Quantitative Genetics of Postponed Aging in *Drosophila melanogaster*. II. Analysis of Selected Lines

Edward W. Hutchinson,[1] Alison J. Shaw[2] and Michael R. Rose[3]

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1, and Department of Ecology and Evolutionary Biology, School of Biological Sciences, University of California, Irvine, California 92717

Manuscript received May 15, 1990
Accepted December 13, 1990

ABSTRACT

Quantitative genetic analyses of *Drosophila melanogaster* stocks with postponed aging have suffered from the problem of a lack of certainty concerning patterns of allelic differentiation. The present experiments were designed to alleviate this difficulty by selecting for enhanced levels of characters known to be related to postponed aging. Selection successfully increased the degree of differentiation of postponed aging stocks with respect to starvation resistance and fecundity, but persistent additive genetic variance suggested that selection did not result in fixation of alleles. The artificially selected stocks were subjected to crosses to test for patterns of dominance and maternal effects. There was little evidence for these effects in the inheritance of the characters underlying postponed aging, even with the increased differentiation of the selected stocks.

GENETIC analysis of postponed aging in laboratory stocks of *Drosophila melanogaster* that have been cultured using older females has indicated that the selected lines combine additively (CLARE and LUCKINBILL 1985; HUTCHINSON and ROSE 1991). However, these analyses have been compromised by their use of stocks whose genetic makeup is only indirectly known. The chromosome substitution experiments of LUCKINBILL *et al.* (1988) provide more direct information about the genetics of one strain with postponed aging. They found that most chromosomes affected the character, but that some chromosomes from the stocks exhibiting postponed aging actually decreased longevity. This suggests the possibility of inconsistent differentiation between loci, a considerable problem for the genetic analysis of segregating populations. Another concern is the retention of genetic polymorphism among the postponed-aging stocks and their controls. This problem together with that of inconsistent differentiation over loci suggests that further selection may be necessary for useful genetic analysis. The rationale is that further selection should yield selected stocks that are more differentiated from their controls, stocks in which genetic polymorphism is reduced, and thus stocks which should permit more reliable genetic analysis.

In the present article, we report experiments in which: (i) the genetic variability present in postponed aging stocks was assayed by means of a sib analysis; (ii) artificial selection was applied to both control and postponed-aging stocks to make them diverge farther; (iii) sib analysis was used to assess the degree to which selection reduced genetic variability; and (iv) diallel analysis and other types of population crosses were performed to check the findings of CLARE and LUCKINBILL (1985) and HUTCHINSON and ROSE (1991). Taken together, the present results are largely consistent with the results obtained in previous studies.

MATERIALS AND METHODS

Stocks: The present study used the same postponed-aging, called "O," type of stocks as those of HUTCHINSON and ROSE (1991). The controls were also the same, called "B"s. However, while that study employed five independent lines of each stock-type, the present study employed only three.

Culture media, assays and statistical procedures: The culture methods, assays, and statistical procedures were the same as those given in HUTCHINSON and ROSE (1991).

Sib analysis: Sib analysis was performed on the stocks before and after selection. There were six such stocks, and each was subject to sib analysis twice, making a total of 12 sib analyses. Before selection, sib analyses of fecundity were not performed, because more extensive data were already available in earlier studies (*e.g.* ROSE and CHARLESWORTH 1981a) of similar populations. For each sib analysis, 50 sires were mated to 12 dams each, the dams laid eggs on charcoal medium individually, and then 30 eggs were harvested from the charcoal medium for rearing in the normal banana medium. One full sibling of each sex was assayed for starvation resistance from each rearing vial. In the sib analyses performed after selection, the fecundity of one of the sibs was assayed as well. Components of variance were calculated using the standard quantitative genetics half-sib design (FALCONER 1981), from which heritabilities are readily determined.

Artificial selection: The O stocks and B stocks were

[1] Present address: Institute for Behavioral Genetics, Box 447, University of Colorado, Boulder, Colorado 80309.
[2] Present address: Department of Ecology and Systematics, Cornell University, Carson Hall, Ithaca, New York 14853-0999.
[3] To whom correspondence should be addressed at the University of California.

TABLE 1

F diallel experiments

Experiment	Character assayed	Populations assayed per character	Mean No. assayed per population	Total No. assayed
DF1				
	Fecundity	3 + 3 = 6	60.0	360
	Conditional fecundity	3 + 3 = 6	59.5	357
DF2				
	Fecundity	3 × 3 = 9	30.9	278
	Conditional fecundity	3 × 3 = 9	29.7	267
	Female starvation	3 × 3 = 9	34.0	306
	Male starvation	3 × 3 = 9	34.0	306
DF3				
	Fecundity	3 × 3 = 9	51.9	467
	Conditional fecundity	3 × 3 = 9	50.7	456
	Female starvation	3 × 3 = 9	52.4	472
	Male starvation	3 × 3 = 9	52.3	471
Total		84		3740

TABLE 2

S diallel experiments

Experiment	Character assayed	Populations assayed per character	Mean No. assayed per population	Total No. assayed
DS1				
	Fecundity	3 + 3 = 6	59.7	358
	Conditional fecundity	3 + 3 = 6	54.7	348
DS2				
	Fecundity	3 × 3 = 9	46.1	415
	Conditional fecundity	3 × 3 = 9	43.9	395
	Female starvation	3 × 3 = 9	47.8	430
	Male starvation	3 × 3 = 9	47.6	428
Total		48		2374

