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EVOLUTION OF LATE-LIFE MORTALITY IN *DROSOPHILA MELANOGASTER*

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Abstract.—Aging appears to cease at late ages, when mortality rates roughly plateau in large-scale demographic studies. This anomalous plateau in late-life mortality has been explained theoretically in two ways: (1) as a strictly demographic result of heterogeneity in life-long robustness between individuals within cohorts, and (2) as an evolutionary result of the plateau in the force of natural selection after the end of reproduction. Here we test the latter theory using cohorts of *Drosophila melanogaster* cultured with different ages of reproduction for many generations. We show in two independent comparisons that populations that evolve with early truncation of reproduction exhibit earlier onset of mortality-rate plateaus, in conformity with evolutionary theory. In addition, we test two population genetic mechanisms that may be involved in the evolution of late-life mortality: mutation accumulation and antagonistic pleiotropy. We test mutation accumulation by crossing genetically divergent, yet demographically identical, populations, testing for hybrid vigor between the hybrid and nonhybrid parental populations. We found no difference between the hybrid and nonhybrid populations in late-life mortality rates, a result that does not support mutation accumulation as a genetic mechanism for late-life mortality, assuming mutations act recessively. Finally, we test antagonistic pleiotropy by returning replicate populations to a much earlier age of last reproduction for a short evolutionary time, testing for a rapid indirect response of late-life mortality rates. The positive results from this test support antagonistic pleiotropy as a genetic mechanism for the evolution of late-life mortality. Together these experiments comprise the first corroborations of the evolutionary theory of late-life mortality.

Key words.—Aging, antagonistic pleiotropy, force of natural selection, late life, mortality plateau, mutation accumulation.

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A variety of species, including medflies, wasps, nematodes, yeast, and humans, exhibit decelerating mortality rates at late ages, sometimes referred to as late-life ''mortality-rate plateaus'' (Greenwood and Irwin 1939; Comfort 1964; Gavrilov and Gavrilova 1991; Carey et al. 1992; Curtsinger et al. 1992; Fukui et al. 1993; Brooks et al. 1994; Curtsinger et al. 1995; Charlesworth and Partridge 1997; Vaupel et al. 1998). Although there was some initial controversy about late-life mortality rate plateaus (Carey et al. 1993a,b; Gavrilov and Gavrilova 1993; Kowald and Kirkwood 1993; Nusbaum et al. 1993; Robine and Ritchie 1993; Vaupel and Carey 1993; Curtsinger et al. 1994; Vaupel et al. 1994), they have been reproduced in experiments performed in a diversity of organisms and laboratories (e.g., Curtsinger et al. 1995; Vaupel et al. 1998; Drapeau et al. 2000). Late-life mortality rate plateaus have been accepted as one of the most curious findings in biology of the last decade and of significance for the fields of gerontology, geriatrics, demography, and evolution.

At present, there is no widely accepted explanation for late-life mortality plateaus. Two main theories have been proposed: the heterogeneity theory and the evolutionary theory based on the force of natural selection. The heterogeneity theory proposes that late-life mortality rates plateau because individuals that are less robust throughout life die off at earlier ages, leaving individuals with life-long superiority for robustness preponderant among those that reach extremely late ages (Vaupel et al. 1979; Vaupel 1988, 1990). According to the heterogeneity theory, the continued survival of individuals with greater life-long robustness will slow increases in average mortality later in life because the individuals who are always less robust are no longer around to contribute to the population's average mortality rate. Theoretical analysis has shown that, when heterogeneity in life-long robustness is extremely pronounced, such effects can arise (Kowald and Kirkwood 1993; Service 2000a). The experimental evidence bearing on the validity of the theory is mixed (e.g., Curtsinger et al. 1992; Fukui et al. 1996; Khazaeli et al. 1998; Drapeau et al. 2000; but see Mueller et al. 2000; Service 2000b).

The main alternative theory for late-life mortality rate plateaus is the evolutionary theory of late life based on the force of natural selection acting on age-specific survival probabilities. In evolutionary theory, mortality rates are expected to increase exponentially during the first part of adulthood, because the force of natural selection declines rapidly during those ages. Research on the evolution of aging corroborates this prediction, as mortality rates in the first part of adult life have been shown experimentally to follow the force of natural selection (Rose 1991). However, the decline in the force of natural selection is expected to end late in life, after the last age of reproduction in the population's evolutionary history (Hamilton 1966; Charlesworth 1994, 2001). Mortality-rate patterns are nevertheless still predicted to follow the pattern of the force of natural selection and plateau sometime after the force of natural selection plateaus in late life (Mueller and Rose 1996; Rose and Mueller 2000; Charlesworth 2001). The correspondence between the start of the plateau in the force of natural selection and the onset of mortality-rate plateaus is not expected to be exact, however, because beneficial gene effects that continue from early to late ages will sustain survival somewhat longer than the last age of reproduction. Nevertheless, the age when mortality-rate acceleration stops or slows should evolve in accordance with large changes in the age at which the force of natural selection hits zero. Therefore, the mortality-rate pattern in late life is predicted to evolutionarily follow the pattern of prior selection on the population's last age of reproduction.

