability after the onset of the plateau. That is, we compared parental ages one and two with three and four in the ACO populations and ages 1–5 with ages six and seven in the CO populations. The analysis and model were analogous to that described above for all age classes, except that we divided the data within each selection regime according to mid- or late-life parental ages. We determined the effect of parental age on offspring viability for mid- and late-life parental age classes independently, using a linear regression model. Lastly, we compared mid- to late-life viability within each selection regime.

Testing for antagonistic pleiotropy

Four NRCO populations were derived from corresponding CO populations and were subjected to selection for early reproduction using procedures similar to those used routinely with the ACO populations, for only 24 generations. At the end of the 24 generations of selection for accelerated development and early reproduction, fecundity assays were performed as described above for all pair-wise comparisons between the NRCO populations and their corresponding CO populations. Statistical analyses were also performed as described above, except with the NRCO populations replacing the ACO populations. Evolutionary theory predicts that the NRCO populations will evolve an earlier plateau in fecundity, compared to the CO populations, if antagonistic pleiotropy is a genetic mechanism shaping late-life fecundity patterns. Because antagonistic pleiotropy does not simply depend on early fecundity, selection for multiple early fitness characters (i.e. early reproduction and/or accelerated development) in the NRCO populations encompasses all types of antagonistic pleiotropy. Therefore, a shift in late age fecundity in response to this selection will implicate antagonistic pleiotropy as a genetic mechanism shaping late-life fecundity patterns, regardless of which particular early-life fitness components are involved.

Results

Late-life fecundity plateaus at late ages and evolves according to evolutionary theory

Mid- and late-life fecundity was measured in each of the 10 populations (Fig. 2). The amount of variation in fecundity within each population steadily declined with age. Every data point at each age starting in mid-life was used to fit the two-stage linear model to each of the five ACO and five CO populations independently (Fig. 3) to test whether fecundity plateaus at late ages and whether these plateaus evolve according to evolutionary theory.

We determined the height of the late-life fecundity plateau for each population, along with the estimated 95% confidence interval, using the parameter estimates obtained from the two-stage linear model (eqns 3–4, Table 1), to test whether fecundity plateaus at some number of eggs per female greater than zero. For all 10 populations, the height of the plateau, or the number of eggs per female per day after the break day, was significantly greater than zero (Fig. 3). This result further demonstrates that fecundity plateaus at late ages.

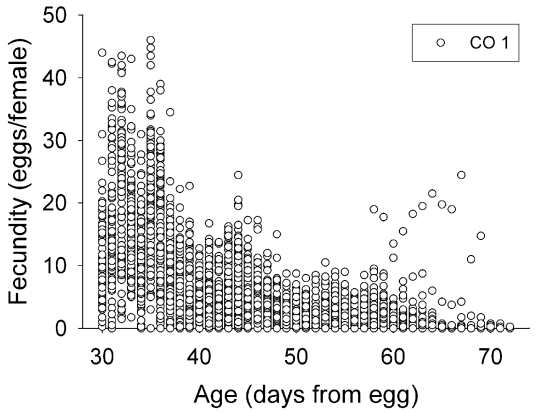

Fig. 2 Mid- and late-life fecundity data collected from the CO_1 population. Each circle represents the mean fecundity from one vial, containing four females. One hundred vials were randomly sampled from the population daily until the total number of vials fell below 100, after which all vials were sampled. The variance in fecundity steadily decreased with age.

We utilized pair-wise comparisons between laboratory-evolved populations selected for different ages of reproduction in order to test whether late-life fecundity plateaus would start later in the later-reproducing CO populations compared to the early reproducing ACO populations. The age of onset of the late-life fecundity plateau for a population, or the break day, was estimated from the two-stage model (Table 1) and used to test whether late-life fecundity plateaus evolve according to the age at which the force of natural selection acting on fecundity plateaus. To test this hypothesis we estimated the significance of the break day parameter (φ_3; eqn 2c) from the non-linear mixed effects two-stage linear model fit by maximum likelihood and found that late-life fecundity plateaus start significantly later in the CO populations (mean = 49.86 days) compared to the ACO populations (mean = 36.06 days; $P < 0.0001$; Table 2), an average pair-wise difference of 13.80 days between the two selection regimes. This result indicates that fecundity plateaus evolve according to the last age of survival in the population's evolutionary history, as predicted by the evolutionary theory of late life based on the force of natural selection (Fig. 4).