subjected to selection for two different characters, starvation resistance and early fecundity, respectively. The rationale for this is that O stocks have enhanced starvation resistance relative to B stocks (SERVICE *et al.* 1985), while B stocks have enhanced early fecundity relative to O stocks (ROSE 1984). More extreme differentiation is thereby obtainable by selecting further in those directions. In addition, since there is a negative additive genetic correlation of large magnitude between these characters (SERVICE and ROSE 1985), selecting up on fecundity should depress starvation resistance and conversely. In both cases, selection proceeded with three control lines matched to each of the three selection lines for the first 13 generations. Over that same period, 250 flies or pairs of flies, in the case of starvation resistance, were assayed for the selected character from each selected line in each generation, while 120 were assayed from each control line. The character which was *not* selected was also observed in 120 flies from both selected and control lines. Both selected and control lines were maintained using 50 separately reared couples as parents of the next generation, the control-line parents being chosen at random. The selected-line parents were chosen from those individuals in the top 50 of their generation. The control lines were discarded after 13 generations, but selection was continued for another 12 generations, at reduced intensity (90 selected out of 160). These later generations of selection cannot, because of the lack of controls, be used for quantitative genetic hypothesis testing. Selection was continued in order to produce more extremely differentiated stocks. The total number of observations made in the course of the selection experiments was 75,982.

TABLE 3

F and S crossing experiments

Experiment	Character assayed	Populations assayed per character	Mean No. assayed per population	Total No. assayed
FS1				
	Fecundity	4 × 3 = 12	66.8	801
	Conditional fecundity	4 × 3 = 12	65.3	783
	Female starvation	4 × 3 = 12	71.5	858
	Male starvation	4 × 3 = 12	71.9	863
	Female longevity	3 × 3 = 9	98.0	882
	Male longevity	3 × 3 = 9	97.6	878
FS2				
	Fecundity	4 × 3 = 12	68.3	820
	Conditional fecundity	4 × 3 = 12	66.9	803
	Female starvation	4 × 3 = 12	70.4	845
	Male starvation	4 × 3 = 12	70.5	846
FS3				
	Fecundity	4 × 3 = 12	70.2	842
	Conditional fecundity	4 × 3 = 12	68.5	822
	Female starvation	4 × 3 = 12	70.2	842
	Male starvation	4 × 3 = 12	70.1	841
	Female longevity	4 × 3 = 12	73.8	886
	Male longevity	4 × 3 = 12	73.8	885
FS4				
	Fecundity	4 × 3 = 12	69.3	832
	Conditional fecundity	4 × 3 = 12	68.3	820
	Female starvation	4 × 3 = 12	70.9	851
	Male starvation	4 × 3 = 12	70.9	851
	Female longevity	4 × 3 = 12	58.3	699
	Male longevity	4 × 3 = 12	58.0	696
FS5				
	Fecundity	4 × 1 = 4	59.0	236
	Conditional fecundity	4 × 1 = 4	57.8	231
	Female starvation	4 × 1 = 4	60.0	240
	Male starvation	4 × 1 = 4	60.0	240
	Female longevity	4 × 1 = 4	60.0	240
	Male longevity	4 × 1 = 4	60.0	240
Total		282		19,373

The lines eventually produced by selection for fecundity are designated "F" lines. The lines eventually produced by selection for starvation resistance are designated "S" lines.

Diallel analysis: The same principles of diallel analysis as those of HUTCHINSON and ROSE (1991) were practiced. Again, the experiments were coded: D in the first position indicating a diallel design; F or S in the second position indicating the nature of the populations analyzed; and the third position numeral indicating the particular experiment. Table 1 and Table 2 give the experiment codes, the characters assayed, the number of populations assayed, and the number of individuals assayed. In the DF1 and DS1 experiments, reciprocal crosses were not followed. The DF2 and DS2 experiments were performed in order to remedy this deficiency. The DF3 experiment was performed because of a lack of numbers in some of the cells of experiment DF2. See HUTCHINSON and ROSE (1991) for more detail on the types of diallel design.

Transmission pattern experiments: The series of experiments on transmission patterns in the F and S stocks is outlined in Table 3. These experiments are coded with "FS" in the first two positions, indicating crosses of F and S

TABLE 4

Heritabilities and variance components of selected characters in B and O populations before selection

Character and population	$h^2 \pm$ SE	V_P	V_A (% of V_P)	V_R[a] (% of V_P)	Total No. assayed
Female starvation					
B1	0.47 ± 0.18	33.4	15.5 (46.5%)	17.9 (53.5%)	368
B2	1.39 ± 0.25	117.6	163.9 (139.4%)	−46.3 (−39.4%)	404
B3	0.38 ± 0.16	30.9	11.8 (38.1%)	19.1 (61.9%)	400
O1	0.68 ± 0.19	77.9	53.3 (68.4%)	24.6 (31.6%)	441
O2	1.37 ± 0.31	84.4	115.8 (137.3%)	−31.5 (−37.3%)	239
O3	0.47 ± 0.17	66.9	31.4 (47.0%)	35.5 (53.0%)	415
Male starvation					
B1	0.00 ± 0.11	26.5	−0.1 (−0.3%)	26.6 (100.3%)	368
B2	0.40 ± 0.16	30.6	12.2 (39.9%)	18.4 (60.1%)	404
B3	0.14 ± 0.13	24.8	3.4 (13.6%)	21.4 (86.4%)	400
O1	0.38 ± 0.15	42.7	16.2 (37.9%)	26.5 (62.1%)	441
O2	1.14 ± 0.30	69.2	79.3 (114.5%)	−10.1 (−14.5%)	239
O3	0.25 ± 0.14	43.9	11.0 (25.1%)	32.9 (74.9%)	415
Starvation					
B1	0.28 ± 0.16	15.1	4.3 (28.2%)	10.9 (71.8%)	366
B2	1.38 ± 0.25	43.0	59.3 (137.9%)	−16.3 (−37.9%)	404
B3	0.37 ± 0.16	13.9	5.2 (37.4%)	8.7 (62.6%)	400
O1	0.82 ± 0.20	37.9	31.2 (82.3%)	6.7 (17.7%)	441
O2	1.07 ± 0.29	44.2	47.4 (107.3%)	−3.2 (−7.3%)	239
O3	0.35 ± 0.15	30.9	10.8 (34.8%)	20.2 (65.2%)	415

[a] V_R, residual variance, contains V_D, the dominance variance, V_I, the interaction variance, and V_E, the environmental variance.

populations. The numerals then refer to the sequence of experiments. In experiments FS1 and FS4, larvae were reared at a density of 90 per vial. In experiments FS2, FS3 and FS5, larvae were reared at 30 per vial. In experiment FS5, synthetic crosses were performed involving all F or all S lines, to create multiply hybrid F and S populations. These two populations were then crossed to test for their transmission patterns.