As for the evolution of aging itself, two population genetic mechanisms can explain plateaus for age-specific mortality rates, separately or together: mutation accumulation and antagonistic pleiotropy (Charlesworth 1994, 2001; Mueller and Rose 1996). Mutation accumulation affects the evolution of aging when alleles that are deleterious at later ages, but neutral at all earlier ages, accumulate by mutation pressure and genetic drift (Medawar 1952; Rose 1991; Charlesworth 1994, 2001). Such mutations are expected to be unique to each evolving population. They are also expected to be somewhat recessive on average, because that is usually the heterozygous effect of deleterious mutations (Simmons et al. 1978). However, these recessive, deleterious mutations are able to persist in populations because they increase mortality rates only later in life, when the force of natural selection is relatively weak. These features of mutation accumulation are expected to produce hybrid vigor in crosses of populations subject to mutation accumulation. Not all alleles are expected to foster hybrid vigor with mutation accumulation, and mutation accumulation is not the only possible cause of hybrid vigor (Charlesworth and Hughes 1996). However, the demonstration of hybrid vigor in crosses between populations influenced by mutation accumulation provides at least indirect support for the hypothesis of mutation accumulation as a genetic mechanism in the evolution aging, as argued by Mueller (1987).

Another genetic mechanism that may explain the evolution of late life is antagonistic pleiotropy, specifically when alleles that are beneficial early in life are deleterious later in life (Williams 1957; Rose 1985; Charlesworth 1994). Alleles that are deleterious and cause increased mortality rates late in life can persist because these same alleles enhance reproduction earlier in life, when the force of natural selection is much stronger. This genetic mechanism for late-life evolution can be experimentally distinguished from mutation accumulation and genetic drift by subjecting long-established, late-reproducing populations to an evolutionary reversion to much earlier ages of reproduction, an experimental protocol that has been of value in the study of the evolution of aging (e.g., Service et al. 1988). As long as this reverse evolution (cf. Teotónio and Rose 2001) is imposed on large populations for a small number of generations, there is too little evolutionary time for mutation accumulation or genetic drift to act significantly. Switching to a selection regime with an earlier last age of reproduction should lead to an earlier onset age for late-life mortality rate plateaus, if this genetic mechanism is active in the evolution of late life. As selection for early reproduction increases the frequency of alleles enhancing early-fitness characters, those alleles with antagonistic pleiotropy between early and late ages will increase mortality rates before the start of the plateau, causing an earlier plateau onset. Therefore, if there is a detectable shift of the mortality plateau to earlier ages over a small number of generations of reverse selection, antagonistic pleiotropy is implicated as a genetic mechanism underlying late-life mortality patterns.

In this paper, we use computer simulations of populations with various last ages of reproduction evolving with recurrent mutation to simulate the evolution of late-life mortality-rate plateaus, as age of reproduction varies. We then test this theoretical analysis of late-life mortality plateaus experimentally using outbred *Drosophila melanogaster* populations that have long had substantially different ages of last reproduction. We predict these populations should have corresponding differences in the age at which late-life mortality undergoes reduced acceleration, and thus plateaus. If such experimental populations do not conform to this pattern, then the evolutionary theory for late-life mortality based on the force of natural selection would be falsified. Finally, we test two population genetic mechanisms that may shape the evolution of late-life mortality: mutation accumulation and antagonistic pleiotropy.

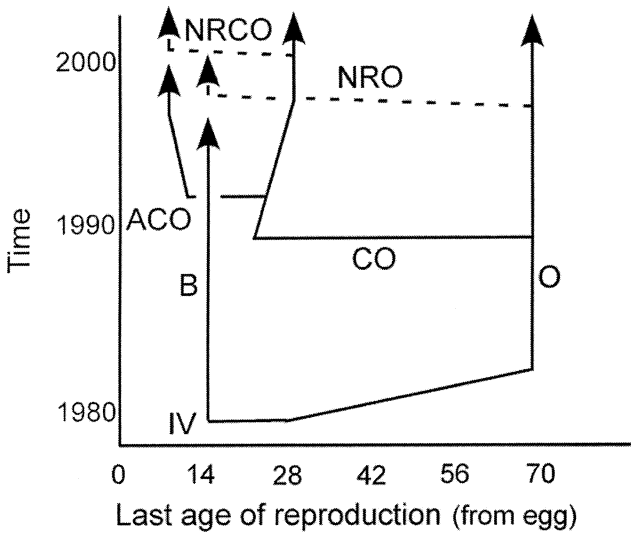

FIG. 1. Selection histories of the experimental populations. The ancestral population was the IV population, sampled from nature in 1975, which was used as the ancestor of the five B and five O populations in 1980. In 1989, the five CO populations were derived from the five individual O populations, and the five ACO populations were in turn derived from each of the CO populations in 1991. The five NRO populations were derived from the individual O populations in 1998. All experimental assays took place from late 1997 to early 2000.

MATERIALS AND METHODS

Populations Employed

All stocks used in these experiments were ultimately derived from a sample of the Amherst, Massachusetts, Ives population (e.g., Ives 1970) that was collected in 1975 and cultured at moderate to large population sizes ever since. Individual populations have been subjected to a series of selection regimes, as indicated in Figure 1 (Rose 1984; Chippindale et al. 1994). Each of five stocks differs in their age of last reproduction and each stock in turn consists of five outbred replicate populations. The five stocks are $B_{1–5}$, $O_{1–5}$, $CO_{1–5}$, $ACO_{1–5}$, and $NRO_{1–5}$. The ACO and B populations have an early age of last reproduction (nine and 14 days, respectively), the CO populations have an intermediate last age of reproduction (28 days), and the O populations have a late last age of reproduction (70 days). The five NRO populations were derived from their respective O populations and have a last age of reproduction of 14 days. The NRO culture procedure was like that of the O populations, except that flies were placed in cages

at about 10 days from the egg stage, with egg collection at 14 days, after feeding with yeast. Except for the NRO group, these populations have each been maintained for more than 100 generations at effective population sizes ≥1000. Together, these populations define a spectrum of selection on the age of reproduction, and thus a spectrum of patterns for the age-specific force of natural selection.