In addition to testing whether selection had an effect on the age of onset of late-life fecundity plateaus, we tested whether selection had an effect on the other parameter estimates determined from the two-stage

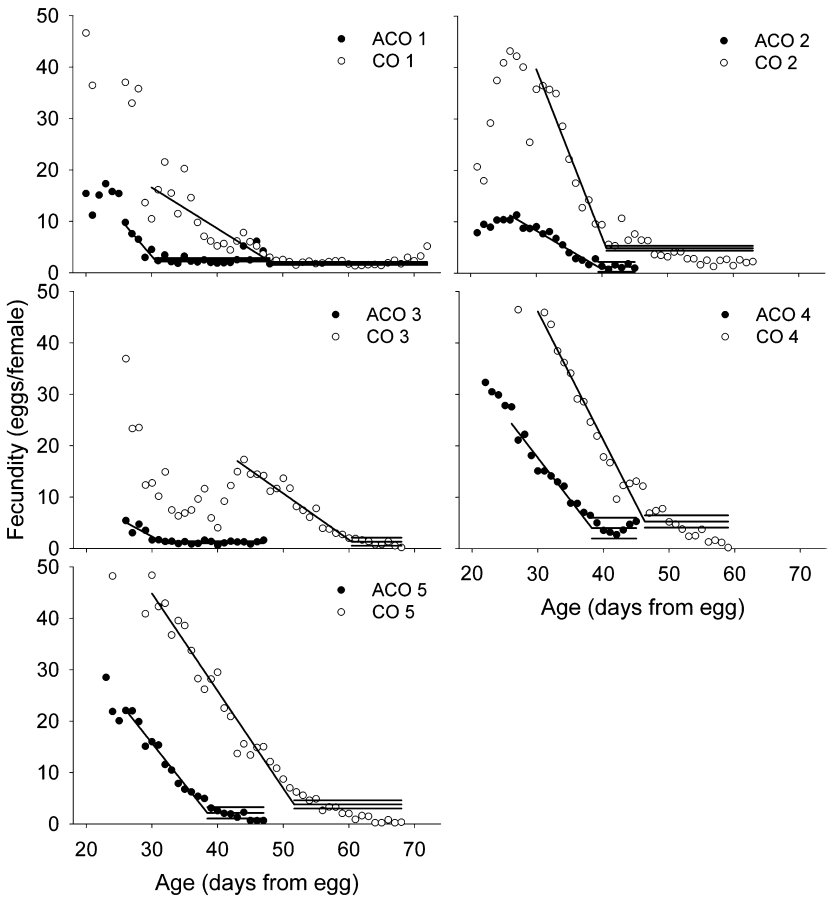

Fig. 3 Mean mid- and late-life fecundity as a function of age for each of the ACO_{1-5} (early reproducing) and CO_{1-5} (late reproducing) populations. Fecundity was measured during the ACO_i and CO_i pair-wise comparisons. The two-stage linear model was fit to each population independently. For all 10 populations, plateau height was significantly greater than zero.

Table 1 Parameter estimates from the two-stage linear model fitted to mid- and late-life fecundity data from the early reproducing ACO populations and the later reproducing CO populations.

Population	First-stage		Break day (φ_3)	Plateau height (φ_4) (eggs/female/day)
	y-int (φ_1)	Slope (φ_2)		
ACO_1	48.11	−1.49	30.52	2.50
ACO_2	30.61	−0.75	39.44	1.22
ACO_3	22.97	−0.69	31.62	1.21
ACO_4	67.44	−1.66	38.24	3.98
ACO_5	63.99	−1.61	38.44	2.16
CO_1	40.54	−0.80	48.55	1.80
CO_2	137.41	−3.26	40.67	4.86
CO_3	55.74	−0.90	60.43	1.32
CO_4	121.26	−2.51	46.30	5.27
CO_5	101.66	−1.89	51.66	3.81

The height of the fecundity plateau was computed from eqn 4 and the estimated height was significantly different from zero ($P < 0.05$ for each population).
Parameter estimates for φ_1, φ_2 and φ_3 were all significantly different from zero; $P < 0.001$.

Table 2 Results from the test comparing the fecundity-model parameters of the early reproducing ACO populations and the later-reproducing CO populations.

