Quantitative Genetics of Postponed Aging in *Drosophila melanogaster*. I. Analysis of Outbred Populations

Edward W. Hutchinson[1] and Michael R. Rose[2]

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, 1989
Accepted for publication December 13, 1990

ABSTRACT

Selection has been used to create replicated outbred stocks of *Drosophila melanogaster* with increased longevity, increased later fecundity, and increased levels of physiological performance at later ages. The present study analyzed the quantitative transmission patterns of such stocks, employing extensive replication in numbers of stocks, individuals, and assayed characters. The populations used derived from five lines with postponed aging and five control lines, all created in 1980 from the same founding base population. The following characters were studied: early 24-hr fecundity, early ovary weight, early female starvation resistance, early male starvation resistance, female longevity and male longevity. Numerous crosses were performed to test for non-Mendelian inheritance, average dominance, maternal effects, sex-linkage and between-line heterogeneity. There was only slight evidence for any of these phenomena arising reproducibly in the characters studied. These findings suggest the value of this set of stocks for studies of the physiological basis of postponed aging.

DROSOPHILA melanogaster is one of only two species for which stocks having genetically postponed aging are available (ROSE 1984; LUCKINBILL *et al.* 1984), the other being *Caenorhabditis elegans* (JOHNSON and WOOD 1982; JOHNSON 1987; FRIEDMAN and JOHNSON 1988). These *D. melanogaster* stocks were not created by mutagenesis, but by selection on quantitative genetic variability. It has not proven practical to use mutagenesis to produce *Drosophila* stocks with postponed aging (*e.g.* ROBERTS and IREDALE 1985), with one possible exception (LEFFELAAR and GRIGLIATTI 1984). This limitation on genetic analysis arises because *Drosophila* spp. are subject to inbreeding depression for fitness-related characters, like longevity (CLARKE and MAYNARD SMITH 1955), making the isolation of mutant strains with postponed aging very difficult.

The use of selection is feasible for two reasons. First, there is abundant quantitative genetic variability for life-historical characters in outbred stocks of *D. melanogaster* (ROSE and CHARLESWORTH 1981a,b). Secondly, an indirect selection procedure can be used, in which natural selection is directed to act at later ages by the use of eggs laid by older females exclusively. When applied repeatedly over a number of generations, this type of selective screen leads to the evolution of postponed aging (WATTIAUX 1968a,b; ROSE and CHARLESWORTH 1981b; ROSE 1984; LUCKINBILL *et al.* 1984). This procedure also involves population sizes large enough (300–3000) that population size alone should not radically limit the gains made by selection (*cf.* YOO 1980; WEBER 1990; WEBER and DIGGINS 1990).

The *D. melanogaster* populations that have been created using these methods have been analyzed in two different ways. First, these populations have been compared with control populations, of identical origin but lacking postponed aging, with regard to morphology (ROSE *et al.* 1984; LUCKINBILL *et al.* 1988a) and physiology (SERVICE *et al.* 1985; SERVICE 1987; LUCKINBILL *et al.* 1988a; GRAVES, LUCKINBILL and NICHOLLS 1988). This research has endeavored to find mechanisms that could causally account for the postponed aging of the selected lines. Second, one of these populations has been examined genetically by means of biometrical analysis (CLARE and LUCKINBILL 1985; LUCKINBILL *et al.* 1987) and chromosomal substitution (LUCKINBILL *et al.* 1988b). The present article combines both of these avenues of research in an attempt to ascertain the quantitative genetic basis of postponed aging in *D. melanogaster*.

Of particular concern for the present work is the degree of replication in the studies of CLARE and LUCKINBILL (1985) and LUCKINBILL *et al.* (1987). Those studies used a single pair of long-lived and control populations, measuring longevity and fecundity alone among the characters that have responded to selection for postponed aging (*cf.* SERVICE *et al.* 1985; LUCKINBILL *et al.* 1988a). The present study analyzes five different stocks having postponed senescence, comparing them with five different control stocks. In addition, these stocks were not derived from

[1] Present address: Institute for Behavioral Genetics, Box 447, University of Colorado, Boulder, Colorado 80309.
[2] To whom all correspondence should be addressed at the University of California.