RESULTS

Sib analysis before selection: Table 4 gives heritability and variance estimates from the B and O populations used for selection. In the case of the O populations, the heritability and additive genetic variance estimates suggest that there has not been fixation of alleles affecting the characters studied in the longer-lived lines. The results for the B populations were not as clear, but still did not inspire confidence that they were close to fixation for the relevant alleles. These findings motivated our artificial selection study.

Artificial selection; creation of F and S lines: Artificial selection produced significant direct responses to selection, as shown in Figures 1 and 2, which plot the generations for which the controls were retained. The realized heritability (FALCONER 1981) for selection on fecundity was 0.115 ± 0.003 (mean ± standard error). The realized heritability for selection on starvation resistance was 0.203 ± 0.004. Note that these standard errors reflect the variance *between* replicated selection lines, not the error term *within* each selection line. (See Table 5 for more detail.)

These findings qualitatively corroborated the previous sib analyses, indicating that the B and O populations were indeed polymorphic for the alleles involved, although these realized heritability estimates are much smaller than the heritability values obtained in the sib analyses. Starvation resistance indirectly responded to selection on fecundity, the regression of starvation resistance on the cumulative selection differential applied to fecundity being −0.016 ± 0.006. The same result for the indirect response fecundity to selection on starvation resistance was not statistically significant, being −0.022 ± 0.025, though in the expected direction (cf. SERVICE and ROSE 1985).

After 25 generations of selection, the starvation-selected lines derived from the O's were designated S's, while the fecundity-selected lines derived from the B's were designated F's. In terms of numbering, the F_i population was obtained by selection from a derivative of the B_i population, and similarly for the S_i relative to the O_i. While there is always some variation in population averages from assay to assay, the O's have mean starvation resistance levels from 30–40 hr, while the S's have starvation resistances of 50–60 hr, almost a doubling. The F fecundities were increased by about ten eggs per day over the mean fecundities of the B populations.

Sib analysis after selection: In spite of the considerable increases in starvation resistance among the S's and in fecundity among the F's, Table 6 indicates that there has been no statistically consistent reduction in

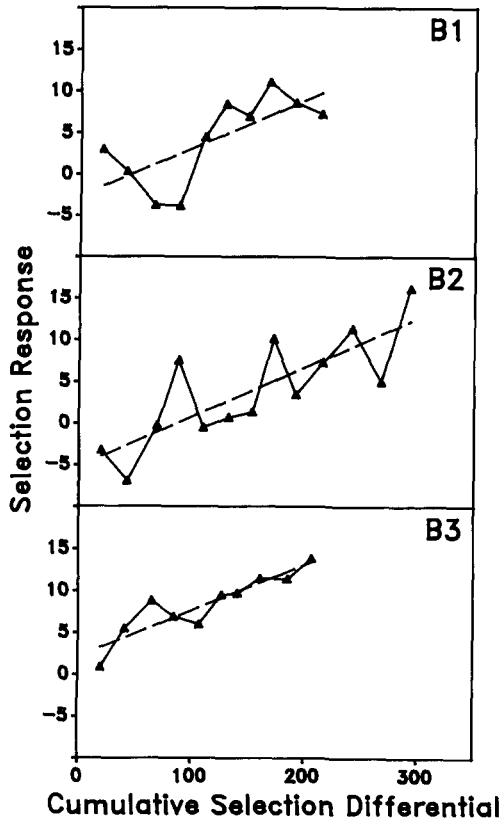

FIGURE 1.—The cumulative response to selection, measured relative to controls, for increased fecundity is plotted against the cumulative selection differential (FALCONER 1981) in the three B lines used. Twice the slope of the regression is the realized heritability, because selection is imposed on one sex only.

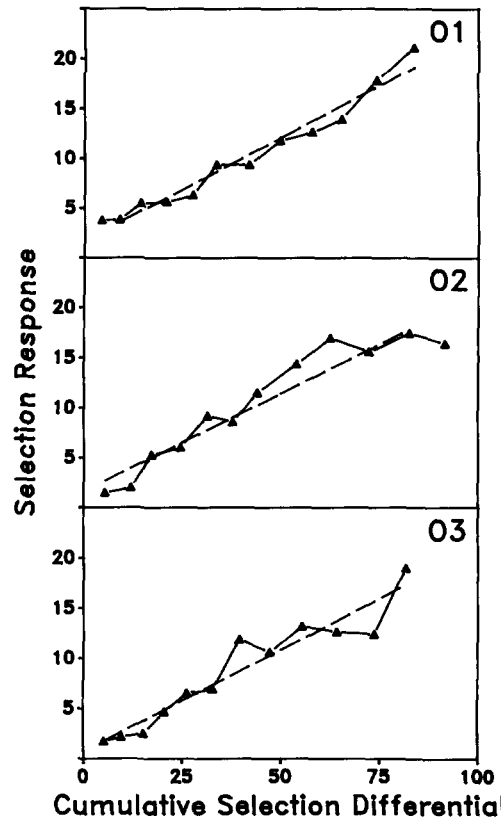

FIGURE 2.—The cumulative response to selection, measured relative to controls, for increased starvation resistance is plotted against the cumulative selection differential in the three O lines used. The slope of the regression is the realized heritability, because selection acts on both parents.

heritabilities or additive genetic variances among these populations. (Analysis not shown.) Artificial selection failed to eliminate genetic variability within the F and S lines. However, these lines are considerably farther apart after selection, offering some hope of clearer results from population crosses.

