

Testing the heterogeneity theory of late-life mortality plateaus by using cohorts of *Drosophila melanogaster*

Mark D. Drapeau*, Erin K. Gass, Michael D. Simison,
Laurence D. Mueller, Michael R. Rose

Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525, USA

Received 24 June 1999; received in revised form 4 November 1999; accepted 5 November 1999

Abstract

Variation among individuals in robustness has been posed as a general explanation for the lack of increase in late-life mortality rates. Here, we test corollaries of this heterogeneity theory. One is that populations that have undergone strong laboratory selection for differentiated stress resistance should show significant differences in their late-life mortality schedules. To test this corollary, we employed 40 410 flies from three groups of *Drosophila melanogaster* populations that differ substantially in their resistance to starvation. No significant differences between these groups were found for late-life mortality. Another corollary of the heterogeneity theory is that there should be late-life plateaus in stress resistance that coincide with the plateau stage of the mortality curve. In 20 994 flies from six replicate outbred laboratory populations, we measured mortality rates every other day and starvation and desiccation resistance every 7 days. Both male and female starvation and desiccation resistance clearly decreased with time overall. There was no late-life plateau in male desiccation resistance. A late-life plateau in male starvation resistance may exist, however. Together, these two experiments generally constitute evidence against heterogeneity as a major contributor to the phenomenon of late-life mortality plateaus. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Fruit fly; Medfly; Demography; Negative aging; Longevity; Frailty; Stress; Survival; Ageing

1. Introduction

Recently, some evolutionary biologists, demographers, and gerontologists have directed their attention toward the now well-documented plateau in age-specific mortality

* Corresponding author. Tel.: +1-949-824-5994; fax: +1-949-824-2181.

E-mail address: drapeau@darwin.bio.uci.edu (M.D. Drapeau)

rates late in the life of *Drosophila* fruit flies and a number of other organisms, including medflies, wasps, nematodes, yeast, and humans (Charlesworth and Partridge, 1997; Vaupel et al., 1998). Because late-life plateaus in cohort mortality rates indicate that there is no “wall of death” past which no organism can survive, some individuals can theoretically live indefinitely. Understandably, researchers have been intrigued by this phenomenon since its first experimental demonstration (Carey et al., 1992; Curtsinger et al., 1992). Indeed, late-life mortality plateaus seem to challenge current definitions and theories of aging (see Finch, 1990; Rose, 1991). Since 1992, some theorists have risen to this challenge of explaining late-life mortality plateaus by using population genetic theories of senescence (e.g., Mueller and Rose, 1996).

In addition, non-population genetic theories have been proposed that purport to fully account for mortality patterns in late life (Vaupel et al., 1979; Abrams and Ludwig, 1995; reviewed in Charlesworth and Partridge, 1997). One such theory postulates that the differential ability of individuals in a population to resist stress can account for the occurrence of late-life mortality plateaus (Vaupel et al., 1979; Vaupel, 1988, 1990). This “heterogeneity theory” or “frailty theory” predicts that stress resistance is intimately tied to mortality: individuals in a population who die early in life will tend to be those who are less resistant to stresses in their environment. Those individuals with high longevity are those who are extremely resistant, and these individuals as a group may have a very slowly increasing mortality rate, yielding an approximate mortality plateau as the less-resistant groups die off. The required heterogeneity may be genetic or environmental (cf. Curtsinger et al., 1992; Fukui et al., 1993).

Currently, little experimental evidence exists that supports individual heterogeneity as a general explanation of late-life mortality patterns. Although a theoretical analysis of the Carey et al. (1992) medfly mortality data has shown that it can be fitted post hoc to a cohort heterogeneity model (Kowald and Kirkwood, 1993), this is weak support at best. Brooks et al. (1994) demonstrated that mortality rate in an isogenic population of the nematode *Caenorhabditis elegans* showed a less-distinct plateau than in a genetically heterogeneous population, but Vaupel et al. (1994) were quick to point out that the isogenic line was grown at a higher temperature and in higher food concentration than the heterogeneous line, greatly complicating the interpretation of the mortality patterns. In addition, isogenic versus heterogenous populations of *Drosophila melanogaster* do not consistently show these same patterns (Curtsinger et al., 1992; Fukui et al., 1996). Furthermore, although the relevance of individual *environmental* heterogeneity to late-life mortality rates is extremely difficult to test effectively (Curtsinger et al., 1992; Fukui et al., 1993; Vaupel and Carey, 1993), an intricate experiment by Khazaeli et al. (1998) found no evidence to support the hypothesis that environmental heterogeneity among individual flies is a primary factor in determining late-life mortality rates. In another experiment, Khazaeli et al. (1995b) seemingly demonstrated environmental heterogeneity for stress resistance by subjecting genetically homogenous populations to a 24-hr heat stress and comparing their poststress mortality patterns to control lines; however, this paper was later retracted after new research revealed that the experimental method was flawed (Curtsinger and Khazaeli, 1997; Khazaeli et al., 1997; Tatar et al., 1997).

