Evolution, 55(9), 2001, pp. 1822–1831

DECLINE IN OFFSPRING VIABILITY AS A MANIFESTATION OF AGING IN *DROSOPHILA MELANOGASTER*

STEFANIE KERN, MARTIN ACKERMANN, STEPHEN C. STEARNS, AND TADEUSZ J. KAWECKI[1]
Unit for Ecology and Evolution, Department of Biology, University of Fribourg, Chemin du Musee 10, CH-1700 Fribourg, Switzerland

Abstract.—The evolutionary explanation of senescence proposes that selection against alleles with deleterious effects manifested only late in life is weak because most individuals die earlier for extrinsic reasons. This argument also applies to alleles whose deleterious effects are nongenetically transmitted from mother to progeny, that is, that affect the performance of progeny produced at late ages rather than of the aging individuals themselves. We studied the effect of maternal age on offspring viability (egg hatching success and larva-to-adult survival) in two sets of *Drosophila melanogaster* lines (HAM/LAM and YOUNG/OLD), originating from two long-term selection experiments. In each set, some lines (HAM and YOUNG, respectively) have been selected for early reproduction, whereas later reproduction was favored in their counterparts (LAM and OLD). In the HAM and LAM lines, both egg hatching success and larval viability declined with mother's age and did so with accelerating rates. The hatching success declined significantly faster with maternal age in HAM than in LAM lines, according to one of two statistical approaches used. Egg hatching success also declined with maternal age in YOUNG and OLD lines, with no difference between the selection regimes. However, the relationship between mother's age and offspring larva-to-adult viability differed significantly between these two selection regimes: a decline of larval viability with maternal age occurred in YOUNG lines but not in OLD lines. This suggests that the rate with which offspring viability declines with mother's age responded to selection for early versus late reproduction. We suggest broadening the evolutionary concept of senescence to include intrinsically caused declines in offspring quality with maternal age.

Key words.—Aging, maternal age, maternal effects, offspring quality, offspring viability, senescence.

Received November 1, 2000. Accepted April 26, 2001.

Evolutionary biology defines aging as an irreversible, intrinsically caused decline in survival rate and fertility with age (Rose 1991; Kirkwood et al. 1999). Partridge and Barton (1996) proposed to quantify senescence as an intrinsic decline with age of reproductive value, which measures the expected future contribution of an individual to the future gene pool. Reproductive value depends not only on the survival and fecundity schedules, but also on the quality of the offspring produced (e.g., Caswell 1989; Houston and McNamara 1992). In this paper we suggest broadening the concept of senescence to include any intrinsic decline in offspring quality with parental age. We study this aspect of senescence in two sets of lines of *Drosophila melanogaster*, asking two questions. First, does offspring performance decline with maternal age? Second, do experimental populations that have evolved different rates of senescence, as manifested in the mortality and fecundity rates, also differ with respect to the rate with which offspring performance declines with maternal age?

Evolutionary theory explains senescence as a consequence of the force of natural selection declining with age (Medawar 1952; Hamilton 1966; Emlen 1970). Alleles with deleterious effects late in life are favored if they improve survival and fecundity early in life (antagonistic pleiotropy theory; Williams 1957). Furthermore, even in the absence of beneficial effects at early ages, deleterious mutations manifested only late in life are only weakly counter-selected and are likely to accumulate (mutation accumulation theory; Medawar 1952). Both theories have some support in experimental evidence (e.g., Luckinbill et al. 1984; Rose 1984; Service et al. 1988; Partridge and Fowler 1992; Partridge and Barton 1993; Hughes and Charlesworth 1994; Zwaan et al. 1995; Promislow et al. 1996; Stearns and Partridge 2001).

A crucial assumption of the evolutionary theories of aging is that the phenotypic effects of senescence do not accumulate from generation to generation: the clock is reset for each newly born individual. If this were not the case, the deleterious effects of genes involved in aging would become manifested at progressively younger ages and thus increasingly more strongly counter-selected, leading to their elimination from the gene pool. The irreversible damage due to aging is limited to the soma in organisms with separation of soma and germ line (Kirkwood and Holliday 1979; Rose 1991).

On the other hand, the parents affect the phenotype of their offspring not only through the germ line genes they pass to them, but also by the quantity and quality of provisioning and care and by the cytoplasmic factors involved in early development. Such maternal (and paternal) effects have been widely documented (reviewed in Mousseau and Fox 1998). They are manifested most strongly in juvenile traits of the offspring, and although they sometimes can be detected after two generations (e.g., Hercus and Hoffmann 2000), they do not seem to accumulate from generation to generation.

Because parental effects often reflect variation in parental condition, it is conceivable that older, senescent parents produce offspring of lower quality, i.e., of lower probability of survival or slower growth rate. A decline in offspring viability or developmental rate with maternal age has indeed been observed in *Drosophila melanogaster* (Fleuriet and Vageille 1982; Cadieu 1983; Barnes 1984; Rose 1984; Kerver and Rotman 1987), *D. serrata* (Hercus and Hoffmann 2000), and some other species (Fox 1993; Kennedy et al. 1994; Mohaghegh et al. 1998; Fox and Czesak 2000). This could

[1] Corresponding author: E-mail: t.kawecki@unibas.ch.

be seen as a mechanistic consequence of the general decline in maternal performance due to senescence. It should be noted, however, that the reasoning underlying the evolutionary theory of senescence not only applies to age-specific survival and fertility rates, but also to offspring quality. Alleles that result in increased investment in offspring produced early in life at the cost of reduced quality of offspring produced late in life are expected to be favored. Even in the absence of positive effects early in life, mutations adversely affecting the quality of offspring produced late in life are only weakly counter-selected and are likely to accumulate. In principle, one could imagine an organism that evolves senescence that is only manifested in declining offspring quality.

In the experiments described here we investigated how offspring viability (egg hatching success and larva-to-adult survival) change with parental age in *Drosophila melanogaster*. We tested two sets of selection lines: the HAM (High Adult Mortality)/LAM (Low Adult Mortality) lines from Basel (Stearns et al. 2000) and the YOUNG/OLD lines from London (Partridge et al. 1999). Using these lines allowed us to address two questions. First, does offspring viability deteriorate with increasing age of the mothers, and is this pattern consistent across populations originating from different laboratories and exposed to different selection regimes? Second, did the relationship between maternal age and offspring viability respond to selection for early versus late reproduction, either direct (YOUNG/OLD lines) or indirect (HAM/LAM lines)? More precisely, does offspring performance decline with maternal age faster in the YOUNG and HAM lines than in the OLD and LAM lines, respectively? These lines had responded to their different selection regimes by evolving differential rates of senescence manifested in mortality and fecundity rates (Partridge et al. 1999; Stearns et al. 2000). Here we tested if a parallel evolutionary response had occurred with respect to offspring viability.

