

Experimental evolution of aging, growth, and reproduction in fruitflies

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We report in this paper an evolutionary experiment on *Drosophila* that tested life-history theory and the evolutionary theory of aging. As theory predicts, higher extrinsic mortality rates did lead to the evolution of higher intrinsic mortality rates, to shorter lifespans, and to decreased age and size at eclosion; peak fecundity also shifted earlier in life. These results confirm the key role of extrinsic mortality rates in the evolution of growth, maturation, reproduction, and aging, and they do so with a selection regime that maintained selection on fertility throughout life while holding population densities constant.

life-history evolution | lifespan | age at maturity | body size | *Drosophila*

In this paper, we report a case study in experimental evolution with the fruit fly *Drosophila melanogaster* that is designed to test predictions of life-history theory (1–6) and the evolutionary theory of aging (7–11). It did confirm those predictions. The change in the environment that drove phenotypic change was a difference in extrinsic adult mortality rates. What is different in this experiment is that mortality was applied in a way closely resembling natural conditions rather than by using traditional artificial selection. Treatments differed only in adult mortality applied twice each week, which maintained selection on fertility throughout life.

Evolutionary Theory of Aging and Life Histories. Evolutionary theory predicts the impact of a difference in extrinsic mortality on intrinsic mortality rates (hence, lifespan) and on growth, maturation, body size, and reproduction. When extrinsic mortality rates increase, they lower the probability of survival to a given age and cause the strength of selection to decline faster with age, making an increase in intrinsic mortality rates with age “more affordable” or “less avoidable.” From this concept follows a central prediction of the evolutionary theory of aging (11): Higher extrinsic mortality rates should lead to higher intrinsic mortality rates and a decrease in lifespan, which is a prediction adumbrated by Weismann (7) and Medawar (8), explicit in the work of Williams (9), quantitative in the research of Hamilton (10) and Charlesworth (1), and consistent with comparative evidence (11–13).

Extrinsic mortality rates also affect the evolution of other life-history traits. Higher extrinsic adult mortality rates should lead to higher reproductive effort early in life and, for age- but not stage-dependent life histories (5), to more rapid development and eclosion at an earlier age and a smaller size (1–6).

Experimental Evolution and Artificial Selection. Recently, a new tool has been exploited to test such predictions: experimental evolution (14). In contrast to artificial selection, in which the experimenter determines which trait is selected, in experimental evolution, the experimenter creates the conditions under which a prediction should hold and lets the evolving population determine with which traits the problem will be solved. This approach has yielded important insights with bacteria (14–16), algae (17), poeciliid fish (18), and temperature adaptation in

flies (19–22). It succeeds because evolution is more rapid than until recently was expected (23, 24).

Previous artificial selection experiments on *Drosophila* (25–31) suggested that the evolution of lifespan is constrained by genetic links between early-life traits, such as early fecundity and larval competitive ability, and late-life mortality and fecundity rates. The longest running artificial selection experiment on the evolution of aging in fruit flies (25) started in 1976. One set of lines is only allowed to reproduce early in life, another only late in life; fecundity and survival are the targets of selection, and the evolutionary responses in traits such as intrinsic mortality are measured. The late-reproducing lines have evolved a much longer lifespan and lower intrinsic mortality rates than the early-reproducing lines. This experiment has been repeated in another laboratory with a design that avoids unintentional selection and controls larval density (31). In this second case, the late-reproducing lines have improved survival and decreased early-life fertility, but there has been no improvement in late-life fertility, and no correlated responses to selection in the preadult period have been observed. In the best field test of life-history theory, still continuing in Trinidad (18, 24), extrinsic mortality rates of guppies are manipulated by exposing them to different predators. Significant genetically based changes in age at maturity and fecundity in the predicted direction have been observed, and changes in lifespan are expected.

Our Experiment: High Versus Low Adult Mortality. Our experiment subjected a stock of wild *D. melanogaster* to two treatments: high adult mortality (HAM) and low adult mortality (LAM). In contrast to previous experiments, we knew age-specific extrinsic mortality precisely, because we imposed it twice weekly. Thus, the problem posed by selection to the organisms differed from the problems posed by previous work (25, 31), because the LAM flies were contributing offspring to future generations throughout their lives, not just at the ends of their lives. This selection regime, which maintains selection on fertility throughout adult life, is a much closer approximation of the kind of selection that is likely to be found in nature than is selecting for flies that lay eggs only very early or very late in life (25, 31). Moreover, because the population density of both the adults (200 per population cage) and the larvae (250 per unit of larval medium) was the same in both treatments, the responses can be attributed solely to differences in mortality rates with no confounding effects of larval or adult density.