Here, instead of directly testing the heterogeneity theory itself, we indirectly test two of its corollaries. One corollary is that populations that are greatly differentiated for stress resistance should show great differences in their late-life mortality schedules. To test this corollary, we estimated mortality rates of cohorts derived from populations of *Drosophila melanogaster* that have evolved very different levels of resistance to starvation (Rose et

al., 1992). Variation for stress resistance between these three types of populations is far greater than that found within natural populations of *D. melanogaster*, and therefore, this is a conservative test of the heterogeneity theory. It has been established that the level of stress resistance affects mortality in *Drosophila* (Rose et al., 1992; Chippendale et al., 1993), with greater stress resistance giving increased longevity in most experiments. This makes the *Drosophila* stress-resistance system an appropriate one for testing theories about the relationship between late-life mortality and robustness. In particular, the invocation of differential robustness by the heterogeneity theory in turn implies that significant differences should be found in late-life mortality between populations having highly differentiated levels of stress resistance. This is because the genotypes contained within populations selected for increased starvation resistance are a rarefied subset of the genotypes contained in the outbred starting population which the selected lines are all derived from. Indeed, they will tend to be the “hearty” genotypes. We note that we have estimated previously that starvation resistance accounts for approximately 22% of the difference in longevity between long-lived laboratory selection lines and their controls (Rose et al., 1992). This calculation was based on lines in which selection was imposed specifically on starvation resistance and no other character. Thus the present study is not one in which the relationship between robustness and survival is purely conjectural; it has already been shown experimentally. Therefore, it is reasonable to choose starvation resistance as a character for the present study because it plays a major role in determining the longevity of the *Drosophila* populations used here. If heterogeneity of robustness is indeed central to late-life mortality rate plateaus, then this is a good experimental system in which to demonstrate it empirically, as opposed to merely assuming it.

A second testable corollary of the heterogeneity theory is that age-specific stress resistance should be directly related to age-specific mortality. Depending on the assumptions that are made under the general heterogeneity model, we expect a variety of results. If we assume that within-individual stress resistance is constant with age, but that between-individual stress resistance can differ, less-resistant individuals will die before more-resistant ones. From these assumptions, we predict that when a cohort of individuals is sampled throughout its “lifetime,” the mean level of stress resistance in the sample will increase with the age of the flies. Alternatively, we might assume that overall stress resistance decreases in every individual with age. Therefore, overall stress resistance will decrease with the age of a cohort. However, because according to frailty theories the population will be purified of the less-frail individuals with time (age), the theory predicts a steep decline in stress resistance before the time of onset of a mortality plateau, and a less steep decline postonset due to the hypothesized predominance of a highly robust subgroup within the aging cohort. This reduction could in fact also be thought of as a plateau in stress resistance late in life.

By using cohorts of ~3500 *D. melanogaster* taken from six outbred laboratory-selected populations with identical demography, we investigated these predicted relationships between stress resistance and mortality rates. Sex-specific mortality rates for each cohort were estimated every other day, and measures of sex-specific starvation and desiccation resistance were made every 7 days. It is to be expected under the heterogeneity theory that either: (1) starvation and desiccation resistance will increase overall; or (2) although starvation and desiccation resistance will decrease overall, as the mortality rate plateaus, there will be no significant change in either form of stress resistance during the plateau. Hence, before the plateau, the stress resistance should change, whereas during the plateau stage of life there should be no significant change in either character, if stress resistance

is closely linked to mortality rate. Again, we stress that Rose et al. (1992) calculated that both starvation resistance and desiccation resistance account for large amounts of the difference in longevity between long-lived laboratory selection lines and their controls (22% and 11%, respectively), making these characters reasonable choices for this experiment.

In sum, we found that populations differentially selected for stress resistance show no differences in day of plateau onset nor mortality rate on the plateau, that both overall starvation and desiccation resistance decrease with age, that there is no late-life male desiccation resistance plateau, and that there possibly is a late-life male starvation resistance plateau. In its entirety, the data collected in this study constitutes additional evidence against heterogeneity theories as general explanations for late-life mortality plateaus. Alternative theories to explain such plateaus are then discussed briefly.

2. Materials and Methods

2.1. *Stress resistance and mortality plateaus*

Three groups of 5-fold replicated populations that previously had undergone different stress selection regimes were used in this experiment. Populations undergoing the SO selection regime have evolved an increased ability to resist starvation; these populations are designated SO₁₋₅. Populations CO₁₋₅ have the same demography as the SO populations (Rose et al., 1992), but they are different from the SO lines in that they are not selected for starvation resistance. SO₁₋₅ and CO₁₋₅ were derived from the O₁₋₅ populations (respectively) of Rose (1984). At the time of the study, the SO and CO populations were in generation 118 of selection. RSO₁₋₅ populations were derived from SO₁₋₅ (respectively) 25 generations before the start of this experiment. They were then maintained exactly like populations CO₁₋₅.

For each SO and RSO replicate population, 70 8-dram glass banana–agar–corn syrup food vials each containing 60 ± 5 eggs were prepared. For each CO population, 50 food vials containing 60 ± 5 eggs were prepared. On the ninth and tenth days after the egg collection (but within a 24-hr period), all eclosed adult flies were sexed and placed into new food vials in groups of 24 (1 : 1 male : female) that would be used to start the mortality assay.