TABLE 1

B diallel experiments

Experiment	Character assayed	Populations assayed per character	Mean No. assayed per population	Total No. assayed
DB1	Ovary weight	5 × 5 = 25	29.6	740
	Female starvation	5 × 5 = 25	54.7	1368
DB2	Ovary weight	3 × 3 = 9	26.1	235
Total		59		2343

TABLE 2

O diallel experiments

Experiment	Character assayed	Populations assayed per character	Mean No. assayed per population	Total No. assayed
DO1	Ovary weight	5 × 5 = 25	28.0	700
	Female starvation	5 × 5 = 25	55.4	1384
DO2	Female starvation	5 × 5 = 25	52.8	1321
DO3	Ovary weight	3 × 3 = 9	26.0	234
DO4	Female starvation	5 × 5 = 25	25.6	639
	Male starvation	5 × 5 = 25	25.4	636
Total		134		4914

TABLE 3

B and O crossing experiments

Experiment	Character assayed	Populations assayed per character	Mean No. assayed per population	Total No. assayed
BO1	Ovary weight	3 × 5 = 15	29.5	443
	Female starvation	4 × 5 = 20	31.2	624
	Male starvation	4 × 5 = 20	31.2	624
BO2	Ovary weight	4 × 5 = 20	30.0	600
	Fecundity	4 × 5 = 20	58.9	1,177
	Conditional fecundity	4 × 5 = 20	58.9	1,177
	Female starvation	4 × 5 = 20	54.0	1,080
	Female longevity	4 × 5 = 20	57.0	1,140
BO3	Ovary weight	4 × 3 = 12	45.2	542
	Female starvation	4 × 3 = 12	40.0	480
	Female longevity	4 × 3 = 12	49.9	599
BO4	Female starvation	4 × 3 = 12	33.7	404
BO5	Fecundity	4 × 3 = 12	76.5	918
	Conditional fecundity	4 × 3 = 12	75.3	903
	Female starvation	4 × 3 = 12	78.3	940
	Male starvation	4 × 3 = 12	78.3	940
BO6	Fecundity	4 × 3 = 12	77.4	929
	Conditional fecundity	4 × 3 = 12	75.9	911
	Female starvation	4 × 3 = 12	78.8	945
	Male starvation	4 × 3 = 12	78.8	945
	Female longevity	3 × 3 = 9	98.9	890
	Male longevity	3 × 3 = 9	98.9	886
BO7	Fecundity	4 × 3 = 12	70.1	841
	Conditional fecundity	4 × 3 = 12	69.3	831
	Female starvation	4 × 3 = 12	71.8	863
	Male starvation	4 × 3 = 12	71.8	861
	Female longevity	4 × 3 = 12	58.5	702
	Male longevity	4 × 3 = 12	57.8	694
BO8	Fecundity	4 × 1 = 4	59.0	236
	Conditional fecundity	4 × 1 = 4	58.0	232
	Female starvation	4 × 1 = 4	60.0	240
	Male starvation	4 × 1 = 4	60.0	240
	Female longevity	4 × 1 = 4	58.2	233
	Male longevity	4 × 1 = 4	57.3	229
Total		413		24,299

the stocks analyzed by CLARE and LUCKINBILL (1985) and LUCKINBILL *et al.* (1987). Results which are general to all these lines might therefore be general to the species as a whole.

The basic techniques used in the present analysis involve crosses of populations, both within the selected and control groups of lines and between the selected and control groups of lines. With some exceptions, we find that our results tend to indicate additive inheritance among populations and little differentiation between lines within a given type, postponed aging or control.

MATERIALS AND METHODS

Stocks: The postponed-aging stocks, called "O"s, that were used in the present study were derived by culture selection from the Ives population studied by ROSE and CHARLESWORTH (1981a,b). The date of founding for these stocks, and their controls, was February, 1980. The culture selection procedure involved stock maintenance using discrete generations and females of progressively greater age. Eventually, the females used were 70 days of age from the egg. For more details, consult ROSE (1984) or SERVICE *et al.* (1985). These stocks exhibit increased male and female longevities, decreased early fecundity, decreased early ovary weight, increased resistance to certain stresses, decreased early metabolic rate, increased lipid content, decreased early locomotor activity, and increased later locomotor activity (ROSE 1984; ROSE *et al.* 1984; SERVICE *et al.* 1985; SERVICE 1987), relative to the control stocks. The control stocks, called "B"s, were maintained using the same media and procedures as the O stocks, except that females of 14 days of age were used to start each generation. Population sizes for all stocks were of the order of 10^3.

Culture media: The medium used for culture maintenance and serial transfer of adults, when fecundity was not measured, was banana-molasses, as in ROSE (1984). Charcoal high-agar medium with yeast paste on the surface was used for fecundity counts, as in ROSE and CHARLESWORTH (1981a). The flies were kept in shell vials when they were cultured and handled, except for maintenance of the O culture adults, which were kept in cages with the medium replenished every 2–3 days.

