Demography of Genotypes: Failure of the Limited Life-Span Paradigm in Drosophila melanogaster

James W. Curtsinger, Hidenori H. Fukui, David R. Townsend, James W. Vaupel

Science, New Series, Volume 258, Issue 5081 (Oct. 16, 1992), 461-463.

raphy **16**, 3 (1979); K. G. Manton, *Milbank Mem. Fund Q.* **60**, 183 (1982); J. W. Vaupel and A. Yashin, *Am. Stat.* **39**, 176 (1985); _____, K. G. Manton, *Math. Popul. Stud.* **1**, 21 (1988); A. Rogers, *Demography* **29**, 31 (1992).
16. R. S. Pearl, L. Parker, B. M. Gonzalez, *Am. Nat.* **57**, 153 (1923); J. Miquel, P. R. Lundgren, K. G. Bensch, H. Atlan, *Mech. Ageing Dev.* **5**, 347 (1976); J. W. Curtsinger, H. H. Fukui, D. R. Townsend, J. W. Vaupil, *Science* **258**, 461 (1992).
17. M. Rockstein and H. M. Lieberman, *Gerontologia* **3**, 23 (1959).
18. Pooled data on 1999 medfly adults reared from different fruit hosts and held in cages of 30 to 80 individuals. D. A. Krainacker, thesis, University of California, Davis (1986); _____, J. R. Carey, R. Vargas, *Oecologia* **73**, 583 (1987).
19. M. Tatar, in preparation.
20. R. E. Beard, in *Biological Aspects of Demography*, W. Brass, Ed. (Taylor and Francis, London, 1971), pp. 75–68.
21. V. Kannisto, *Popul. Stud.* **42**, 389 (1988).
22. A. R. Thatcher, *J. Inst. Actuaries* **109**, 346 (1982).
23. L. A. Gavrilov and N. S. Gavrilova, *The Biology of Life Span: A Quantitative Approach* (Harwood Academic, Chur, Switzerland, 1991), p. 129.
24. A. C. Economos, *Age* **2**, 74 (1979); *Arch. Gerontol. Geriatr.* **1**, 3 (1982).
25. J. F. Fries, *Milbank Mem. Fund Q.* **61**, 397 (1983); *Gerontol. Perspecta.* **1**, 5 (1987).
26. We thank J. Reyes, director of the Moscamed Program in Mexico, directors J. L. Zavala, A. Villaseñor, and W. Enkerlin for use of the facilities in Metapa; H. Celedonio, A. Oropeza, R. Rincon, M. Reyes, H. Esquinca, and C. Fredersdorff for technical assistance; L. X. Li for assistance with data analysis; and A. Clark, M. Tatar, and T. Prout for critical review of the report. Research supported by the National Institute on Aging (grant AG08761-01).

21 May 1992; accepted 18 August 1992

Demography of Genotypes: Failure of the Limited Life-Span Paradigm in *Drosophila melanogaster*

James W. Curtsinger,* Hidenori H. Fukui, David R. Townsend, James W. Vaupel

Experimental systems that are amenable to genetic manipulation can be used to address fundamental questions about genetic and nongenetic determinants of longevity. Analysis of large cohorts of ten genotypes of *Drosophila melanogaster* raised under conditions that favored extended survival has revealed variation between genotypes in both the slope and location of age-specific mortality curves. More detailed examination of a single genotype showed that the mortality trajectory was best fit by a two-stage Gompertz model, with no age-specific increase in mortality rates beyond 30 days after emergence. These results are contrary to the limited life-span paradigm, which postulates well-defined, genotype-specific limits on life-span and brief periods of intense and rapidly accelerating mortality rates at the oldest ages.

A limited life-span paradigm underlies much gerontological thinking (*1*). Individuals are assumed to be born with a maximum life-span potential that is "genetically fixed" (*2*, p. 5). If an individual survives the various hazards that might result in premature death, life will be "terminated by a sharp decline mandated by senescence" (*2*), ending in "natural death" (*2*). Environmental improvement—including better health care and more salubrious behavior in the case of humans—can reduce premature death but cannot delay senescent death (*2*). Fries (*3*) provides the most specific hypothesis for humans: Each individual is genetically endowed with a maximum life-span potential that is approximately normally distributed among individuals with a mean of 85 years and a standard deviation of 7 years.

