

LABORATORY EVOLUTION OF POSTPONED SENESCENCE IN *DROSOPHILA MELANOGASTER*

MICHAEL R. ROSE

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

Received May 24, 1983. Revised December 31, 1983

Evolutionary genetics seems to have found the fundamental cause of senescence: the decline in the sensitivity of natural selection to gene effects expressed at later ages in most populations of organisms with separate somatic and germ-line tissue. (Here "senescence" refers to decline in age-specific fitness-components after the onset of reproductive maturity.) This idea traces back to Haldane (1941) and Medawar (1946, 1952), with considerable elaboration and elucidation since then (Williams, 1957; Hamilton, 1966; Edney and Gill, 1968; Emlen, 1970; Charlesworth and Williamson, 1975; Charlesworth, 1980; Rose, 1983a). While there are still clear limitations to the mathematical formulation of this theory (cf. Hamilton, 1966; Charlesworth, 1980), the basic formal analysis leads to a straightforward conclusion: the first partial derivative of fitness with respect to appropriately scaled changes in age-specific life-history characters usually declines in magnitude with the age of these changes. The force of natural selection thus declines with age.

This overall theory and its particular subsidiary variants lead to a number of empirically testable corollaries (Rose, 1983a, 1983b). Some of these corollaries are specific to the subsidiary variants of the theory (Rose and Charlesworth, 1980, 1981a, 1981b; Rose, 1983b), so that tests of them individually do not test the theory as a whole. Fortunately, there are two corollaries which follow from the general theory itself: the reproductive schedule of an outbred population will give rise to natural selection acting to (i) *accelerate* senescence in populations with a relatively earlier age of reproduction and (ii) *postpone* senescence in populations with

a relatively *later* age of reproduction (Edney and Gill, 1968; Rose, 1983a). The former prediction has been corroborated by Sokal (1970) using *Tribolium castaneum*, while the latter has been corroborated by Wattiaux (1968a, 1968b) and by Rose and Charlesworth (1980, 1981b), using *Drosophila* species.

Once a theory has been well-developed mathematically and then empirically corroborated, attention turns to experiments in which the theory either is not clearly corroborated or is ostensibly refuted. It would be misleading to claim that all relevant experimental results directly corroborate the evolutionary theory of senescence. Sokal (1970) and Mertz (1975) using *Tribolium castaneum* and Taylor and Condra (1980) using *Drosophila pseudoobscura* found heterogeneity between lines in experiments with replication, such that some lines did not exhibit the predicted response to the imposed selective regime. Taylor and Condra (1980) also found a difference in the response of the sexes which was later attributed to the pattern of female mating preference (Taylor et al., 1981). More problematic still are the studies from the Lints laboratory, one of which failed to obtain a direct response to artificial selection for longevity (Lints et al., 1979), while another gave puzzling fluctuations in life-history attributes (Lints and Hoste, 1974, 1977). Lints (1978, 1983) has made a great deal of these problems, contending that they cast doubt on all proposed evolutionary theories of senescence.

While it can be argued that these puzzling results are due to technical artifacts such as inbreeding, genetic disequilibrium, and inadequate controls (cf. Rose and Charlesworth, 1981b), the only ef-

fective way to resolve such empirical ambiguities is persistent repetition of these experiments under well-defined conditions. Here I report experiments which reprise a previous experiment of this kind (Rose and Charlesworth, 1981*b*) both by wholesale repetition and by internal replication, in an attempt to remove any question about the potential reproducibility of the predicted results under conditions in which artifacts are forestalled.

MATERIALS AND METHODS

The experimental population used in these experiments was an outbred laboratory population derived from the wild South Amherst, Massachusetts, *Drosophila melanogaster* population studied by Ives (1970), as outlined in Rose and Charlesworth (1981*a*). This population was intensively studied during 1977–1979, when it was found to have a great deal of additive (selectable) genetic variability for many adult life-history characters (Rose and Charlesworth, 1981*a*, 1981*b*). In particular, there were several lines of evidence indicating negative additive genetic correlation between early and late life-history characters, such as early fecundity and longevity.