We checked that the predictions of both the evolutionary theory of aging and the reproductive-effort model held for our experimental conditions by analyzing computer models simulating the evolutionary dynamics of phenotypes endowed with

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Abbreviations: HAM, high adult mortality; LAM, low adult mortality.

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different life-history traits and age schedules of intrinsic mortalities. In the simulations, these phenotypes were subjected to selection regimes with different adult mortalities that corresponded to the regimes used in the experiment. The models assumed the standard constraining relations among development time, body size, and fecundity that are known to *Drosophila* researchers (5). The prediction that the HAM treatment should evolve higher intrinsic mortalities held for a range of assumptions about the shapes of intrinsic-mortality curves, including Gompertz (32) and sigmoid. The predictions of the reproductive-effort model also were confirmed. The computer models predicted that HAM flies would evolve shorter development times, smaller size at eclosion, higher fecundity early in life, and lower fecundity later in life than did LAM flies.

These expectations have the following intuitive explanation. In this experiment, extrinsic adult mortality starts when flies enter a population cage at the age of 14 days from egg. Under the high mortality in the HAM treatment, most flies can only reproduce for a few days before they are killed; they should evolve high fecundity at the age of 14 days by eclosing early and by being at the peak of their age-fecundity curve when they enter the cage. In the LAM treatment, most flies can reproduce for several weeks; fecundity late in life is important and can be increased by eclosing later at a larger size, which, however, delays maturity and lowers fecundity at age 14 days. Note that if the shift in mortality rates occurs precisely at eclosion, rather than at a particular age, then an increase in adult mortality selects for a longer juvenile period (at low risk) and a larger size with higher fecundity at eclosion.

Methods

The founder stock of 820 flies consisted of 10 virgin males and 10 virgin females from each of 41 isofemale lines that were collected in and near Basel, held as isofemale lines for several years, then bred together in a single cage for about five generations to yield a genetically variable population. To found the selection lines, we collected eggs once a week for 5 weeks to get five age classes and to avoid the cycles of reproduction and age classes that persist in populations founded with flies of one age. The experiment started November 15, 1993, is continuing, and is maintained on a day-night cycle of 12 h of light, 25°C/12 h of dark, 20°C, and at 70% relative humidity. Eggs for recruits are collected from the population cages on Tuesdays and Fridays, at which time two bottles for each line are established, each with 250 eggs in a fixed amount of larval medium. The adults that hatch from these eggs are used 14 days later to replace flies that die or are killed in the population cages.

Each treatment consists of three replicates, and each replicate has 100 male and 100 female adults, enough to avoid inbreeding. Adult mortality rates are imposed and densities are reestablished by hand with 14-day-old flies on Mondays and Thursdays (Fig. 1); larvae are maintained at the same density in the same medium for both treatments. We could maintain constant larval densities because of excess production of eggs and constant adult densities, because we reared more flies than were needed. Extrinsic mortality was adjusted so that intrinsic plus extrinsic mortality reached the target level.

The mortality rates imposed and the rearing conditions have changed twice since the start of the experiment. In the first 13.5 months in the HAM treatment, 90% of the flies in the cage were killed and replaced twice per week; the probability of surviving 1 week as an adult was $P = 0.01$. In the LAM treatment, the probability of surviving 1 week as an adult was $P = 0.64$. In January 1995, larval density was raised from 6.25 to 10.40 larvae per ml, and food quality was lowered from 1% to 0.75% yeast in the larval medium. In January 1996, larval density was raised to 12.50 larvae per ml, and the adult mortality rate in the LAM treatment was lowered, raising the probability of surviving 1