Materials and Methods

Selection Lines

The details of the history of the HAM/LAM lines are given by Stearns et al. (1996, 2000). Briefly, the HAM and the LAM selection regimes contain three replicate lines each, derived from a founder stock consisting of 10 virgin males and 10 virgin females from each of 41 isofemale lines collected around Basel, Switzerland, and held in the laboratory for several years. Because the selection experiment began in November 1993, HAM and LAM lines have been exposed to different levels of extrinsic adult mortalities: the probability of surviving one week has been 0.01 for HAM flies and 0.81 for LAM flies. The HAM and LAM lines are maintained in population cages at a constant density of 100 females and 100 males per line, at 12h/12h, 25°C/20°C day/night cycle. Killed flies are replaced by 14 day old flies (counted from egg), reared in bottles with 250 larvae/25 ml food. By the time of this experiment the LAM flies had evolved longer developmental time, larger size at eclosion, lower fecundity at age 13–15 days from egg, and lower intrinsic mortality late in life (between 40 and 75 days counted from egg) than HAM flies (Stearns et al. 2000).

The origin of the YOUNG/OLD lines is described by Partridge et al. (1999). The YOUNG and OLD selection regimes each contain five lines initiated from the random bred Dahomey base stock. Selection began in late 1992 and is continuing. Flies have been kept in population cages on Lewis medium, with 250 flies for each YOUNG line and 450 flies for each OLD line. Egg collection for the next generation is performed seven days after the last adult has eclosed in the YOUNG lines and 3–4 weeks later in the OLD lines. Time of egg collection in OLD lines depends upon mortality, because eggs are collected when the number of flies alive reaches 250, which equals the number of flies reproducing in the YOUNG lines. By the time of this study, the OLD lines had evolved greater longevity and lower fertility early in life than the YOUNG lines (Partridge et al. 1999).

Experimental Design

The assays were carried out in March 1998 for HAM/LAM lines and in July 1998 for YOUNG/OLD lines. Before the assay the flies were maintained in our laboratory in Basel on a two-week cycle for three generations, with five vials per line. Each vial contained 7 ml of medium, on which more than 100 flies were allowed to oviposit for one day. From the offspring produced by the third generation 60 (HAM/LAM lines) or 50 (YOUNG/OLD lines) virgin females per line collected within a 10-h interval were used as the experimental individuals (mothers). The assays were carried out at 25°C and under 12:12 h photoperiod, that is, at the same conditions as those that the populations had experienced during the selection experiment. The assays were always done on the medium, on which the flies had evolved (HAM/LAM medium for HAM/LAM flies, Lewis medium for YOUNG/OLD flies).

To obtain the offspring whose traits we would measure, the mothers were placed individually in 52 × 18 mm vials with two males from the same line and of the same age. Each vial contained 1 ml of larval medium for HAM/LAM flies (Stearns et al. 2000) or 2 ml of Lewis medium for YOUNG/OLD flies (Partridge et al. 1999), covered with one drop of yeast. Vials were refreshed every third day, and food was not a limiting factor.

Eggs for the assay were collected over a 12-h interval (0800–2000 h). For HAM and LAM lines the eggs were collected when the mothers were 16, 21, 32, 43, 50, and 56 days old (all ages reported in this paper are counted from egg, not from eclosion). For YOUNG and OLD lines, the eggs were collected from females aged 16, 23, 30, 37, and 44 days old.

Males that died during the experiment were replaced by mated males from the same line. Initially the males added to replace those that had died were of the same age as the females. However, not enough surplus males survived until the late stages of the experiment, when younger males had to be used as replacement; these younger males could have fathered some of the offspring produced by the females in the last two age classes.

Immediately following oviposition, up to 12 eggs per female in the HAM and LAM lines, and up to 10 eggs per female in the YOUNG and OLD lines were transferred to a different vial. As before, for HAM/LAM lines each vial contained 1 ml of freshly prepared HAM/LAM medium and for

YOUNG/OLD lines each vial contained 2 ml of Lewis medium. These larval densitites were chosen to mimic the densities the lines had experienced under their respective selection regimes. One day after oviposition the number of hatched larva was recorded for each vial. If needed, larvae of a white eyed *D. melanogaster* mutant of the same age were added to keep the larval density in each vial constant (12 or 10 larvae per vial in HAM/LAM and YOUNG/OLD lines, respectively). Eclosed adults were counted about 14 days later.

Statistics

We analyzed two components of offspring viability: egg hatching success (the number of hatched larvae divided by the number of transferred eggs) and larva-to-adult viability (the number of eclosed adults divided by the number of hatched larvae). We also analyzed the overall egg-to-adult viability defined as the proportion of transferred eggs that resulted in an eclosed adult. The egg-to-adult viability is a product of the two other proportions so it contains little additional information, but it might reveal some patterns not detectable in its components. We used two alternative statistical approaches to investigate how these viability characters change with maternal age and differ between selection regimes: repeated measures analysis of variance of angularly transformed proportions and Gompertz regression.

Repeated measures ANOVA.—This approach assumes a normal distribution of errors but allows treating individual mothers as subjects and maternal age as a within-subject effect. Therefore, the test for the effect of maternal age is not confounded by differential mortality of mothers that produce offspring of different quality. For this analysis we calculated a value of the hatching success, larva-to-adult viability and egg-to-adult viability as the appropriate proportions for each vial (i.e., for each mother at each age). The proportions were angular-transformed (arcsin($\sqrt{x}$); Sokal and Rohlf 1995) and treated as raw data points in an analysis of variance (ANOVA). The analysis was carried out with PROC GLM of SAS statistical software, version 6.08 (SAS Institute 1989). We used a repeated-measures analysis of variance on the angular-transformed proportions to test two hypotheses: (1) that these components of offspring viability decline with mother's age, and (2) that the decline is faster in HAM and YOUNG lines than in LAM and OLD lines, respectively. The generally recommended multivariate approach to repeated-measured analysis cannot handle subjects with missing observations at some levels of the repeated factor (SAS Institute 1989). We therefore used the univariate approach to the repeated-measures ANOVA, analyzing it as a split-plot design (PROC GLM of SAS without the REPEATED statement; Littell et al. 1991, pp. 272–274). Because the proportions used as data for the analysis were obtained from a variable number of offspring (the intended 10 or 12 eggs/female were not always obtained), we used a weighted least-squares approach (WEIGHT statement in GLM). For hatching success and egg-to-adult survival the weight was the number of eggs in a given vial, for larval viability the number of hatched larvae. The individual mother was treated as a subject, with the viability of offspring produced at different ages constituting the repeated measures. The mothers were nested within line, which was in turn nested within the selection regime. The interaction of mother's age with selection regime and mother's age with line (nested within selection regime) was also included in the ANOVA model. Because mother and line were random factors, we used line MS as the denominator in the *F*-test for the main effect of selection regime and mother MS as the denominator in the *F*-test for the effect of line. Mother's age and mother's age × selection regime interaction were tested over mother's age × line(selection regime) MS.