In February, 1980, after more than 130 generations of laboratory culture at 25 C with unlimited food and 14 day discrete generations, ten experimental populations were derived from a single generation of the base population. Five of these were then maintained for about 50 generations in the same fashion as the base population. Here they will be referred to as the “B” populations, with subscripts 1–5 to indicate replicate number. The remaining five populations were kept under the same culture conditions as the B’s, but the day on which eggs were collected for the next generation was progressively postponed. For four generations, eggs were collected from adults that were 28 days of age. Then, there were two generations of 35 days, followed by six generations of 42 days. The next two generations lasted 56 days, and finally, there was a generation of 70 days, taking

the population to December, 1981. These populations were thus subject to 15 generations of laboratory evolution in which only surviving older females were able to reproduce. Here they are called “O” populations, with subscripts as before.

The culture conditions changed over the two-year period of laboratory evolution. For the first year and three months, the populations were maintained on cornmeal-molasses medium and a 12L:12D light regime, at the Laboratory of Genetics of the University of Wisconsin–Madison.

The populations were then transported to Dalhousie University, where they were maintained on banana-molasses medium and a 24L:0D light regime. Except for the period of transport, the flies were kept at 25 C. Food was always abundant in quantity, and populations experienced no severe crowding. Population sizes were in the thousands at the start of each generation, except for the O₂ population, which accidentally fell in numbers at one point. One generation of the O₅ population was accidentally terminated early. Since O₂ and O₅ were thereby aberrant, they were excluded from the life-history assays reported here. For the purpose of matching B’s with O’s, populations B₄ and B₅ were also dropped from the assays.

Two major life-history assays were performed from January to June, 1982. Both used adults obtained after two generations of controlled density sampling, with 14-day generations, from both B and O populations, as in Rose and Charlesworth (1981*a*). This was done to remove parental effects. The major difference in technique was that 20 eggs were harvested using a dull scalpel for each rearing tube. The first assay was of lifetime female daily fecundity and the longevity of both sexes, using the media and transfer procedures of Rose and Charlesworth (1981*a*). Complete fecundity records were obtained for 416 females, for a total of over 700,000 eggs counted. A total of 791 longevities, 424 female and 367 male, were recorded. The second assay was of

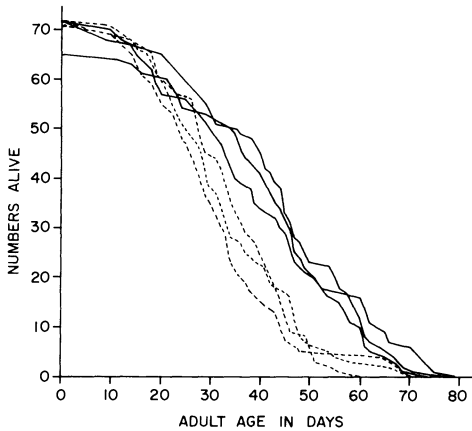


FIG. 1. Surviving numbers of females from the start of the adult life-history assay period. B population samples are shown as dashed lines, O samples as solid lines.

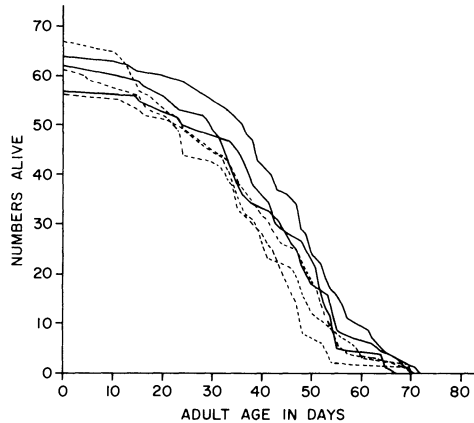


FIG. 2. Surviving number of males from the start of the adult life-history assay period. B population samples are shown as dashed lines, O samples as solid lines.

the viability of eggs laid by females over the first 8 weeks of adult life, as ascertained by the survival of 50 eggs reared together in one tube from oviposition to 14 days of age. These eggs were obtained weekly from mass layings by females kept with males in population cages. The total number of larvae assayed in this experiment was 81,250.