Simulations of Mortality Rate Evolution

The simulated populations initially had mortality rates similar to those of either the B populations or the O populations. They were then switched from early to late reproduction or from late to early reproduction. Mortality rates evolved by the complete substitution of mutants with age-specific effects on mortality, as in Mueller and Rose (1996). The mutants affected the survival of two periods of 10 contiguous days, chosen at random. Fitness, however, was computed as the product of survival to and fecundity on a specific day of adult life. When the ancestral population was B-type, the age of reproduction in the simulated populations was shifted from day 20 to one of day 31, 41, 51, or 61. When the ancestral population was O-type, the last age of reproduction was shifted from day 60 to one of day 21, 31, 41, or 51. After 10,000 mutations were simulated, the evolved mortality rates were used to determine the predicted day of onset of the late-life mortality plateau.

Testing Whether Mortality Plateaus Shift with the Last Age of Reproduction

We tested the prediction from evolutionary theory, and our simulations, that the onset of mortality plateaus should evolve in accordance with the last age of reproduction in the population's evolutionary history, using the B, O, ACO, and CO *Drosophila* populations described above (see Fig. 1). The B and ACO populations have earlier last ages of reproduction and shorter life spans. The CO populations have an intermediate last age of reproduction and an intermediate life span. Finally, the O populations have a later age of reproduction and a longer life span. However, these average life span patterns by themselves do not indicate the timing or nature of the mortality-rate plateaus of these populations.

Two comparisons using these populations provide independent tests of the evolutionary theory for late-life mortality plateaus. These are the comparisons of the B with the O populations and the ACO with the CO populations. Specifically, these comparisons allow us to test the prediction concerning the effects of last age of reproduction on the start of mortality-rate plateaus.

The B and O populations share a common ancestor, but have long had a 56-day difference in their last age of reproduction. They had evolved separately for more than 17 years (450 B generations) at the time that we estimated the age-specific mortality rates presented here. The mortality data of the B and O populations were fit to two-stage Gompertz equations by maximum-likelihood techniques, allowing, but not assuming, a late-life mortality rate plateau (see estimation of mortality rate plateaus, below). This model fitting was not performed to support the Gompertz model, but rather to infer mortality-rate patterns by an objective procedure.

The ACO populations were derived directly from the CO populations, as shown in Figure 1. The ACO populations had a last age of reproduction of nine days, whereas the CO populations had a last age of reproduction averaging about 30 days, in the period before the present experiments. These populations were compared using the same statistical methodology as for the B and O populations, except that the B-O comparison was unpaired, whereas the CO-ACO was a paired comparison because each ACO population was derived from the CO population having the same numeric subscript.

Collection of Mortality-Rate Data

For each replicate population, at least 80 8-dram glass banana-agar-corn syrup food vials each containing 60 ± 5 eggs were prepared. On days 9 and 10 after the egg collection (but within a 24-h period), all eclosed adult flies were sexed and placed into new food vials in groups of 24 (12:12 male: female) that would be used to start the mortality assay.

Adult survival was determined and living flies were transferred to new vials with fresh food every other day. Any flies that were living but irretrievably stuck to a part of the vial were scored as dead two days later. When necessary, flies within replicates were recombined to maintain a density of approximately 24 flies/vial, to rule out any possible density effects on mortality rates (cf. Carey et al. 1993a,b; Graves and Mueller 1993, 1995; Nusbaum et al. 1993; Curtsinger 1995a,b; Khazaeli et al. 1995, 1996). Survival assays were continued until every fly was dead. We used high cohort sizes (at least 2000 individuals per replicate) to reduce sampling variance in our estimations of mortality rates (Promislow et al. 1999; Pletcher 1999; see Tables 1, 2 for sample sizes).

Estimation of Mortality-Rate Plateaus

Mortality rate plateaus were estimated by allowing d^* to be the age at which mortality rates become constant with age (the breakday). Then, at ages x less than d^*, age-specific mortality rates were modeled by the continuous-time Gompertz equation and set equal to $A \exp(\alpha x)$, where A is the age-independent rate of mortality and α is the age-dependent rate of mortality increase. For $x \geq d^*$, mortality rates were assumed to equal $\tilde{A}$. $\tilde{A}$ is independent of age, but different from A. For a particular value of d^*, A, α, and $\tilde{A}$ were estimated by maximum likelihood. This was repeated for a range of d^* values, and the value of d^* that yielded the largest likelihood value was chosen as the best estimate of the breakday between early and late mortality.

The likelihood function was constructed from ages at death of the N members of a cohort following methods similar to Mueller et al. (1995). In this experiment vials are checked every two days. Thus, the raw data consists of the number of dead flies recorded every two days, which might be zero. We number the two-day checks sequentially and let the t_N be the last check during which the last fly died. Then the number of dead flies in each two-day period is $d_1, d_2, \ldots, d_{t_N}$. Likewise the number of flies alive at the start of each census period is N_1 $(=N)$, $N_2, \ldots, N_{t_N}$ $(=d_{t_N})$. Let $q(i)$ be the probability that an individual that lived to census period i, dies by census period $i \pm 1$. Then the likelihood function is defined as