Diallel analysis of F and S lines: The results of the diallel analysis among F and S lines are shown in Tables 7, 8 and 9. (The missing entries arise from the design variations discussed in the MATERIALS AND METHODS.) There is little evidence for consistent between-line heterogeneity, maternal effects, or heterosis, since only 4 of 108 tests are significant at the 0.05 level, fewer than would be expected by chance. As was found in the analysis of the B and O populations, additive average combinations seem to arise when lines are crossed.

Transmission pattern: The crosses of F with S lines again can be used to test for the presence of: (i) differentiation between lines within treatments; (ii) differences between treatments; (iii) maternal effects; and (iv) directional dominance and the like.

Tables 10 and 11 give two different analyses of line differentiation, the first within experiments, the second over all experiments. While the first analysis indicates considerable differentiation between lines within individual experiments, the second analysis indicates such differentiation only for starvation resistance in the S lines and male longevity in the F lines.

With the more differentiated F and S lines, there is the prospect of greater clarity in the transmission pattern results. These results are shown in Table 12. Most of the tests for significant differentiation of F and S populations yield statistical significance, particularly those for fecundity. Most of the tests for maternal effects and dominance, shown in Tables 13 and 14, give nonsignificant results. There are five results with $P < 0.05$ out of 102 hypothesis tests, and one with $P < 0.01$, about what would be expected by chance.

An analysis of variance that combines results over all experiments is summarized in Table 15. All treatment differences remain significant, while dominance effects remain insignificant. A change is that one out of ten of the maternal and dominance effects tests gives a significant result, that for maternal effects on male starvation resistance. This result could be due to the effect of the *X* chromosome, however, rather than a nongenetic maternal effect, particularly in that the individual experimental results for FS2, FS3, and FS4 given in Table 13 indicate that the maternal genotype

TABLE 5

Realized heritabilities and variance components of selected characters in B and O populations during 11–14 generations of directional selection

Character and population	$h^2 \pm$ SE	V_P	V_A (% of V_P)	V_R[a] (% of V_P)	Total No. assayed
Fecundity					
B1	0.116 ± 0.040	379.6	44.0 (11.6%)	335.6 (88.4%)	4284
B2	0.120 ± 0.026	370.8	44.5 (12.0%)	326.3 (88.0%)	4962
B3	0.110 ± 0.018	422.6	46.5 (11.0%)	376.1 (89.0%)	3813
Female starvation					
O1	0.293 ± 0.016	87.5	25.6 (29.3%)	61.9 (70.7%)	4596
O2	0.211 ± 0.019	65.3	13.8 (21.1%)	51.5 (78.9%)	4434
O3	0.251 ± 0.020	60.2	15.1 (25.1%)	45.1 (74.9%)	4572
Male starvation					
O1	0.114 ± 0.013	88.7	10.1 (11.4%)	78.6 (88.6%)	4588
O2	0.188 ± 0.026	125.3	23.6 (18.8%)	101.7 (81.2%)	4433
O3	0.174 ± 0.024	105.2	18.3 (17.4%)	86.9 (82.6%)	4572
Mid-parent starvation					
O1	0.209 ± 0.013	47.7	10.0 (20.9%)	37.7 (79.0%)	9172
O2	0.196 ± 0.020	50.6	9.9 (19.6%)	40.7 (80.4%)	8866
O3	0.204 ± 0.020	44.4	9.1 (20.4%)	35.3 (79.5%)	9144

The number of generations with controls varied among the populations. B1 had 11 generations, B2 had 14 generations, B3 had 11 generations, and O1–O3 had 13 generations.

[a] V_R, the residual variance, contains V_D, the dominance variance, V_I, the interaction variance, and V_E, the environmental variance.

TABLE 6

Heritabilities and variance components of selected characters in F and S populations

Character and population	$h^2 \pm$ SE	V_P	V_A (% of V_P)	V_R[a] (% of V_P)	Total No. assayed
Fecundity					
F1	0.24 ± 0.14	455.7	109.2 (24.0%)	346.4 (76.0%)	387
F2	0.35 ± 0.17	382.6	134.1 (35.1%)	248.4 (64.9%)	341
F3	0.27 ± 0.16	475.8	128.9 (27.1%)	346.9 (72.9%)	323
S1	1.27 ± 0.24	451.2	573.7 (127.2%)	−122.5 (−27.2%)	405
S2	0.92 ± 0.25	305.0	281.1 (92.2%)	23.9 (7.8%)	312
S3	0.07 ± 0.14	453.3	32.5 (7.2%)	420.8 (92.8%)	302
Female starvation					
F1	0.50 ± 0.18	23.8	11.8 (49.6%)	12.0 (50.4%)	398
F2	0.45 ± 0.18	46.7	20.9 (44.7%)	25.9 (55.3%)	356
F3	0.51 ± 0.19	29.1	14.9 (51.3%)	14.2 (48.7%)	364
S1	0.78 ± 0.20	198.0	155.0 (78.3%)	43.0 (21.7%)	418
S2	0.11 ± 0.14	71.0	8.0 (11.2%)	63.1 (88.8%)	333
S3	0.65 ± 0.21	66.9	43.6 (65.2%)	23.3 (34.8%)	347
Male starvation					
F1	0.34 ± 0.16	17.0	5.8 (34.1%)	11.2 (65.9%)	398
F2	0.39 ± 0.17	27.5	10.6 (38.5%)	16.9 (61.5%)	356
F3	0.20 ± 0.14	20.1	3.9 (19.5%)	16.2 (80.5%)	364
S1	0.57 ± 0.18	108.0	61.1 (56.6%)	46.9 (43.4%)	418
S2	0.37 ± 0.18	82.4	30.4 (36.9%)	52.0 (63.1%)	333
S3	0.45 ± 0.18	48.2	21.9 (45.3%)	26.3 (54.7%)	347
Starvation					
F1	0.51 ± 0.18	12.5	6.4 (51.4%)	6.1 (48.6%)	398
F2	0.51 ± 0.19	21.5	10.9 (50.6%)	10.6 (49.4%)	365
F3	0.51 ± 0.19	12.8	6.6 (51.5%)	6.2 (48.5%)	364
S1	0.98 ± 0.22	82.8	81.4 (98.3%)	1.4 (1.7%)	418
S2	0.54 ± 0.20	44.4	24.1 (54.3%)	20.3 (45.7%)	333
S3	0.81 ± 0.23	36.1	29.1 (80.6%)	7.0 (19.4%)	347