The limited life-span paradigm as well as Fries's specific hypothesis have come under increasing scrutiny (*4*). We have now performed experiments that directly test the predictions of the limited life-span paradigm in a model system. Our findings are based on a new approach that might be called the experimental demography of genotypes: We have applied methods of demographic analysis to survival data from large cohorts of genetically identical *Drosophila melanogaster* reared under controlled laboratory conditions. The combination of demography and genetics in an experimental setting creates a hybrid perspective that may provide insights beyond those attainable by either field in isolation (*5*).

If there were well-defined limits on life-span, they should produce rapid acceleration in mortality rates and corresponding sharp declines in survivorship at advanced ages in large, single-genotype cohorts raised under conditions that favor survival. The absence of brief periods of intense mortality at advanced ages would constitute evidence against the limited life-span paradigm. Previous experimental studies provide little information on this issue because, as noted by Finch (*6*, p. 16), "survivorship curves are often based on small samples, the curves may not be smooth, and the resulting mortality estimates are not very precise." It is customary in experimental gerontology to use 100 or fewer individuals per strain or treatment, yielding ten or fewer individuals in the oldest 10% of cohorts. Our experiments were designed to provide estimates based on hundreds of individuals in the tail of the survivorship curve.

We used genetically homogeneous lines because age-specific and genotype-specific mortality rates are estimable in cohorts but not in individuals. By studying highly inbred lines, we were able to estimate mortality rates for single genotypes—a feat that cannot be accomplished by studies of heterogeneous populations. We also studied crosses between inbred lines, which are genetically homogeneous in the F_1 generation but lacking in the depression of vigor and life span often associated with complete homozygosity (*7*).

Four highly inbred lines (*8*) of *D. melanogaster* were cultured under standard conditions (*9*) for three generations and then crossed within and between lines to produce ten genotypes: four inbred and six F_1. Genotype "$i \times j$" was produced by crossing females from line i with males from line j. Males of all ten genotypes were collected within 12 hours of emergence, lightly anesthetized with CO_2, and placed in groups of five in 4-dram shell vials with medium. Vials were assigned random locations in a single incubator and were examined daily; the numbers of both live and dead flies were recorded. Flies were transferred to fresh medium once (blocks I, II, and IV) or twice (block III) per week. Four experimental blocks were set up: three with all ten genotypes and one with a large sample of a single genotype.

Average life-spans (days after emergence) for ten genotypes studied in three nonoverlapping experiments are shown in Table 1. Sample sizes varied from block to block because of genotypic variations in fertility. There is statistically significant variation in mean life-span between blocks and between genotypes, as well as block × genotype interaction (*10*). The significant genotypic effect demonstrates genetic variation between lines that influences average life-span and is consistent with previous demonstrations of genetic variation for this character in *Drosophila* (*6*, *11*). The significant interaction, largely due to line 3 × 2 in block III where the estimated longevity was roughly 20 days lower than expected, indicates that genotypes responded differently to microenvironmental variations from block to block. Inbred lines tended to have lower longev-

J. W. Curtsinger, H. H. Fukui, D. R. Townsend, Department of Ecology, Evolution, and Behavior, University of Minnesota, Minneapolis, MN 55455.
J. W. Vaupel, Odense University Medical School, Odense, Denmark; and Center for Demographic Studies, Duke University, Durham, NC 27706.
*To whom correspondence should be addressed.

Table 1. Mean longevities (days after emergence) and sample sizes (*n*) for ten genotypes of *D. melanogaster* studied in three experimental blocks. SE = standard error.

