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Cultural artifacts: a comparison of senescence in natural, laboratory-adapted and artificially selected lines of *Drosophila melanogaster*

Catherine Linnen,[1‡] Marc Tatar[2] and Daniel Promislow[1]*

[1]*Department of Genetics, University of Georgia, Athens, GA 30602-7223 and*
[2]*Department of Ecology, Evolution and Behavior, Brown University, Providence, RI 02912, USA*

ABSTRACT

When animals are kept in the laboratory in non-overlapping generations, a high load of mutations with late-acting effects can accumulate over time. This may lead, in turn, to a gradual but steady decline in life expectancy. Selection experiments designed to extend longevity in laboratory-adapted lines may, in fact, simply restore the vigour that was lost from the original lines after they were introduced to the laboratory environment. To test this idea, we compared longevity in virgin males from six strains in the fruit fly, *Drosophila melanogaster*, including a wild-caught strain recently obtained from the wild, a laboratory strain that has been housed in the laboratory with a 2 week generation time for almost a century, two lines that had been selected for long life span, and two lines that had served as controls in the longevity selection experiment. We found that life span in the wild-caught strain was almost identical to that of the line that had been under selection for long life span for nearly 20 years. Life span in the laboratory strain and the two control lines was also quite similar. In contrast to previous studies, we found that variation in life span among strains was due almost entirely to variation in the intercept of the Gompertz mortality trajectory. Gompertz slopes among all strains were quite similar. We suggest that the effects of laboratory culture may be responsible, in part, for the trade-offs observed between late survival and early fecundity in selection experiments designed to test the antagonistic pleiotropy model of senescence.

Keywords: antagonistic pleiotropy, artificial selection, *Drosophila*, laboratory culture, mutation accumulation, senescence.

INTRODUCTION

Since the 1940s, evolutionary biologists have argued that the age-related decrease in the force of natural selection leads inexorably to the evolution of ageing (Medawar, 1946; Williams, 1957; Hamilton, 1966). Medawar (1946) showed how natural selection is less able

* Author to whom all correspondence should be addressed. e-mail: promislow@arches.uga.edu
‡ *Present address:* Department of Organismic and Evolutionary Biology, Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA.

to select against deleterious germ-line mutations if they are only expressed at late ages. As these late-acting mutations accumulate, they lead to a decline in age-related survival, fertility and fecundity. In his 'antagonistic pleiotropy' model, Williams (1957) extended Medawar's theory by suggesting that late-acting deleterious mutations may be favoured by natural selection if they have beneficial pleiotropic effects at early ages. A series of studies in the fruit fly, *Drosophila melanogaster*, has examined evidence for mutation accumulation and antagonistic pleiotropy as a cause of ageing (Rose and Charlesworth, 1981a; Luckinbill *et al.*, 1984; Rose, 1984; Mueller, 1987; Partridge and Fowler, 1992; Partridge and Barton, 1993; Hughes and Charlesworth, 1994; Zwaan *et al.*, 1995; Charlesworth and Hughes, 1996).

If ageing is due to alleles that segregate with antagonistic pleiotropic effects, one would expect to see negative genetic correlations between early-age and late-age fitness traits (Rose, 1982; Charlesworth, 1990; Curtsinger *et al.*, 1994). However, a series of studies in the early 1980s (Murphy *et al.*, 1983; Stearns, 1983; Bell, 1984a,b) found positive correlations between fitness traits at different ages. This prompted Service and Rose (1985) to suggest that natural populations were not a suitable starting material for the analysis of life-history covariance structure. They argued that by placing organisms in a novel environment, one might observe artifactual positive correlations despite underlying negative genetic correlations between early-age and late-age fitness traits. Genotypes that happen to do relatively well (or poorly) in a novel laboratory environment are likely to do so at all ages. To address this problem, Service and Rose (1985) argued that studies on ageing should instead use organisms that are well adapted to the environment in which genetic correlations will be determined.