Assays: With the exceptions indicated below, all assayed flies were reared at a density of 30 larvae per vial. Longevity was measured in groups of four to five mated pairs, transferred every four to five days. Fecundity was measured as the total number of eggs laid in 24 hr by one female, aged three to five days from pupal eclosion, kept with a single male. The term "conditional fecundity" (ROSE and CHARLESWORTH 1981a) refers to fecundity data in which zero fecundity is treated as a missing data point. Ovaries were obtained from females also aged 3–5 days, dried for 24 hr,

TABLE 4

B and O diallel heterosis effects

Character and experiment	Mean ± SEM		*t*-test		ANOVA
	Parentals	Crosses	Separate *t*	Pooled *t*	*F*
Ovary weight (mg)					
DB1	0.136 ± 0.011	0.142 ± 0.002	0.54	0.78	0.62
DB2	0.172 ± 0.008	0.163 ± 0.010	0.75	0.61	0.33
DO1	0.068 ± 0.014	0.083 ± 0.005	0.98	1.18	1.40
DO3	0.136 ± 0.021	0.136 ± 0.007	0.03	0.03	0.001
Female starvation (hr)					
DB1	37.9 ± 2.2	38.1 ± 0.7	0.09	0.12	0.01
DO1	36.1 ± 1.7	37.0 ± 0.9	0.47	0.46	0.21
DO2	65.3 ± 4.0	59.9 ± 1.5	1.27	1.53	2.28
DO4	35.5 ± 1.5	34.5 ± 0.7	0.58	0.61	0.52
Male starvation (hr)					
DO4	27.4 ± 1.0	28.4 ± 0.4	0.97	1.02	1.52

TABLE 5

B and O diallel line differentiation

Character and experiment	ANOVA		
	Mother *F*	Father *F*	Combined *F*
Ovary weight			
DB1	0.28	1.15	0.71
DB2	0.75	0.57	0.67
DO1	4.72*	1.06	2.89*
DO3	34.03**	0.06	17.10**
Female starvation			
DB1	1.38	2.63	2.04
DO1	1.86	1.60	1.74
DO2	2.44	5.27*	3.91**
DO4	0.98	1.45	1.25
Male starvation			
DO4	1.03	2.74	1.92

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

TABLE 6

B and O diallel maternal effects

Character and experiment	ANOVA	
	Method 1 *F*	Method 2 *F*
Ovary weight		
DB1	0.24	0.98
DB2	1.32	2.45
DO1	4.46	14.78*
DO3	576.84**	40.98*
Female starvation		
DB1	0.53	0.67
DO1	1.17	0.96
DO2	0.46	0.41
DO4	0.67	0.54
Male starvation		
DO4	0.38	0.57

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

and then weighed separately in the pairs that came from single females using a Cahn electronic microbalance, as in ROSE *et al.* (1984). Except as noted, starvation resistance was measured as the number of hours that a fly aged 3–5 days would respond to provocation in a vial prepared without medium, but provided with rayon saturated with water to sustain humidity, sealed with Parafilm, as in SERVICE *et al.* (1985).

Diallel analysis: A series of diallel analyses (MATHER and JINKS 1982) were performed to test for heterogeneity among lines of a given type. In a diallel design, all possible crosses are performed between a set of populations, including the distinct reciprocal crosses. The coding for these experiments involves three character positions: in the first position, *D* indicates a diallel experiment; in the second position, *B* or *O* indicates whether the populations used were B or O stocks; and the character in the third position is a numeral indicating which experiment it was, in chronological order. Thus the first experiment performed was DB1. Tables 1 and 2 give the experiment codes, the characters assayed, the populations assayed (*e.g.* 5 × 5 indicating all possible crosses of the five replicate populations of a type; 3 × 3 indicating all possible crosses of the first three populations), and the number of individuals assayed. In experiment DO2, starvation was assayed in individuals of 17–20 days of age from pupal eclosion. Each indicated experiment is entirely independent from any other; the same data are not incorporated in more than one experiment.

Transmission pattern experiments: The series of experiments on transmission of postponed aging characters is outlined in Table 3. These experiments are coded with "BO" in the first two positions, indicating crosses of B and O populations. The numerals then refer to the specific experiments. In all these experiments, but one (BO1), B and O parental populations were assayed together with both their reciprocal crosses. (This gives rise to the "4×" terms.) In most experiments, either all five replicates of each type were used or three replicates of each type were used. Crossings used the correspondingly subscripted populations (which has no significance), so that B_1 was always crossed to O_1 and so on for stocks two to five of each stock-type. In this way, each cross was independent of every other cross of the same type. In experiment BO4, starvation was assayed at 17–20 days of age. In experiment BO7, the flies for assay were reared at a density of 90 per vial. In experiment BO8, the parental B and O lines were obtained by a synthetic

TABLE 7

B and O line differentiation–1-way ANOVA

Character and experiment	ANOVA B lines	ANOVA O lines
Ovary weight		
BO1	3.03*	
BO2	5.06**	5.98**
BO3	0.30	23.18**
Fecundity		
BO2	29.45**	49.13**
BO5	12.32**	2.65
BO6	2.09	2.79
BO7	74.54**	27.98**
Conditional fecundity		
BO2	29.45**	49.13**
BO5	11.63**	5.81**
BO6	7.13**	1.20
BO7	115.67**	43.95**
Female starvation		
BO1	3.90**	1.02
BO2	7.20**	27.35**
BO3	3.12*	1.58
BO4	1.65	1.58
BO5	8.66**	4.71**
BO6	8.78**	14.34**
BO7	4.08*	5.95**
Male starvation		
BO1	2.86*	2.95*
BO5	3.72*	0.55
BO6	41.35**	18.76**
BO7	4.47*	0.90
Female longevity		
BO2	12.34**	8.06**
BO3	8.07**	8.27**
BO6	11.33**	5.45**
BO7	2.03	0.74
Male longevity		
BO6	0.24	2.77
BO7	3.12*	0.11

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

cross of three B and three O stocks, respectively.