Line	Mean longevity (*n*)					
	Block I		Block II		Block III	
1×1	36.4	(629)	35.2	(701)	46.1	(235)
2×1	47.0	(574)	27.2	(139)	59.5	(180)
2×2	31.5	(496)	18.3	(293)	33.9	(155)
2×4	36.9	(484)	25.8	(362)	56.3	(59)
3×1	50.4	(559)	35.1	(202)	59.7	(221)
3×2	46.8	(604)	29.5	(143)	34.8	(225)
3×3	29.6	(623)	19.6	(662)	35.3	(248)
3×4	41.3	(597)	41.7	(548)	58.5	(185)
4×1	45.4	(489)	40.6	(165)	55.6	(266)
4×4	39.9	(566)	28.6	(176)	52.5	(236)
Inbred mean	34.4	(2314)	25.4	(1832)	42.0	(874)
SE	4.7		8.0		8.9	
F_1 mean	44.6	(3307)	33.3	(1559)	54.1	(1136)
SE	4.8		6.9		9.6	
Total mean	40.5	(5621)	30.2	(3391)	49.2	(2010)
SE	6.9		8.0		10.8	

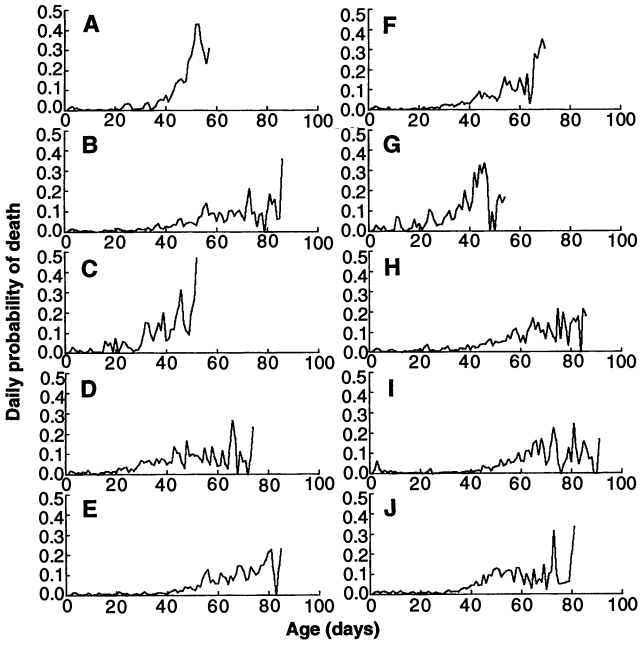

Fig. 1. Age-specific daily probabilities of death for ten male genotypes of *D. melanogaster* pooled over three experimental blocks (ten final deaths for each genotype not shown). Genotypes are (**A**) 1 × 1; (**B**) 2 × 1; (**C**) 2 × 2; (**D**) 2 × 4; (**E**) 3 × 1; (**F**) 3 × 2; (**G**) 3 × 3; (**H**) 3 × 4; (**I**) 4 × 1; and (**J**) 4 × 4.

ity than their F_1 crosses (Table 1).

Gompertz mortality rate parameters (*12*) for the ten genotypes are shown in Table 2. Differences between genotypes in both *a* and *b* parameters are generally much greater than the standard errors of the estimates, indicating that genotypes differed in both the location (*a*) and the slope (*b*) of mortality rate functions. Mortality rate doubling time (MRDT), expressed in terms of years to facilitate comparison with other studies (*13*), varied twofold between lines. Thus, *Drosophila* genotypes differ not only in average life-span, which is well documented, but also in the shape and location of the age-specific mortality curves when approximated by the Gompertz model.

Estimates of age-specific daily probabilities of death for each genotype are shown in Fig. 1. Although there is some suggestion of sharply increasing mortality rates for two genotypes (Fig. 1, A and C), the data show no general pattern of dramatic acceleration of mortality rates at the most advanced ages as would be expected if there were a well-defined cap for each genotype. It is possible that caps beyond observed life-spans exist that could only be detected with even larger experiments. This objection could be raised for any experiment, no matter how large, that failed to detect caps. Our conclusion therefore applies only to the range of life-spans actually observed. We have failed to detect a general pattern of caps in large experiments in which the oldest flies survived more than 100 days after emergence. Considering the life-span of the founding population for the preceding 160 generations (2 weeks in laboratory culture), the likely life expectancy of *Drosophila* in the wild (1 to 2 weeks), and the observation that most males are sterile by 40 days after emergence (*14*), we conclude that, if there are limits to life-span in *Drosophila*, they occur at several times the life-spans experienced by the populations in their recent evolutionary history.