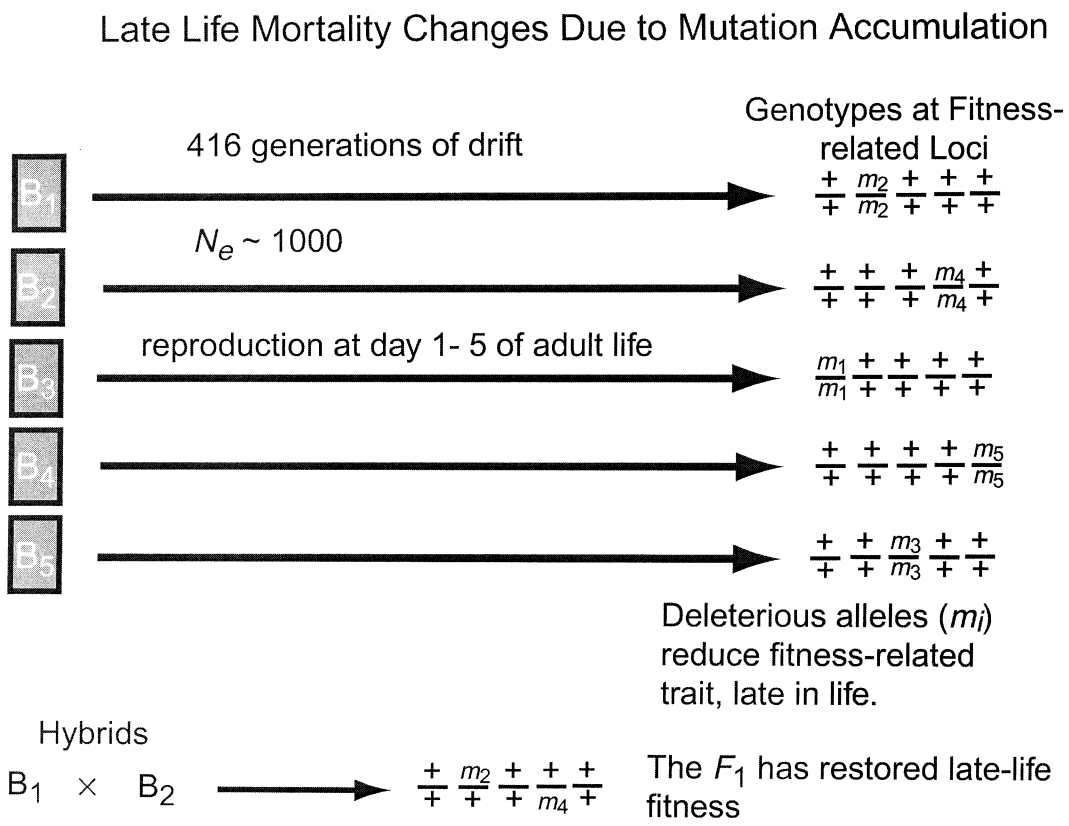

FIG. 2. Expected results from hybrid crosses under the mutation accumulation hypothesis of late-life evolution. The expected result if mutation accumulation was the most important determinant of late-life fitness is shown on the top. Five independent populations are maintained on a two-week generation cycle for 416 generations at an effective population size of about 1000. This culture regime removes the action of selection on traits expressed after about day 5 of adult life. We assume that at the start of the experiment the populations were polymorphic for deleterious, late-acting recessive alleles at multiple loci. In this case, we have shown five such alleles, m_1, m_2, m_3, m_4, and m_5 at five different loci. One of these deleterious alleles becomes fixed by genetic drift in each of the five populations. When late-life fitness is examined in the five B-populations at the end of the experiment, they show a depression. When the hybrids are made from any two B-populations, they show elevated late life fitness, because they are now heterozygous for the dominant wild-type allele at the relevant loci.

$$L = \prod_{i=1}^{i=t_N} \binom{N_i}{d_i} q(i)^{d_i}[1 - q(i)]^{(N_i - d_i)}. \quad (1)$$

For a particular breakday, d^*, $q(i)$ is then estimated as

$$\begin{cases} 1 - \exp\left\{\dfrac{A[\exp(\alpha 2i) - \exp(\alpha 2[i+1])]}{\alpha}\right\} & \text{if } 2i < d^* \\ 1 - \exp(-2\tilde{A}) & \text{if } 2i \geq d^*. \end{cases} \quad (2)$$

Test for Mutation Accumulation as a Genetic Mechanism

To test whether late-life mortality does indeed reproducibly undergo some type of mutation accumulation, we generated 25 distinct outbred populations of *D. melanogaster* by making all possible crosses of the five B populations. These five B populations were derived from a common ancestral population in February 1980, and since then have been kept on a two-week culture regime with effective population sizes of approximately 1000 individuals (Rose 1984; Leroi et al. 1994). About 18 years, or 465 generations, had elapsed since their founding when the present experiments were performed. We think it unlikely that a substantial number of new mutations affecting survival have arisen in these replicate B populations since their founding. Rather, it is more likely that each B population started with a large number of rare alleles that were deleterious in their effects on late-life survival while in nature, but were subsequently made neutral by laboratory culture. A fraction of these neutral alleles are expected to increase in frequency by random genetic drift. Molecular studies of the B populations show that they are genetically differentiated (Fleming et al. 1993).

In this study we have created conditions in the B populations for mutation accumulation. Such an accumulation will depend on several factors: (1) the elimination of selection in late life; (2) the finite population size; and (3) the existence of late acting deleterious alleles for the life history characters we examine. The first two factors are part of the experimental design, the third factor constitutes the biological hypothesis of mutation accumulation. The dynamic aspects of the process of mutation accumulation in the B populations are shown in Figure 2. This process presumes multiple loci affecting the trait of interest. It further assumes that some existing deleterious alleles will rise to high frequency in each population and others will not. The particular alleles that rise to

high frequency in one population are also likely to be different from one population to the next.