General statistical procedures: All data analysis was performed using SAS (SAS Institute 1988) and SYSTAT (WILKINSON 1988) software packages, which treat data analysis with unbalanced designs, but do not interpolate data. Data were analyzed without transformation and with both WRIGHT's (1968) and Taylor power law (DOWNING 1979) transformations. Neither transformation changed the results of any hypothesis test, so only the data analysis with untransformed data is reported. Throughout the tables, a single asterisk (*) is used to indicate a result with $P < 0.05$, while a double asterisk (**) is used to indicate a result with $P < 0.01$.

RESULTS

Diallel analysis: There are three separate questions which we will address in the diallel analysis. First, is there any evidence for heterosis, or conversely inbreeding depression, in crosses between lines within either B or O stocks? The data were analyzed using both *t*-tests and a nested analysis of variance, the null hypothesis under test being no difference between the average of the two parental lines and the average of the two reciprocal crosses. The results are shown in Table 4. The two types of *t*-test in this table differ with respect to both the pooling of the sample variances in the calculation of the *t*-statistic and the number of degrees of freedom. The null hypothesis is not rejected, indicating an absence of heterosis, whatever way the data are analyzed.

TABLE 8

B and O line differentiation–2-way ANOVA

Character	ANOVA B lines *F*	ANOVA O lines *F*
Ovary weight	0.56	0.35
Fecundity	0.66	1.86
Conditional fecundity	0.59	1.77
Female starvation	0.99	2.61
Male starvation	0.77	0.49
Female longevity	2.44	1.81
Male longevity	1.28	2.88

Second, to what extent are the lines within a stock-type differentiated from each other? This was analyzed using a mixed analysis of variance model in which the maternal and paternal lines used in each cross appear as factorial design components. In this design, the *F*-statistic determines whether the ratio of the line-component variance to the within-line component is significantly greater than expected by chance. The results are shown in Table 5. In some cases, there were significant differences between lines, but these were not reproducible between experiments. This statistical design confounds the effects of a line with the choice of parents from that line. This can lead to the erroneous inference of a line effect when none is present. Therefore, the absence of reproducible line effects suggests that the results are ambiguous in significance. More light is shed on this question in the analysis of the B × O experiments discussed below.

Third, to what extent do maternal effects outweigh paternal effects, within stock-types? This question is addressed with greater experimental power in the transmission pattern experiments, but there is the possibility that it arises in the diallel designs. This question was examined using the *F*-statistic produced by the ratio of female parent to male parent variance components in the diallels. ANOVA methods 1 and 2 differ with respect to inclusion (method 1) or exclusion (method 2) of the uncrossed parental lines in the data analysis. The results from the two methods differed only in the analysis of experiment DO1. As shown in Table 6, significant maternal effects arise only in the cases in which there were significant line