To reduce the likelihood of uncontrolled biases, B and O populations were handled as pairs following a consistent order. Thus, in the first assay, first B₁ and O₁ were transferred together, then B₂ and O₃, and finally B₃ and O₄. For the fecundity characters, the relative order of pair handling made a substantial difference. This was probably due to diurnal variability in laboratory temperature. These data were accordingly analysed using general mixed model maximum-likelihood analysis of variance methods, as they take into account the possibility of such design effects. For the longevity characters, there was no evidence for such an effect from mixed model analysis, so that an unbalanced, random effects, hierarchical model was used for the analysis of variance instead. All mixed model analysis proceeded using the BMDPV.3 program documented in Dixon (1983).

The hierarchical analysis used the standard equations for such models (cf. Kempthorne, 1957).

RESULTS

Longevity

Figures 1 and 2 show the pattern of survivorship differences between O and B populations for females and males, respectively. The overall mean female longevities for O and B populations are 42.81 and 33.28 days, respectively, while the overall mean male longevities for O and B populations are 44.14 and 38.49 days, respectively. Using a hierarchical analysis of variance, the null hypothesis of no longevity difference between O and B treatments can be rejected ($P < .01$) for both males and females. Longevity data from this population tend to be positively skewed and leptokurtic, but not so much as to render an analysis of variance invalid.

Another test of survivorship differentiation is the comparison of mortality rates. This was done as follows: (i) Age-specific mortality rates were calculated for each 10-day interval from day 1 to day 50 of the assay. (After day 50, there were too few B individuals.) (ii) the mortality rate difference between the paired

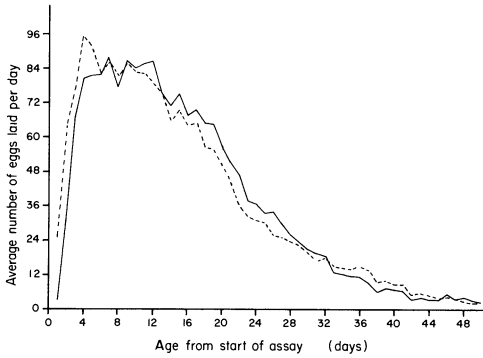


FIG. 3. Treatment means of daily fecundity plotted against days of age from the start of the adult life-history assay. The B treatment means are shown as a dashed line, the O means as a solid line.

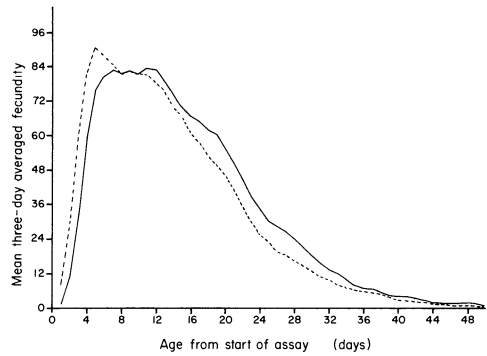


FIG. 4. Treatment means of three-day averaged daily fecundity, irrespective of survival, plotted against days of age from the start of the adult life-history assay. The B treatment means are shown as a dashed line, the O means as a solid line.

B and O populations was calculated for each of the three pairs. (iii) This difference was then divided by the total mortality rate of that pair in that age-interval.

This was done in order to compensate for the scale effect arising from the substantial increase in mortality with age. For the purpose of data analysis, the mortality rate differences of each pair are subject to serial correlation problems. This problem can be surmounted by treating the parameters of the distribution of the mortality rate differences of each pair as raw data. There are two obvious hypotheses to test concerning mortality rate differences. First, do O's have lower mortality rates than B's, on average? The mean of the average mortality rate difference over the three B-O pairs is $.2821 \pm .0911$ (standard error) in females and $.2440 \pm .1181$ in males. Both are significantly greater than zero ($P < .05$), allowing us to reject the null hypothesis of no mortality rate differences. Second, is there any trend with respect to age in mortality rate differences? This was tested using least-squares linear regression through each pair's mortality rate data. The slopes were $-.0617 \pm .0703$ and $-.0700 \pm .0699$ for females and males, respectively. Both of these are within about one standard error of zero, giving no justification for the rejection of the null hypothesis. Thus O's were mor-

tality-resistant compared to the B's, with no apparent age-specificity in this differentiation.