Survival was determined every other day. Any flies that were living but stuck to a part of the vial were scored as dead 2 days later. Living flies were transferred to new vials with fresh food. When necessary, flies within replicates were recombined in order to maintain a density of approximately 24 flies/vial, to rule out any possible density effects on both early- and late-life mortality rates (cf. Graves and Mueller, 1993, 1995; Curtsinger, 1995a,b; Khazaeli et al., 1995; 1996). Survival assays were continued until every fly was dead. Mean (\pm SD) sample sizes for the populations of each group were the following: CO, 2121 ± 397 ; RSO, 2906 ± 373 ; SO, 3054 ± 249 . Detailed sample sizes are provided in Table 1. We deliberately used high sample sizes to reduce sampling variance in our estimations of mortality rates (cf. Promislow et al., 1999).

The data for each cohort were then fit to a two-stage Gompertz equation by using maximum likelihood techniques, as follows. Let d^* 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

Table 1

Data on mortality plateau breakday, mortality rate on the plateau, mean longevity, and sample size for males and females of 15 populations diverged for resistance to starvation. See text for statistical analysis

Breakday of plateau						
Males			Females			
Replicate	CO	RSO	SO	CO	RSO	SO
1	42	76	44	50	78	46
2	42	44	44	50	66	52
3	46	68	70	46	52	72
4	44	44	44	50	52	46
5	44	44	72	52	52	46
Plateau mortality rate						
Males			Females			
Replicate	CO	RSO	SO	CO	RSO	SO
1	0.10	0.21	0.07	0.10	0.14	0.07
2	0.10	0.07	0.05	0.13	0.15	0.08
3	0.13	0.11	0.10	0.12	0.07	0.16
4	0.12	0.08	0.07	0.15	0.10	0.09
5	0.10	0.08	0.19	0.12	0.09	0.07
Mean lifetime (days)						
Males			Females			
Replicate	CO	RSO	SO	CO	RSO	SO
1	44.0	54.1	44.0	42.1	54.4	34.6
2	45.6	49.6	53.9	46.2	46.7	42.2
3	45.9	58.9	53.7	43.9	52.6	37.6
4	43.3	51.5	46.5	40.6	45.6	38.9
5	45.5	50.4	46.0	46.3	49.1	32.7
Cohort size						
Males			Females			
Replicate	CO	RSO	SO	CO	RSO	SO
1	595	1212	974	857	1815	2258
2	800	1176	1118	1266	1855	2022
3	995	915	828	1352	1330	1813
4	937	1269	1124	1475	1811	1890
5	936	1176	952	1394	1972	2293

A exp (αx), where A is the age-independent rate of mortality and α is the age-dependent rate of mortality. For ages greater than d^* , mortality rates were assumed to equal a constant value \bar{A} , specific to each population. \bar{A} is independent of age but different from A . All of these parameters were estimated by maximum likelihood techniques (Mueller et al., 1995). The Pascal program used can be obtained from the corresponding author.

Six one-way analyses of variance (ANOVAs) were performed to determine the presence of significant differences between both the males and females of the SO, CO, and RSO populations for plateau breakday, mortality rate on the plateau, and average lifetime. In addition, an *a posteriori* nonparametric binomial test was used to test whether the SO

populations exhibited higher mortality rates than the CO and RSO populations during the first 30 days of the assay.

2.2. Early- and late-life stress resistance patterns

Six outbred *Drosophila melanogaster* populations, derived from a common ancestor in 1980 and maintained under identical demographic laboratory selection, were used in this experiment. These are the B₁₋₅ and IV populations of Rose (1984). These stocks have undergone no previous stress or longevity selection. They have been maintained on a 14-day life cycle at low densities, 25°C, and constant light for over 500 generations. On Day 14 after egg collection, 2–3-day old adults were dumped into Plexiglas cages at densities of approximately 200 per cage. For 4 days, flies were given yeasted food to increase their fecundity. On the fifth day, approximately 60 eggs were collected for each of 130 vials, for a total of ~8000 eggs per replicate. These eggs developed and became the adults assayed in this experiment.

On Day 0 of the experiment, adult flies were lightly anesthetized with carbon dioxide and separated into new 8-dram food vials, in groups of 12 males and 12 females per vial. The mortality assay for each replicate started with ~3500 adults, a total of 20 994 in the whole experiment. These 20 994 flies were used not only in the mortality assay but also as a source for flies to be used for the stress assays each week. Both starvation resistance and desiccation resistance were assayed for males and females. The same assay procedures were followed every 7 days.

