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Evolution

Evolution of accelerated senescence in laboratory populations of *Drosophila*

(semelparity/life histories/mutation accumulation)

LAURENCE D. MUELLER

Department of Zoology and Program in Genetics and Cell Biology, Washington State University, Pullman, WA 99164-4220

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ABSTRACT **Ecological theories of life history evolution predict that natural selection should favor semelparous life histories in environments where juvenile survival is high relative to adult survival and rates of population growth are high. That is, organisms should complete their entire reproductive effort in a short period of time following maturation. Direct empirical verification of this idea has been lacking. Six independent populations of *Drosophila melanogaster* were maintained in two different environments, called *r* and *K*, for more than 120 generations. In the *r* environment population size was small, larval survival and rates of population growth were high, and reproduction was limited to a few days after eclosion. In the *K* environment population size was large and larval survival low, but adults were allowed to reproduce indefinitely. The fecundity of females of different sizes from each environment was measured daily for 4 weeks. No differences in fecundity were seen during the first week of adult life for females from the two environments. By the fourth week, however, the fecundity of large females from the *r* environment was 47–83% less than that of females from the *K* environment. The accelerated senescence exhibited by females from the *r* environment appears to be due to the accumulation of deleterious alleles whose effects are expressed late in life, which is consistent with the mutation accumulation hypothesis for the evolution of senescence.**

Semelparity is the life history characteristic of many plants and animals in which reproduction and death are restricted to a short interval following maturation. In contrast, iteroparous organisms reproduce repeatedly after maturation and show a gradual decline in survivorship and fecundity, hereafter called senescence, with advancing age. Two schools of theory have developed to explain the evolution of these different life history patterns. The genetic theory seeks to understand the general nature of genes that may contribute to the process of senescence. The two most prominent genetic theories are the mutation accumulation theory and the pleiotropy theory. The mutation accumulation theory (1, 2) states that senescence is due to the accumulation of deleterious alleles, in mutation–selection balance, whose effects are expressed late in life. Such alleles will have only small effects on fitness and thus be only weakly acted upon by natural selection. The pleiotropy theory (3–5) suggests that alleles with beneficial effects for survival or fecundity early on in life will have deleterious pleiotropic effects late in life. The early beneficial effects of such alleles substantially outweigh these deleterious late effects, and consequently such alleles are favored by natural selection.

In contrast to the genetic theories are ecological theories that attempt to outline environmental conditions which may be conducive to the evolution of the alternative life histories iteroparity and semelparity (6–10). Two kinds of environments should be favorable for the evolution of semelparity: (*i*) environments in which juvenile survival is high relative to adult survival and the rate of population growth is high, and (*ii*) environments in which there are large fluctuations in adult survival (11).

Studies with *Drosophila melanogaster* have shown that the normal pattern of senescence present in this iteroparous organism is probably due to pleiotropy (12), although some recent evidence (13) on male virility indicates higher frequencies of deleterious late-acting alleles than early-acting ones in natural populations. Here I show that evolution of accelerated senescence occurs in small populations of *D. melanogaster* with abundant resources when reproduction is restricted to a few days after eclosion. The genetic mechanism of this evolution appears to be the accumulation of late-acting deleterious alleles rather than antagonistic pleiotropy.

MATERIALS AND METHODS

These ideas have been tested by examining female fecundity in six populations of *D. melanogaster* that have been undergoing independent evolution in two different environments for more than 120 generations. The methods by which these populations are maintained were described previously (14). In the selection regime three populations (*r*-1, *r*-2, and *r*-3) have been maintained with an adult population of 50 per half-pint culture bottle and abundant resources for both larvae and adults. The females used to produce the next generation were usually between 3 and 6 days old. The remaining three populations (*K*-1, *K*-2, and *K*-3) were kept at very high adult densities (about 1000 adults per half-pint culture bottle) and larval crowding and mortality were quite high. Females in the reproducing adult population were allowed to live indefinitely although the average life span was probably 2–3 weeks.