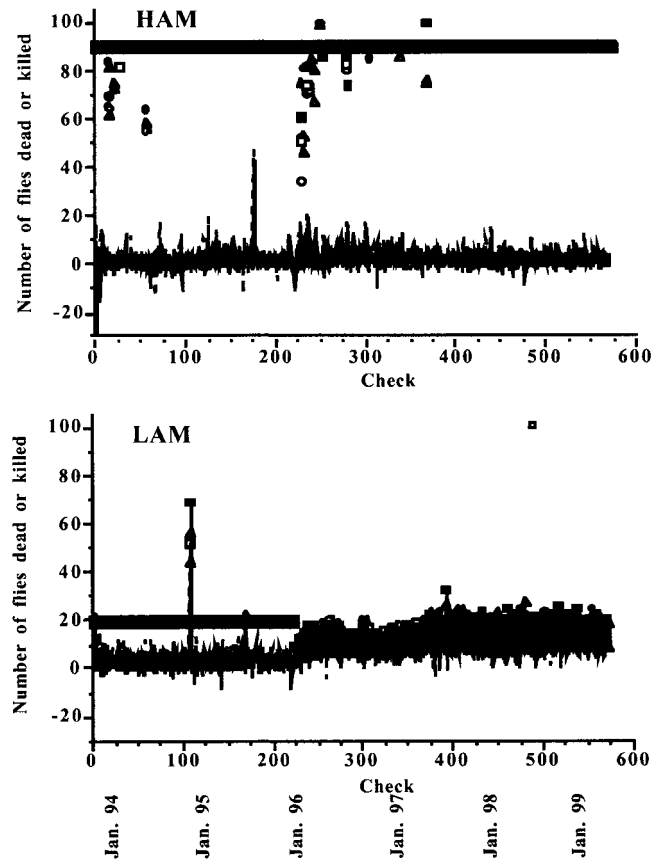


Fig. 1. Intrinsic and total mortality in the population cages. Intrinsic mortality is indicated by the irregular lower lines, one line per replicate. Negative mortalities indicate that too many flies accidentally were added in the last check. Total mortality (intrinsic plus extrinsic mortality) is given by the thick straight line. The symbols indicate when there were not enough flies available to achieve the target mortality because not enough eggs had been laid 14 days previously. □, Replicate 1; ○, replicate 2; △, replicate 3. Filled symbols indicate females, and open symbols indicate males. (Upper) HAM. The symbols above the total-mortality line indicate that 4 times a replicate was lost because of mislabeling and was replaced from backup stocks 2 weeks behind the current history of selection. At the start of the experiment and after larval density was increased in January 1996, there were sometimes not enough replacement flies to achieve the target mortality (23 times for females, 2.1% of the checks; 20 times for males, 1.8% of the checks). (Lower) LAM. Before January 1996, natural mortality rates twice exceeded 20%. After January 1996, when the target mortality was 10%, there were more old flies in the cages, and intrinsic mortality increased. From January 1996 to July 1998, intrinsic mortality was 11.3% for females (vs. 2.8% in HAM) and 10.3% for males (vs. 3.6% in HAM).

week as an adult to $P = 0.81$. Those changes increased both the physiological stress on the larvae and the difference between the two mortality regimes. We have achieved the target adult mortality rates in the two treatments (Fig. 1).

Traits were assayed in vials, not in the population cages themselves, in measurements made parallel to the main selection experiment. For each of the three replicates in both selection regimes, 4 × ≈50 14-day-old flies (age measured from egg) were collected in 250-ml glasses and fed fresh yeast for 4 h, then allowed to lay eggs into Petri dishes for 3 h. About 26 h after egg laying, 10 vials with 25 larvae each were set up for each replicate. One day before eclosion began, the vials were positioned in random order in a machine that collected newly hatched flies at 2-h intervals. After eclosion (8–12 h) males and females were separated. Males were frozen, dried for 3 h at 50°C, and weighed to ± 1 μg. Females were weighed alive (±10 μg) and set up in

