The Effects of Spontaneous Mutation on Quantitative Traits. I. Variances and Covariances of Life History Traits

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ABSTRACT

We have accumulated spontaneous mutations in the absence of natural selection in *Drosophila melanogaster* by backcrossing 200 heterozygous replicates of a single high fitness second chromosome to a balancer stock for 44 generations. At generations 33 and 44 of accumulation, we extracted samples of chromosomes and assayed their homozygous performance for female fecundity early and late in adult life, male and female longevity, male mating ability early and late in adult life, productivity (a measure of fecundity times viability) and body weight. The variance among lines increased significantly for all traits except male mating ability and weight. The rate of increase in variance was similar to that found in previous studies of egg-to-adult viability, when calculated relative to trait means. The mutational correlations among traits were all strongly positive. Many correlations were significantly different from 0, while none was significantly different from 1. These data suggest that the mutation-accumulation hypothesis is not a sufficient explanation for the evolution of senescence in *D. melanogaster*. Mutation-selection balance does seem adequate to explain a substantial proportion of the additive genetic variance for fecundity and longevity.

MUTATION is fundamentally important for evolution as the ultimate source of adaptive variation. On the other hand, the average new mutation clearly has deleterious effects on fitness. There is ample evidence that deleterious alleles are maintained in populations of higher organisms as a result of mutation pressure, and contribute to a significant reduction in the average fitness of individuals in the population compared with that of mutation-free individuals (KONDRASHOV 1988; CROW 1993a). Despite the low mutation rates at individual loci, this high genetic load is sustained because the total number of loci in a higher organism is very large. Direct estimates of the genomic mutation rate in *Drosophila melanogaster* (MUKAI 1964; MUKAI *et al.* 1972; OHNISHI 1977b; CROW and SIMMONS 1983), and indirect estimates from selfing plants (CHARLESWORTH *et al.* 1990) suggest that there is likely to be an average of at least one new deleterious mutation per individual per generation in these species. The per genome mutation rate in organisms with larger genome sizes, such as mammals, is likely to be even greater, although no direct estimates are available (KONDRASHOV 1988; CROW 1993b; KONDRASHOV and CROW 1993).

This constant flux of mutations has many important implications for evolution. First, mutation maintains a significant amount of additive genetic variance in fitness components (MUKAI *et al.* 1974; MUKAI 1988). This additive genetic variance may provide a basis for selection in favor of choice of mates by females (CHARLESWORTH 1987; POMIANKOWSKI 1988; RICE 1988). In addition, pleiotropic effects of these alleles probably contribute to the maintenance of genetic variation for other quantitative traits (ROBERTSON 1967; KEIGHTLEY and HILL 1988; BARTON 1990; KONDRASHOV and TURELLI 1992). Second, the partially recessive nature of most deleterious mutations (CROW and SIMMONS 1983) means that they contribute to inbreeding depression, which creates a selective pressure in favor of outbreeding devices (LANDE and SCHEMSKE 1985; CHARLESWORTH *et al.* 1990). Third, mutation load can provide an evolutionary advantage to sexual reproduction and genetic recombination (CROW 1970; FELDMAN *et al.* 1980; KONDRASHOV 1988; CHARLESWORTH 1990a). In addition, the stochastic decline in fitness under mutation pressure in non-recombining finite populations by the process known as Muller's ratchet (FELSENSTEIN 1974) may cause the extinction of asexual populations (HAIGH 1978; LYNCH and GABRIEL 1990; CHARLESWORTH *et al.* 1993), and lead to the degeneration of *Y* chromosomes (CHARLESWORTH 1978).

Finally, theory suggests that accumulation of deleterious mutations may cause the evolution of senescence (MEDAWAR 1952; CHARLESWORTH 1994). All evolutionary theories of senescence depend on the fact that the effects of alleles on fitness early in life are more strongly selected than effects late in life (HAMILTON 1966; CHARLESWORTH 1990b; ROSE 1991; PARTRIDGE and BARTON 1993).

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If there is substantial mutation pressure for age-specific deleterious effects, then alleles with deleterious consequences late in life will reach a higher equilibrium frequency than those with effects early in life, thus leading to a disproportionate decrease in late-life fitness components. As mutation pressure depresses late-life performance, this further decreases the effects of subsequent late-acting mutants on relative fitness, accelerating the rate of decline. If mutation pressure is strong enough, it can lead to a truncation of later parts of the life cycle (CHARLESWORTH 1990b, 1994; PARTRIDGE and BARTON 1993). The other major hypothesis for the evolution of senescence, the antagonistic pleiotropy theory, notes that alleles which increase fitness early in life, but have deleterious effects late in life are often favored by selection (WILLIAMS 1957; HAMILTON 1966; CHARLESWORTH 1994). If alleles with such effects have often been fixed over evolutionary time, this hypothesis would explain the prevalence of senescence; if such alleles are maintained in a polymorphic state they would both cause senescence, and allow a response to selection for increased life span.