	Population	
	ACO	CO
Sample size (*x*, *y*-values)	6540	12 873
First-stage *y*-int (φ_1)	43.58	88.31*
First-stage slope (φ_2)	−1.14	−1.80
Break day (φ_3)	36.06	49.86**
Plateau height (φ_4) (eggs/female/day)	2.21	3.41

Plateau height was computed from eqn 4. The *x*, *y*-values used in the regression were from the 100 vials (400 females) randomly sampled daily from an initial population size of 4000 vials (16 000 females).
**$P < 0.0001$; *$P < 0.05$.

linear model. We found that the *y*-intercept of the line from the first stage (φ_1) was statistically significant ($P < 0.05$) and the slope of the line from the first stage (φ_2) was not affected by selection regime ($P = 0.14$; Table 2). Lastly, a simple paired *t*-test showed that the estimated plateau heights (φ_4) were not affected by selection regime ($P = 0.18$).

Eggs laid in late life are viable

The effect of parental age on offspring viability was tested over all age classes in both the ACO_{3-5} and CO_{3-5} populations, using a linear regression model fit to the angular-transformed viability proportions (Fig. 5). The estimates for the intercept and slope of the line fit to the early reproducing ACO populations were significant

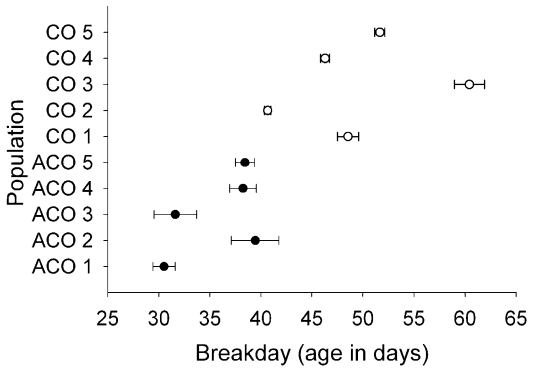

Fig. 4 Late-life fecundity plateau age of onset (break day) for all five pair-wise comparisons of CO and ACO populations. The fecundity plateau started significantly later in the later reproducing CO populations compared to the early-reproducing ACO populations ($P < 0.0001$). The break day and 95% confidence intervals were estimated for each population from the two-stage linear model using a non-linear least squares regression function.

(1.120 ± SE 0.040; $P < 0.001$ and −0.078 ± SE 0.016; $P < 0.001$, respectively; Table 3), and indicate that offspring viability decreased with increasing parental age. Similarly, the parameter estimates for the intercept and slope of the line fit to the later-reproducing CO populations were significant (1.211 ± SE 0.017; $P < 0.001$ and −0.022 ± SE 0.004; $P < 0.001$, respectively; Table 3), also indicating that offspring viability decreased with increasing parental age.

We tested whether the change in offspring viability with parental age differs between the two selection treatments (ACO vs. CO populations), by including the selection regime in the linear model as a covariate,

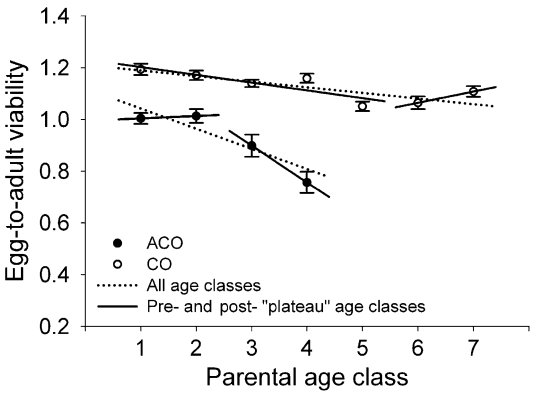

Fig. 5 Egg-to-adult offspring viability as a function of parental age class in flies from the ACO_{3-5} and CO_{3-5} populations. Mean viability (±SE) was calculated within each selection regime using angular-transformed egg-to-adult viability data. Each selection regime is fitted with a regression line determined from a linear model for all age classes and for those age classes that occur before and after the onset of the late-life fecundity plateau.

Table 3 Results from the test of the effects of parental age and selection treatment on offspring viability.