A significant effect of maternal age in this analysis implies that offspring viability differs among maternal age classes, but not necessarily that it systematically declines with mother's age, which was our first hypothesis. To address this hypothesis specifically we tested for the significance of the linear trend in the main effect of mother's age (a planned linear contrast). We also tested for the quadratic trend to see if the decline tends to accelerate or decelerate with mother's age (CONTRAST statement of PROC GLM, SAS Institute 1989; for the description of the logic of this approach, see Sokal and Rohlf 1995, pp. 671–672).

Similarly, a significant mother's age × selection regime interaction only implies that the difference between the two selection regimes varies among maternal age classes. Thus, it does not specifically test our second hypothesis that the difference between the least-square means of the two selection regimes changes systematically with mother's age. We addressed this hypothesis by testing for the significance of a linear trend in the mother's age × selection regime interaction (see Sokal and Rohlf 1995, pp. 673–677). The coefficients for the linear and quadratic contrasts were obtained from SAS procedure GLM, REPEATED statement with the option POLYNOMIAL. We used the mother's age × line interaction MS as the denominator in *F*-tests for all three contrasts.

Gompertz regression.—Analysis of variance assumes a normal error distribution whereas the true error distribution for proportions is binomial. To check the robustness of the results we also fitted a Gompertz regression model using a Generalized Linear Model implemented in SAS procedure GENMOD (SAS Institute 1997). This approach assumes that $\ln(-\ln(x))$ is a linear function of the explanatory variables, where x is a proportion (e.g., egg hatching success as defined above) with a binomial error distribution. The negative logarithm of the proportion of survivors measures the instantaneous mortality rate (or ''force of mortality'') operating over a given life stage. The Gompertz curve is a standard model fitted to age-specific survival rates in aging studies (Elandt-Johnson and Johnson 1980), and thus it is also interesting to see how well it describes the changes of offspring viability with maternal age. The disadvantage of this approach is that the model containing the individual mother as a factor (subject) could not be fitted because offspring viability of some mothers was 100% at all ages—that would lead to an infinite estimate of the corresponding parameter. Therefore for the purpose of this analysis we had to pool data from all mothers within replicate lines. To separate the effect of maternal age from the possibility that mothers producing offspring with higher or lower viability were more likely to contribute to late age classes, we included mother's reproductive life span as an additional explanatory variable in the Gompertz regression. A mother's reproductive life span

was defined as the last age at which she laid at least one egg. The model thus included six terms: selection regime, line nested within selection regime, mother's age and mother's reproductive life span as continuous covariates, and mother's age × selection regime, and mother's age × line interaction terms. For the purpose of this analysis the origin of the age axis was set to 15 days from egg (i.e., 15 days were substracted from all maternal ages). The intercept of the Gompertz regression therefore estimates ln(−ln) of the viability of offspring produced at age 15 days counted from egg, roughly corresponding to peak fecundity; a significant effect of selection regime implies that offspring viability produced at that age differed between selection regimes. The choice of the origin of the age axis does not affect the estimated slopes of Gompertz curves or the signicance tests of the effects involving mother's age. The significance of the mother's age × selection regime interaction (or mother's age × line interaction) implies that the slopes of the Gompertz regression differ between selection regimes (or among lines within selection regimes). Significance of the effects was tested using Type 3 likelihood ratio analysis (SAS Institute 1997, p. 288).

To check if the replacement of males that had died with fresh males had an effect on offspring viability and thus could have biased our results, we repeated the above analyses including an additional factor that contrasted viability of offspring fathered by the original males with those produced after at least one male was replaced. Unfortunately, due to an oversight male replacement was not recorded for the YOUNG/OLD flies, and we could thus only do this additional analysis for HAM/LAM flies.

Results

HAM/LAM Lines

The offspring viability characters of HAM/LAM flies declined with mother's age (Fig. 1). The repeated measures ANOVA (Table 1) indicates that this decline was highly significant for the total egg to adult survival as well as for both its components, the hatching success and larval survival (significant linear contrast in age). Furthermore, the quadratic contrast in age was also significant for all three characters (Table 1), indicating that the decline in offspring viability accelerated with mother's age. A marked decline in larval viability was only observed for mothers older than 44 days. The means of the three viability characters did not differ between the selection regimes. A tendency for hatching success to decline faster with age in HAM than LAM lines was nearly significant in the ANOVA (linear contrast for selection regime × age interaction, $P = 0.09$, Table 1); the interaction contrasts for the other two characters were not significant. For hatching success and the overall egg to adult viability, but not for larval viability, line (within selection regime) was significant both as a main factor and in interaction with maternal age, whereas large variation among individual mothers was detected for all three characters.

The Gompertz regression (Fig. 1C) confirmed that offspring viability declined with maternal age—all slopes were significantly positive (positive slope implies decreasing viability on the Gompertz scale). In contrast to the repeated measures ANOVA, the Type 3 analysis of the Gompertz regression revealed a highly significant difference between the selection regimes in the intercept and slope of the regression for hatching success and egg-to-adult viability (Table 2). This would imply that offspring viability was initially higher in HAM lines, but then declined faster with maternal age than in LAM lines, in accordance with the prediction formulated in the introduction. For larva-to-adult viability the slope of the Gompertz regression did not differ between the selection regimes, but the intercept did (Table 2), implying that larva-to-adult viability was higher in HAM lines irrespective of maternal age. The analysis also suggested that there was significant variation among replicate selection lines with respect to both intercept and slope of the Gompertz regression. Because mother's age was treated as a continuous variable in the Gompertz regression, but as a within-subject classification variable in the repeated measures ANOVA described above, the interpretation of the significance of the line effect and mother's age × line interaction differs between these two analyses. However, the linear interaction contrast in the repeated-measures ANOVA (Table 1) and the mother's age × selection regime interaction in the Gompertz regression analysis (Table 2) were performed to test the same biological hypothesis. We return to this discrepancy in the Discussion. Mother's reproductive life span had a significant effect on the hatching success—mothers that produced offspring with higher hatching success were more likely to contribute to late age classes. However, the tests for other factors, including the mother's age × selection regime interaction, remained virtually unchanged when mother's reproductive life span was excluded from the analysis. Finally, it should be noted that the Gompertz model fits the data rather poorly, particularly for larva-to-adult viability.

In the additional analysis of the effect of male replacement, neither statistical approach detected any effect of male replacement on any offspring viability character (all $P > 0.5$ for the repeated measures ANOVA; all $P > 0.2$ for the Gompertz regression, detailed results not reported). Including male replacement as a factor in the analyses had no influence on the tests of other effect. Also, even though obviously the proportion of males replaced increased with female's age, it did not differ between the selection regimes and was not affected by age differently in HAM than in LAM lines (both $P = 0.9$, logistic regression). Our results are thus unlikely to be an artifact of male replacement.