Fecundity

The overall pattern of B and O fecundity is shown in Figure 3. Initially, B's have greater fecundity, while O's have greater fecundity from about day 10 to day 30. B's again have greater fecundity from days 33 to 44, after which the pattern is unclear.

One encounters many problems when analyzing data of this kind: (a) It is known that fecundity is serially correlated among individuals (Rose and Charlesworth, 1981a). Thus one cannot compare fecundities day-by-day. (b) There are problems with the fecundity character of dead individuals, which bias the data analysis if such individuals are simply excluded. (c) Handling gave rise to day-to-day cycles in the fecundity of individual females. To deal with the last two problems, three-day average fecundities were calculated for each female irrespective of survival. These gave the fecundity curves of Figures 4 and 5. Differential survival plays little role for the first 20 days, the survivorship difference amounting to only 5.3% at age 20 days, while the three-day averaged fecundity difference is 20.9%. The problem of serial correlation is more

severe. One way around such difficulties is to pick a pair of days, early and late, using the significance levels for those two days only to evaluate the null hypothesis of no treatment differences early or late. For many of the first six days, this hypothesis can be rejected, at the $\alpha = .05$ level. Similarly, from day 17 to day 37, this hypothesis can also be rejected, as well as sporadically thereafter, at the $\alpha = .05$ level. Ideally, one would want a single parametric test for the entire fecundity pattern, but any such parameter would have to be derived by complicated model-fitting, given the nonlinearity of the fecundity patterns. However, it seems reasonable to conclude that a pattern of postponement in reproductive output is present in the O's relative to the B's.

Mean total fecundities for the B and O treatments were 1635.01 and 1733.41, respectively. This is not a statistically significant difference at the $\alpha = .05$, or even $\alpha = .1$, level. There is certainly no evidence for an overall depression in O population fecundity. Thus the disparity in daily fecundities appears to arise from the redistribution of an approximately fixed total reproductive output.

Viability

Unlike the fecundity and longevity data, there was no apparent differentiation between treatments, with mean viabilities of .793 and .744 for B's and O's, respectively. Statistical analysis corroborates this inference. (In fact, the probability of getting this much treatment differentiation by chance alone is .879.) Nor were members of the pairs appreciably different from each other. The only statistically discernable pattern was a fall in viability with parental age.

DISCUSSION

The overall thrust of the results is in accord with those of earlier studies of the same population (cf. Rose and Charlesworth, 1980, 1981a, 1981b). For example, Figure 5 may be compared with Figure 1 of Rose and Charlesworth (1981b). Essentially the same pattern of an initial

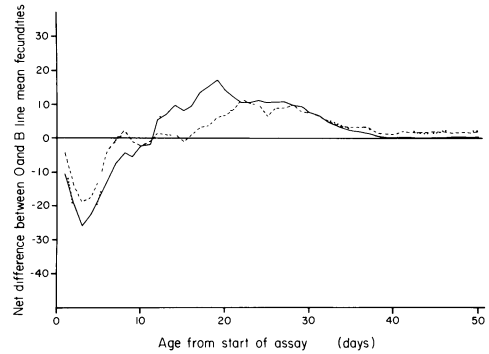


FIG. 5. Net difference between population sample means for three-day averaged daily fecundity plotted against days of age from the start of the adult life-history assay. The solid line gives the difference between B₁ and O₁. The dashed line gives the difference between B₂ and O₃. The dotted line gives the difference between B₃ and O₄.

dip below the zero-axis followed by a rise above it is present in both figures. This study thus underscores conclusions found before: (i) female longevity is increased by culturing populations at later ages, (ii) late fecundity is similarly enhanced, and (iii) these changes are associated with depression of early fecundity. In terms of life-history characters, senescence has been postponed.