Parameters (d.f.)	Estimated value ± SE (all age classes)
ACO_{3-5}	
Intercept (77)	1.120 ± 0.040**
Age [slope] (77)	−0.078 ± 0.016**
CO_{3-5}	
Intercept (185)	1.211 ± 0.017**
Age [slope] (185)	−0.022 ± 0.004**
ACO_{3-5}–CO_{3-5}	
Intercept (262)	1.029 ± 0.067**
Selection regime (3)	0.091 ± 0.037*
Age [slope] (262)	−00.134 ± 0.026**
Selection regime: age (262)	0.056 ± 0.014**
Mid-LIFE parameters (d.f.)	Estimated value ± SE (mid-life age classes)
ACO_{3-5}	
Intercept (45)	0.995 ± 0.054**
Age [slope] (45)	0.009 ± 0.034
CO_{3-5}	
Intercept (145)	1.233 ± 0.020**
Age [slope] (145)	−0.030 ± 0.006*
'Plateau' parameters (d.f.)	Estimated value ± SE ('plateau' age classes)
ACO_{3-5}	
Intercept (30)	1.326 ± 0.225**
Age [slope] (30)	−0.142 ± 0.067*
CO_{3-5}	
Intercept (38)	0.804 ± 0.212**
Age [slope] (38)	0.043 ± 0.033

Offspring viability decreased significantly with parental age regardless of selection regime, and was significantly affected by selection regime. Viability decreased in the CO populations *before* the onset of the fecundity plateau (mid-life), and decreased in the ACO population *after* the onset of the fecundity plateau. Parameters were estimated using a linear regression model.
**$P < 0.001$; *$P < 0.05$.

interacting with age. The selection regime and the selection regime × age interaction estimates were significant, demonstrating that selection treatment had an effect on offspring viability (Table 3). The intercepts and slopes of the best-fit lines fit to the two selection regimes independently indicate that offspring viability is higher in the CO populations at all ages and that viability declines faster in the ACO populations compared to the CO populations.

To test whether the eggs laid after the onset of the fecundity plateau are viable we compared mid-life viability to late-life viability using a linear regression model fit to the angular-transformed viability proportions (Fig. 5). The effect of parental age on offspring viability for the mid-life parental age classes was not significant in the ACO populations (slope = 0.009 ± SE 0.034), but highly significant in the CO populations (slope = −0.030 ± SE 0.006; $P < 0.001$; Table 3). Conversely, the effect of parental age on offspring viability for the late-life parental age classes was significant in the ACO populations (slope = −0.142 ± SE 0.067; $P < 0.05$), but not in the CO populations (slope = 0.043 ± SE 0.033; Table 3).

Lastly, we tested the effect of mid-life parental age classes compared to late-life age classes on offspring viability within each selection regime by grouping the age classes before and after the onset of the late-life fecundity plateaus. We found that late-life viability was not significantly different than mid-life viability in the ACO populations, but only marginally non-significant ($P = 0.093$). However, in the CO populations, late-life viability was significantly different than mid-life viability ($P < 0.05$).

Antagonistic pleiotropy implicated in late-life fecundity

Antagonistic pleiotropy was tested as a genetic mechanism involved in late-life fecundity by subjecting the newly derived NRCO populations to selection for accelerated development and early reproduction for only 24 generations, and subsequently comparing the onset of late-life fecundity plateaus to the onset in the corresponding CO populations. Figure 6 shows the average population fecundity for the four pair-wise comparisons between the NRCO and CO populations, along with the fitted two-stage linear model. As Table 4 shows, the break day, estimated from the two-stage model, was significantly earlier in the early reproducing NRCO populations (mean = 49.59 days; $P < 0.0001$) compared to the late-reproducing CO populations (mean = 56.16 days). This result suggests that late-life fecundity plateaus rapidly respond to selection, and that antagonistic pleiotropy connects late-life fecundity to early life fitness characters, resulting in the evolution of an average pair-wise difference of 6.57 days in only 24 generations. Note, however, that the late-life fecundity plateaus of the NRCOs are ill-defined, compared to those of the ACO and CO populations, a pattern that arises because a strictly flat plateau is theoretically expected to occur only at evolutionary equilibrium; the NRCOs are of course far from evolutionary equilibrium.