Table 2. Level parameter *a* and slope parameter *b* for Gompertz mortality functions estimated for ten genotypes of *D. melanogaster* in three experimental blocks. Standard errors for all estimates are less than 0.00009. MRDT is the arithmetic average over three blocks.

Line	Block I		Block II		Block III		MRDT (years)
	a	*b*	*a*	*b*	*a*	*b*	
1×1	0.0007	0.1225	0.0021	0.0920	0.0001	0.1644	0.0150
2×1	0.0008	0.0882	0.0081	0.0683	0.0012	0.0547	0.0190
2×2	0.0026	0.0993	0.0175	0.0786	0.0053	0.0646	0.0235
2×4	0.0026	0.0813	0.0127	0.0538	0.0014	0.0558	0.0298
3×1	0.0005	0.0931	0.0079	0.0449	0.0008	0.0641	0.0282
3×2	0.0010	0.0833	0.0124	0.0406	0.0042	0.0718	0.0291
3×3	0.0027	0.1062	0.0130	0.0855	0.0019	0.0966	0.0198
3×4	0.0030	0.0636	0.0046	0.0479	0.0010	0.0598	0.0333
4×1	0.0019	0.0660	0.0057	0.0433	0.0023	0.0451	0.0369
4×4	0.0019	0.0809	0.0110	0.0481	0.0016	0.0605	0.0301

To obtain more detailed information about mortality rates at advanced ages, we performed an additional experiment with 5751 males from line 3 × 3 (Fig. 2, A and B). This line was chosen for further examination because it appeared to have the most nearly rectangular survivorship in preliminary experiments, as judged by low variance of the life-span distribution. The age-specific mortality rate (*15*) increased linearly on the log scale until day 30, and then remained constant at ~30% at later ages (*16*). A two-stage mortality model, with Gompertz-type acceleration of mortality until day 30 and then constant mortality rate, fit the data better than either a single-stage Gompertz model or a Weibull model (*16*). Thus, for flies of this genotype older than 30 days, estimated mortality rate was approximately constant and independent of age (Fig. 2B).

Occasional experimental studies have described mortality rate reduction at advanced ages (*17*). Carey *et al.* (*18*) document a dramatic leveling off of mortality rates in a heterogeneous population of 1.2 million medflies (*Ceratitis capitata*). As noted by these investigators, there are several possible explanations for the leveling off of mortality rate with age. One explanation depends on genetic heterogeneity; if genetically frailer individuals die at earlier ages, then the population at the oldest ages may be genetically "robust" and have a reduced mortality rate (*19*). However, because our experiments were performed with highly inbred lines and

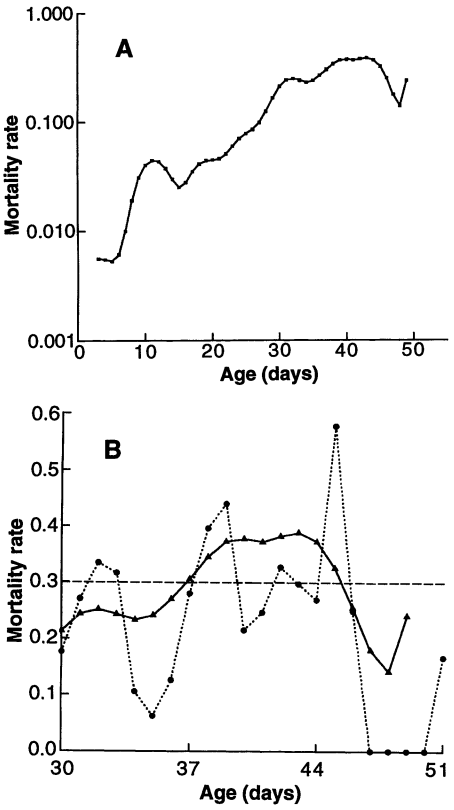

Fig. 2. (**A**) Semilogarithmic plot of mortality rates observed in block IV for 5751 male *D. melanogaster* from inbred line 3 × 3. (**B**) Detail of mortality rate (arithmetic scale) of last 1906 deaths in block IV. Dotted line: Observed daily probabilities of death (five final deaths not shown). Solid line: Mortality rates estimated by smoothing (*14*). Dashed line: Maximum likelihood estimate of mortality rates from two-stage Gompertz model (*16*).