TABLE 9

B and O differences

Character and experiment	Mean ± SEM		*t*-test		ANOVA
	B	O	Indep. *t*	Paired *t*	*F*
Ovary weight (mg)					
BO2	0.109 ± 0.009	0.057 ± 0.006	4.61**	3.75*	14.09*
BO3	0.177 ± 0.003	0.112 ± 0.022	2.94*	3.14	9.65
Fecundity (eggs/24 hours)					
BO2	102.1 ± 3.8	92.3 ± 6.0	1.38	3.86*	14.74*
BO5	80.7 ± 4.3	85.0 ± 2.0	0.92	1.91	3.70
BO6	94.2 ± 1.9	93.3 ± 2.5	0.26	0.42	0.18
BO7	81.9 ± 9.7	87.3 ± 6.3	0.47	0.82	0.68
BO8	95.2 ± 4.0	98.6 ± 2.2			0.57
Conditional fecundity (eggs/24 hr)					
BO2	102.1 ± 3.8	92.3 ± 6.0	1.38	3.86*	14.74*
BO5	82.2 ± 3.6	86.2 ± 2.6	0.90	3.16	10.42
BO6	96.3 ± 2.9	95.6 ± 1.4	0.22	0.26	0.06
BO7	83.2 ± 10.1	88.9 ± 6.6	0.47	0.80	0.64
BO8	98.9 ± 3.5	98.6 ± 2.2			0.01
Female starvation (hr)					
BO1	28.7 ± 0.9	32.7 ± 0.6	3.74**	2.88*	8.25*
BO2	47.3 ± 2.7	68.2 ± 6.3	3.05*	4.10*	16.65*
BO3	44.8 ± 1.6	55.5 ± 1.3	5.19**	7.10*	50.40*
BO4	39.6 ± 2.5	47.2 ± 2.0	2.38	10.99*	116.65**
BO5	27.9 ± 1.3	35.8 ± 1.2	4.42*	7.47*	56.19*
BO6	27.3 ± 1.0	33.8 ± 2.0	2.89*	6.41*	40.92*
BO7	34.3 ± 1.0	48.1 ± 1.8	6.54*	6.62*	43.78*
BO8	27.2 ± 0.8	35.4 ± 0.9			45.51**
Male starvation (hr)					
BO1	19.9 ± 0.8	25.6 ± 0.9	5.04**	10.45**	109.94**
BO5	17.8 ± 0.6	26.7 ± 0.4	12.96**	53.07**	2799.13**
BO6	20.0 ± 1.8	31.0 ± 2.6	3.48*	13.86**	191.88**
BO7	24.3 ± 0.8	36.4 ± 0.5	12.45**	11.62**	135.08**
BO8	21.3 ± 0.8	26.1 ± 0.6			25.02**
Female longevity (days)					
BO2	50.1 ± 2.7	62.5 ± 2.7	3.26*	9.15**	76.07**
BO3	25.2 ± 2.9	48.3 ± 2.7	5.86**	5.86*	34.32*
BO6	40.4 ± 2.3	51.4 ± 2.2	3.48*	2.48	6.15
BO7	36.6 ± 1.1	48.6 ± 0.9	8.33**	8.41*	71.22*
BO8	34.9 ± 1.5	44.9 ± 2.0			16.46**
Male longevity (days)					
BO6	31.9 ± 0.3	52.0 ± 1.4	13.83**	11.92**	142.15**
BO7	31.8 ± 1.3	48.0 ± 0.4	11.87**	9.90**	98.67**
BO8	31.1 ± 1.2	45.0 ± 1.6			50.77**

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

effects (from Table 5), and those were not reproducible in any case. It would seem dubious to draw any firm conclusions from these maternal-effect results alone, without the maternal-effect tests using the transmission pattern results, below.

Transmission pattern experiments: Four features of the transmission data are of importance: (i) differences between B and O lines, within treatments; (ii) differences between B and O treatments; (iii) average dominance, as measured by the deviation of the crosses from the mid-parent value; and (iv) maternal effects, as measured by differences between the two reciprocal cross means.

Tables 7 and 8 summarize the analysis of the between-line, within-treatment, variance components. Two types of analysis were performed, one-way model II analysis of variance for each experiment and an overall two-way analysis that combined data over experiments. In the latter case, the relevant *F*-statistics are obtained from the ratio of the variance between replicate lines divided by the variance component for the replicates in interaction with experiments. That is, the first analysis of variance, shown in Table 7, tests for a significant deviation of particular lines of a type within each experiment, while the second analysis of variance, shown in Table 8, tests for a significant deviation of particular lines that is sustained over all experiments. Evidently, the results of these two analyses suggest that specific lines can be significantly deviant within particular experiments, but these deviations are not sustained over repeated experiments. This also makes some sense of the diallel results for