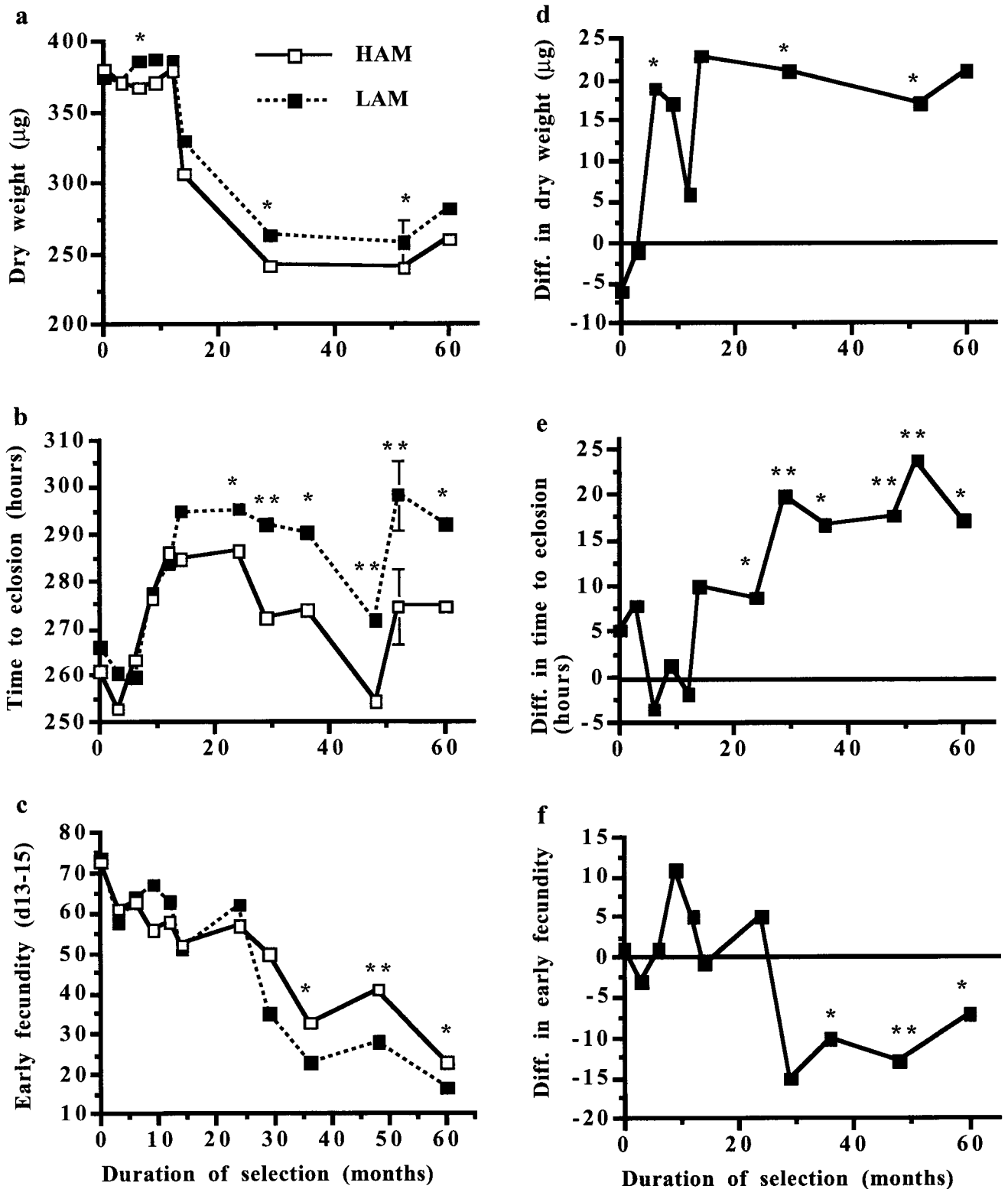


Fig. 2. Changes in dry weight (a), time to eclosion (b), and early fecundity (c), 1993–1997. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The bars at the end of the series in a and b are the standard errors of three sets of measurements made in the spring of 1998. Divergence between HAM and LAM (LAM minus HAM) in dry weight (d), time to eclosion (e), and early fecundity (f), 1993–1997.

individual vials (52×18 mm) with two males apiece of the same age and line to determine fecundity. The medium in the vial caps was covered with 1:10 (vol/vol) diluted fresh yeast; caps were

changed every day. Eggs were counted automatically with imaging software until 95% of the females had died; dead and trapped males were replaced until all females had died.

Table 1. Variation among replicates and differences between treatments

Trait	HAM					LAM					P values	
	n	Repl. 1	Repl. 2	Repl. 3	Total	n	Repl. 1	Repl. 2	Repl. 3	Total	Treat/ repl., ms*	Repl./ error, ms*
Females												
Development time, h	389	252	255	256	254	345	268	271	277	272	0.0041	0.0009
Dry weight, μg	90	250	236	240	242	90	257	261	265	261	0.0156	0.0468
Fecundity												
Days 13–15	340	43.8	39.7	38.8	40.8	322	28.5	28.1	24.8	27.0	0.0035	0.1071
Days 31–33 (late)	124	31.7	29.9	26.2	29.8	104	31.9	31.3	35.1	33.2	0.1008	0.7189
Males												
Development time, h	389	259	259	261	260	334	270	280	277	276	0.0061	0.0001
Dry weight, μg	388	206	192	194	197	332	221	212	217	217	0.0182	0.0054

Mean values of traits measured in November 1997, 4 years after the experiment started, except female dry weights, which were measured February 1998. Repl., replicate; ms, mean square.