Service and Rose's (1985) paper has been enormously influential. However, at least for selection experiments, by not using recently wild-caught flies we may obtain erroneous estimates of natural genetic variation for life span and of the genetic covariance structure between life span and other traits (Clark, 1987; Promislow and Tatar, 1998; Harshman and Hoffman, 2000). Studies of numerous traits in *Drosophila* have found that laboratory culture can lead to rapid changes in life-history (Matos *et al.*, 2000), behavioural (e.g. Gromko and Markow, 1993; Ritchie and Kyriacou, 1994) and biochemical (e.g. Toolson and Kuper-Simbrón, 1989) factors.

Flies in laboratory culture are typically maintained in a 2 week generation time, in which flies older than 5 or 6 days do not have the opportunity to contribute genetically to future generations. This culture regime can affect life-history evolution in two ways. First, it will favour high early reproductive effort and potentially increase the frequency of genes that contribute to rapid reproduction. Negative genetic correlations between early fecundity and survival can then lead to a reduction in longevity. Second, novel deleterious mutations whose effects are confined to ages later than 5 or 6 days are effectively neutral. In this case, drift overwhelms selection and, over many generations, the number and frequency of these mutations will increase over time (Promislow and Tatar, 1998). Thus, we expect that flies maintained in the laboratory in 2 week culture will have a high load of late-age mutations, an increase in alleles (possibly with pleiotropic effects) that increase early fecundity and a concomitant decrease in longevity (Promislow and Tatar, 1998). Experiments that select for long life span by breeding from older flies may achieve life extension by removing accumulated mutations and effectively restoring wild-type alleles.

Recent work by Sgrò and Partridge (2000), in which wild-caught flies were compared with flies from the same population that had been maintained in the laboratory for several

generations, lends strong support to the suggestion that laboratory culture will lead to an increase in early fecundity and a decline in longevity.

Here we focus on the effects of laboratory culture on rates of ageing. To examine the effect of laboratory culture on life span, we compared age-specific mortality rates in flies that had recently been caught in the wild with a standard laboratory strain (Canton-S), two lines of flies that had been selected for extended life span (O1 and O2; Rose, 1984) and two control lines derived from the same source as the long-lived strains (B1 and B2; Rose, 1984), but maintained in 2 week culture. If wild-caught strains live a significantly longer time than laboratory-adapted strains, this would suggest that the response to selection on longevity in laboratory-adapted populations may be a poor indicator of the genetic variance–covariance structure of longevity in the wild.

MATERIALS AND METHODS

Lines and culture conditions

We analysed survival rates in six different strains of the fruit fly, *Drosophila melanogaster*. These included a wild-caught population (UGA98) obtained in August 1998 from a peach orchard at the University of Georgia horticultural farm in Watkinsville, GA, a laboratory strain [Canton Special (Canton-S), collected in the 1920s in Canton, OH by Calvin Bridges] and four selected strains obtained from M.R. Rose (University of California, Irvine). These selected lines included two strains (O1 and O2) that had been under selection for long life span since 1980 (Rose, 1984) and two strains (B1 and B2) that had been maintained as control lines in 2 week culture since 1975, when they were caught in Amherst, MA by P.T. Ives (Rose and Charlesworth, 1981b). We obtained Rose's lines in April 1997. Since then, the lines have been maintained in half-pint (0.275 litre) glass bottles on standard medium under a relaxed selection regime, with a fluctuating 2–4 week generation time. All lines were kept at 24°C and 60% relative humidity on a 12:12 light/dark cycle.

Adult mortality measurements

To create large same-age populations for mortality measurements, we set up 72 six-ounce (~180 ml) plastic bottles (Applied Scientific) each for the UGA98 and Canton-S lines, and 6 six-ounce plastic bottles each for the O1, O2, B1 and B2 strains, with approximately 15 females and 15 males in each bottle. Flies were allowed to lay eggs for 2 days on yeasted medium. We collected virgin males under light CO_2 anaesthesia at room temperature no more than 8 h after eclosion. We obtained 4200 UGA98 flies, 4200 Canton-S flies and 600 flies for each of the Rose lines. With these flies, we set up 36 mortality cages with approximately 300 virgin males in each: 14 UGA98 cages, 14 Canton-S cages and two cages for each of the Rose lines. The mortality cages that we used were based on a modified 'thanatometer' (Fukui, 1992). The cages were maintained at 24°C and 60% relative humidity on a 12:12 light/dark cycle.