TABLE 10

B and O maternal effects

Character and experiment	Mean ± SEM		*t*-test		ANOVA
	B female × O male	O female × B male	Indep. *t*	Paired *t*	*F*
Ovary weight (mg)					
BO1	0.122 ± 0.008	0.124 ± 0.006	0.28	0.27	0.07
BO2	0.098 ± 0.009	0.090 ± 0.009	0.47	0.86	0.75
BO3	0.143 ± 0.014	0.161 ± 0.009	1.04	0.81	0.67
Fecundity (eggs/24 hr)					
BO2	97.9 ± 4.8	97.0 ± 3.6	0.15	0.47	0.22
BO5	88.0 ± 3.7	85.3 ± 5.6	0.41	1.41	2.15
BO6	91.1 ± 3.2	88.2 ± 3.0	1.67	0.160	2.60
BO7	87.7 ± 4.3	84.5 ± 3.3	0.54	10.64**	114.70**
BO8	98.0 ± 5.2	102.9 ± 3.7			0.28
Conditional fecundity (eggs/24 hr)					
BO2	97.9 ± 4.8	97.3 ± 3.6	0.10	0.33	0.11
BO5	89.0 ± 3.3	86.7 ± 4.9	0.39	1.18	1.46
BO6	91.9 ± 3.0	90.3 ± 3.8	0.31	1.43	1.98
BO7	87.7 ± 4.3	85.1 ± 4.9	0.40	3.77	14.04
BO8	99.7 ± 5.0	102.9 ± 3.7			0.13
Female starvation (hr)					
BO1	31.8 ± 1.1	30.5 ± 1.2	0.81	0.66	0.44
BO2	58.6 ± 4.8	50.5 ± 3.8	1.34	3.06	7.70
BO3	50.8 ± 1.8	52.2 ± 2.9	0.41	0.48	0.23
BO4	47.0 ± 3.6	42.8 ± 5.9	0.60	0.53	0.28
BO5	29.2 ± 1.4	32.1 ± 2.6	0.98	1.99	3.89
BO6	27.7 ± 0.9	28.7 ± 2.0	0.44	0.76	0.58
BO7	38.3 ± 0.7	40.7 ± 0.7	2.34	2.46	6.05
BO8	29.6 ± 1.0	29.7 ± 1.3			0.00
Male starvation (hr)					
BO1	22.0 ± 0.6	22.3 ± 0.8	0.30	0.50	0.25
BO5	20.9 ± 0.8	25.3 ± 0.7	4.05	17.51**	300.16**
BO6	25.1 ± 0.7	27.1 ± 2.0	0.94	1.30	1.71
BO7	31.0 ± 0.7	32.0 ± 0.1	1.52	1.40	1.97
BO8	23.6 ± 0.6	22.9 ± 1.0			0.32
Female longevity (days)					
BO2	57.7 ± 2.5	55.9 ± 2.3	0.53	0.59	0.32
BO3	36.3 ± 1.7	37.9 ± 1.5	0.73	0.68	0.47
BO7	41.5 ± 2.7	41.4 ± 2.1	0.03	0.04	0.00
BO8	41.4 ± 2.0	40.6 ± 2.2			0.07
Male longevity (days)					
BO7	35.6 ± 2.3	39.4 ± 2.3	1.17	1.66	2.75
BO8	37.1 ± 1.7	38.4 ± 1.6			0.30

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

line effects, which were not consistent over experiments (Tables 5 and 6). Such effects evidently can arise, but do not seem to reflect a durable differentiation of lines within treatments.

Table 9 gives the results of the analysis of between-treatment differences. The *t*-tests compare the means of the assayed groups either using the conventional test for differences of two group means ("Indep.") or using a test for a mean paired difference ($B_i - O_i$) greater than zero ("Paired"). The ANOVA model is mixed, with treatments taken as fixed effects and populations within treatments taken as random effects. In this model, treatment effects are tested using the *F*-statistic defined by the ratio of the treatment mean-square divided by the mean-square for the interaction of treatments and populations. Most of the known character differences have apparently been preserved, except for early fecundity. This last result is discussed further below. For longevity and starvation resistance, at least, there is consistent duplication of earlier findings.

Tables 10 and 11 give the results for dominance and maternal effects. There are only a few cases of statistical significance, some of which can be expected to arise by chance with repeated statistical tests.

As with the line-effects analysis of Table 8, the data were also analyzed using a three-way analysis of variance, with treatment differences (DIF), maternal effects (MAT), and average dominance (DOM) all modeled as fixed effects. The mean square for each of these effects ("eff") was divided by

$$MS_{EXP \times eff} + MS_{REP \times eff} - MS_{EXP \times REP \times eff}$$

TABLE 11

B and O average dominance effects

Character and experiment	Mean ± SEM		*t*-test		ANOVA
	Parentals	Crosses	Indep. *t*	Paired *t*	*F*
Ovary weight (mg)					
BO2	0.083 ± 0.004	0.094 ± 0.008	1.27	1.89	3.58
BO3	0.142 ± 0.010	0.152 ± 0.004	0.92	1.91	3.66
Fecundity (eggs/24 hr)					
BO2	97.2 ± 4.9	97.4 ± 4.1	0.04	0.18	0.03
BO5	82.7 ± 3.2	86.7 ± 4.6	0.71	1.64	2.62
BO6	93.7 ± 0.9	89.7 ± 3.1	1.08	0.82	0.67
BO7	84.7 ± 8.0	86.1 ± 4.3	0.16	0.29	0.08
BO8	96.9 ± 2.3	99.2 ± 4.0			0.30
Conditional fecundity (eggs/24 hr)					
BO2	97.2 ± 4.9	97.6 ± 4.2	0.07	0.28	0.08
BO5	84.1 ± 3.1	87.9 ± 4.1	0.73	1.73	2.91
BO6	95.9 ± 1.8	91.1 ± 3.4	1.25	1.02	1.03
BO7	86.1 ± 7.8	86.4 ± 4.6	0.04	0.07	0.01
BO8	98.7 ± 2.0	100.5 ± 3.8			0.19
Female starvation (hr)					
BO1	30.4 ± 0.3	31.1 ± 0.6	0.94	1.40	2.05
BO2	59.0 ± 4.4	53.9 ± 4.1	0.84	3.36*	11.21*
BO3	50.1 ± 1.2	51.5 ± 1.9	0.59	1.92	3.67
BO4	44.3 ± 2.4	45.1 ± 3.1	0.21	0.89	0.80
BO5	31.9 ± 1.1	30.7 ± 2.0	0.51	0.84	0.71
BO6	30.5 ± 1.5	28.3 ± 0.6	1.03	14.33**	202.54**
BO7	41.2 ± 1.1	39.5 ± 0.5	1.41	2.50	6.23
BO8	31.3 ± 0.7	29.6 ± 0.8			2.22
Male starvation (hr)					
BO1	22.3 ± 0.8	22.1 ± 0.6	0.22	0.38	0.14
BO5	22.2 ± 0.5	23.1 ± 0.7	0.99	4.28	18.12
BO6	25.5 ± 2.2	26.3 ± 1.2	0.31	0.70	0.50
BO7	30.3 ± 0.4	31.5 ± 0.3	2.28	3.21	10.29
BO8	23.7 ± 0.5	23.4 ± 0.5			0.13
Female longevity (days)					
BO2	55.9 ± 2.8	56.9 ± 1.9	0.30	0.42	0.19
BO3	36.7 ± 2.0	37.1 ± 1.0	0.16	0.24	0.06
BO6	45.9 ± 0.3	47.2 ± 0.9	1.28	1.84	3.39
BO7	42.6 ± 0.8	41.4 ± 1.9	0.57	0.60	0.36
BO8	40.0 ± 1.3	41.0 ± 1.5			0.25
Male longevity (days)					
BO6	42.0 ± 0.7	39.1 ± 0.9	2.64	3.03	9.16
BO7	40.2 ± 0.3	37.5 ± 2.0	1.33	1.53	2.35
BO8	37.8 ± 1.1	37.8 ± 1.2			0.00