YOUNG/OLD Lines

The mothers from YOUNG/OLD lines have shorter life span than HAM/LAM lines (Partridge et al. 1999; Stearns et al. 2000), and this was also the case in this experiment. No females aged 44 days laid eggs in one of the OLD lines and very few did in the remaining lines, which is why we could not estimate the planned contrasts in the repeated-measures ANOVA. Therefore, the offspring of the last two age classes were pooled for the ANOVA (but not for Gompertz regression). The mean maternal age for eggs included in this pooled age class was 38.2 days; that of hatched larvae 37.9 days. We therefore assigned 38 days as the maternal age of the

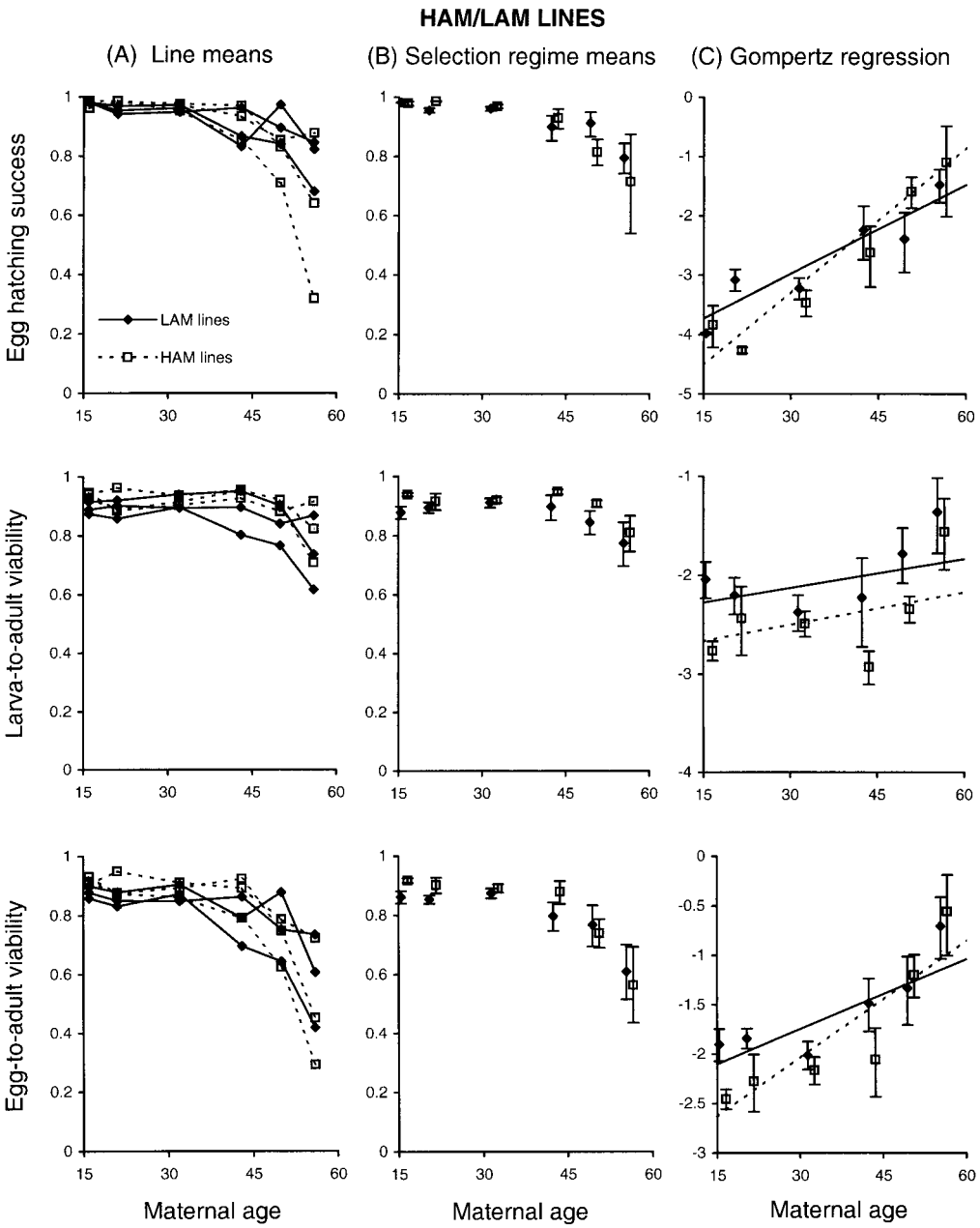

FIG. 1. Offspring viability characters as functions of maternal age in flies from HAM and LAM selection regimes. (A) Means of replicate lines. (B) Selection regime means ± standard errors. (C) Selection regime means (±SE) transformed as ln(−ln(proportion of survivors)), with fitted Gompertz regression lines. The selection regime means are based on angularly-transformed proportions of survivors weighted by the sample sizes; standard errors on variance among angularly-transformed line means. Age is counted from egg. Filled diamonds and solid lines: LAM; open squares and broken lines: HAM.

pooled age class to determine the coefficients of polynomial contrasts.

As in HAM/LAM flies, hatching success and the overall egg to adult viability of flies from YOUNG and OLD lines declined significantly with maternal age, but the quadratic trend was only marginally significant for egg to adult survival and just above the 5% significance level for hatching success (Fig. 2, Table 3). This was confirmed by the highly significant effect of mother's age in the Gompertz regression analysis (Table 4). No effect involving selection regime was significant for these two viability characters and the Gompertz intercepts and slopes did not differ significantly between selection regimes (Fig. 2C, Table 4).

The linear contrast of the effect of age on larval viability was also significant, but so was the selection regime × mother's age interaction contrast (Table 3). This result was confirmed by the the Gompertz regression analysis (Table 4), implying that larva-to-adult viability of offspring produced early in life did not differ between the selection regimes, but it declined faster with maternal age in YOUNG than in OLD lines. This pattern was responsible for the significant main effect of selection regime in Table 3, which is averaged over

TABLE 1. Repeated measures ANOVA (univariate approach) on angular-transformed offspring viability in HAM/LAM lines. All tests based on type IV sums of squares. The denominators of *F*-test were Line for the main effect of Selection regime, Mother for the effect of Line, and Mother's age × Line for the main effect of Mother's age, Mother's age × Selection regime interaction, and all contrasts. The assayed offspring were produced when the mothers were 16, 21, 32, 43, 50, and 56 days old (counted from egg). The corresponding vector of the coefficients of the contrasts linear in age is (1, 0.754, 0.213, −0.328, −0.672, −0.967); that of the contrast quadratic in age (−1, −0.008, 1.160, 0.931, 0.058, −1.140).