I examined differences in the life histories of *Drosophila* kept in the *r* vs. *K* environments by measuring female fecundity as a function of both age and size. Adult flies were taken directly from the selection regime to lay eggs, which were raised under common conditions. These adults were used to produce 100 first-instar larvae, which were placed in vials with nonnutritive agar and various amounts of yeast. The thorax lengths of females from these nonnutritive agar cultures were measured and daily egg counts were obtained by using standard methods (15) except that females were transferred without anesthetization. Egg counts were made for a maximum of 28 days or until the female died. All experiments were at 23°C with a cycle of 12 hr of light and 12 hr of darkness.

To ascertain whether any increased senescence is due to pleiotropy or mutation accumulation I also examined the fecundity of F_1 hybrids. The *r*-F_1 population was produced by crossing (♂ × ♀) *r*-1 × *r*-2, *r*-1 × *r*-3, *r*-2 × *r*-3, and the reciprocal matings. Females from like crosses (e.g., *r*-1 ♂ ×

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r-2 ♀ and r-1 ♀ × r-2 ♂) were paired and allowed to lay eggs. Equal numbers of first-instar larvae were taken from each cross and placed in vials, 100 per vial. The vials differed in the amount of yeast available for the larvae to feed on. Thus, the adult females that emerged from different vials were of different sizes and were used for the fecundity measurements reported here. The K-F_1 population was created in a like manner. If rapid senescence is due to the accumulation of deleterious recessive alleles by the processes of genetic drift and mutation we would expect the F_1 progeny to be heterozygous for these deleterious alleles and hence "cured" of this rapid senescence. Alternatively, the decline in late fecundity may have been due to natural selection favoring certain alleles with beneficial early effects and deleterious late effects. Since the three r populations were derived from a single source population they should each have had similar sorts of genetic variation. Thus, we would have expected selection to have caused the fixation of the same or nearly the same sets of alleles. F_1 individuals of these populations would remain homozygous for alleles with beneficial early effects and deleterious late effects and hence should continue to exhibit reduced late fecundity relative to the K populations. Fecundity was measured for populations run in matched pairs: r-1 and K-1, r-2 and K-2, etc. The numerical index of each population was assigned randomly to each population when they were first created 8 years ago. Thus, these matched pairs can be considered random assignments with respect to the phenotypes measured here. Except for the hybrid populations, fecundity measurements were made at two different times.

RESULTS

Only the total fecundity for the first and fourth weeks is shown in Fig. 1, since these results sufficiently illustrate the main conclusions. The well-known relationship between female size and fecundity was evident (Fig. 1) until the fourth week after eclosion, after which the regression of fecundity on size was frequently not significant. During the first week of life the fecundity of females from each environment was quite similar. By the fourth week, however, the fecundity of females from the K environment was substantially higher than that of those from the r environment. It was found that fecundity measurements made on the same population but at different times could sometimes differ significantly. Since it was impossible to run all populations at the same time the most appropriate way to analyze these data is to compare counts of eggs collected at the same time. Since this procedure will reduce the sample sizes for each test it should make it more difficult to find significant differences between these populations. An analysis of covariance (ANCOVA) was performed on each matched pair of populations, using only those data that had been collected at the same time, to address whether the regression of weekly fecundity on size is affected by the environment from which the female originated. In four comparisons the standard ANCOVA could not be performed because the sample size was too small, the regression was not significant, or the slopes were heterogeneous. The following alternative tests were used instead: r-2 vs. K-2, week 4, the mean fecundity of all females was tested for significant differences; r-1 vs. K-1, week 4, the mean fecundity of large females only (thorax length = 1.04 mm) was tested for significant differences; r-1 vs. K-1, weeks 2 and 3, all the data were used and time of the sample and environment of origin were treated as qualitative variables. Since there are four pairs of populations and four weeks of fecundity data, for each pair, there are a total of 16 ANCOVAs. The r and K environments do not produce any significant differences in the regressions during the first week (4 of the 16 tests). Of the remaining 12 tests all but two (r-3 vs. K-3 in weeks 3 and 4) showed that the fecundity of the K females was significantly greater than that of the r females. The decline in fecundity of the r females is quite dramatic by the fourth week of adult life (Fig. 1). Among the r females 38% laid ≤10 eggs during the fourth week as compared with only 11% in the K populations ($\chi_1^2 = 5.22, P < 0.05$). These results indicate that r females are becoming more semelparous.