We assume that most deleterious alleles that rise to high frequency by drift will be recessive or partially recessive. This conclusion follows from the simple population genetic considerations that suggest that in the ancestral population recessive deleterious alleles will be at a much higher equilibrium frequency than dominant alleles. Consequently, in the simplest case, which involves fixation of the deleterious alleles, the parental populations will show a depression in these late-life characters that will be elevated in the F_1 hybrids (Fig. 2). If, however, fitness-characters early and late in life are determined wholly by alleles with antagonistic effects, we expect to see little difference between the hybrids and parents for their late-life fitness characters.

However, there is nothing about the design of this experiment that guarantees or assumes that these deleterious alleles will be fixed after the 416 generations of drift. For neutral alleles at an initial frequency of p and with an effective population size of N, it will take on average $-4N(1 - p)\ln(1 - p)/p$ generations to fix the allele (assuming it is fixed, which will occur with probability p; Ewens 1979, p. 77). In the B populations $N_e \cong 1000$, and if $p = 0.05$, the average time to fixation would be 3898 generations.

Although 416 generations is not sufficiently long for most initially rare neutral alleles to be fixed, there may be some that have risen to sufficiently high frequencies that late-life fitness will begin to suffer. For instance, using the stationary distribution of neutral alleles we can calculate the chance of finding neutral alleles in certain frequency ranges (Crow and Kimura 1970, p. 383). In the B populations 4–9% of the neutral alleles are expected to be at a frequency of 0.4, or greater (assuming $N_e = 1000$, and the initial frequencies are between 0.01 and 0.1). At final frequencies above 0.4, there will be sufficient numbers of homozygotes with deleterious effects at late ages, yet still neutral under B conditions, to reduce late-life fitness-characters.

Every pairwise combination of the cross $B_i \times B_j$ (both i and j varying from 1 to 5) was performed and nonhybrid cross progeny were handled in the same manner as hybrid cross progeny. Rearing was parallel for two generations prior to assay. Densities were standardized for parental and experimental fly cultures. The progeny from these 25 crosses were assayed for mortality by complete census of deaths every other day, similar to the mortality assays described above. Sample sizes were approximately 880 flies/sex/population, with a total of 21,702 males and 22,235 females. Mortality-rate estimations were done in the same manner described above for both the hybrid and nonhybrid populations. Of the 20 hybrid crosses produced, 14 crosses were included in the final analysis because there were missing observations when collecting survival data.

Test for Antagonistic Pleiotropy

The NRO populations were subjected to selection for early reproduction for 24 generations, after derivation from the O populations (see Fig. 1). The NRO populations have a last age of reproduction of 14 days from egg, like the B populations. Mortality assays and mortality-rate estimations were performed as described above for all pairwise comparisons between the NRO populations and their corresponding O populations. Evolutionary theory predicts that the NRO populations will eventually evolve an earlier plateau in mortality rates when compared to the O populations.

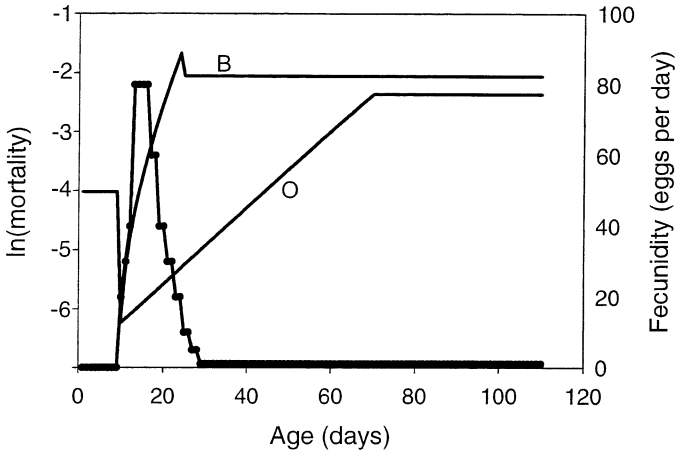

FIG. 3. The initial mortality rates and female fecundity used in the computer simulations. The solid lines are age-specific mortalities for either the B or O populations. The line with solid circles is the common, age-specific fecundity of the B and O females. The x-axis shows age starting from the egg-stage. Reproduction is assumed to start at day 10 and continue until day 110, albeit at a very low level.

RESULTS

Mortality Plateaus Shift with Last Reproduction in Simulations

We simulated populations with mortality rates initially similar to B and O populations and then switched them from early to late or late to early reproduction. Female fertility was assumed to be the same in both the B and O populations. The initial mortality rates and female fecundity are shown in Figure 3. Mortality rates were allowed to evolve with the substitution of mutants with antagonistic pleiotropic effects on mortality, as well as genetic drift.

The process of generating these mutants followed the procedures described in Mueller and Rose (1996). In these simulations fitness for the mutant and resident genotypes were determined as the product of the chance of surviving to the age of reproduction times the fecundity of females at that age. Drift with $N_e = 1000$ was also incorporated in these simulations as described in Mueller and Rose (1996). When a mutant appeared with greater fitness than the resident genotype it was assumed to sweep through the population and replace the resident unless it was lost by drift.

Figure 4 summarizes the results of these computer simulations of populations with various last ages of reproduction evolving with recurrent mutation. The results confirm the intuitive expectation of the evolutionary theory for late-life mortality: The last age of reproduction determines the first age at which late-life mortality rates plateau. As the day of last reproduction increases, the age at which mortality rates plateau also increases.