[a] V_R, the residual variance, contains V_D, the dominance variance, V_I, the interaction variance, and V_E, the environmental variance.

TABLE 7

F and S diallel line differentiation

Character and experiment	ANOVA Mother *F*	Father *F*	Combined *F*
Fecundity			
DF2	0.34	0.90	0.58
DF3	1.60	1.75	2.00
DS2	1.32	0.71	1.04
Conditional fecundity			
DF2	1.15	2.00	1.24
DF3	1.63	1.93	2.11
DS2	0.97	1.25	0.94
Female starvation			
DF2	2.58	0.20	1.47
DF3	0.41	0.03	0.21
DS2	0.92	0.43	0.82
Male starvation			
DF2	0.26	0.14	0.15
DF3	7.37*	4.77	5.83
DS2	1.35	0.17	0.82

* $P < 0.05$.

TABLE 8

F and S diallel maternal effects

Character and experiment	ANOVA Method 1 *F*	Method 2 *F*
Fecundity		
DF1	7.15	
DF2	0.38	0.11
DF3	0.91	1.55
DS1	0.79	
DS2	1.86	0.06
Conditional fecundity		
DF1	2.95	
DF2	1.15	0.33
DF3	0.85	1.38
DS1	1.18	
DS2	0.78	0.03
Female starvation		
DF2	13.21	1.04
DF3	11.79	5.56
DS2	2.13	0.86
Male starvation		
DF2	1.80	0.42
DF3	1.50	1.30
DS2	7.78	2.08

(the first in the strain coding) has more influence than the paternal genotype. Quantitatively, this is plausible, because the average male starvation resistance difference between F and S lines is 19.36 hr, whereas the average maternal effect over these experiments is 2.4 hr, or 12.4% of the total difference. It seems reasonable to invoke loci on the *X* chromosome to explain this difference, because the *X* constitutes about 23% of the *D. melanogaster* genome (ASHBURNER 1989).

TABLE 9

F and S diallel heterosis effects

Character and experiment	Mean ± SEM Parentals	Crosses	*t*-test Separate *t*	Pooled *t*	ANOVA *F*
Fecundity (eggs/24 hr)					
DF1	46.2 ± 1.8	47.4 ± 0.8		0.62	0.39
DF2	88.3 ± 3.7	86.7 ± 3.6	0.30	0.27	0.06
DF3	70.0 ± 15.7	64.6 ± 7.0	0.31	0.37	0.14
DS1	27.2 ± 1.1	30.2 ± 0.6		2.51	6.27
DS2	73.6 ± 2.5	78.6 ± 1.7	1.65	1.68	3.08
Conditional fecundity (eggs/24 hr)					
DF1	47.0 ± 2.2	47.4 ± 0.8		0.17	0.03
DF2	91.4 ± 4.9	91.6 ± 2.4	0.04	0.14	0.001
DF3	71.7 ± 14.9	65.3 ± 6.9	0.39	0.45	0.21
DS1	28.1 ± 1.0	30.7 ± 0.3		2.51	6.36
DS2	78.7 ± 0.9	81.8 ± 1.5	1.71	1.31	2.24
Female starvation (hr)					
DF2	26.3 ± 1.0	27.2 ± 0.7	0.72	0.73	0.51
DF3	31.1 ± 0.4	28.6 ± 0.6	3.32*	2.62*	9.58*
DS2	40.6 ± 5.1	44.3 ± 3.7	0.59	0.58	0.36
Male starvation (hr)					
DF2	20.4 ± 1.1	22.7 ± 0.8	1.70	1.63	2.94
DF3	21.2 ± 0.6	22.1 ± 0.9	0.90	0.71	0.68
DS2	33.6 ± 5.1	34.4 ± 3.2	0.13	0.13	0.12

* $P < 0.05$.

TABLE 10

F and S line differentiation–1-way ANOVA

Character and experiment	ANOVA F lines	S lines
Fecundity		
FS1	8.21**	2.83
FS2	13.23**	31.58**
FS3	6.67**	1.11
FS4	11.12**	8.40**
Conditional fecundity		
FS1	8.11**	5.43**
FS2	11.25**	32.36**
FS3	2.84	2.70
FS4	16.68**	14.23**
Female starvation		
FS1	10.65**	69.37**
FS2	602.16**	42.90**
FS3	5.90**	71.10**
FS4	43.87**	24.53**
Male starvation		
FS1	12.66**	57.28**
FS2	700.41**	71.92**
FS3	2.93	73.42**
FS4	30.10**	18.45**
Female longevity		
FS1	2.22	26.94**
FS3	16.05**	5.96**
FS4	1.68	2.84
Male longevity		
FS1	11.48**	3.88*
FS3	4.34*	0.16
FS4	13.00**	1.18

* $P < 0.05$; ** $P < 0.01$.