Discussion

The results support a general evolutionary theory of late life

Most evolutionary theories suggest a rapid rise in age-specific fecundity at early ages followed by a long decline after some peak value. Our interpretation of the evolutionary theory of late life, based on the decline in the force of natural selection, was that population fecundity will plateau at very late ages, like age-specific mortality rates (Rauser *et al.*, 2003). We made this prediction because the force of natural selection acting on age-specific fecundity asymptotically falls to such a low level that it can no longer distinguish fitness differences in

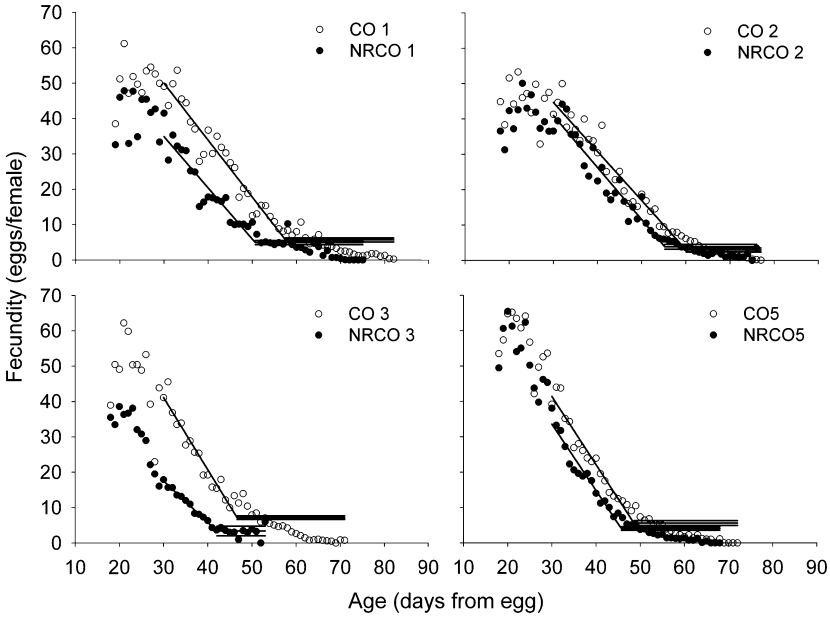

Fig. 6 Mean mid- and late-life fecundity as a function of age for each of the newly derived $NRCO_{1-3,5}$ (early reproducing) and $CO_{1-3,5}$ (late reproducing) populations. Fecundity was measured during the $NRCO_i$ and CO_i pair-wise comparisons. The two-stage linear model was fit to each population independently. For all eight populations, plateau height was significantly greater than zero. The earlier onset of the late-life fecundity plateau in the NRCO populations after only 24 generations of selection implicates antagonistic pleiotropy as a genetic mechanism working to shape late age fecundity patterns.

Table 4 Results from the test of antagonistic pleiotropy demonstrate that the onset of the late-life fecundity plateau starts significantly earlier in the NRCO populations, selected for earlier reproduction for just 24 generations, compared to the CO populations.

	Population	
	NRCO	CO
Sample size (x, y-values)	9750	13 587
First-stage y-int (φ_1)	71.24	84.80
First-stage slope (φ_2)	−1.36	−1.44
Break day (φ_3)	49.59	56.16*
Plateau height (φ_4) (eggs/female/day)	3.97	3.94

Plateau height was computed from eqn 4. The x, y-values used in the regression were from the 100 vials (400 females) randomly sampled daily from an initial population size of 3200 vials (12 800 females).
*$P < 0.0001$.

fecundity at different ages. Our experimental work supports this interpretation. We found that the rate of the decline in fecundity greatly slows at late ages, or plateaus, in 10 independent populations at some number of eggs laid per day greater than zero. Although some populations show a more defined plateau in fecundity than others, the two-stage model, with a second-stage plateau, fit the data in all 10 populations. The data do not have to fit the model, but they do. Note that if there was not a slowing in the decline in fecundity at late ages, the model would not have fit, or only the first stage of the model would have fit the data (the way the model-fitting algorithm was written, a failure to fit was allowed). This, however, was never the case. It is also important to note that each of the five populations from a selection regime are replicates and that we expect to see subtle differences between these replicate populations because of differential mutation and drift effects and because each pair-wise comparison was performed at different times. Thus, not all replicates will seem, on visual inspection, to fit a plateau model; this is sampling variation. Furthermore, the law of large numbers guarantees that the right end of the plateau will show 'disintegration', with a tendency to values much above or below the inferred plateau. Lastly, we do not necessarily expect to observe an exact plateau for each of these 10 populations because they are not that old (approximately 150 and 360 generations old for the CO and ACO populations, respectively) and thus are not fully converged. This line of reasoning suggests that the CO and the newly derived NRCO populations should have a less defined late-life plateau compared to the ACO populations, which have been maintained for many more generations. Upon visual inspection, this is exactly what we observe (Figs 3 and 6). Rose *et al.* (2002) observed a similar result for late-life mortality-rate plateaus. That is, the B populations, which had been maintained for 450 generations, showed the most well defined late-life mortality-rate plateau compared to the O, CO and ACO populations.