Dead flies were removed daily from cages and counted. From these data, we were able to determine instantaenous mortality rate $\mu_x \approx \ln[p_x]$, where p_x is the probability of survival from age x to age $x + 1$ (Elandt-Johnson and Johnson, 1980). In many organisms, age-specific mortality increases exponentially over most of the life course (Finch *et al.*, 1990;

Promislow, 1991; Carey *et al.*, 1992; Curtsinger *et al.*, 1992). The simplest model that describes this mortality trajectory was first suggested by Gompertz (1825):

$$\mu_x = ae^{bx} \quad (1)$$

where a is age-independent mortality and e^b is the rate at which mortality increases as a function of age.

To fit Gompertz parameters to each cohort, to test for differences in parameter values between cohorts and to determine the source of differences in longevity among cohorts, we used WinModest, a maximum likelihood estimator program designed by S. Pletcher (Pletcher, 1999). The model fits parametric mortality models to data on age at death. By using maximum likelihood to fit data to the Gompertz model, we avoid a number of potential pitfalls that arise with standard regression approaches. We are able to include estimates of mortality where $\mu_x = 0$ and $\ln(\mu_x)$ is undefined, we can correct for biased estimates that arise when few deaths occur (Promislow *et al.*, 1999) and we can include censored (escaped) individuals in the mortality analysis.

To test for significant differences in parameter values between cohorts, we forced a single Gompertz model to fit two separate cohorts (i.e. each cohort had identical values of a and b) and obtained a log-likelihood value for the model. We then allowed the intercept for the two cohorts to vary, but maintained a common slope, and obtained a new log-likelihood value. To test for differences in slope, we fitted a model with fixed slope and variable intercept. We then tested this against a model with variable slope and variable intercept ($a_1 \neq a_2$, $b_1 \neq b_2$). In each case, twice the difference between the log-likelihood values is distributed as χ^2 with k degrees of freedom, where k is the number of additional parameters in the model (Edwards, 1972). The results of tests for both intercept and slope differences between cohorts are presented in Table 1.

Two cohorts may differ in life expectancy due to variation in slope or intercept. To determine the relative contribution of these two factors to life expectancy, we use Pletcher's longevity decomposition technique built into WinModest. By taking the partial derivative of mean longevity with respect to the parameters in a particular mortality model (in this case, a and b), one can determine the relative contributions of a and b to mean longevity.

Dry weight

In July 1999, we collected 25 males from each of the stocks that had been used in our mortality experiment. These flies were dried at 50°C for 24 h. Flies from each line were then weighed in groups of five to the nearest 10 μg. We compared weights among lines using a two-tailed t-test, with a Bonferroni correction for multiple comparisons.

RESULTS

Life expectancy

A clear pattern emerged from measures of life expectancy at eclosion. Three lines (Canton-S, B1 and B2) had life expectancies between 53.0 and 59.8 days (Table 1). In marked contrast, the other three lines (UGA98, O1 and O2) had life expectancies between 83.3 and 88.4 days (Table 2). All lines in the short-lived group were significantly different from all lines in the long-lived group.