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

TABLE 12

B and O differences, maternal effects, and average dominance effects–3-way ANOVA

Character	ANOVA		
	DIF *F*	MAT *F*	DOM *F*
Ovary weight	51.65**	0.32	0.33
Fecundity	0.27	1.01	0.12
Conditional fecundity	0.33	0.67	0.02
Female starvation	9.34*	0.44	1.74
Male starvation	100.78*	3.29	1.39
Female longevity	45.77*	0.01	0.03
Male longevity	57.68*		4.77

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

to obtain the *F*-statistics shown in Table 12. As may be seen, the only effects that are significant are the treatment effect differences. Of these treatment effect differences, only the two fecundity characters do not give significant results. It is not immediately clear why this should occur, particularly given that ovary weight, which essentially reflects egg content, remains significantly differentiated between B and O treatments. Our interpretation is that potential fecundity is different between B and O treatments, but a difference in actual fecundity is not always elicited in particular experiments. With respect to the general transmission pattern, we conclude that the data indicate additive inheritance among populations averaged over loci without maternal effects. This does *not* indicate an

absence of dominance in the transmission patterns of the individual loci involved in postponed senescence.

DISCUSSION

Overall, the present results suggest the absence of consistent directional dominance or maternal effects in the inheritance of postponed aging in the *D. melanogaster* stocks studied. Maternal or line effects, when present, are not consistent over experiments. It cannot be said that any of the postponed-aging stocks is consistently superior to any other. On average, hybrids of postponed-aging and control stocks appear to be intermediate; there is no reproducible heterosis, inbreeding depression, or directional dominance.

Like CLARE and LUCKINBILL (1985), who studied fewer characters, fewer lines, and far fewer individuals, we find essentially additive inheritance in population crosses. Since the stocks involved are independent, the conclusions of CLARE and LUCKINBILL (1985) seem to be strongly supported.

But these conclusions may be weakened by the possibility that the populations that have been analyzed are still highly polymorphic for the alleles involved in postponed aging. In particular, crosses of highly polymorphic populations will not give clean tests of average dominance of differentiated alleles. In addition, parental lines that aren't extremely differentiated will not be as distinguishable from their F_1's, as indeed fecundity was not so differentiated in these experiments. For these reasons, we set about creating selected lines that would be more differentiated with respect to at least some of the characters involved in postponed aging, in the hope of then performing a more refined genetic analysis. The results of that study are reported in a companion paper by HUTCHINSON, SHAW and ROSE (1991) (in this issue).

However, some features of the present results are of importance as they stand. One of the outstanding problems affecting research on aging has been the persistence of disbelief that aging is made up of normal phenotypes amenable to genetic analysis and selection (*e.g.* LINTS 1978; LINTS and HOSTE 1974, 1977; LINTS *et al.* 1979). The present results together with those of Luckinbill and his colleagues (CLARE and LUCKINBILL 1985; LUCKINBILL and CLARE 1985) indicate that aging phenotypes are typical quantitative characters.