Mortality was estimated every 2 days. The data were collected by transferring the living flies into a new vial, leaving any dead flies in the old vial stuck to either the fly media or the walls. Living flies that were trapped in the food or on the vial wall were counted as dead 2 days later. The starvation assay procedure began by removing a random sample from the common pool. A total of 40 flies per sex per replicate population were assayed each week for starvation. These samples were lightly anesthetized using carbon dioxide and then placed into 8-dram vials as described by Service et al. (1985). Four flies (either male or female, never mixed) were constrained to the bottom 15 mm of space with a foam sponge. Two cotton balls were placed at the top of each vial, and 3 ml of water was pipetted onto the cotton. The vials were then securely sealed with Parafilm. Death counts took place every 6 hours until all flies were dead. This procedure was repeated for 5 weeks for males and 6 weeks for females. The means for each sex of each replicate population were tabulated and graphed. To compare resistance before the plateau and resistance on the plateau, two slopes were calculated. The data points before the breakday were used to determine a preplateau slope and the data points collected postbreakday were used to calculate a slope on the plateau. The significance of any difference between mean hours to death before the plateau and mean hours to death on the plateau were analyzed by using Mann–Whitney *U* tests.

Desiccation assays were also carried out in a manner similar to Service et al. (1985). The flies were lightly anesthetized using carbon dioxide and placed at densities of four flies per sex per vial. A total of 40 flies per sex per replicate were assayed each week. A sponge was placed into the vial, confining the flies to the bottom 20 mm of space, and approximately 10 g of Drierite desiccant was then added to each vial. The vials were then sealed with Parafilm and checked every hour for deaths until no flies were alive. Mean times to death for each sex of each replicate were calculated and graphed. Analysis of the

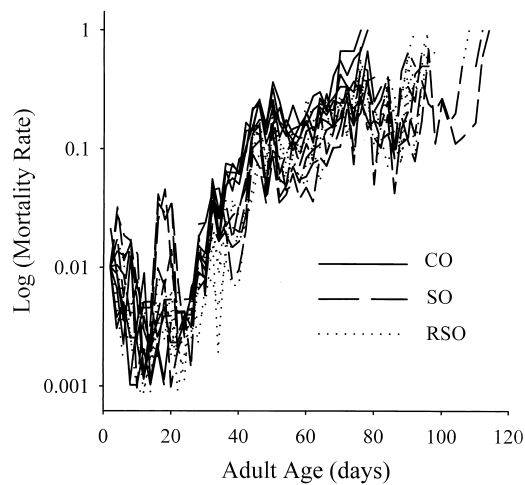


Fig. 1. Male age-specific mortality rates in 15 cohorts differentiated with respect to starvation resistance. Solid lines, CO₁₋₅; dashed lines, SO₁₋₅; dotted lines, RSO₁₋₅. There are no significant differences between the different sets of populations with respect to either plateau breakday or mortality rate on the plateau, the opposite of the prediction made by the heterogeneity theory. Early male mortality rates (Days 1–30) were significantly higher in the SO populations.

mean hours to death and the pre- and postbreakday slopes of the replicate populations before the plateau and on the plateau was done with *t*-tests.

2.3. Presentation of mortality rates

Age-specific mortality rates are presented in Figs. 1-4, as a way of conveying the inherent variability of our raw data. These figures do not include “smoothed” mortality rates, which are weighted averages from a number of adjacent ages (Carey et al., 1998). Because the age-specific mortality rates are not used to directly estimate any statistical parameters used in this paper, we feel that smoothing would only obscure the true nature of these raw data.

3. Results

3.1. Do differences in stress resistance result in differences in mortality schedules?

One corollary of the heterogeneity theory of late-life mortality plateaus is that populations with large differences in average stress resistance should have different mortality schedules because laboratory-selected populations that have evolved high levels of resistance to stress are equivalent to a “hearty” subset of the starting population. This corollary was tested by using long-term outbred laboratory-selection lines that were greatly differentiated with regard to resistance to starvation. Lifetime age-specific mortality rates of the SO₁₋₅, RSO₁₋₅, and CO₁₋₅ populations are shown in Figs. 1 (males) and 2 (females). No significant differences were found between the age at which mortality plateaus started in the SO, CO, and RSO population breakdays (One-way ANOVA: $P = 0.29$, males; $P =$

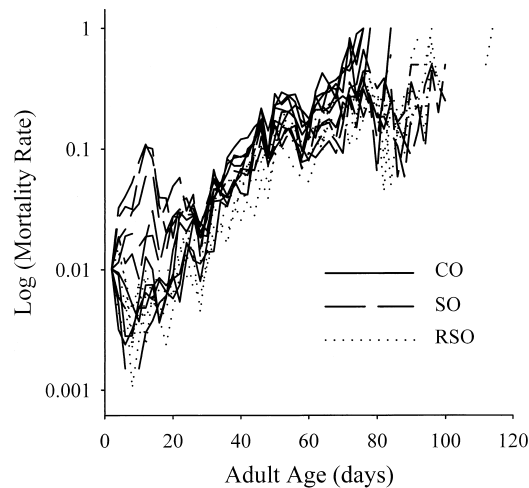


Fig. 2. Female age-specific mortality rates in 15 cohorts differentiated with respect to starvation resistance. Solid lines, CO₁₋₅; dashed lines, SO₁₋₅; dotted lines, RSO₁₋₅. There are no significant differences between the different sets of populations with respect to either plateau breakday or mortality rate on the plateau, the opposite of the prediction made by the heterogeneity theory. Early female mortality rates (Days 1–30) were significantly higher in the SO populations.