Table 1. The diagonal entries, in **bold**, show the intercept (above) and slope (below) for the MLE-fitted Gompertz equation (see text). The off-diagonal entries give χ^2-values for comparisons of intercept (assuming common slope) and slope (assuming different intercept) on the upper and lower diagonals, respectively, based on a likelihood ratio test, with χ^2 equal to twice the difference in the log-likelihoods. *P*-values are calculated after a table-wide Bonferroni correction for multiple comparisons

	UGA98	Canton-S	O1	O2	B1	B2
UGA98	**2.94E-05** **0.0836**	4042.6***	120.6***	35.6***	1287.2***	1566.4***
Canton-S	12.8*	**2.72E-04** **0.0782**	2329.4***	1843.3***	75.3***	211.1***
O1	6.4	0.7	**3.55E-05** **0.0756**	5.4	916.8***	1048.0***
O2	10.9*	2.9	0.4	**5.40E-05** **0.0727**	822.8***	957.2***
B1	12.6*	3.0	0.3	0.0	**5.55E-04** **0.0734**	23.7***
B2	21.5***	8.0	1.7	0.3	0.6	**8.75E-04** **0.0706**

* $P < 0.05$; *** $P < 0.001$.

Mortality patterns

The original data for the mortality curves, with a 3 day bin to reduce zero values, are presented in Fig. 1. Initial mortality rates were relatively high and a clear Gompertz-type mortality pattern did not emerge until approximately 25 days. Accordingly, in our maximum likelihood estimates of the Gompertz parameter values, we censored the first 25 days. We censored an average of 5% of the individuals in each cohort (number of individuals censored out of total per cohort: UGA98 = 116/3888; Canton-S = 449/4099; O1 = 11/557; O2 = 13/565; B1 = 45/600; B2 = 34/587).

Values for the Gompertz slope and intercept parameters for each of these curves are shown in Table 1. A relatively small intercept implies a low rate of age-independent mortality. As with life expectancy, the six lines are clearly delineated into two groups with respect to intercept, with UGA98 < O1 < O2 << Canton-S < B1 < B2. Intercept *a* in UGA98, O1 and O2 is significantly different from that in Canton-S, B1 and B2 (Table 2). Within each of these two groups (UGA98/O1/O2 and Canton-S/B1/B2), the intercepts were relatively similar, differing by no more than a factor of 3.2 (with an average difference of 1.9-fold). The picture is rather different between these two groups. For example, the intercept for UGA98 differs 19-fold for B1 and 30-fold for B2. Among lines with similar life expectancies, the intercepts were also significantly different from one another in all but one case (O1 *vs* O2), although the difference was not nearly as great as that for the comparisons between short-lived and long-lived lines (Table 1). Variation among lines in rates of ageing (*b*) was far less dramatic than variation in the intercept for the Gompertz curve (Table 1). In most cases, the slopes did not differ significantly among lines (Table 1).

Although all mortality curves generally increased exponentially with age over most of the life span, as anticipated from previous studies, there was one anomalous increase in

Table 2. The diagonal entries, in **bold**, show the life expectancy (± standard error) for each of the six lines analysed. The entries above the diagonal show the amount of the difference in longevity (in days) that is accounted for by variation in the intercept of the Gompertz model (see text). The entries below the diagonal show the amount of the difference in longevity that is accounted for by variation in the slope of the Gompertz model. For comparison, we have italicized the entry (slope or intercept) that accounts for most of the effect on longevity. Note that, in most cases, most of the variation between lines in longevity is due to differences in slope, rather than intercept

	Variation due to the effects of intercept					
	UGA98	Canton-S	O1	O2	B1	B2
Variation due to the effects of slope						
UGA98	**83.3 ± 0.3**	*27.3*	2.4	7.8	*37.1*	*43.6*
Canton-S	–4.4	**59.8 ± 0.3**	*26.3*	*21.2*	*–9.2*	*–15.3*
O1	*–8.0*	2.3	**88.4 ± 0.9**	*–5.6*	*–36.5*	*–43.0*
O2	*–10.8*	4.7	3.0	**83.6 ± 0.8**	*–31.4*	*–38.2*
B1	–8.1	3.1	1.9	–0.6	**56.2 ± 0.7**	*–6.1*
B2	–10.1	4.8	4.2	1.8	1.7	**53.0 ± 0.7**

mortality at day 87, when the incubator temperature increased to 35°C for several hours (see Fig. 1). This increase occurred relatively late in the experiment, by which time most of the flies of Canton-S, B1 and B2 had died. The heat shock led to a transient increase in mortality rates in all three surviving lines. In a single day, the mortality rate was 23% in UGA98, 28% in O1 and 55% in O2. However, all three lines quickly recovered to pre-heat shock mortality rates.