The present study provides the following new conclusions. First, replication can give rise to the same *relative* changes, so that results of this kind can be reproducible (cf. Mertz, 1975). Second, the variability over replicates shows that there need be no assayable absolute changes in such characters, making paired control and selected populations during phenotypic assay essential for proper hypothesis evaluation. Third, male longevity is also increased by late culturing, although not to the same extent (cf. Taylor and Condra, 1980; Taylor et al., 1981). Fourth, there do not appear to be any large changes in viability arising from postponement of senescence. None of these new conclusions clash with those found before.

The scientific significance of these conclusions is that senescence in this popu-

lation of *Drosophila melanogaster* appears to be due to antagonistic pleiotropy, such that genes which postpone senescence appear to depress early fitness-components. Put another way, these results corroborate the hypothesis of a cost to reproduction (Williams, 1957, 1966), since prolonged life seems to require reduced early reproductive output. (However, it should be pointed out that total reproductive output is not reduced with increased lifespan, so that the reproductive cost is not "absolute" or irrespective of timing.) Thus this set of interrelated hypotheses has received another experimental corroboration.

What then of other experiments which have not corroborated these hypotheses? There are a number of ways in which one could fail to get results of the present kind. First, an initially inbred population need not exhibit any appropriate response to changes in reproductive schedule, as the requisite genetic variability may not be present. Second, because small differences in assay conditions can have large effects on life-history characters, as has been shown here experimentally, careful pairing of "experimental" and control samples is important. Third, the development of inbreeding depression during an experiment of this kind could also obscure any selection response. Fourth, because these experiments take on the order of 30–50 normal generations (and thus years with insect species), they may be prematurely terminated, before sufficient opportunity has been given for selection to act. It is therefore not surprising that some of the experiments of this type should have failed to corroborate the evolutionary theory of senescence, though which technical difficulty was involved in which experiment would be difficult to ascertain.

Lints (1983) has attacked previous results which have been regarded as corroborations of the evolutionary theory of senescence. First, he has suggested that the mean longevity of the base population studied here is abnormally low. There is no force in this criticism, as this pop-

ulation has been deliberately selected for high initial fertility, with no selection for later survival. That it should have low longevity is therefore not problematic, but corroborative. Second, Lints draws attention to evolutionarily uninterpretable differences in *absolute* fecundities and longevities in the experiments of Rose and Charlesworth (1981*b*), but, as has been shown here, these are of no necessary significance. Third, Lints points out that experiments from his laboratory (Lints and Hoste, 1974, 1977) have shown that the absolute means of life-history characters can vary in odd ways over generations. There are two rejoinders to this criticism: again there are no clear expectations for absolute responses of the characters, and the choices of the generations for assay in this experiment and its predecessor (Rose and Charlesworth, 1981*b*) were arbitrary, yet the results were consistent. All told, Lints provides no cogent critique of the experimental evidence in support of the evolutionary theory of senescence.

Another criticism of experiments like the present one has been that they do not provide information concerning the epigenetic mechanisms underlying the postponement of senescence (Hart and Turturro, 1983). In effect, the present experiments treat the organism as an evolving black-box. This need not remain the case. The B and O populations developed here, with their different rates of senescence, provide suitable material for the analysis of the biological basis of postponed senescence.

SUMMARY

A general corollary of population-genetic theories of senescence is that the culture of outbred laboratory populations using females of later ages should lead to the evolution of postponed senescence. This has been tested before, but the predicted results have not been consistently reproducible. An experiment of this kind was both repeated and replicated, using a previously studied *Drosophila melanogaster* population. The

results uniformly corroborated the evolutionary theory of senescence. Senescence was consistently postponed. Other, anomalous, results are explained in terms of experimental artifact.