In addition to our experimental work, we have preliminary computer simulations of populations evolving with recurrent mutations that explore the evolution of late-life age-specific fecundity. These simulations also generate plateaus in fecundity at ages after the point when the force of natural selection falls to zero.

Previously, many studies fit mathematical models to fecundity data *post hoc*, using statistical goodness-of-fit

criteria to determine the best function (e.g. Hoem *et al.*, 1981; Gage, 2001; Müller *et al.*, 2001; Novoseltsev *et al.*, 2002, 2003; Carey, 2003), with no deep theoretical motivation for any of the functions tested (note, however, that these studies are fitting models to individual fecundity data, rather than population fecundity data). Furthermore, these studies did not observe late-life fecundity plateaus because they fitted their models to data sets that started with far fewer females than our own ($n = 3200$ per cohort), greatly reducing the likelihood of females surviving to ages late enough to observe the occurrence of late-life plateaus in population fecundity.

In this study, we not only demonstrate that population fecundity plateaus at late ages, but we show that these plateaus evolve according to the age of last survival in these populations' evolutionary history. Together, these results corroborate the basic evolutionary theory of late life and its prediction that fecundity, just like mortality-rates, should plateau some time after the force of natural selection acting on fecundity plateaus. Our experiments could have refuted the basic evolutionary theory of late life if there had been no difference between populations in the age at which their late-life fecundity plateaus commence, after long maintenance with very different last ages of reproduction. The theory could have also been refuted if the difference between these break days had been in the opposite direction from the difference in the day of last reproduction. However, neither of these outcomes occurred.

As for the viability of the eggs laid throughout life, including late life, we found that viability declines over all parental ages, regardless of selection regime. This result is different from what Kern *et al.* (2001) found in similarly selected populations. They found that offspring viability did not decline in some of the later reproducing populations. This discrepancy may have arisen because they did not include very late age viability in their analysis. However, like the results of Kern *et al.* (2001), we found that viability declines more rapidly in populations selected for earlier reproduction relative to those selected for later reproduction. Although they concluded that offspring viability is a general feature of senescence, we found that it does not follow the same pattern of senescence as mortality and fecundity. That is, viability does not deteriorate so rapidly, nor plateau at late ages.

We also separated mid-life from late-life parental ages and found that viability did not decline during mid-life in the populations selected for earlier reproduction, but that it did decline in the populations selected for later reproduction. The opposite result was true for parental ages that occurred after the onset of late-life fecundity plateaus. Our results indicate that aging is reflected in offspring quality, regardless of selection regime, when very late ages are also considered. The contrast we observed between mid- and late-life parental ages for viability (changing in opposite directions for the two selection regimes) suggests that there may be a trade-off in age-specific fitness characters. Offspring viability declines in mid-life, but stops declining late in life in those populations selected for later reproduction. Note, however, that when performing the analysis for viability we pooled the data from the replicated populations within each selection regime. Therefore, any random effects associated with each replicated population were not taken into account.

Lastly, we tested antagonistic pleiotropy as a genetic mechanism affecting late-life fecundity. With antagonistic pleiotropy between early and late ages, some of the alleles that enhance early reproduction will depress later survival or fecundity (Williams, 1957,1966; Rose, 1985; Charlesworth, 1994). Natural selection on early reproduction will therefore tend to increase mortality rates and decrease fecundity later in life. We found that late-life fecundity was significantly responsive to selection for early reproduction imposed for a small number of generations. That is, antagonistic pleiotropy between early and late ages resulted in an earlier decline in the force of natural selection acting on fecundity in the NRCO populations, which caused an earlier decrease in fecundity before the start of the plateau. These results are also consistent with our general prediction that the last age of survival and the start of late-life fecundity plateaus should be positively connected.