Variation in both slope and intercept contributed to differences between lines in life expectancy. However, most of the differences in longevity were due to variation in the intercept parameter, a (Table 2).

Dry body mass

There were significant differences among lines in dry mass of males, with the O-lines being heaviest and Canton-S being lightest (Fig. 2). UGA98 weighed significantly less than both O lines, but did not differ significantly from either B line (O1 = O2 > UGA98 = B1 = B2 > Canton-S; after sequential Bonferroni correction, $P < 0.05$ in all cases except O2 *vs* B1 and O2 *vs* UGA98, where $0.5 < P < 0.1$).

DISCUSSION

Mean life span in the long-lived selected lines was approximately 86 days, which is a 60% increase over the control (B) lines, but not significantly different from the UGA98 flies. Mean life span in the established laboratory strain, Canton-S, showed little difference from the control lines.

A previous study of mortality rates in the O and B lines found that they differed in slope but not in intercept (Service, 1998). In marked contrast in this study, most of the variation in longevity between lines was due to variation in the intercept parameter (a in equation 4)

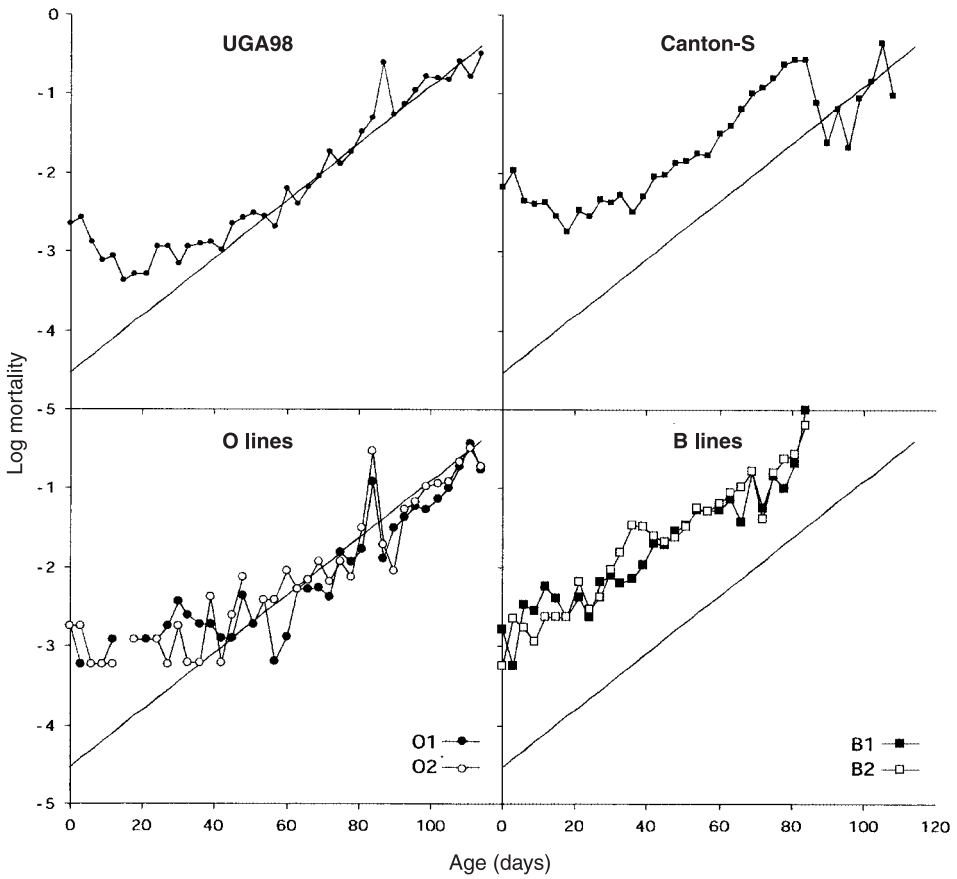

Fig. 1. Log_{10} mortality rate as a function of age for UGA98, Canton-S, B lines and O lines. The solid line in each figure is the maximum likelihood estimated Gompertz line (see text) for UGA98. Note that there is no significant difference between the O lines, which were selected for long life span, and wild-caught flies from Georgia.