their F_1 crosses, the genetic heterogeneity hypothesis cannot explain our *Drosophila* data. A similar objection applies to an explanation based on sexual heterogeneity, given that we studied only male flies. It is conceivable that mortality rates decline with density in experimental vials, but changes in density cannot explain our results (*20*). Two hypotheses remain. First, there could be environmental heterogeneity—in larval feeding rate or quality of pupation site or adult vial, for instance—that leads to heterogeneity of frailty in adults (*21*). Second, the chance of death for individuals may level off at older ages. In either case, if senescence is measured by the increase in age-specific mortality rates in a cohort (*22*), one is led to the unexpected conclusion that the oldest *Drosophila* of at least one genotype do not senesce.

We conclude that the limited life-span paradigm is not supported by observations on genetically homogeneous populations of *Drosophila*. There is little evidence of brief periods of intense mortality at the most advanced ages in large single-genotype cohorts. Indeed, the most detailed observations suggest that age-specific mortality rates level off at advanced ages.

REFERENCES AND NOTES

1. L. A. Gavrilov and N. S. Gavrilova, *Biology of Life Span: A Quantitative Approach* (Harwood Academic, Chur, Switzerland, 1991).
2. J. F. Fries, *Gerontol. Perspect.* **1**, 5 (1987).
3. _____, *N. Engl. J. Med.* **303**, 130 (1980); L. M. Crapo, *Vitality and Aging: Implications of the Rectangular Curve* (Freeman, San Francisco, 1981). The paradigm has also been applied to nonhuman species (*2*).
4. K. G. Manton, *Gerontol. Perspect.* **1**, 23 (1987); J. M. Guralnick and E. L. Schneider, *ibid.*, p. 65; G. C. Myers and K. G. Manton, *Gerontologist* **24**, 346 (1984).
5. J. F. Fries [*Gerontol. Perspect.* **1**, 54 (1987)] noted that "Attempts to prove that the life span is increasing by demographic arguments which ignore the science of genetics will . . . fail" (p. 62). Finch emphasized the integration of various disciplines—including veterinary medicine, toxicology, developmental biology, evolutionary biology, ecology, and wildlife management—to address gerontological issues (*6*, pp. 10–11). Finch also noted that the *Drosophila* mortality figures "are particularly open to refinement using the abundant life table data that have recently been gathered but not analyzed for mortality rate changes" (*6*, p. 46). Recent *Drosophila* studies have demonstrated genetic variation for average longevity and its correlates but generally ignore demographic methods and implications.
6. C. E. Finch, *Longevity, Senescence, and the Genome* (Univ. of Chicago Press, Chicago, 1990).
7. Inbred lines often carry recessive deleterious mutations in the homozygous condition, whereas crosses between inbred lines generally result in hybrid vigor, which is attributable to masking of the deleterious alleles in the heterozygous condition [D. S. Falconer, *Introduction to Quantitative Genetics* (Longman, London, ed. 2, 1981)].
8. The founding population, consisting of 350 isofemale lines collected in Massachusetts in 1981, has been maintained in the laboratory on a 2-week generation schedule at a population size of ~10,000 individuals. In 1987, we began inbreeding the flies and by the beginning of these experiments had completed 30 generations of half-sib mating. The expected inbreeding coefficient at the beginning of the experiments was greater than 99%.
9. Density was controlled at 200 larvae per 110 ml of medium in a half-pint milk bottle for two generations before experiments, as well as at the beginning of the experimental generation. All flies were reared at 24°C on cornmeal-molasses medium.