Random genetic drift and mutation accumulation could not have a significant effect in the 24 generations of selection for earlier reproduction. Drift fixes mutations at a rate of $4N_e$ generations, or 4000 generations in this case, as these populations have been maintained at an effective population size of at least 1000 individuals. Similarly, the impact of mutation accumulation on the differentiation between the CO and NRCO populations, with 24 generations of accumulation of deleterious mutations, will not exceed about 0.1% of the break day differentiation of the ACO and CO stocks, adapting the mutation-accumulation calculations of Passananti *et al.* (2004). This small an effect would be undetectable in experiments of our size. Although our experimental results implicate antagonistic pleiotropy in the evolution of late life, it is important to note that the two genetic mechanisms of antagonistic pleiotropy and mutation accumulation are not mutually exclusive and that a positive result for antagonistic pleiotropy does not necessarily mean that mutation accumulation is not involved in the evolution of late life.

In our antagonistic pleiotropy experiment, we measured fecundity again in four of the five CO populations and determined the break day to be 56.16 days (Table 4), which is later than the break day determined in the ACO-CO pair-wise comparison (49.86 days, Table 2). This discrepancy is most likely due to environmental effects arising because these comparisons were performed at different times. Note that pair-wise comparisons were performed for both sets of experiments because evaluating relative differences in ages of plateau onset is the only

way to properly control for possible environmental effects that may arise from performing replicate assays at different times.

Further implications for late life

Late-life fecundity plateaus are yet another surprising feature of late life. Although these fecundity plateaus are at a low number of eggs per female, they are significantly greater than zero. Thus they are analogous to late-life mortality-rates that plateau below 100%. This finding potentially has profound implications for our understanding of pleiotropy and selection in evolution. Perhaps there are some alleles that generally foster survival and fecundity, at *both* early ages, when the force of natural selection is great *and* at later ages, when the force of natural selection is weak. That is, some alleles may not have an age-specific effect, but may instead have an effect at all ages (Charlesworth, 2001).

Although the evolutionary theory of late life readily explains both the occurrence and evolution of late-life fecundity plateaus, this study does not attempt to test the influence of individual female heterogeneity in fecundity on the occurrence of these plateaus. It is conceivable that individual female fecundity does not plateau, but that the plateaus we observe are aggregate population characteristics. For example, it is possible that high egg-layers die early, leaving only the lifetime low egg-laying females alive at late enough ages to contribute to the population fecundity plateau we observe. This heterogeneity in fecundity idea is analogous to the demographic heterogeneity theories that have been proposed to explain late-life mortality-rate plateaus (e.g. Vaupel *et al.*, 1998). However, unlike the case of mortality-rates, the amount of heterogeneity in individual fecundity patterns can be measured directly. We have data on individual female fecundity trajectories from large out-bred cohorts, not presented here, which shows that heterogeneity in fecundity is not sufficient to cause plateaus in fecundity at late ages in our populations. The present study was designed to test the predictions made by the evolutionary theory, and so does not test this alternative hypothesis.

Other non-evolutionary theories that have been proposed to explain late-life mortality-rate plateaus do not naturally lend themselves as explanations of late-life fecundity plateaus. One such theory is the reliability theory of Gavrilov & Gavrilova (2001). Under this theory, death occurs when the first of several essential physiological blocks fails. Each of these blocks contains redundant systems that can be characterized by the failure rate of their components. The structure of these systems can lead to both exponential rates of increase of mortality rates at young ages and mortality-rate plateaus at advanced ages. The age of onset of these mortality rates is a function of the failure rate of the component systems. Reliability theory explanations do not negate the influence of natural selection on the age of onset of a plateau through its influence on the rate that physiological systems fail. These failure rates could certainly be set by evolutionary forces, like those described by Mueller & Rose (1996). However, it is also clear that the physiological processes that determine the numerical age-specific decline of eggs produced by females would not naturally follow processes described by reliability theory without resorting to a number of *ad hoc* assumptions.

The ability of evolutionary theories based on the force of natural selection to explain the existence and experimental manipulation of *both* mortality-rate (Rose *et al.*, 2002) and fecundity plateaus adds to their plausibility as causal explanations of these phenomena.

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