TABLE 11

F and S line differentiation–2-way ANOVA

Character	ANOVA F lines *F*	ANOVA S lines *F*
Fecundity	0.36	1.32
Conditional fecundity	0.41	1.67
Female starvation	1.56	7.65*
Male starvation	0.40	35.13**
Female longevity	1.55	5.03
Male longevity	12.35*	0.29

* $P < 0.05$, ** $P < 0.01$.

DISCUSSION

For most characters, the present results continue to indicate essentially additive inheritance, averaged over loci, even when selection had produced more extreme differences between strains. The major exception to this conclusion is male starvation, which appears to be more influenced by the maternal than the paternal genotype. This could reflect an effect of the *X* chromsome or it could reflect a nongenetic maternal effect. The results of the present study therefore conform to those reported in HUTCHINSON and ROSE (1991), excepting only male starvation resistance. Since CLARE and LUCKINBILL (1985) and LUCKINBILL *et al.* (1987) did not study starvation resistance, the corresponding results in the present study also fit theirs.

What is the significance of these Drosophila results for our understanding of the genetics of aging in general? First, what of the many known alleles, from that which causes Huntington's chorea in man to those aberrant mutants in Drosophila with shortened lifespan? These alleles are often supposed to cause "accelerated aging," and are taken as evidence for few controlling elements for the aging process. In both man (MARTIN 1978) and Drosophila (HUTCHINSON

TABLE 12

F and S differences

Character and experiment	Mean ± SEM F	Mean ± SEM S	*t*-test Indep. *t*	*t*-test Paired *t*	ANOVA *F*
Fecundity (eggs/24 hr)					
FS1	77.0 ± 2.7	54.5 ± 1.8	6.95**	17.66**	310.58**
FS2	92.6 ± 5.7	72.6 ± 5.3	2.55	18.10**	332.69**
FS3	106.7 ± 5.1	80.6 ± 1.5	4.88**	6.75*	50.06*
FS4	88.4 ± 4.3	72.1 ± 2.7	3.18*	9.55*	90.66*
FS5	122.3 ± 3.3	100.0 ± 2.7			27.38**
Conditional fecundity (eggs/24 hr)					
FS1	78.1 ± 2.2	57.2 ± 1.8	7.29**	32.84**	1070.32**
FS2	96.1 ± 4.1	73.3 ± 5.0	3.52*	10.54**	116.40**
FS3	109.9 ± 2.6	83.9 ± 1.9	7.99**	15.27**	259.25**
FS4	90.2 ± 4.5	72.9 ± 3.1	3.19*	12.49**	150.59**
FS5	125.5 ± 2.4	102.5 ± 2.1			51.05**
Female starvation (hr)					
FS1	35.3 ± 2.0	67.4 ± 7.0	4.42*	3.60	13.02
FS2	32.0 ± 8.7	39.5 ± 3.9	0.79	0.66	0.42
FS3	25.5 ± 1.3	47.3 ± 6.2	3.47*	3.62	11.55
FS4	38.7 ± 3.9	65.7 ± 4.4	3.78*	6.50*	45.41*
FS5	31.7 ± 0.9	51.2 ± 1.6			114.39**
Male starvation (hr)					
FS1	24.2 ± 1.7	51.7 ± 5.4	4.89**	4.71*	22.15*
FS2	23.9 ± 7.3	32.8 ± 4.5	1.03	0.78	0.59
FS3	18.5 ± 0.8	39.3 ± 5.4	3.82*	3.90	13.22
FS4	25.0 ± 2.2	48.7 ± 4.4	10.09**	4.88*	106.33**
FS5	24.5 ± 0.7	40.4 ± 1.4			108.68**
Female longevity (days)					
FS1	35.9 ± 1.1	53.8 ± 4.9	3.52*	3.10	9.64
FS3	32.1 ± 3.3	49.6 ± 2.7	4.09*	3.78	14.28
FS4	33.9 ± 0.9	50.9 ± 1.7	8.49**	11.15*	123.46**
FS5	36.3 ± 1.4	46.2 ± 1.9			17.98**
Male longevity (days)					
FS1	34.4 ± 2.2	53.9 ± 2.1	6.36*	8.68**	74.91*
FS3	31.6 ± 1.3	47.5 ± 0.4	15.08**	23.59**	556.63**
FS4	32.2 ± 2.7	52.9 ± 1.1	7.08**	5.76*	33.33*
FS5	31.8 ± 1.3	49.0 ± 1.9			55.39**

* $P < 0.05$, ** $P < 0.01$.

TABLE 13

F and S maternal effects

Character and experiment	Mean ± SEM FS	Mean ± SEM SF	*t*-test Indep. *t*	*t*-test Paired *t*	ANOVA *F*
Fecundity (eggs/24 hr)					
FS1	68.4 ± 4.5	65.5 ± 5.1	0.43	2.39	0.14
FS2	85.2 ± 6.6	88.2 ± 3.0	0.41	0.69	0.49
FS3	101.2 ± 3.7	101.1 ± 4.5	0.04	0.09	0.01
FS4	76.2 ± 6.8	76.7 ± 3.4	0.08	0.17	0.03
FS5	116.6 ± 4.4	114.0 ± 3.9			0.20
Conditional fecundity (eggs/24 hr)					
FS1	68.4 ± 4.5	66.0 ± 5.0	0.37	3.04	9.23
FS2	87.0 ± 6.0	89.6 ± 3.5	0.37	0.90	0.49
FS3	103.0 ± 3.8	101.0 ± 4.5	0.34	0.93	0.88
FS4	77.3 ± 6.1	77.4 ± 4.0	0.01	0.04	0.00
FS5	119.5 ± 3.4	114.0 ± 3.9			1.15
Female starvation (hr)					
FS1	47.7 ± 3.5	46.9 ± 1.6	0.20	0.36	0.13
FS2	30.6 ± 2.0	31.3 ± 3.2	0.17	0.55	0.31
FS3	34.0 ± 4.8	35.6 ± 4.2	0.81	1.51	2.21
FS4	51.4 ± 2.5	55.4 ± 4.7	0.75	1.75	3.08
FS5	35.4 ± 1.3	34.1 ± 1.4			0.48
Male starvation (hr)					
FS1	36.1 ± 2.5	37.0 ± 1.9	0.52	2.23	4.99
FS2	20.7 ± 2.2	26.2 ± 2.8	1.65	5.61*	30.39*
FS3	24.5 ± 2.9	30.0 ± 2.7	1.40	6.09*	38.29*
FS4	34.2 ± 3.2	39.2 ± 4.6	0.89	2.10	4.35
FS5	33.6 ± 1.9	28.7 ± 1.2			4.75
Female longevity (days)					
FS4	44.4 ± 0.9	42.3 ± 2.1	0.92	1.75	3.04
FS5	42.0 ± 1.5	41.5 ± 2.3			0.09
Male longevity (days)					
FS4	41.2 ± 2.9	42.7 ± 4.1	0.27	1.23	1.49
FS5	38.5 ± 2.0	40.9 ± 1.9			0.74