ACKNOWLEDGMENTS

I thank A. M. Coyle and M. L. Dory for technical assistance. D. Hamilton and the referees provided helpful suggestions concerning the statistical analyses. The research was supported by Natural Sciences and Engineering Research Council of Canada grant number U0178, while the author was a University Research Fellow.

LITERATURE CITED

- CHARLESWORTH, B. 1980. *Evolution in Age-Structured Populations*. Cambridge Univ. Press, London.
- CHARLESWORTH, B., AND J. A. WILLIAMSON. 1975. The probability of survival of a mutant gene in an age-structured population and implications for the evolution of life-histories. *Genet. Res.* 26:1-10.
- DIXON, W. J., ed. 1983. *BMDP Statistical Software*. Univ. California Press, Berkeley.
- EDNEY, E. B., AND R. W. GILL. 1968. Evolution of senescence and specific longevity. *Nature* 220: 281-282.
- EMLEN, J. M. 1970. Age-specificity and ecological theory. *Ecology* 51:588-601.
- HALDANE, J. B. S. 1941. *New Paths in Genetics*. Allen and Unwin, London.
- HAMILTON, W. D. 1966. The moulding of senescence by natural selection. *J. Theoret. Biol.* 12: 12-45.
- HART, R. W., AND A. TURTURRO. 1983. Theories of aging. *Rev. Biol. Res. Aging* 1:5-17.
- IVES, P. T. 1970. Further studies of the South Amherst population of *Drosophila melanogaster*. *Evolution* 24:507-518.
- KEMPTHORNE, O. 1957. *An Introduction to Genetic Statistics*. John Wiley and Sons, N.Y.
- LINTS, F. A. 1978. *Genetics and Aging*. S. Karger, Basel, Switzerland.
- . 1983. Genetic influences on lifespan in *Drosophila* and related species. *Rev. Biol. Res. Aging* 1:51-72.
- LINTS, F. A., AND C. HOSTE. 1974. The Lansing effect revisited. I. Lifespan. *Exp. Geront.* 9:51-69.
- . 1977. The Lansing effect revisited. II. Cumulative and spontaneously reversible parental age effects on fecundity in *Drosophila melanogaster*. *Evolution* 31:387-404.
- LINTS, F. A., J. STOLL, G. GRUWEZ, AND C. V. LINTS. 1979. An attempt to select for increased longevity in *Drosophila melanogaster*. *Gerontology* 25:192-204.
- MEDAWAR, P. B. 1946. Old age and natural death. *Mod. Quart.* 1:30-56.
- . 1952. *An Unsolved Problem of Biology*. H. K. Lewis, London.
- MERTZ, D. B. 1975. Senescent decline in flour beetles selected for early adult fitness. *Physiol. Zool.* 48:1-23.
- ROSE, M. R. 1983a. Evolution of aging. *Rev. Biol. Res. Aging* 1:19-24.
- . 1983b. Theories of life-history evolution. *Amer. Zool.* 23:15-23.
- ROSE, M., AND B. CHARLESWORTH. 1980. A test of evolutionary theories of senescence. *Nature* 287:141-142.
- . 1981a. Genetics of life-history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics* 97:173-186.
- . 1981b. Genetics of life-history in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics* 97:187-196.
- SOKAL, R. R. 1970. Senescence and genetic load: evidence from *Tribolium*. *Science* 167:1733-1734.
- TAYLOR, C. E., AND C. CONDRA. 1980. r- and K-selection in *Drosophila pseudoobscura*. *Evolution* 34:1183-1193.
- TAYLOR, C. E., C. CONDRA, M. CONCONI, AND T. PROUT. 1981. Longevity and male mating success in *Drosophila pseudoobscura*. *Amer. Natur.* 117:1035-1039.
- WATTIAUX, J. M. 1968a. Parental age effects in *Drosophila pseudoobscura*. *Exp. Geront.* 3:55-61.
- . 1968b. Cumulative parental age effects in *Drosophila subobscura*. *Evolution* 22:406-421.
- WILLIAMS, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11:398-411.
- . 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Amer. Natur.* 100:687-690.

Corresponding Editor: R. E. Ricklefs