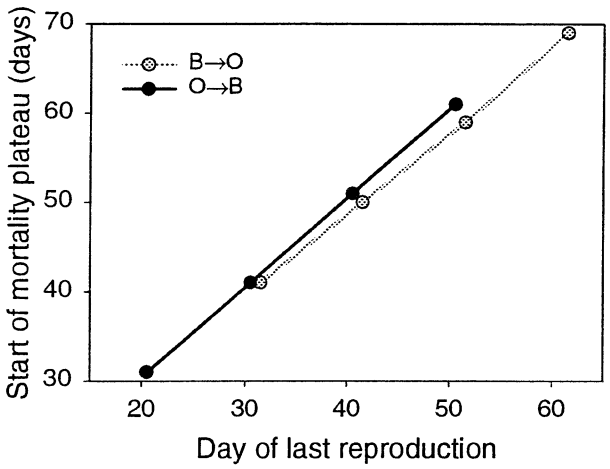

FIG. 4. Simulated evolution of mortality rate plateaus in response to changes in the last age of reproduction. The gray line shows the evolved plateaus when the ancestral population was B type. The black line shows the evolved plateaus when the ancestral population was O type. Lines are plotted slightly offset for clarity.

Mortality Plateaus Shift with the Age of Last Reproduction in Drosophila melanogaster

We used two independent pairwise comparisons between laboratory-evolved populations selected for different last ages of reproduction to test the predictions made by the evolutionary theory of late-life mortality and our computer simulations. Specifically, we predicted that the five O populations, which have a later age of last reproduction compared to the five B control populations, would have a later onset of mortality-rate plateaus. Similarly, we predicted a later onset of mortality-rate plateaus in the five later reproducing CO populations compared to the five ACO populations.

Our experiment with the B and O populations confirmed our theoretical predictions. The adult ages at which late-life mortality plateaus in the O populations (male: 58.0 days; female: 68.4 days) were significantly greater than the age at which mortality plateaus in the B populations (male: 23.6 days; female: 24.0 days; see Fig. 5, Table 1). This result corroborates the evolutionary theory for late-life mortality based on the force of natural selection.

Additional support for our predictions was found in our experiment with the ACO and CO populations. We found that the start of ACO mortality-rate plateaus (male: 42.6 days; female: 40.6 days) occurs earlier than the start of CO plateaus (male: 58.6 days; female: 57.0 days; see Fig. 6, Table 2). All 10 CO and ACO populations derive from O populations, and therefore their disparity does not arise from differences in long-standing genetic background. Importantly, the ACO-CO comparison is a completely independent test of evolutionary theory from the B-O comparison above. Again, the evolution of their late-life mortality conforms to the age at which the force of natural selection falls to zero, their last age of reproduction, as presented in Figure 4.

No Hybrid Vigor for Late-Life Mortality

As a test of mutation accumulation underlying late-life mortality plateaus, we estimated the amount of hybrid vigor between the genetically divergent B laboratory selection lines. We found that the late-life mortality of hybrids created from 14 of the 20 possible crosses of the five B populations exhibited no detectable superiority to the uncrossed cohorts sampled from the parental B populations (see Fig. 7). There was no significant difference between the mortality plateau breakday between the hybridized and the nonhybridized B cohorts (males: *t*-test, $t = -0.44$, df = 17, $P = 0.67$; females: *t*-test, $t = -0.075$, df = 17, $P = 0.46$). Similarly, the mean estimated plateau mortality rate was not significantly different between the hybridized and the nonhybridized B populations (males: *t*-test, $t = -1.56$, df = 17, $P = 0.14$; females: *t*-test, $t = -0.05$, df = 17, $P = 0.96$). Although mutation accumulation was not supported as a genetic mechanism for the evolution of late-life mortality rate plateaus, it was not refuted either, because different patterns of dominance among

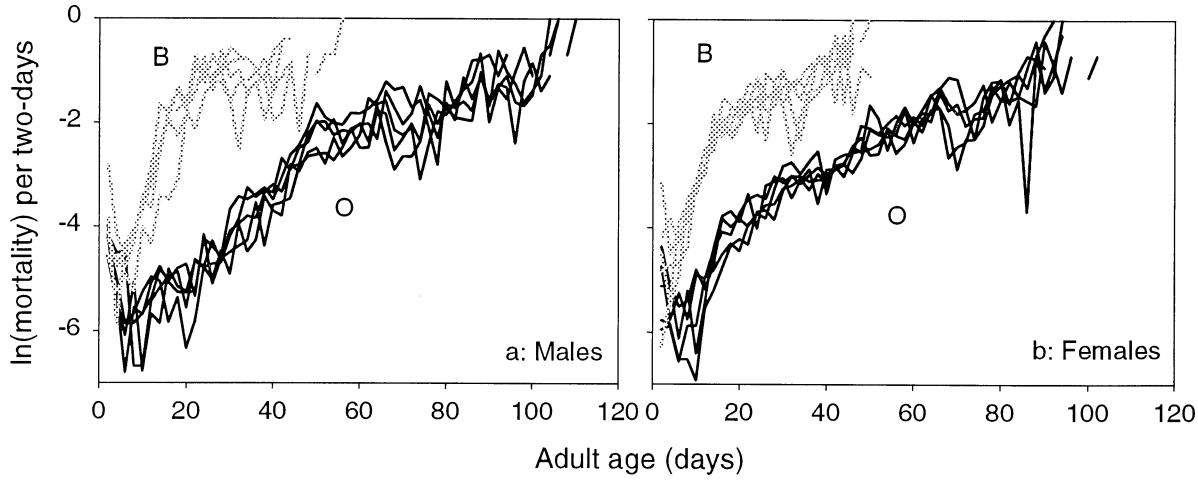

FIG. 5. Two-day mortality rates for 10 cohorts sampled from B and O populations. B populations (early last reproduction) are shown as gray lines and O populations (late last reproduction) are shown as black lines. Occasional regions are missing because mortality rates of zero cannot be properly interpreted on a logarithmic scale.