0.24, females). In addition, no differences were found among populations in the mortality rate on the plateau for either males or females (One-way ANOVA: $P = 0.88$, males; $P = 0.34$, females). The above tests are relatively conservative. However, even when multiple t tests are used to make every pairwise comparison between populations, for both plateau breakday and mortality rate on the plateau, for both males and females, not a single comparison shows significant differences between any pair of populations.

We tested an *a posteriori* hypothesis that there was among-selection line differentiation in early mortality rates (Days 1–30 of adult life). It was determined that the age-specific mortality rates over the first 30 days of the studies were significantly higher in the SO populations (Binomial test, $N_{CO} = N_{RSO} = N_{SO} = 15$; $P = 0.042$, males; $P = 0.0005$, females).

Weighted average lifetimes of the SO, RSO, and CO populations were significantly differentiated (One-way ANOVA: $P = 0.012$, males; $P < 0.0001$, females). Specifically, weighted average lifetimes were related to each other in the following manner (longest to shortest): Males: RSO (53 days) > SO (49) > CO (45); Females: RSO (50) > CO (44) > SO (37).

3.2. Does stress resistance plateau late in life?

Mortality assays

In order to ascertain the relationship between stress resistance (frailty) and mortality, we first measured these characters. Here we present the sex-specific mortality rates measured every two days in cohorts of ~3500 individuals. Mortality rate breakdays and plateau mortality rates for both males and females are presented in Table 2 and Figs. 3 and 4. Females had significantly later breakdays than males (2-tailed t -test: d. f. = 5; $N_{males} = 6$; $N_{females} = 6$; $P = 0.0077$). However, males and females did not have significantly

Table 2

Data on mortality plateau breakday, mortality rate on the plateau, and sample size for males and females of six populations diverged for approximately 500 generations, but under identical demographic selection. Females had significantly later plateau breakdays, but mortality rates on the plateau were not significantly different between them

	Breakday of plateau		Plateau mortality rate		Cohort size	
	Males	Females	Males	Females	Males	Females
B1	18	48	0.145	0.478	1552	1634
B2	18	32	0.203	0.289	1813	1590
B3	24	24	0.291	0.157	1693	1297
B4	24	44	0.203	0.497	2310	2015
B5	24	46	0.203	0.430	1831	1875
IV	18	44	0.175	0.720	1751	1633

different mortality rates on the plateau (2-tailed *t*-test: d. f. = 5; $N_{\text{males}} = 6$; $N_{\text{females}} = 6$; $P = 0.0626$).

Stress resistance

Every 7 days, starvation and desiccation resistance were assayed for 40 males and 40 females from each of the six populations studied in the mortality assay (above). One-way ANOVA detected no significant variation between populations for either males ($P = 0.97$, starvation; $P = 0.99$, desiccation) or females ($P = 0.96$, starvation; $P = 0.73$, desiccation; Figs. 5 and 6).

Relationship between mortality and stress

If within-individual stress resistance is constant with age and there is variation in between-individual stress resistance levels, less-resistant individuals will die before more-resistant ones. From this, we predict that when a cohort of individuals is sampled throughout its “lifetime,” the mean level of stress resistance in the sample will increase

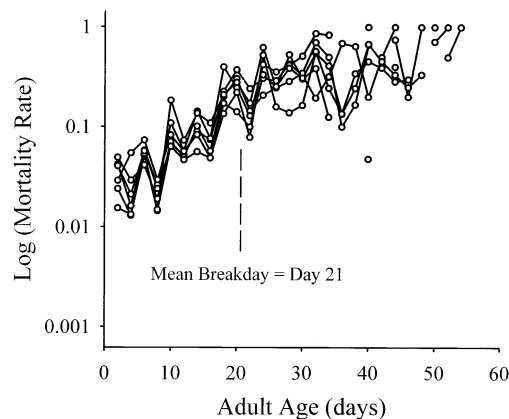


Fig. 3. Male age-specific mortality rates of six demographically identical laboratory selected populations sharing a common ancestor. There is no significant between-line variation with respect to either plateau breakday or mortality rate on the plateau. The mean plateau breakday, Day 21, is indicated by the vertical dashed line.

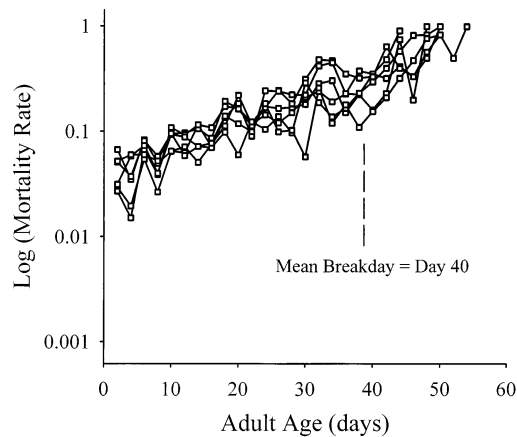


Fig. 4. Female age-specific mortality rates of six demographically identical laboratory selected populations sharing a common ancestor. There is no significant between-line variation with respect to either plateau breakday or mortality rate on the plateau. The mean plateau breakday, Day 40, is indicated by the vertical dashed line.

with the age of the flies. Our data are clearly inconsistent with this prediction: all 20 sets of points have negative slopes as ascertained by standard parametric linear regression (results not shown; Figs. 5 and 6).