Source	df	Hatching success		Larval viability		Egg to adult viability	
		F	*P*	*F*	*P*	*F*	*P*
Selection regime	1	0.40	0.56	2.07	0.22	0.04	0.86
Line (Selection regime)	4	6.90	**0.0001**	1.66	0.16	6.42	**0.0001**
Mother (Line × Selection regime)	343/340/341*	1.32	**0.0008**	1.52	**0.0001**	1.49	**0.0001**
Mother's age	5	9.30	**0.0001**	4.36	**0.0076**	10.71	**0.0001**
Linear contrast	1	44.73	**0.0001**	11.68	**0.0027**	51.52	**0.0001**
Quadratic contrast	1	9.80	**0.0053**	12.58	**0.0020**	19.88	**0.0002**
Selection regime × Mother's age	5	1.04	0.42	1.00	0.44	0.68	0.64
Linear contrast	1	3.19	0.090	0.08	0.79	1.86	0.19
Line (Selection regime) × Mother's age	20	4.08	**0.0001**	1.34	0.15	2.72	**0.0001**
Error	917/895/916*						

* For hatching success/larval viability/egg to adult viability, respectively.

the maternal age classes. The apparent increase in the larval viability in the last age class is based on a very small number of mothers that still laid eggs at that age, likely not a random subset of the original sample (the means in Figures 1 and 2 are not corrected for the effect of individual mothers, but the analysis in Tables 1 and 3 is). Variation among replicate lines manifested as a significant effect of line or as line × mother's age interaction was detected for all three viability characters by both statistical approaches (Tables 3 and 4).

There was a positive relationship between mother's reproductive life span and all three offspring viability characters, but as with the HAM/LAM lines, removing this factor from the analysis had no effect on the significance of other factors. The results are thus not an artifact of differential mortality of the mothers.

DISCUSSION

In this study we tested two hypotheses: (1) that offspring viability declines with parental age, and (2) that this decline is faster in lines that have been selected for early reproduction than in corresponding lines selected for postponed senescence. Our results provide good support for the first hypothesis and weaker support for the second hypothesis.

Parental Age and Offspring Viability

This experiment showed that older flies produce less viable offspring. Both hatching success and larval viability, and thus also overall egg-to-adult viability, declined with mother's age in both sets of lines (HAM/LAM and YOUNG/OLD). In HAM/LAM lines this decline accelerated with age. A similar quadratic trend was detected for egg-to-adult survival in YOUNG/OLD lines, although not for its components, hatching success, and larval viability. However, when the OLD and YOUNG lines were tested separately, a decline in larval viability was only detected in the YOUNG lines.

Because for most offspring both parents were of the same age, we are unable to separate the effects of maternal and paternal age on viability. For example, the decline in egg hatching success could be because of fertilization failure due to paternal aging. However, the strongest decline in offspring viability occurred in the last two age classes when many of the original males were replaced by younger ones. Furthermore, male replacement had no detectable influence on offspring viability in HAM/LAM lines (the data were lost for YOUNG/OLD lines). Thus, the decline of offspring viability we observed seems to be mostly due to maternal aging, at least in HAM/LAM flies.

These findings are consistent with other *Drosophila* studies (Fleuriet and Vageille 1982; Cadieu 1983; Barnes 1984; Rose 1984; Hercus and Hoffmann 2000), which showed a maternal age effect on offspring viability. In a recent study on *Drosophila serrata* (Hercus and Hoffmann 2000), offspring viability began to decline with maternal age from the first day of oviposition, and the decline was approximately linear. In contrast, in our experiment, particularly in the HAM/LAM

TABLE 2. Gompertz regression of offspring viabilities in HAM and LAM flies: Type 3 analysis of deviance.

Source	df	Hatching success		Larval viability		Egg to adult viability	
		χ^2	*P*	χ^2	*P*	χ^2	*P*
Selection regime	1	25.7	0.0000	20.7	0.0000	45.8	0.0000
Line (Selection regime)	4	35.1	0.0000	29.3	0.0000	9.5	0.05
Mother's age	1	497.8	0.0000	13.8	0.0002	279.0	0.0000
Selection regime × Mother's age	1	33.9	0.0000	0.4	0.54	24.1	0.0000
Line (Selection regime) × Mother's age	4	76.1	0.0000	8.9	0.064	29.9	0.0000
Mother's reproductive life span	1	19.0	0.0000	2.4	0.12	0.1	0.75