10. In the analysis of variance of the pooled data, blocks (F = 20.6, $P < 0.001$), genotypes (F = 4.7, $P < 0.01$), and block × genotype interaction (F = 36.8, $P < 0.001$) are statistically significant (error mean square = 54.1). Significant G × E interaction means that the ranking of genotypes with respect to mean longevity changed from block to block; the significance of the G × E term in the analysis of variance suggests that genotypes responded in a nonuniform fashion to microenvironmental differences between blocks. In a small experiment the substantial interaction effect associated with genotype 3 × 2 would be regarded as a statistical fluctuation, but with ~1000 observations on this genotype the interaction can be regarded as real. L. S. Luckinbill and M. J. Clare [*Heredity* **56**, 329 (1986)] have documented the importance of G × E interactions in determining responses to artificial selection on longevity. P. A. Parsons [*Exp. Geront.* **12**, 241 (1977)] has also demonstrated significant G × E interactions for longevity in *Drosophila* (F = 3.3, $P < 0.001$, error mean square = 57.0).
11. M. R. Rose, *The Evolutionary Biology of Aging* (Oxford Univ. Press, Oxford, 1991); L. S. Luckinbill and M. J. Clare, *Heredity* **55**, 9 (1985).
12. The Gompertz formula is $mu(x) = a \exp(bx)$, where $mu(x)$ denotes the force of mortality or hazard of death at age x, a is the location or level parameter, and b is the slope or scale parameter. Mortality rate parameters were estimated by maximum likelihood as implemented in the GAUSS computer software package.
13. Finch (*6*) reports MRDT for a variety of *Drosophila* studies in the range 0.02 to 0.04 year.
14. At 20 days after emergence, ~90% of males were fertile. At 30 days, the figure was ~50%. At 40 days, none were fertile. Data were based on observation of 50 males per age.
15. Mortality rates were estimated from the observed daily probabilities of death by application of the smoothing algorithm of H. Ramlau-Hansen [*Anal. Stat.* **11**, 453 (1983); *Scand. Actuar. J.* **66**, 165 (1983)], which was developed specifically for smoothing demographic data. The algorithm used a 7-day "window" to reduce weekly periodicities associated with transfer to fresh medium.
16. Maximum likelihood fitting of a two-stage mortality model revealed a breakpoint at day 30. Gompertz parameters are a_1 and b_1 before the breakpoint, and a_2 and b_2 after the breakpoint. Parameter estimates are a_1 = 0.0077, b_1 = 0.0902, a_2 = 0.2993, and b_2 = 0.0000. The two-stage model fits the data significantly better than a single-stage Gompertz model (log-likelihood ratio test, $P < 0.001$). Examination of cumulative hazard shows that both the two-stage and single-stage Gompertz models fit the data better than a Weibull model.
17. A. C. Economos, *Age* **2**, 74 (1979); *Arch. Gerontol. Geriatr.* **1**, 3 (1982); M. Witten, *Mech. Age. Dev.* **46**, 175 (1988).
18. J. R. Carey, P. Liedo, D. Orozco, J. W. Vaupel, *Science* **258**, 457 (1992).
19. J. W. Vaupel, K. G. Manton, E. Stallard, *Demography* **16**, 439 (1979); J. W. Vaupel and A. Yashin, in *Sociological Methodology*, N. B. Tuma, Ed. (Jossey-Bass, London, 1985), pp. 179–211; *Am. Stat.* **39**, 176 (1985).
20. Holding age constant, mortality rates increased with decreasing density in these experiments. The density effect was therefore in the wrong direction to explain the leveling off of age-specific mortality rates in the final 22 days of block IV. This observation is explained by microenvironmental effects on longevity attributable to slight variations in the bottles in which larvae were reared and the vials in which adults were assayed. In a nested analysis of variance with effects due to genotypes, bottles, and vials, all three main effects were statistically significant ($P < 0.001$).
21. A. C. Economos [*Arch. Gerontol. Geriatr.* **1**, 3 (1987)] is impressed with ". . . the degree of dissimilarity of individuals, even in inbred populations, all with the same age, particularly in the age-range of appreciable or high mortality. Rather small initial phenotypic differences at young age have been greatly amplified as the individuals aged" (p. 24).
22. "Senescence: a deteriorative change that causes increased mortality" (*6*, p. 678). "Senescence can be quantified using age-related changes in mortality rates in a population" (*ibid.*, p. 12).

23. We thank T. Rorvick, L. Xiu, A. Keso, and M. Ruan for technical assistance and J. Carey and M. Tatar for comments on the manuscript. Supported by National Institutes of Aging, NIH, grant PO1 AG08761, with additional support from NIH KO4 HD 00638.

21 May 1992; accepted 18 August 1992