Alternatively, overall stress resistance may decrease in every individual with age, and overall stress resistance will therefore decrease with the age of a cohort. Because according to heterogeneity theories the population will be purified of the less frail individuals with time (age), we predict a steep decline in stress resistance before the time of onset of a mortality plateau, and a less-steep decline postonset due to the hypothesized predominance of a highly robust subgroup within the aging cohort. This reduction could be thought of as a plateau in stress resistance late in life.

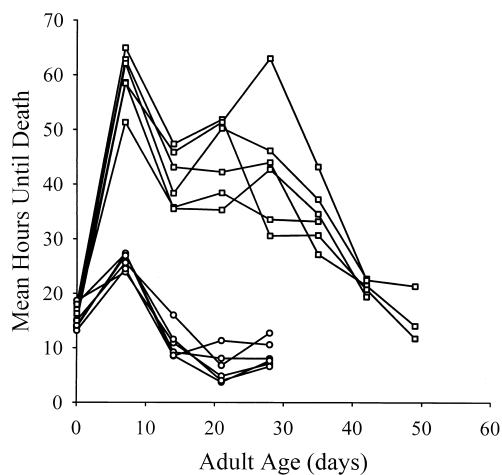


Fig. 5. Male (○) and female (□) age-specific starvation resistance. Mortality under starvation conditions was measured every 6 hours in each assay. Assays were performed once every 7 days.

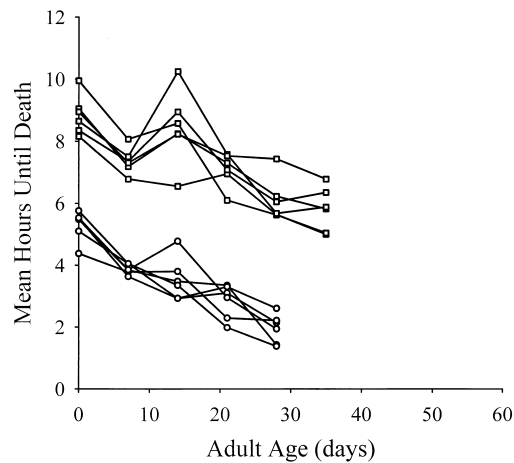


Fig. 6. Male (○) and female (□) age-specific desiccation resistance. Mortality under starvation conditions was measured once per hour in each assay. Assays were performed once every 7 days.

To determine if there were late-life stress resistance plateaus, or any other causative relationship between stress resistance and mortality rate, we divided both the starvation and desiccation data for each sex into two domains based on mean mortality breakdays calculated from data in Table 2 (mean male breakday was Day 21, and mean female breakday was Day 40). These domains will be called the prebreakday and postbreakday domains. Day 0 was excluded from all analyses due to the fact that the density that the assayed flies were contained in immediately before the assay (~ 60 flies/vial) was over twice as high as it was immediately before any other assay (24 flies/vial). Therefore, the number of hours of survival under stress conditions is not due completely to age at this initial stage, but is likely to be confounded with density effects. Standard parametric linear regression through data gathered on Days 7, 14, 21, and 28 was used to calculate slopes for both pre- and post-breakday domains of both assay types. Day 21 was included in the male post-breakday period for the purposes of this analysis.

Because there was an insufficient number of postbreakday female data points to estimate slopes from both starvation and desiccation assays, further analysis was carried out on males only. Mean hours of male starvation resistance were significantly lower post-breakday (t -test: $N_{\text{prebreakday}} = 6$; $N_{\text{postbreakday}} = 6$; $t = 4.81$; $P < 0.005$). In addition, post-breakday starvation resistance slopes were significantly *greater* (i.e. less negative) than prebreakday slopes (Mann–Whitney U test: $N_{\text{prebreakday}} = 6$; $N_{\text{postbreakday}} = 6$; $P < 0.05$; see also Fig. 5). This indicates a possible plateauing of starvation resistance during the same period that mortality rates plateau. Testing the slope of starvation resistance during the plateau period indicates that the preceding decline in starvation resistance has ended, with a marginally significant increase in starvation resistance (two-tailed test: $t = 2.290$; $N = 6$; $P < 0.10$;). Mean hours of male desiccation resistance were also significantly lower post-breakday (t -test: $N_{\text{prebreakday}} = 6$; $N_{\text{postbreakday}} = 6$; $t = 15.2$; $P < 0.005$). However, there was no difference between pre- and postbreakday desiccation resistance slopes (Mann–Whitney U test: $N_{\text{prebreakday}} = 6$; $N_{\text{postbreakday}} = 6$; $P > 0.05$; see also Fig. 6).