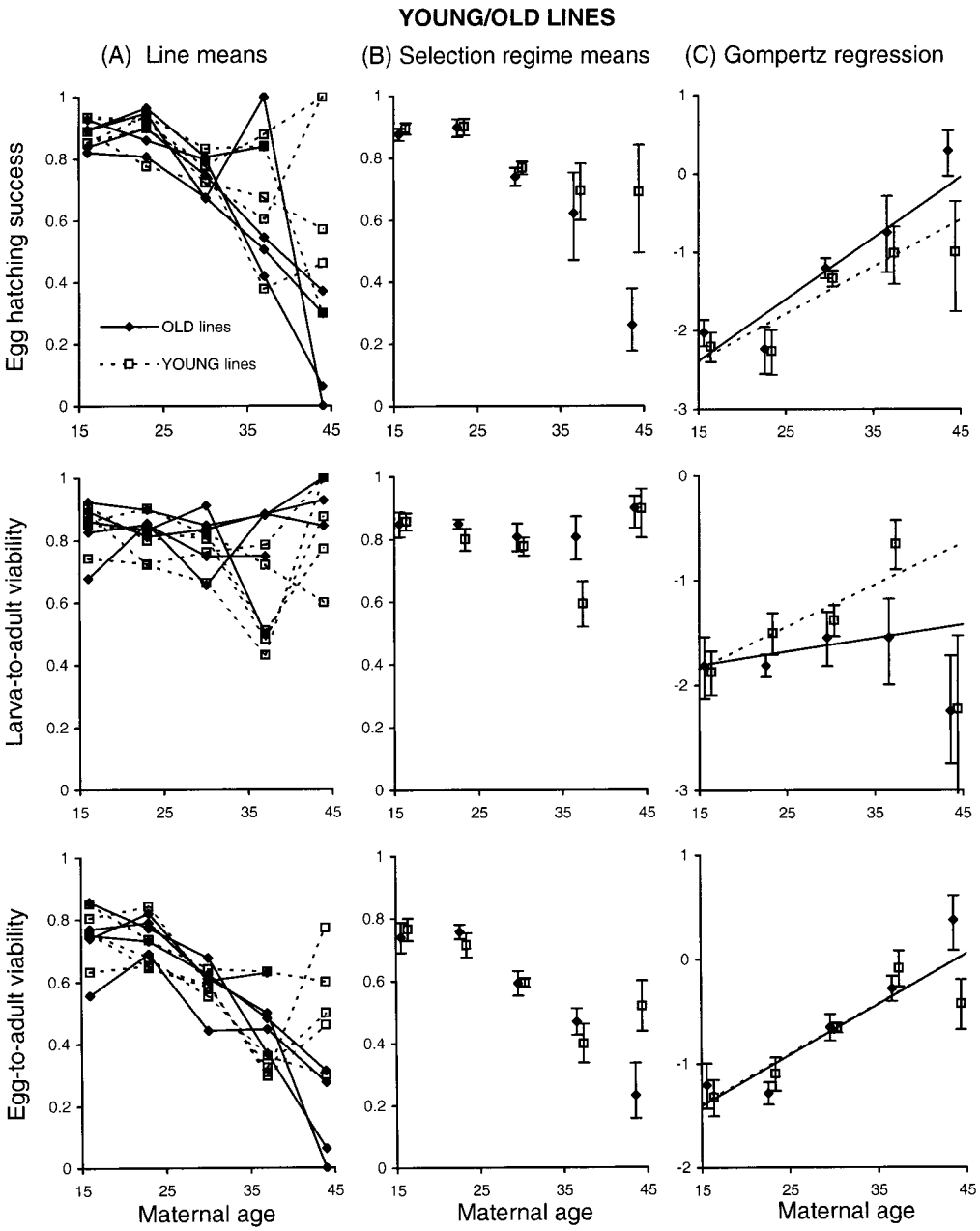

FIG. 2. Offspring viability characters as functions of maternal age in flies from YOUNG and OLD selection regimes. Filled diamonds and solid lines: OLD; open squares and broken lines: YOUNG. Other details as in Figure 1.

lines, the decline of offspring viability became apparent only in older females and seemed to accelerate with age. Whether a relationship is linear or not depends of course on the scale used, and this acceleration was demonstrated on the scale of angularly transformed proportions of survivors. It should be noted, however, that an accelerating decline on the angularly tranformed scale is even more strongly accelerating on the scale of untransformed proportions as long as the proportions are above 0.5.

We can exclude the alternative explanation that mothers producing low quality offspring were more likely to survive to contribute offspring to late age classes—we controlled for this effect by including individual mother as a factor (subject) in the repeated-measure analysis, and by using mother's reproductive life span as a covariate in the Gompertz regression. On the contrary, mothers that early in life produced offspring with high viability were more likely to survive and oviposit at late ages (this held for hatching success in both sets of lines and for larval viability in YOUNG/OLD lines). It is also unlikely that deterioration in experimental conditions could be responsible—a similar decline was observed in both sets of lines even though they were assayed four months apart. Decline in offspring viability thus appears to be a general feature of senescence in *Drosophila.*

TABLE 3. Repeated measures ANOVA (univariate approach) on angularly transformed offspring viability in YOUNG/OLD lines. All tests based on type IV sums of squares; the denominators of *F*-test as in Table 1. The assayed offspring were produced when the mothers were 16, 23, 30, 37, and 44 days old. The last two age classes were pooled and assigned their average age of 39 days, the corresponding contrast vectors used were (1, 0.364, −0.273, −1.091) for the linear trend and (−1, 0.829, 1.082, −0.911) for the quadratic trend.

		Hatching success		Larval viability		Egg to adult viability	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*
Selection regime	1	0.34	0.58	10.47	**0.012**	0.54	0.48
Line (Selection regime)	8	3.51	**0.0006**	1.16	0.32	3.03	**0.0025**
Mother (Line × Selection regime)	469	1.05	0.29	1.07	0.22	1.22	**0.010**
Mother's age	3	11.39	**0.0001**	5.25	**0.0063**	30.50	**0.0001**
Linear contrast	1	28.58	**0.0001**	13.31	**0.0013**	84.36	**0.0001**
Quadratic contrast	1	4.04	0.056	0.26	0.61	5.19	**0.032**
Selection regime × Mother's age	3	0.06	0.98	2.20	0.11	1.52	0.23
Linear contrast	1	0.00	0.98	5.19	**0.032**	3.03	0.095
Line (Selection regime) × Mother's age	24	2.37	**0.0003**	2.04	**0.0026**	1.25	0.19
Error	670/610*						

* Error df = 670 for hatching success and egg to adult viability; 610 for larval viability.

Response to Divergent Selection Regimes

Support for our second hypothesis is equivocal. We did find that larval viability declined significantly faster with mother's age in YOUNG than OLD lines. A similar tendency for hatching success to decline faster with parental age in HAM than LAM lines was detected with one statistical approach (Gompertz regression), but not with the other (repeated measured ANOVA on proportions). These differences paralleled the previously found differences in age-specific mortality and fecundity between HAM and LAM (Stearns et al. 2000), as well as between YOUNG and OLD lines (Partridge et al. 1999). In contrast, mean hatching success in YOUNG/OLD lines tended to show an opposite trend, and even though it was far from significant, it partially compensated for the differences in the rate of decline in larval survival. As a result, no differences in the rate of decline with mother's age could be detected in YOUNG/OLD flies for overall egg-to-adult survival.

It is difficult to interpret the discrepancy between the repeated measures ANOVA and the Gompertz regression concerning the rate with which hatching success declines with mother's age in HAM versus LAM flies. The Gompertz regression is expected to have more power because it treats individual offspring as observations and assumes binomial error distribution. However, the test for selection regime × mother's age interaction in the Gompertz regression seems to be rather insensitive to variation in the way replicate lines respond to mother's age—replicate selection lines are not treated as a nested random factor in the way they are in a ANOVA. In other words, a significant selection regime × mother's age interaction in the Gompertz regression (Table 2) implies that the three HAM lines had on average significantly steeper Gompertz slopes than the three LAM lines, but we cannot exclude that this was due to drift or some other factor unrelated to the selection regimes. In contrast, the *F*-test in the ANOVA treats the lines as the main level of replication and is thus more conservative (Table 1). It should be noted that the interaction between line and maternal age, which we found for hatching success in both sets of lines and for larval survival in YOUNG/OLD lines, suggests that there is genetic variation in the rate with which offspring viability declines with maternal age, and thus suggests that this rate can evolve.

This is, to our knowledge, the first study that attempted to find differences in the rate with which offspring viability declines with mother's age between lines that differ in the rate of aging as manifested in mortality rates as a result of evolving under different selection regimes. Although the results are encouraging, additional studies are needed to find out if differences in the rate of senescence are also reflected in offspring quality.

Offspring Quality and the Evolutionary Theory of Aging

Traditionally, definitions of aging used in evolutionary biology have been limited to effects on mortality and fecundity of the aging individual (Rose 1991; Kirkwood et al. 1999). Although several studies have found that offspring performance declines with maternal age (see below), and the decline

TABLE 4. Gompertz regression of offspring viabilities in YOUNG and OLD flies: Type 3 analysis of deviance.