* $P < 0.05$.

TABLE 14

F and S average dominance effects

Character and experiment	Mean ± SEM Parentals	Mean ± SEM Crosses	*t*-test Indep. *t*	*t*-test Paired *t*	ANOVA *F*
Fecundity (eggs/24 hr)					
FS1	65.7 ± 2.2	66.9 ± 4.7	0.23	0.47	0.22
FS2	82.2 ± 5.8	86.7 ± 4.7	0.60	1.73	2.93
FS3	91.6 ± 4.0	101.1 ± 4.0	1.69	2.08	4.49
FS4	80.7 ± 3.1	76.5 ± 5.0	0.73	2.10	4.39
FS5	111.0 ± 2.3	115.3 ± 2.9			1.24
Conditional fecundity (eggs/24 hr)					
FS1	67.8 ± 2.0	67.2 ± 4.7	0.13	0.25	0.06
FS2	84.0 ± 4.8	88.3 ± 4.7	0.63	1.91	3.67
FS3	94.7 ± 3.4	102.0 ± 4.0	1.38	2.14	4.71
FS4	82.0 ± 3.3	77.3 ± 5.0	0.78	2.75	7.54
FS5	113.9 ± 1.9	116.7 ± 2.6			0.81
Female starvation (hr)					
FS1	51.6 ± 2.8	47.3 ± 2.5	1.13	1.37	1.87
FS2	36.3 ± 3.7	30.9 ± 2.6	1.19	1.56	2.37
FS3	38.3 ± 4.2	34.8 ± 4.5	0.58	1.37	2.00
FS4	51.3 ± 4.4	53.4 ± 3.6	0.38	0.92	0.86
FS5	41.4 ± 1.2	34.8 ± 1.0			13.43**
Male starvation (hr)					
FS1	37.9 ± 2.7	36.9 ± 2.2	0.30	1.51	2.27
FS2	29.0 ± 2.2	23.5 ± 2.4	1.68	1.77	3.06
FS3	30.7 ± 3.7	27.2 ± 2.8	0.76	1.71	2.94
FS4	35.9 ± 3.1	36.8 ± 3.7	0.18	0.80	0.63
FS5	32.5 ± 1.0	31.1 ± 1.1			0.68
Female longevity (days)					
FS2	45.0 ± 2.2	45.3 ± 4.1	0.08	0.16	0.03
FS3	40.7 ± 2.0	47.6 ± 2.9	1.93	3.85	14.83
FS4	42.5 ± 1.2	43.3 ± 1.5	0.44	3.08	9.43
FS5	41.2 ± 1.2	42.0 ± 1.5			0.70
Male longevity (days)					
FS1	44.2 ± 1.9	43.5 ± 1.5	0.32	1.57	2.46
FS3	36.9 ± 0.9	42.8 ± 2.0	2.21	4.29	18.29
FS4	42.5 ± 1.0	41.9 ± 3.5	0.15	0.20	0.04
FS5	40.3 ± 1.3	39.7 ± 1.4			0.77

** $P < 0.01$.

TABLE 15

F and S differences, naternal effects, and average dominance effects–3-way ANOVA

Character	ANOVA DIF *F*	ANOVA MAT *F*	ANOVA DOM *F*
Fecundity	142.64*	0.03	0.48
Conditional fecundity	153.02**	1.55	0.18
Female starvation	16.14*	5.69	2.54
Male starvation	18.14*	16.49*	6.91
Female longevity	56.30*		1.33
Male longevity	560.98*		0.34

* $P < 0.05$, ** $P < 0.01$.

and ROSE 1987), mutants of this kind are only doubtfully aging mutants. They may kill adults, and induce chronic pathologies, but that is not evidence that they affect aging itself. Close inspection of their pathophysiology reveals a number of disparities with respect to "normal aging" (MARTIN 1978). Therefore, such alleles may not be of relevance to the genetic dissection of aging.

Second, are there any known alleles that can postpone aging? Such alleles are known in both *D. subobscura* (MAYNARD SMITH 1958) and *Caenorhabditis elegans* (FRIEDMAN and JOHNSON 1988). In both these studies, lifespan is increased by homozygosity of a single allele as much or more than it is in the *D. melanogaster* stocks of ROSE (1984) or LUCKINBILL *et al.* (1984). Interestingly, in both these cases, reproduction is greatly decreased in the longer-lived mutant strain. The *D. subobscura* mutants are in fact completely sterile (MAYNARD SMITH 1958). In a physiological sense, these other studies corroborate the results of ROSE and CHARLESWORTH (1981a,b), ROSE (1984), and LUCKINBILL and CLARE (1985) in finding a clear association between postponed aging and reduced early reproduction. However, the dramatic effects of these single mutants arise by genetic transmission patterns quite unlike those of the present system, in which no such large effect alleles are apparent.