4. Discussion

In this study, we conducted three critical tests of the heterogeneity theory of late-life mortality plateaus. With respect to the first test, the data presented here do not indicate any clear relationship between late-life mortality rates and large genetic differences in stress resistance. In this respect, our results provide a refutation of the heterogeneity theory of late-life mortality.

With respect to the other two tests, the results are mixed. Male desiccation resistance continues to decline during the period in which late-life mortality has plateaued. This also refutes the heterogeneity theory. On the other hand, male starvation decelerates once the late-life mortality rates plateau, suggesting the possibility that starvation resistance shows a concordant plateau with late-mortality, as required by the heterogeneity theory. There is, however, an important asymmetry between the desiccation resistance and starvation resistance results. It is possible for stress resistance to stabilize for reasons unrelated to the loss of heterogeneity. But the converse is not true. As the heterogeneity theory is based on robustness, and it has been shown that desiccation is a mortality-rate determinant and thus part of robustness, it is a refutation of the heterogeneity theory that desiccation resistance does not follow the required pattern, even though starvation resistance does.

Our general conclusion, then, is that the heterogeneity theory has received another refutation from our work, as it did from the work of Khazaeli et al. (1998). Although the Carey et al. (1992) medfly mortality data have been fitted to a cohort heterogeneity model (Kowald and Kirkwood, 1993), a weak post hoc corroboration, other experiments (Brooks et al., 1994; Vaupel et al., 1994; Curtsinger et al., 1992; Fukui et al., 1996) have not been successful in finding consistent evidence for this theory. Provisionally, we regard it as falsified.

Despite this, other tests that use laboratory cohorts could be performed in order to test further the validity of the heterogeneity theory. First, experiments could be done the purpose of which is to observe age-specific mortality differences between groups of populations that are diverged in other stress-related traits besides the one tested here, resistance to starvation. Such populations are available for traits such as desiccation resistance (Rose et al., 1992; Hoffman and Parsons, 1993) and ethanol tolerance (Kerver et al., 1992). Second, composite cohorts could be constructed and then their aggregate age-specific mortalities could be measured. Such composite cohorts could be created from stocks differentiated for stress resistance. On the frailty theory, the prediction would be that populations that are more heterogenous in frailty should show more pronounced mortality plateaus in comparison to less heterogenous populations. Laboratory-selected populations such as the ones used in this paper would be ideal to use in an experiment of this type. A definitive study of this type would consist of composite cohorts constructed from different subpopulations with different degrees of divergence in frailty. Yet other lines of testing could no doubt be found. Additional work would be helpful either in definitively obliterating the heterogeneity theory or in resuscitating it. However, until good corroborative evidence is found, the heterogeneity theory cannot simply be assumed by theoreticians who find it a convenient assumption for spinning demographic. The theory has no convincing experimental support at this time, despite its primacy in theoretical and empirical research on late-life mortality.

Without a viable heterogeneity explanation for late-life mortality plateaus, evolutionary theories for late-life mortality should receive greater consideration in future empirical work. Mueller and Rose (1996) used population genetic models to demonstrate that the

late-life plateau in the force of natural selection can give cohort mortality plateaus late in life. This particular evolutionary theory of mortality plateaus has, however, recently been challenged on theoretical grounds (Pletcher and Curtsinger, 1998). In any case, evolutionary theory concerning the action of selection and mutation on late-life mortality can still be pursued, because it has not yet been empirically refuted.

Acknowledgments

We thank Curt Adams and Seth Bordenstein for helpful discussions. This research was funded by NIH grant AG11644.