Although the r-F_1 females show a decline in fecundity after the first week, relative to their K-F_1 controls, this decline is not nearly so great as for the individual r populations. For instance, the fecundities of large (thorax length = 1.04 mm) r-1, r-2, and r-3 females (±95% confidence interval) during the fourth week relative to the K controls are 0.17 (±0.31), 0.23 (±0.53), and 0.53 (±0.37), respectively. This same quantity for the r-F_1 females is 0.74 (±0.22).

DISCUSSION

A reasonable interpretation of these results is that the large decline in fecundity of the r populations is due to the fixation of different deleterious recessive alleles whose effects are not expressed in the heterozygous F_1 population. Explanations for the reduced fecundity of the r-F_1 population may be that some of the deleterious alleles are not fully recessive, there has been fixation of the same deleterious alleles in two or more r populations, or there has been an increase in the frequency of alleles, due to selection, with pleiotropic effects on late fecundity. The evolutionary decline in the late fecundity of the r populations was not particularly rapid. An earlier examination of female fecundity between the 20th and 30th generations of selection revealed no differences in fecundity (T. J. Bierbaum, L.D.M., and F. J. Ayala, unpublished data). This is consistent with the notion that drift alone has been responsible for the increase in these deleterious alleles, since the average time until their fixation should be about 4 times the effective population size or 200 generations. Although genetic drift has been a significant force in the evolution of the r populations the phenomenon seen here is not equivalent to inbreeding depression. This conclusion rests on the observation that the decline in fecundity was seen only in females aged 1 week or more. Thus, the action of natural selection for high fecundity during the reproductive age (3–6 days) of females in the r environments was sufficiently strong to counter any deleterious effects due to random genetic drift.

This study provides evidence that is consistent with ecological theories of life history evolution and the genetic theory of mutation accumulation. Important features of the r environment to the evolution of accelerated senescence are probably small population size and restriction of reproduction to a narrow time interval. Small population size accelerates the process of allele fixation even when these alleles have slightly deleterious effects. Indeed, the slight reduction in fecundity of r females during the first week may be due to the same deleterious alleles causing the late decline in fecundity. In large populations even weak selective forces acting during the first week would be sufficient to prevent the increase of such alleles. The restriction of the time of reproduction to a 4-day period during the first week of adult life eliminates the influence of natural selection on alleles whose effects are expressed late in life. In this sense the role of natural selection is different from that ascribed to it by the ecological theories. Although the r environment used here might mimic environments encountered by certain "weedy" species of plants and animals, it is probably atypical of the environments *D. melanogaster* occupy, especially the small population size. Consequently, the results of the current study do not contradict Rose and Charlesworth's (12) con-