The mutation-accumulation hypothesis depends on two strong testable assumptions about mutation (PARTRIDGE and BARTON 1993). First, the rate of production of deleterious mutations must be large, and, second, the age-specific effects of these mutations must be substantially uncorrelated. If mutations have similar effects at all adult ages, then selection for early life performance will also increase performance late in life. While we cannot know how far from maximal the survivorship of a population is, we can observe drastic declines in early-life performance when late-life performance is selected (ROSE and CHARLESWORTH 1981b; LUCKINBILL *et al.* 1984; ROSE 1984b; SERVICE 1993). If the mutation-accumulation hypothesis is a sufficient explanation for the evolution of senescence, similar declines in early-life performance should be observable in the absence of any selection (CHARLESWORTH 1984). No comparable strong assumptions about mutational variance or covariance are necessary for the antagonistic pleiotropy hypothesis. Senescence-increasing alleles may be fixed by selection, or maintained as balanced polymorphisms under this hypothesis, so that there is no requirement that such alleles arise frequently through mutation (PARTRIDGE and BARTON 1993).

We have studied the variance and covariance of life history traits, due to spontaneous mutations accumulated in the virtual absence of natural selection. In order to do this, we have adopted the experimental techniques of MUKAI (1964; MUKAI *et al.* 1972) for studying mutations arising on the second chromosome of *D. melanogaster*. We report here on studies through generation 44 of mutation-accumulation for longevity, and for fecundity and male mating success at two ages. In addition to providing data relevant to the mutation-accumulation theory for the evolution of senescence, this work bears on the other issues raised above. For example, in spite of the importance of the rate at which mutation supplies new genetic variation and covariation for theories of mutation-selection balance (BARTON and TURELLI 1989; CHARLESWORTH 1990b; HOULE 1991; KONDRASHOV and TURELLI 1992), relatively few estimates are available for life-history traits (LYNCH 1988).

MATERIALS AND METHODS

Culture conditions: Flies were reared on a standard brewer's yeast-cornmeal-sucrose-agar medium, with propionic acid added to inhibit growth of microorganisms. No live yeast was added to the medium, although yeast was usually carried from vial to vial by the flies. Antibiotics were occasionally added to the medium to eliminate bacteria (ASHBURNER and THOMPSON 1978). Flies were maintained in temperature-controlled incubators at 25° during experiments, and at 18° at other times. All incubators were maintained on a 12:12 hr light dark cycle. Experimental flies were reared and maintained in 25 × 90-mm shell vials closed with rayon plugs. Other stocks were reared in 60 × 135-mm bottles. Flies were handled under CO_2 anesthesia when necessary.

***IV* population:** The base population from which wild chromosomes were drawn to initiate these experiments was founded from approximately 400 iso-female lines captured in Amherst, Massachusetts, by P. T. IVES in 1975, and reconstituted from 21 inversion-free iso-female lines in 1977 (CHARLESWORTH and CHARLESWORTH 1985). This population has been maintained in 10 half-pint milk bottles at 25° and a 12:12 hr light dark cycle on standard medium since 1977. Paper towels are added to increase the carrying capacity of the bottles. Flies are transferred every 14 days, using a regular scheme where each new bottle is founded by mixing flies from two bottles from the previous generation.

Balancer stock: We used the standard balancer stock *SM1, Cy/bw^D*. *SM1* is a multiply inverted second chromosome, marked with the dominant mutation *Curly wing*, and *bw^D* is a dominant allele of the *brown eye* locus. Both of these chromosomes are lethal when homozygous. To allow detection of contamination, we introduced the fourth chromosome recessive eye mutation *sparkling-poliert* (*spa^{pol}*) into this stock. *X* and third chromosomes from the *IV* population were introduced into this stock by repeated backcrosses of females to *IV*, and selection of the visible markers. More complete descriptions of these chromosomes and mutations may be found in LINDSLEY and ZIMM (1992).