		Hatching success		Larval viability		Egg to adult viability	
Source	df	χ^2	*P*	χ^2	*P*	χ^2	*P*
Selection regime	1	0.2	0.69	0.1	0.81	0.0	0.90
Line (Selection regime)	8	68.4	0.0000	155.3	0.0000	194.6	0.0000
Mother's age	1	268.2	0.0000	69.8	0.0000	354.3	0.0000
Selection regime × Mother's age	1	1.1	0.29	9.4	0.0021	0.6	0.43
Line (Selection regime) × Mother's age	8	46.6	0.0000	71.3	0.0000	62.7	0.0000
Mother's reproductive life span	1	4.3	0.039	11.1	0.0009	13.6	0.0002

has occasionally been considered a by-product of aging (e.g., Mousseau 1991), it has received little attention in the context of the evolution of aging. For example, in a classic paper on trade-offs involved in aging in *Drosophila,* Rose (1984) states in one sentence that in his experiment offspring viability fell with parental age, without discussing the significance of this finding, for example, possible involvement of offspring quality in trade-offs between early and late fitness components.

We propose that a decline in offspring quality should be integrated into the evolutionary definition of aging. Three theoretical arguments can be made to support this proposition. First, offspring quality affects an individual's reproductive value (Caswell 1989; Houston and McNamara 1992). If aging is defined as irreversible intrinsic decline of reproductive value with age (Partridge and Barton 1996), a decline in offspring viability due to deteriorating maternal condition will contribute to it. Second, early offspring performance may be more affected by its mother's genotype and phenotype than by its own, i.e., may be treated as the mother's trait, her ''extended phenotype''. This is obvious in organisms with parental care, but even in its absence the mother provisions the egg with cytoplasm and resources. Because offspring performance becomes independent of maternal effects only gradually, the problem cannot be avoided by defining fertility as the number of offspring surviving to a certain point (e.g., weaning). Third, the quality of offspring produced late in life is likely to be involved in trade-offs with survival, fertility, or offspring quality early in life.

Several empirical issues are relevant to our proposition. How commonly does a decline in offspring quality accompany parental aging? Does it involve similar proximate mechanisms as the decline in age specific survival and fertility rates? Is there genetic variation for the rate of decline? Are there trade-offs between the quality of offspring produced late in life and fitness components early in life?

As it has now been demonstrated in a number of independent studies (this study, Fleuriet and Vageille 1982; Cadieu 1983; Barnes 1984; Rose 1984; Kerver and Rotman 1987), there seems to be little ground to doubt that a decline in offspring viability is a typical feature of *Drosophila melanogaster.* A similar pattern has been discovered in several other arthropod systems (Fox 1993; Mohaghegh et al. 1998; reviewed by Fox and Czesak 2000) and in yeast (Kennedy et al. 1994). An early demonstration by Lansing (1947) that rotifer cultures propagated from older mothers had reduced life span is most likely an artifact of his experimental conditions (Rose 1991). It should also be noted that in several organisms offspring performance *increases* with maternal age. This could be due to maternal growth in organisms with indeterminate growth (e.g., Ebert 1993; Bridges 1996), gaining experience in species with parental care (e.g., Alados and Escos 1991), or ''terminal reproductive investment'' at the end of the season (e.g., Dixon et al. 1993).

We can only speculate about the proximate mechanisms responsible for the decline in *Drosophila.* It could be a consequence of smaller egg size or lower nutrient content. In several other insects the decline in offspring quality is at least partially caused by a decline in egg size (Fox and Czesak 2000). In most of those cases, a resource needed for reproduction is only gathered in the prereproductive phase (e.g., protein in most butterflies). Begon and Parker (1986) argue that a decline in investment per offspring with maternal age is an optimal strategy for such organisms. In this case the proximate mechanism of the decline in offspring viability with paternal age would be very different from the proximate factors usually associated with aging. This does not apply to *Drosophila,* however, which gathers resources for reproduction throughout adult life. Nonetheless, egg size or nutrient content could still decline with maternal age in *Drosophila,* which in the nutrient-rich assay environment would reflect senescence of the reproductive organs. Egg size in *Drosophila* has an effect on offspring viability (Azevedo et al. 1997), but we did not find published evidence for a decline of egg size with maternal age. There is evidence, however, that vitellogenesis declines with maternal age, and that the decline can be slowed down in response to selection for late reproduction (Carlson and Harshman 1999). Another potential mechanism involves weakening of the microtubule network during meiosis in aging oocytes, which can result in increasing frequency of aneuploidy or trisomic zygotes with maternal age (Schatten et al. 1999). An age-dependent shift in heteroplasmy (the presence of more than one type of mtDNA within a cell) has been described in *D. melanogaster* and is transmitted to the offspring (Kann et al. 1998), but whether this affects offspring viability remains unknown.

To our knowledge, genetic variation in the rate with which offspring quality declines with age has not been measured in any organism. Our results—the difference in the rate of decline in larval viability between YOUNG and OLD lines and the significant line × maternal age interaction—provide the first evidence for such genetic variance. We hope that this paper will stimulate research on this problem. Similarly, research is needed on possible trade-offs between the quality of offspring produced late in life and early fitness components. Although the trade-off between offspring size and number has been extensively studied (reviewed in Stearns 1992), a potential trade-off between performance early in life and the quality of offspring produced late in life was neglected.

Conclusion

In this paper we argue that a decline of offspring quality with parental age should receive more attention in the context of the evolution of aging. It should not be regarded as just a by-product of the loss of function associated with parental aging. Rather, offspring quality should be treated as a maternal (and paternal) fitness component that can evolve in an age-specific way, that may be involved in trade-offs with itself and other fitness components across age classes, and that may be affected by deleterious mutations with a late age of onset carried by the mother (or father). In addition to confirming that a decline in offspring viability with maternal age is a normal feature of *Drosophila,* our results suggest that the rate of decline can respond to the same selection pressures that also results in changes in the rate of aging as manifested in mortality and fertility rates.

Acknowledgments

We are grateful to L. Partridge for providing us with the YOUNG/OLD experimental lines, the product of a long-term

selection experiment supported by the Natural Environment Research Council. We thank M. Kaiser, A. Mislin, H. Zingerli, P. Ramseier, and C. Kern for help with the experiments and D. Ebert and two anonymous referees for comments. This work has been supported by Swiss National Science Foundation grants to TJK and SCS.

Literature Cited

Alados, C. L., and J. Escos. 1991. Phenotypic and genetic characteristics affecting lifetime reproductive success in female Cuvier, dama, and dorcas gazelles (*Gazella cuvieri, G. dama,* and *G. dorcas*). J. Zool. 223:307–321.

Azevedo, R. B. F., V. French, and L. Partridge. 1997. Life-history consequences of egg size in *Drosophila melanogaster*. Am. Nat. 150:250–282.