*Significance levels from ANOVA.

Intrinsic mortalities were measured precisely in 1998, when the HAM treatment had experienced ≈ 90 and the LAM treatment ≈ 50 generations, on 5,000 flies of each sex for each replicate in two cages per replicate with 2,500 males and 2,500 females per cage ($5,000 \times 2 \text{ sexes} \times 3 \text{ replicates} \times 2 \text{ treatments} = 60,000$ flies). Dead flies were sexed, counted, and replaced every 3 days with white-eyed mutants to hold the density constant throughout the assay. At the end, the number of flies remaining in each cage was counted to estimate the number of escapees, which averaged about 4% of the flies. We distributed escapees uniformly over age classes in proportion to the number of flies surviving.

Fecundity, development time, and weight were analyzed by ANOVA [SAS Institute (Cary, NC) procedure GLM] with replicate lines nested within treatments and, where appropriate, replicate vials nested within lines. Line MS was used to test treatment effects. Mortality curves were analyzed with Cox regressions (SAS procedure PHREG) with the same design as the ANOVA. Data were right-censored for escapees.

Results and Discussion

The female flies responded in the first year of their new life in population cages by taking longer to develop, becoming smaller at eclosion, and decreasing slightly in early fecundity (male response was similar), regardless of the treatment. Time to eclosion increased in both treatments from 266 to 285 h. Dry weights at eclosion held constant at about $380 \mu\text{g}$. Early (days 13–15) fecundity dropped from ≈ 75 eggs per day to ≈ 50 eggs per day (Fig. 2 a–c). The assay methods were improved during the first year of the experiment, and the changes in the absolute values of life-history traits during the first year can mostly be attributed to changes in the assay methods.

Divergence between the two treatments in time to eclosion and dry weight began after 1 year when larval food quality was lowered and larval density was increased; divergence in early fecundity began after 2 years when larval density was raised again and the adult mortality rate in the LAM treatment was lowered. In all three traits, divergence continued for 1–2 years, then slowed considerably or stopped (Fig. 2 d–f). The slow change at the beginning of the experiment can be attributed to two factors: the larvae were not food-stressed enough to express the genetic variation that allows a response to selection, and the difference in the adult mortality rates was not yet large enough to provoke a rapid response. After the stress on the larvae was increased in 1995 and again in 1996, and the difference in mortality rates was increased in 1996, the lines diverged rapidly,

as predicted (Table 1). The HAM flies developed more rapidly, eclosed at a smaller size, and reached peak fecundity more rapidly. Then the dynamics slowed, either because the lines had reached new adaptive peaks or because genetic variation for improvements was exhausted by selection and inbreeding.

The results qualitatively confirmed the assumptions we made in the computer model: Flies that eclosed earlier were lighter ($r = 0.86$; $P = 0.02$) than flies that eclosed later and had higher early fecundity (day 13–15 after egg; $r = 0.99$; $P < 0.0001$, calculated with the six-line means).

Intrinsic mortality was significantly higher for HAM than for LAM flies from the 40th to the 75th day of life (Fig. 3). Thus, differences in extrinsic mortality rates did lead to the evolution of the expected differences in intrinsic mortality rates. Recently, there has been considerable discussion (33–36) about the shape of mortality curves, stimulated by the observation of decelerating mortalities late in life (37). In this study, female age-specific intrinsic mortality (d_x) stopped increasing after about 65 days of age, then decreased, then increased again (Fig. 3), suggesting that mortality rates do slow their rate of increase and may decrease late in life.