Selection of the stem chromosome: In 1988, 50 wild-type second chromosomes were extracted from the *IV* population. In the course of this, each chromosome stock was also rendered homozygous for *spa^{pol}*. Each chromosome was assayed for homozygous egg-to-adult viability relative to *SM1/+*. The 10 chromosomes with the highest viability were then assayed for fitness in competition with *SM1* in population cages (SVED 1971; HOULE *et al.* 1992), and for longevity and female fecundity of homozygotes. Considerable variation between chromosomes in these traits was detected, despite similar egg-to-adult viabilities. One chromosome (line 55), identified as having good overall performance in these measures, including relatively high survival and reproductive performance late in life, was chosen for use in the mutation-accumulation experiment. The remaining lines were pooled to form the *IV; spa^{pol}* stock. The line 55 and *SM1/bw^D* stocks were found to be of *P/I* cytotype, thus precluding hybrid dysgenesis on crossing with *P/I* males (ASHBURNER 1989).

Mutation accumulation: Two hundred replicate mutation-accumulation (MA) lines were founded from line 55, by backcrossing a single male $SM1/+_{55}$ fly to three virgin $SM1/bw^{D}$ females. In each subsequent generation, two backcross vials were set up within each line from a single $SM1/+$ male and three virgin $SM1/bw^{D}$ females. One of these two crosses was then chosen arbitrarily to supply males for the following generation. If this cross failed to produce offspring, offspring were taken from the second vial. First vials failed to produce offspring less than 1% of the time. This procedure has two effects: first, the MA chromosomes were never in homozygous condition, so recessive effects were not subject to natural selection. Second, each MA chromosome forms a population with an effective size of 1/2. The combined result is that fixation of new mutations with small fitness effects within lines occurs close to the mutation rate. Selection would only be effective against alleles with very large heterozygous effects.

In generations 11, 22, 33 and 44, chromosomes were extracted from a sample of lines and checked for lethality. Lethal lines were discarded. In the first 62 generations, an additional 20 lines were accidentally lost. At generations 33 and 44 other fitness components were studied in the homozygous lines. To control for potential effects of variation in genetic background, as well as cross-generation environmental effects, two independent extractions of each line were carried out for each experimental line. In addition to being isogenic for the second chromosome, replicate extractions have a coancestry of 0.042 for the third and fourth chromosomes, and 0.021 for the *X*, due to the fact that males used to initiate the replicate extractions were half sibs 2/3 of the time, and full sibs 1/3 of the time.

Expression of *Cy*: In generation 11 we discovered that in our genetic background, the *Cy* marker on *SM1* and related balancers had incomplete penetrance. It is likely that, in selecting for a stem chromosome with high viability and fitness, we inadvertently selected for modifiers of *Cy*. Problems with the expression of *Cy* have been noted previously (NOZAWA 1956). In all lines, *Cy* was expressed in the majority of individuals, so the chief difficulties this caused were uncertainty in the estimation of egg-to-adult viability, detection of recessive sterile mutants, and in obtaining homozygous second chromosome stocks. During extractions, stocks were raised at 27° during the late pupal period critical for expression of *Cy*. This improved penetrance, but did not entirely correct the problem. To counteract this, and allow detection of lethal mutations, replicate single pair crosses of flies with wild-type wings were set up, and their offspring examined for *Cy*. Only crosses where no *Cy* offspring were detected were retained for subsequent analyses. Chromosomes were classified as lethal if approximately 10 such single pair matings failed to eliminate the *Cy* marker. In practice, this only occurred in lines where the frequency of wild-type wings was already low.

Lack of a control population: We originally formed a control population isogenic for the line 55 chromosome, and minimized the accumulation of mutations in this population by maintaining it at large effective size. However, in the course of attempts to select this population for increased expression of an introduced *Cy* marker, this population was reduced to a census size on the order of 20 for a number of generations. Previous results make it likely that this is small enough that substantial mutation-accumulation could have occurred (MUKAI *et al.* 1972). Following generation 33 assays, a new population was formed by pooling the six MA lines with the highest average rank for the fecundity and longevity traits. Replicate extractions of each line were pooled separately, yielding two "control" populations. Following generation 62, analyses of transposable element positions have revealed that both replicates of this "control" population were contaminated by another second chromosome (S. NUZHDIN and T. MACKAY, personal communication). The contamination of both replicates suggests that one of the generation 33 chromosomes may have been the source of the contamination, thus making it likely that the control stock was contaminated during the generation 44 assays. This population was used as a control in a previous publication (HOULE *et al.* 1992), but we now regard results involving this stock as suspect. Consequently, we have no control population which would allow us to compare the performance of the original line 55 and the MA lines.