TABLE 1. Results from a test of the evolutionary theory for late-life mortality using a comparison between the early reproducing B populations and the late reproducing O populations with respect to onset of mortality-rate plateaus. The plateau mortality rate is computed from equation (2).

	Males			Females		
	B	O		B	O	
Sample size	4867	8855		5143	10,037	
Breakday	23.6	58.0	***	24.0	68.4	***
Plateau mortality rate	0.338	0.161	***	0.240	0.195	***
A	0.00339	0.00124	*	0.00542	0.00307	
α	0.198	0.0711	***	0.173	0.0577	***
Mean longevity	20.6	52.3	***	20.8	48.2	***

* $P < 0.1$; *** $P < 0.01$.

alleles with effects specific to late life could eliminate hybrid vigor (cf. Charlesworth and Hughes 1996).

Antagonistic Pleiotropy Involved in Late-Life Mortality

To test antagonistic pleiotropy as a genetic mechanism involved in late-life mortality, we derived the NRO populations from the corresponding O populations by reverse evolution under conditions identical to those of the ancestral IV population, with reproduction 14 days from egg. As Table 3 shows, there is significant evidence of a rapid response to selection in the NRO populations with regard to the start of mortality-rate plateaus. Indeed, the response to selection for the starting age of the male mortality-rate plateau is remarkably rapid and highly significant—a net response of more than 20 days in 24 generations. In addition, female response to selection for the start of the mortality-rate plateau was nearly significant and showed a net response of 13 days in the predicted direction. Again, these results taken together are consistent with an evolutionary model in which last age of reproduction and mortality-rate plateaus have a positive relationship (see Fig. 4). The highly significant male result with respect to the breakday is sufficient to support antagonistic pleiotropy as a genetic mechanism involved in late-life mortality. Just 24 generations of reverse evolution are extremely unlikely to produce an effect of drift in populations of this size.

DISCUSSION

The Results Support the Basic Evolutionary Theory

Both comparisons using *Drosophila* populations that evolved with different ages of reproduction support the evolutionary theory for late-life mortality rates based on the force of natural selection. Notably, both experiments could have refuted the evolutionary theory if there had been no difference between populations in the breakday of their plateaus, after long maintenance of very different terminal ages for reproduction, or if the difference between these breakdays had been in the opposite direction from the difference in the day of terminal reproduction. The evolution of the populations that were switched to an earlier last age of reproduction also supported the force of natural selection theory, notwithstanding the nonsignificance of the female data, which is explicable in terms of the short duration of the new evolutionary regime. Of three tests using experimental evolution, the basic evolutionary theory is supported by data from all three.

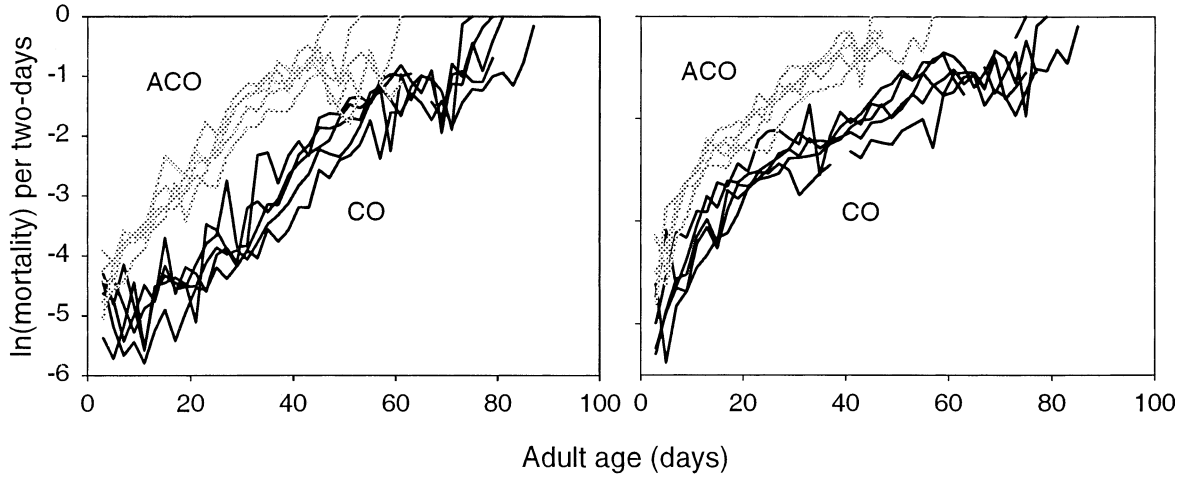

FIG. 6. Two-day mortality rates for 10 cohorts sampled from CO and ACO populations. In each case, ACO populations (selected for early-life fecundity) are shown by gray lines and CO populations (selected for midlife fecundity) are shown by black lines. Occasional regions are missing because mortality rates of zero cannot be properly interpreted on a logarithmic scale, except for one case, which was a result of experimental error. Left: Male mortality; right: female mortality.

TABLE 2. Results from an independent test of the evolutionary theory for late-life mortality using a comparison between the early reproducing ACO populations and the later reproducing CO populations with respect to onset of mortality-rate plateaus. Because each ACO population derives from a single CO population, paired-difference *t*-tests were used to test for significant differences between characters. The plateau mortality rate is computed from equation (2).

	Males			Females		
	ACO	CO		ACO	CO	
Sample size	12,444	11,987		14,084	12,361	
Breakday	42.6	58.6	***	40.6	57.0	***
Plateau mortality rate	0.363	0.286	**	0.520	0.330	***
A	0.00500	0.00156	***	0.00710	0.00465	**
α	0.106	0.0813	***	0.105	0.0644	***
Mean longevity	26.2	44.2	***	23.5	37.2	***

* $P < 0.05$; *** $P < 0.01$.