References

- Abrams, P. A. & Ludwig, D. (1995). Optimality theory, Gompertz' law, and the disposable soma theory of senescence. *Evolution* 49, 1055–1066.
- Brooks, A., Lithgow, G. J., & Johnson, T. E. (1994). Mortality rates in a genetically heterogeneous population of *Caenorhabditis elegans*. *Science* 263, 668–671.
- Carey, J. R., Liedo, P., Mueller, H.-G., Wang, J.-L., & Vaupel, J. W. (1998). Dual modes of aging in Mediterranean fruit fly females. *Science* 281, 996–998.
- Carey, J. R., Liedo, P., Orozco, D., & Vaupel, J.W. (1992). Slowing of mortality rates at older ages in large medfly cohorts. *Science* 258, 457–461.
- Charlesworth, B. & Partridge, L. (1997). Ageing: levelling of the grim reaper. *Current Biology*, 7, R440–R442.
- Chippendale, A. K., Leroi, A. M., Kim, S. B., & Rose, M. R. (1993). Phenotypic plasticity and selection in *Drosophila* life-history evolution. 1. Nutrition and the cost of reproduction. *J Evol Biol* 6, 171–193.
- Curtsinger, J. W. (1995a). Density and age-specific mortality. *Genetica* 96, 179–182.
- Curtsinger, J. W. (1995b). Density, mortality, and the narrow view. *Genetica* 96, 187–189.
- Curtsinger, J. W., Fukui, H. H., Townsend, D. R., & Vaupel, J. W. (1992). Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*. *Science* 258, 461–463.
- Curtsinger, J. W. & Khazaeli, A. A. (1997). A reconsideration of stress experiments and population heterogeneity. *Exp Gerontol* 32, 727–729.
- Finch, C. E. (1990). *Longevity, Senescence, and the Genome*. Chicago: University of Chicago Press.
- Fukui, H. H., Ackert, L., and Curtsinger, J. W. (1996). Deceleration of age-specific mortality rates in chromosomal homozygotes and heterozygotes of *Drosophila melanogaster*. *Exp Gerontol* 31, 517–531.
- Fukui, H. H., Xiu, L., & Curtsinger, J. W. (1993). Slowing of age-specific mortality rates in *Drosophila melanogaster*. *Exp Gerontol* 28, 585–599.
- Graves, J. L., Jr. & Mueller, L. D. (1993). Population density effects on longevity. *Genetica* 91, 99–109.
- Graves, J. L., Jr. & Mueller, L. D. (1995). Population density effects on longevity revisited. *Genetica* 96, 183–186.
- Hoffman, A. A. & Parsons, P. A. (1993). Direct and correlated responses to selection for desiccation resistance: a comparison of *Drosophila melanogaster* and *D. simulans*. *J Evol Biol* 6, 643–657.
- Kerver, J. W. M., Wolf, W., Kamping, A., & Van Delden, W. (1992). Effects on *Adh* activity and distribution following selection for tolerance to ethanol in *Drosophila melanogaster*. *Genetica* 87, 175–183.
- Khazaeli, A. A., Pletcher, S. D., & Curtsinger, J. W. (1998). The fractionation experiment: reducing heterogeneity to investigate age-specific mortality in *Drosophila*. *Mech Ageing Dev* 105, 301–317.
- Khazaeli, A. A., Tatar, M., Pletcher, S. D., & Curtsinger, J. W. (1997). Heat-induced longevity extension in *Drosophila melanogaster*. I. Longevity, mortality, and thermotolerance. *J Gerontol* 52A, B48–B52.
- Khazaeli, A. A., Xiu, L., & Curtsinger, J. W. (1995a). Effect of adult cohort density on age-specific mortality in *Drosophila melanogaster*. *J Gerontol* 50A, B262–B269.
- Khazaeli, A. A., Xiu, L., & Curtsinger, J. W. (1995b). Stress experiments as a means of investigating age-specific mortality in *Drosophila melanogaster*. *Exp Gerontol* 30, 177–184.
- Khazaeli, A. A., Xiu, L., & Curtsinger, J. W. (1996). Effect of density on age-specific mortality in *Drosophila*: a density supplementation experiment. *Genetica* 98, 21–31.

- Kowald, A. & Kirkwood, T. B. L. (1993). Explaining fruit fly longevity *Science* 260, 1664–1665.
- Mueller, L. D., Nusbaum, T. J., & Rose, M. R. (1995). The Gompertz equation as a predictive tool in demography. *Exp Gerontol* 30, 553–569.
- Mueller, L. D. & Rose, M. R. (1996). Evolutionary theory predicts late-life mortality plateaus. *Proc Natl Acad Sci USA* 93, 15249–15253.
- Pletcher, S. D. & Curtsinger, J. W. (1998). Mortality plateaus and the evolution of senescence: why are old-age mortality rates so low? *Evolution* 52, 454–464.
- Promislow, D. E. L., Tatar, M., Pletcher, S., & Carey, J. R. (1999). Below-threshold mortality: implications for studies in evolution, ecology, and demography. *J Evol Biol* 12, 314–328.
- Rose, M. R. (1984). Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* 38, 1004–1010.
- Rose, M. R., Vu, L. N., Park, S. U., & Graves, J. L. (1992). Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Exp Gerontol* 27, 241–50.
- Rose, M. R. (1991). *Evolutionary Biology of Aging*. Oxford: Oxford University Press.
- Service, P. M., Hutchinson, E. W., MacKinley, M. D., & Rose, M. R. (1985). Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol Zool* 58, 380–389.
- Tatar, M., Khazaeli, A. A., & Curtsinger, J. W. (1997). Chaperoning extended life. *Nature* 390, 30.
- Vaupel, J. W. (1988). Inherited frailty and longevity. *Demography* 25, 277–287.
- Vaupel, J. W. (1990). Relative's risks: frailty models of life history. *Theor Popul Biol* 37, 220–234.
- Vaupel, J. W. & Carey, J. R. (1993). Compositional interpretations of medfly mortality. *Science* 260, 1666–1667.
- Vaupel, J. W., Carey, J. R., Christensen, K., Johnson, T. E., Yashin, A. I., Holm, N. V., Iachine, I. A., Kannisto, V., Khazaeli, A. A., Liedo, P., Longo, V. D., Zeng, Y., Manton, K. G., & Curtsinger, J. W. (1998). Biodemographic trajectories of longevity. *Science* 280, 855–860.
- Vaupel, J. W., Johnson, T. E., & Lithgow, G. J. (1994). *Science* 266, 826.
- Vaupel, J. W., Manton, K. G., & Stallard, E. (1979). The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography* 16, 439–454.