rather than the rate of ageing (*b* in equation 4). This pattern of change in intercept but not slope is common for a variety of genetic and environmental traits that influence mortality (Promislow and Tatar, 1998). The difference between Service's results and our own could be due to the fact that we held males in single-sex cages, whereas Service kept flies in mixed-sex cages. Given the difference in costs of reproduction, it is perhaps not surprising that maximum life span in both the B and O lines was about 30 days longer in our laboratory than in that of Service.

A variety of other factors may have also influenced our results. We obtained flies from a locale in northeast Georgia, whereas Rose's lines were originally caught in Amherst, MA. However, recent comparison of Florida and Vermont flies by Eanes and colleagues (W. Eanes, personal communication) suggests that, if anything, flies from more northerly latitudes may live longer. In addition, the O and B lines had been kept under a relaxed selection regime for approximately 18 months before the experiment. However, the average difference in life expectancy of about 58% that we observed between the B1/B2 and O1/O2

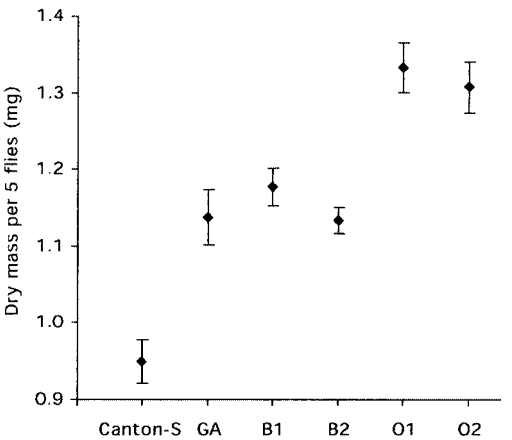

Fig. 2. After Bonferroni corrections for multiple comparisons, O lines were significantly heavier than all other lines, and Canton-S was significantly lighter than all other lines. The UGA98 and B lines did not differ from each other.

lines was comparable to the estimate of 64% from a previous analysis of these same lines (Chippindale *et al.*, 1993). Furthermore, our results are confined to an analysis of virgin males. Future work should determine if similar patterns hold up in virgin females and in reproductively active males and females.

Finally, the non-selected lines may have differed in amounts of inbreeding. Previous studies have shown that inbreeding can reduce life expectancy in *Drosophila* dramatically (e.g. Clarke and Maynard Smith, 1955). The variation among lines examined here may be explained, at least in part, by variations in inbreeding. Although Canton-S is likely to be more inbred than the wild-caught Georgia line, there is no reason to expect that the O lines were any less inbred than the B lines. Furthermore, work on other lines selected for extended life span suggests that variation between long- and short-lived lines is maintained even after intense inbreeding (J. Curtsinger, personal communication). Thus, we think it unlikely that the patterns we observed here were due to the effects of inbreeding.

Laboratory culture and mutation accumulation

Sgrò and Partridge (2000) showed that laboratory culture can lead to a steady decline in life expectancy. They argued that this decrease in life expectancy occurred because laboratory culture selects for increased early fecundity, which increases costs of reproduction, thereby increasing mortality. In addition to costs of reproduction, the decline in life expectancy may also arise from the accumulation of late-acting deleterious mutations. Although the costs of reproduction may be higher in short-lived strains, which have been selected for high early fecundity (Rose and Charlesworth, 1981a; Clark, 1987), the fact that we observe a strong distinction in life expectancy between virgin males from a wild and laboratory-maintained population suggests that the effect could not be due to costs of reproduction alone.