Genetic Mechanisms for the Evolution of Late Life

For all five B populations, the force of natural selection falls to zero at 14 days of adult age. Although these five B populations experienced independent evolution for 18 years before this experiment, their demographic selection regimes were identical. Our expectation was that, whereas the B populations evolved under the aegis of the same demographic selection, mutation accumulation might have produced enough divergence among the five populations to give hybrid vigor upon crossing. But we failed to obtain this result. This does not, however, preclude mutation accumulation as an evolutionary mechanism, because it can occur without producing hybrid vigor. In any case, the absence of hybrid vigor is interesting in itself, because it suggests an absence of inbreeding depression in these populations.

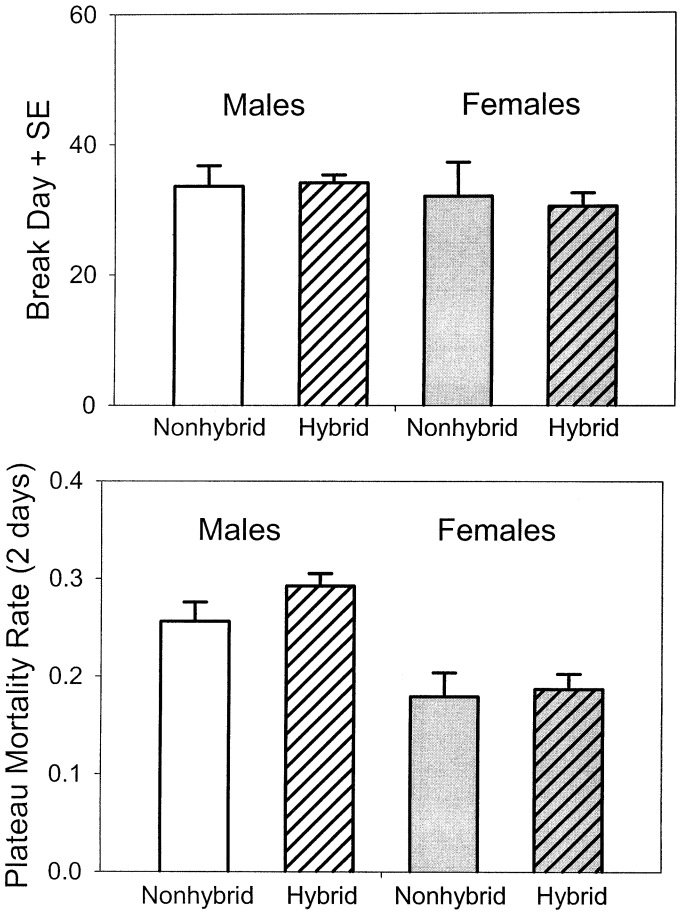

FIG. 7. Test for hybrid vigor between the genetically divergent yet demograpically identical B laboratory selection lines. Top: Mean estimated plateau breakday, the day on which a slope of zero better describes the data than a nonzero slope. The B lines that were not hybridized are not phenotypically distinct from the B lines that were hybridized. Error bars are 95% confidence intervals. Bottom: Mean estimated mortality rate on the plateau. Again, the B lines that were not hybridized are not distinct from the B lines that were hybridized. Error bars are 95% confidence intervals.

Antagonistic pleiotropy was strikingly corroborated by the speed with which male late-life mortality rates evolved over just 24 generations of early last ages of reproduction. Specifically, with antagonistic pleiotropy between early and late ages, an earlier truncation of the force of natural selection should increase mortality rates before the start of the mortality-rate plateau, causing an earlier plateau onset if this increase raises mortality rates up to the level of the plateau. The results of Sgrò and Partridge (1999) suggest how this phenomenon can occur: their unirradiated and nonsterile females show a significant difference between young and old *Drosophila* lines in mortality rates at late ages, in contrast to the irradiated and sterile females, illustrating the connection between early fertility and late life mortality rates.

Further Implications of Late-Life Evolution

There are a number of surprising features of late life. One is that late-life mortality rates are not 100% in the studies that have been performed to date, despite a complete lack of age-specific natural selection at these very late ages (cf. Pletcher and Curtsinger 1998). This finding potentially has profound implications for our understanding of pleiotropy and selection in evolution. Perhaps there are some alleles that generally foster survival, at both early, selected ages and late, unselected ages?

Another surprise in the present experiments is the speed with which late-life mortality evolves. For example, the starting age of the plateau in male mortality rates in the reverse-selected stocks evolved by about one day per generation, which is extremely rapid for an organism that has an average adult life span of a few weeks. All of the populations that we have studied have mortality rate plateaus that have evolved quickly in the direction expected by evolutionary

TABLE 3. Results from a test for response to a brief period of reverse selection in the NRO stocks. Because each NRO population derives from a single O population, paired-difference *t*-tests were used to test for significant average differences between the O and NRO groups. The plateau mortality rate is computed from equation (2).

	Males			Females		
	O	NRO		O	NRO	
Sample size	7343	9072		12,784	13,445	
Breakday	68.6	48.2	***	67.8	54.6	*
Plateau mortality rate	0.28	0.22		0.26	0.24	
A	0.0015	0.0021		0.0019	0.0033	**
α	0.062	0.081	**	0.063	0.076	
Mean longevity	53.3	41.8	***	50.4	39.2	***

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$.

theory. Late-life mortality appears to be evolutionarily responsive to a marked degree. Is this because it is free of selective constraints?

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