Barnes, P. T. 1984. A maternal effect influencing larval viability in *Drosophila melanogaster*. J. Hered. 75:288–292.

Begon, M., and G. A. Parker. 1986. Should egg size and clutch size decrease with age? Oikos 47:293–302.

Bridges, T. S. 1996. Fitness consequences of maternal effects in *Streblospio benedicti* (Annelida: Polychaeta). Am. Zool. 36: 132–146.

Cadieu, N. 1983. Maternal influence and the effect of heterosis on the viability of *Drosophila melanogaster* during different preimaginal stages. Can. J. Zool. 61:1152–1155.

Carlson, K. A., and L. G. Harshman. 1999. Extended longevity lines of *Drosophila melanogaster*: Characterization of oocyte stages and ovariole numbers as a function of age and diet. J. Gerontol. A-Biol. 54:B432–B440.

Caswell, H. 1989. Matrix population models. Sinauer Associates, Sunderland, MA.

Dixon, A. F. G., R. Kundu, and P. Kindlmann. 1993. Reproductive effort and maternal age in iteroparous insects using aphids as a model group. Funct. Ecol. 7:267–272.

Ebert, D. 1993. The trade-off between offspring size and number in *Daphnia magna*: the influence of genetic, environmental, and maternal effects. Arch. Hydrobiol. 4:453–473.

Elandt-Johnson, R., and N. L. Johnson. 1980. Survival models and data analysis. Wiley, New York.

Emlen, J. M. 1970. Age specificity and ecological theory. Ecology 51:588–601.

Fleuriet, A., and M. C. Vageille. 1982. On the maintenance of the polymorphism at the ref (2)P locus in population of *Drosophila melanogaster.* Genetica 59:203–210.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia 96:139–146.

Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods. Annu. Rev. Ecol. Syst. 45:341–369.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. J. Theor. Biol. 12:12–45.

Hercus, M. J., and A. A. Hoffmann. 2000. Maternal and grandmaternal age influence offspring fitness in *Drosophila*. Proc. R. Soc. Lond. B 267:2105–2110.

Houston, A. I., and J. M. McNamara. 1992. State-dependent life-history theory, and its implications for phenotypic plasticity and clutch size. Evol. Ecol 6:243–253.

Hughes, K. A., and B. Charlesworth. 1994. A genetic analysis of senescence in *Drosophila.* Nature 367:64–66.

Kann, L. M., E. B. Rosenblum, and D. M. Rand. 1998. Aging, mating, and the evolution of mtDNA heteroplasmy in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA 95:2372–2377.

Kennedy, B. K., N. R. Austriaco, and L. Guarente. 1994. Daughter cells of *Saccharomyces cerevisiae* from old mothers display a reduced life-span. J. Cell Biol. 127:1985–1993.

Kerver, W. J. M., and G. Rotman. 1987. Development of ethanol tolerance in relation to the alcohol dehydrogenase locus in *Drosophila melanogaster*. II. The influence of phenotypic adaptation and maternal effect on alcohol supplemented media. Heredity 58:239–248.

Kirkwood, T. B. L., and R. Holliday. 1979. The evolution of aging and longevity. Proc. R. Soc. Lond. B 205:531–546.

Kirkwood, T. B. L., G. M. Martin, and L. Partridge. 1999. Evolution, senescence, and health in old age. Pp. 219–230 *in* S. C. Stearns, ed. Evolution in health and disease. Oxford Univ. Press, Oxford, U.K.

Lansing, A. I. 1947. A transmissible, cumulative and reversible factor in aging. J. Gerontol. 2:228–239.

Littell, R. C., R. J. Freund, and P. C. Spector. 1991. SAS system for linear models, third edition. SAS Institute, Cary, NC.

Luckinbill, L. S., R. Arking, M. J. Clare, W. C. Cirocco, and S. A. Buck. 1984. Selection for delayed senescence in *Drosophila melanogaster.* Evolution 38:996–1003.

Medawar, P. B. 1952. An unsolved problem of biology. H. K. Lewis, London.

Mohaghegh, J., P. De Clercq, and L. Tirry. 1998. Effects of maternal age and egg weight on developmental time and body weight of offspring of *Podisus maculiventris* (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 91:315–322.

Mousseau, T. A. 1991. Geographic variation in maternal-age effects on diapause in a cricket. Evolution 45:1053–1059.

Mousseau, T. A., and C. W. Fox. 1998. Maternal effects as adaptations. Oxford Univ. Press, Oxford, U.K.

Partridge, L., and N. H. Barton. 1993. Optimality, mutation and the evolution of ageing. Nature 362:305–311.

———. 1996. On measuring the rate of ageing. Proc. R. Soc. Lond. B 263:1365–1371.

Partridge, L., and K. Fowler. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46:76–91.

Partridge, L., N. Prowse, and P. Pignatelli. 1999. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster.* Proc. R. Soc. Lond. B 266: 255–261.

Promislow, D. E. L., M. Tatar, A. A. Khazaeli, and J. W. Curtsinger. 1996. Age-specific patterns of genetic variance in *Drosophila melanogaster.* I. Mortality. Genetics 143:839–848.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38:1004–1010.

———. 1991. Evolutionary biology of aging. Oxford Univ. Press, Oxford, U.K.

SAS Institute. 1989. SAS/STAT user's guide. Vers. 6, 4th ed. SAS Institute, Inc., Cary, NC.

SAS Institute. 1997. SAS/STAT software: changes and enhancements through release 6. 12. SAS Institute, Inc., Cary, NC.

Schatten, H., A. Chakrabarti, and J. Hedrick. 1999. Centrosome and microtubule instability in aging *Drosophila* cells. J. Cell. Biochem. 74:229–241.

Service, P. M., E. W. Hutchinson, and M. R. Rose. 1988. Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. Evolution 42:708–716.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd ed. W. H. Freeman, New York.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, Oxford, U.K.

Stearns, S. C., and L. Partridge. 2001. The genetics of aging in *Drosophila.* Pp. 345–360 *in* E. J. Masoro and S. Austad, eds. The handbook of aging. 5th ed. Academic Press, London.

Stearns, S. C., M. Kaiser, and A. Blarer. 1996. A case study in experimental evolution: reproductive effort and induced responses in *Drosophila melanogaster*. Plant Species Biol. 11: 97–105.

Stearns, S. C., M. Ackermann, M. Doebeli, and M. Kaiser. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. Proc. Natl. Acad. Sci. USA 97:3309–3313.

Williams, G. C. 1957. Pleiotropy, natural selection and the evolution of senescence. Evolution 11:398–411.

Zwaan, B., R. Bijlsma, and R. F. Hoekstra. 1995. Direct selection on life span in *Drosophila melanogaster*. Evolution 49:649–659.

Corresponding Editor: T. Mousseau