In any case, our results lend further support to the argument that life expectancy in the laboratory evolves in a downward direction over time (Promislow and Tatar, 1998). The

Canton-S line and the B lines have been kept in standard 2 week laboratory culture for hundreds of generations. In this regime, there is little or no selection against late-acting deleterious mutations, or against the late-age pleiotropic negative effects of early-beneficial alleles. In 2 week laboratory culture, 'late age' may actually refer to ages as young as 5 or 6 days. If this decrease in life expectancy is due to mutation accumulation, then the enormous success of selection experiments to extend life span may be due primarily to the removal of deleterious mutations that have accumulated in the laboratory in 2 week culture, and may not be a reflection of natural variation for life span.

Laboratory culture and tests of antagonistic pleiotropy

Based on the observation of genetic trade-offs between early-age fecundity and late-life survival, it has been argued that antagonistic pleiotropy may play a key role in the evolution of ageing (e.g. Rose and Charlesworth, 1981a; Luckinbill *et al.*, 1984; Zwaan *et al.*, 1995). Previous studies (Zwaan *et al.*, 1995) have suggested that the observed trade-off between early fecundity and late-age survival could be due to the nature of the selection regime. The results of the present study reiterate a possibility raised some time ago (Charlesworth, 1984), that the observed trade-offs might be created by the effects of laboratory culture. In 2 week culture, the control lines experience mutation accumulation for late-age survival and inadvertent selection for relatively high early fecundity. For example, the effect of laboratory culture on early fecundity was demonstrated recently by Matos *et al.* (2000), who found that, under laboratory culture, early fecundity increased and age at first reproduction decreased in *Drosophila subobscura*. At the same time that control lines would experience selection for high early fecundity, lines selected for long life span would experience mutation accumulation with respect to early-age fecundity. These combined effects could contribute to the observed negative correlations between early-age and late-age fitness traits (Rose and Charlesworth, 1981a; Promislow and Tatar, 1998).

Of course, this critique may not apply to all studies. There is evidence from sib analysis for negative genetic correlations between early-age and late-age fitness traits (Rose and Charlesworth, 1981b), and some selection studies have found trade-offs based on freshly caught strains (Luckinbill and Clare, 1985). Our results call for a re-evaluation of Service and Rose's (1985) argument that we must use laboratory-adapted strains to avoid spurious positive correlations. Unfortunately, if one uses stocks that have been maintained in standard laboratory culture, one is at risk of finding negative genetic correlations that might not otherwise exist in the wild.

Experimental life-history studies and laboratory culture

The results presented here, as well as recent findings by Sgrò and Partridge (2000), lead us to conclude that variation for longevity in the laboratory cannot necessarily be used as evidence for genetic variance and covariance for longevity in natural populations. Much of the variation we observe may be due to novel deleterious mutations that have arisen in the laboratory. We need to determine the amount of genetic variation for longevity that exists in natural populations that have not yet adapted to laboratory culture.

The present work, while bolstered by the work of Sgrò and Partridge (2000), suffers from the fact that we compared selected lines with only one wild-caught line and one laboratory strain. We have shown definitively that at least one wild-caught strain can live as long as

laboratory strains that have been under selection for extended life span for decades; just what accounts for the relatively short life span of laboratory-reared control strains remains to be determined.

Furthermore, we need to determine whether genes that have been identified in laboratory culture to influence life span (see, for example, Johnson, 1990; Lin *et al.*, 1998; Rogina *et al.*, 2000; Tatar *et al.*, 2001) might simply be wild-type revertants of deleterious alleles that have accumulated in laboratory strains. At least some genes that have been identified in the laboratory as having a substantial effect on longevity may have little or no effect on variation of longevity in natural populations. To give an analogy, imagine that the gene for Huntington's disease were fixed in all human populations. If one were to restore the disease-causing alleles to their wild-type state in a few individuals, one might claim erroneously that one had discovered the 'gene for ageing'. Of course, one had simply cured some individuals in a very sick population. The same may be true for laboratory-based organisms.

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