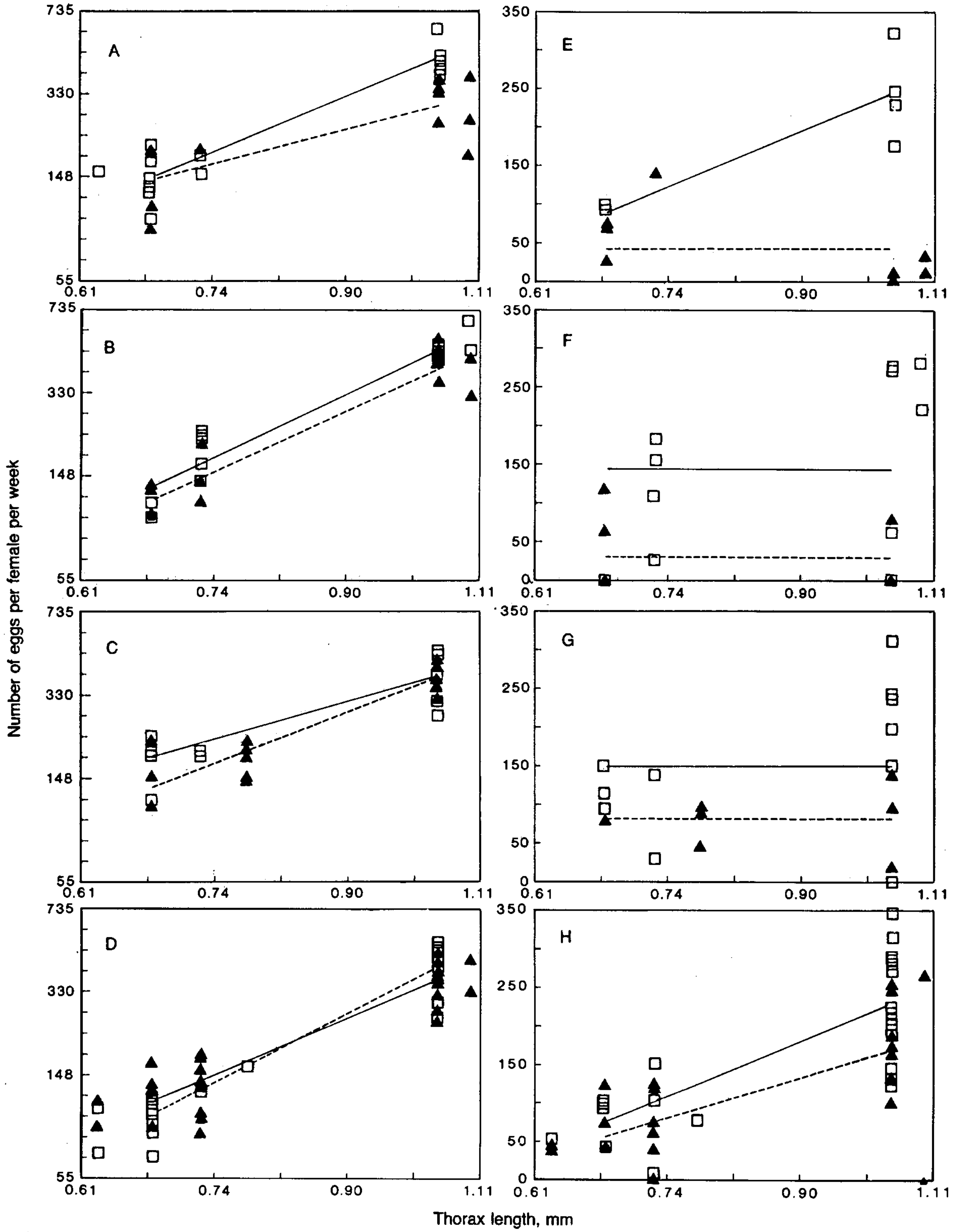

FIG. 1. Female fecundity vs. size during the first (*A–D*) and fourth (*E–H*) weeks of adult life. ▲, Fecundity of individual females from the *r* environments; □, fecundity of those from the *K* environments. The linear regressions of fecundity on size for these data are given for *r* females (– – –) and *K* females (——). For regressions that were not significant horizontal lines have been drawn through the mean fecundity for the appropriate population. The populations are as follows: *r*-1 and *K*-1 (*A*, *E*), *r*-2 and *K*-2 (*B*, *F*), *r*-3 and *K*-3 (*C*, *G*), and *r*-F_1 and *K*-F_1 (*D*, *H*).

clusion that the normal process of senescence in *D. melanogaster* is due to antagonistic pleiotropy. However, this study has delineated ecological conditions that permit the accumulation of alleles that accelerate senescence.

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