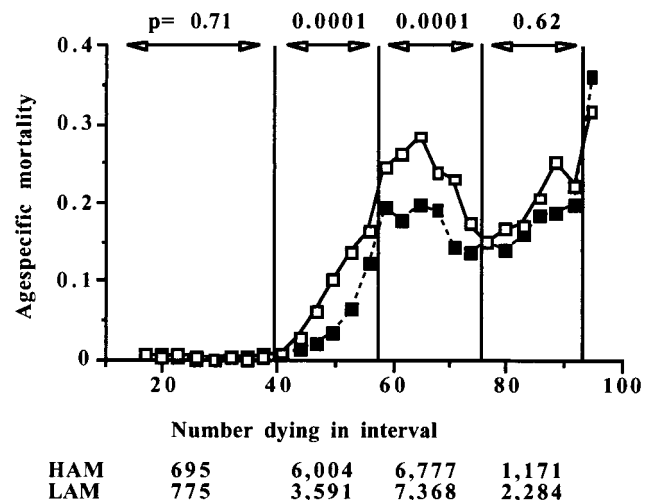


Fig. 3. Intrinsic mortality rates per 3-day interval. Number dying in each period determines statistical power. Results show averages for the two treatments. Mortality curves were measured April–July 1998.

We rarely have measured a significant difference between treatments in late fecundity in 5 years of yearly assays. In the HAM treatment, late fecundity is not under selection, but may be subject to reduction through mutation accumulation. In the LAM treatment, late fecundity is under direct selection in all survivors. The fact that late fecundity has not decreased more dramatically in the HAM flies suggests that the effects of mutation accumulation, if any, or of an antagonistic pleiotropic connection with early fecundity, if any, are not strong. Thus, our results do not support the existence of a genetic connection between early and late fecundity as suggested by Rose (25) but not by Partridge *et al.* (31).

Was there a connection between fecundity and subsequent mortality, a cost of reproduction? In 1996, we checked for a relationship between early fecundity and subsequent adult mortality by calculating the correlation of early fecundity with mortality rates defined on subsequent 5-day intervals (Fig. 4). With measurements on only six lines, power was low, but correlations were consistently strongly positive for about 2 weeks after the fecundity measurement and, for one age class, they were significantly so. Thus, we confirmed the positive relationship between early fecundity and subsequent mortality found by Rose (25) and Partridge *et al.* (31) that is a cornerstone of the life-history theory and the evolutionary theory of aging (5, 6, 11). However, in our case, the strength of the correlations arises because the HAM lines had (i) high early fecundity and (ii) high later mortality, whereas the LAM lines had (iii) low early fecundity and (iv) low later mortality, a pattern explicable as direct responses to selection by both traits in three (i, iii, iv) of four cases. Only the relatively high late mortality of the HAM flies (ii) is not selected directly and suggests either a link to early fecundity, decreased maintenance, or effects of mutation accumulation. Our results do not contradict stronger evidence from other experiments (e.g., ref. 38) in which the relationship measured could not have been caused by direct responses to selection.

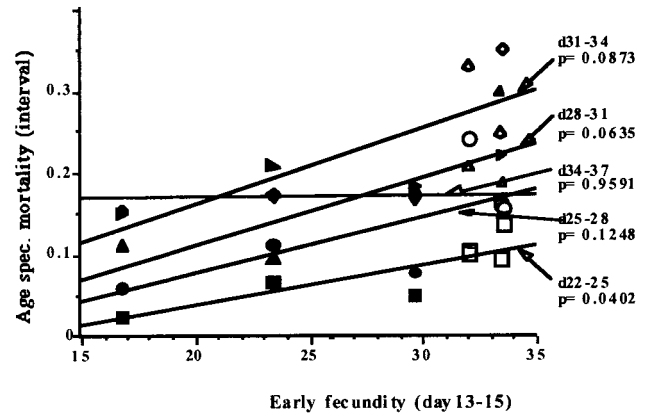


Fig. 4. Correlations of early fecundity with subsequent age-specific mortality defined on 5-day age classes. Open symbols, HAM; closed symbols, LAM. Traits were measured in November 1996.

This experiment tested and confirmed the prediction that higher extrinsic mortality rates lead to the evolution of higher intrinsic mortality rates, something that had been done previously (25–31), but never before with such a realistic and precisely measured difference in mortality regimes and with such precise control of both adult and juvenile population densities. It also confirmed all of the major predictions of life-history theory in a case in which mortality rates were known precisely and where the confounding effects of density variation in both adults and larvae were excluded. The interaction of extrinsic mortality rates with intrinsic constraining relations was sufficient to explain these results.

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