Third, is there any likelihood that the *D. melanogaster* results indicating mostly additive combination

of lines will prove to be generally true of the genetics of aging? We would argue that the finding of additive inheritance is likely to hold generally. Many loci should affect later survival and reproduction, because survival and reproduction are the ends which natural selection strives toward. Loci that do not have alleles that directly or indirectly foster survival or reproduction are not going to be preserved, because natural selection will not oppose the accumulation of silencing mutations at those loci. Maintenance of polymorphism at some of those loci affecting aging is likely, because both of the population genetic mechanisms of aging, antagonistic pleiotropy (WILLIAMS 1957; ROSE 1985) and mutation accumulation (MEDAWAR 1952; EDNEY and GILL 1968; CHARLESWORTH 1980), act to maintain genetic polymorphism. Antagonistic pleiotropy can do so by generating overdominance and its higher-order analogs (ROSE 1982, 1985). Mutation accumulation can do so because it allows mutations affecting later survival and reproduction to drift to high frequencies, because of the weakness of natural selection at later ages (CHARLESWORTH 1980). Therefore, almost all outbred species are likely to have allelic variation affecting aging at a great many loci, allelic variation which could be selected so as to postpone aging. With many loci comes the expectation that all their individual dominance patterns will average out to give additivity at the level of population crosses and responses to selection.

The authors are grateful to P. M. SERVICE for help in the planning and execution of these experiments. We are also grateful to L. D. MUELLER for his comments on an earlier draft of the manuscript. We thank H. GILLIS, K. GRIMM, L. E. JOHNSTON, S. JOHNSON, D. M. LANE, J. JUDAH, B. MUSGRAVE, J. NELSON, D. PRINGLE and D. STEWART for technical assistance. This research was supported by Natural Sciences and Engineering Research Council of Canada grant U0178 and U.S. Public Health Service grant AG06346 to M.R.R., as well as U.S. Public Health Service grant AG08322 to THOMAS E. JOHNSON.

LITERATURE CITED

ASHBURNER, M., 1989 *Drosophila, A Laboratory Handbook.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

CHARLESWORTH, B., 1980 *Evolution in Age-structured Populations.* Cambridge University Press, Cambridge.

CLARE, M. J., and L. S. LUCKINBILL, 1985 The effects of gene-environment interaction on the expression of longevity. Heredity **55:** 19–29.

EDNEY, E. B., and R. W. GILL, 1968 Evolution of senescence and specific longevity. Nature **220:** 281–282.

FALCONER, D. S., 1981 *Introduction to Quantitative Genetics,* Ed. 2. Longman, New York.

FRIEDMAN, D. B., and T. E. JOHNSON, 1988 A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphroditic fertility. Genetics **118:** 75–86.

HUTCHINSON, E. W., and M. R. ROSE, 1987 Genetics of aging in insects. Rev. Biol. Res. Aging **3:** 63–70.

HUTCHINSON, E. W.., and M. R. ROSE., 1991 Quantitative genetics of postponed aging in *Drosophila melanogaster.* 1. Analysis of outbred populaions. Genetics **127:** 719–727.

LUCKINBILL, L. S., and M. J. CLARE, 1985 Selection for life span in *Drosophila melanogaster.* Heredity **55:** 9–18.

LUCKINBILL, L. S., R. ARKING, M. J. CLARE, W. C. CIROCCO and S. A. BUCK, 1984 Selection for delayed senescence in *Drosophila melanogaster.* Evolution **39:** 996–1003.

LUCKINBILL, L. S., M. J. CLARE, W. L. KRELL, W. C. CIROCCO and P. A. RICHARDS, 1987 Estimating the number of genetic elements that defer senescence in *Drosophila.* Evol. Ecol. **1:** 37–46.

LUCKINBILL, J. L. GRAVES, A. H. REED and S. KOETSAWANG, 1988 Localizing genes that defer senescence in *Drosophila melanogaster.* Heredity **60:** 367–374.

MARTIN, G. M., 1978 Genetic syndromes in man with potential relevance to the pathobiology of aging. Natl. Found. **14:** 5–39.

MAYNARD SMITH, J., 1958 The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura.* J. Exp. Biol. **35:** 832–842.

MEDAWAR, P. B., 1952 *An Unsolved Problem of Biology.* H. K. Lewis, London.

ROSE, M. R., 1982 Antagonistic pleiotropy, dominance, and genetic variation. Heredity **48:** 63–78.

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

ROSE, M. R., 1985 Life history evolution with antagonistic pleiotropy and overlapping generations. Theor. Popul. Biol. **28:** 342–358.

ROSE, M. R., and B. CHARLESWORTH, 1981a Genetics of life his tory in *Drosophila melanogaster.* I. Sib analysis of adult females. Genetics **97:** 173–186.

ROSE, M. R., and B. CHARLESWORTH, 1981b Genetics of life his tory in *Drosophila melanogaster.* II. Exploratory selection experiments. Genetics **97:** 187–196.

SAS INSTITUTE INC., 1988 SAS/STAT User's Guide: Release 6.03. SAS Institute Inc., Cary, N.C.

SERVICE, P. M., and M. R. ROSE, 1985 Genetic covariation among life-history components: the effect of novel environ ments. Evolution **39:** 943–945.

SERVICE, P. M., E. W. HUTCHINSON, M. D. MACKINLEY and M. R. ROSE, 1985 Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. Physiol. Zool. **58:** 380–389.

WILKINSON, L., 1988 SYSTAT: The System for Statistics. Systat Inc., Evanston, Ill.

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

Communicating editor: A. G. Clark