While a number of Drosophila stocks exhibit different aging patterns, upon crossing there is often extensive heterosis (CLARKE and MAYNARD SMITH 1955; GOWEN and JOHNSON 1946), indicating inbreeding depression. This made the study of *C. elegans* aging particularly attractive, because frequent self-fertilization in that species appears to prevent inbreeding depression for aging (JOHNSON and WOOD 1982). The lack of hybrid vigor in the extensive crosses performed in the present study indicates that stocks with postponed aging created using the methods of ROSE (1984) will not suffer from the problem. Thus they can be used as material for the investigation of physiological hypotheses concerning mechanisms for the postponement of normal aging (ROSE *et al.* 1984; SERVICE *et al.* 1985; SERVICE 1987; LUCKINBILL *et al.* 1988a).

The authors are grateful to P. M. SERVICE for help in the planning and execution of the reported experiments. R. E. LENSKI and L. D. MUELLER commented upon earlier drafts of the manuscript. We thank D. ARAB, H. GILLIS, G. GLAZOV, K. GRIMM, L. E. JOHNSTON, S. JOHNSON, D. M. LANE, J. JUDAH, M. D. MACKINLEY, B. MUSGRAVE, J. NELSON, S. O'KEEFE, D. PRINGLE, A. J. SHAW, B. SINGH, D. STEWART and B. TREMBLAY 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

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

CLARKE, J. M., and J. MAYNARD SMITH, 1955 The genetics and cytology of *Drosophila subobscura*. XI. Hybrid vigour and longevity. J. Genet. **53:** 172–180.

DOWNING, J. A., 1979 Aggregation, transformation and the design of benthos sampling programs. J. Fish. Res. Board Can. **36:** 1454–1463.

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.

GOWEN, J. W., and L. E. JOHNSON, 1946 On the mechanism of hete rosis. I. Metabolic capacity of different races of *Drosophila melanogaster* for egg production. Am. Nat. **80:** 149–179.

GRAVES, J. L., L. S. LUCKINBILL and A. NICHOLS, 1988 Flight duration and wing beat frequency in long- and short-lived *Drosophila melanogaster*. J. Insect Physiol. **34:** 1021–1026.

HUTCHINSON, E. W., A. J. SHAW and M. R. ROSE, 1991 Quantitative genetics of postponed aging in *Drosophila melanogaster*. II. Analysis of selected lines. Genetics **127:** 729–737.

JOHNSON, T. E., 1987 Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **84:** 3777–3781.

JOHNSON, T. E., and W. B. WOOD, 1982 Genetic analysis of life-span in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **79:** 6603–6607.

LEFFELAAR, D., and T. A. GRIGLIATTI, 1984 A mutation in Drosophila that appears to accelerate aging. Dev. Genet. **4:** 199–210.

LINTS, F. A., 1978 *Genetics and Aging, Interdisciplinary Topics in Gerontology*, Vol. 14, S. Karger, Basel.

LINTS, F. A., and C. HOSTE, 1974 The Lansing effect revi sited. I. Life-span. Exp. Gerontol. **9:** 51–69.

LINTS, F. A., and C. HOSTE, 1977 The Lansing effect revisited. II. Cumulative and spontaneously reversible parental age effects on fecundity in *Drosophila melanogaster*. Evolution **31:** 387–404.

LINTS, F. A., J. STOLL, G. GRUWEZ and C. V. LINTS, 1979 An attempt to select for increased longevity in *Drosophila melanogaster*. Genetics **25:** 192–204.

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

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, L. S., J. L. GRAVES, A. TOMKIW and O. SOWIRKA, 1988a A qualitative analysis of life-history characters in *Drosophila melanogaster*. Evol. Ecol. **2:** 85–94.

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

MATHER, K., and J. L. JINKS, 1982 *Biometrical Genetics*, Ed. 3. Chapman & Hall, London.

ROBERTS, P. A., and R. B. IREDALE, 1985 Can mutagenesis reveal major genes affecting senescence. Exp. Gerontol. **20:** 119–121.

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

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

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

ROSE, M. R., M. L. DOREY, A. M. COYLE and P. M. SERVICE, 1984 The morphology of postponed senescence in *Drosophila melanogaster*. Can. J. Zool. **62:** 1576–1580.

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

SERVICE, P. M., 1987 Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. Physiol. Zool. **58:** 380–389.

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.

WATTIAUX, J. M., 1968a Parental age effects in *Drosophila pseudoobscura*. Exp. Gerontol. **3:** 55–61.

WATTIAUX, J. M., 1968b Cumulative parental age effects in *Drosophila subobscura*. Evolution **22:** 406–421.

WEBER, K. E., 1990 Increased selection response in larger populations. I. Selection for wing-tip height in *Drosophila melanogaster*. Genetics **125:** 579–584.

WEBER, K. E., and L. T. DIGGINS, 1990 Increased selection response in larger populations. II. Selection for ethanol vapor resistance in *Drosophila melanogaster* at two population sizes. Genetics **125:** 585–597.

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

YOO, B. H., 1980 Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. I. Response to selection. Genet. Res. **35:** 1–17.

Communicating editor: A. G. CLARK