Subsequent analyses of transposable element insertion sites on generation 62 MA lines have not revealed any contamination of the MA lines themselves. We examined insertion sites for the elements *297* and *roo* (LINDSLEY and ZIMM 1992), following the methods of MONTGOMERY *et al.* (1987) and CHARLESWORTH *et al.* (1992). Element sites were scored over the entire second chromosome, with the exception of the proximal portion of 2L. A total of six MA lines were examined, and for two of them we examined both replicate extractions. For each extraction, we examined one to three slides with an average of 2.33. Testing took place approximately 22 generations after extraction, and thus over 80 generations since mutation-accumulation began. For *297*, we detected a total of six invariant sites, and two apparent element insertions. For *roo*, we detected 20 invariant sites, and three element insertions. Each insertion was only found in a single line. As an independent check, S. NUZHDIN kindly examined a different six lines for *297* only, finding 11 sites across the whole chromosome, with no gains or losses. This sample included the lines with the highest and lowest fitnesses in a Sved cage test of 40 lines at generation 62 (D. HOULE and E. BROWN, unpublished). These are the lines most likely to be contaminated. The number of element gains is consistent with previous estimates of transposition rates, while the lack of any element losses strongly argues against contamination of any of these lines. While it seems likely that at least one MA line was contaminated, based on the fact that both replicates of the control stock were contaminated, the proportion of lines affected was probably very small. Since no lines had anomalously high estimates for any fitness component, the effect of such contamination on among line variance is probably small.

Production of assay flies: Assays were carried out in three experimental blocks at generation 33, and two blocks at generation 44. Starting two generations before each block's assays, vials were set up with 4 female and 4 male flies between 3 and 7 days post-eclosion. These were allowed to lay eggs for 2–4 days, depending on the experiment. The number of parental vials set up per line-extraction combination varied from 5 to 10, depending on the block. In block two of generation 33 and in both generation 44 blocks, each group of four parental flies was allowed to lay eggs in two vials. Flies were collected for longevity assays from the first, or L, laying, and for fecundity and male mating from the second, or F, laying.

Fecundity: We assayed female fecundity on days 5 and 6, and days 27 and 28 post-eclosion. Early fecundity is the sum of eggs laid by an individual on days 5 and 6, while late fecundity is the sum of eggs laid on days 27 and 28. Females used in early fecundity experiments were always drawn from flies eclosing on the same day. For the late fecundity experiment, we obtained all flies from the target eclosion date, where possible, although we frequently used some flies up to 2 days older or younger than this. Subsequent analyses indicated that there were no significant differences in fecundity of flies within this age spread (data not shown). Flies used in the early fecundity experiment were never used for late fecundity assays.

Prior to assay, females were mated with males of the outbred *IV; spa^{pol}* stock. In most experiments, no special attempt was made to collect virgin females (although most probably were), on the assumption that any effects of early matings with males of their own genotype would be negligible following remating with *IV; spa^{pol}* males. For both early and late fecundity assays, flies were held in groups of 15–20 females per vial, with a comparable number of *IV; spa^{pol}* males. Mating with *IV; spa^{pol}* males always took place on day 2 post-eclosion for early females, and between 2 and 5 days after the target date for late females. Flies held for late fecundity were transferred to fresh food every 2–3 days for the first 2 weeks, and every 3–4 days thereafter.

Three days before a fecundity assay, flies were transferred to fresh vials, seeded with a live yeast solution. In the late fecundity assay, the *IV; spa^{pol}* males were replaced with males between 3 and 5 days old at the same time. On the first assay day, flies were anesthetized and sorted into male-female pairs, and each pair placed on fresh yeasted standard medium in disposable plastic shell vials with a diameter of 2.5 cm. Green food coloring was added to the medium to make the eggs more visible. After 24 hr, flies were transferred to fresh medium without anesthetization. After another 24 hr, the flies were discarded. Vials in which the female died were discarded. Following removal of the adults, vials were frozen to await egg counting.

A total of nine observers counted eggs from fecundity assays, and the counts were assigned so that vials with eggs from the same female were generally counted by different observers. Some eggs were difficult to observe on the surface of the food for several reasons, including hatching of some larvae before the vials were frozen and residual yeast on the surface. Consequently it proved difficult to get observers to agree on counts. Although the means obtained by each counter are within 10% of each other, analyses of variance show significant counter effects. To adjust for these effects, we assumed that counters tended to over- or underestimate counts by a constant proportion. Counts were log-transformed and residuals obtained from analyses of variance (ANOVAs) for each generation, with blocks, day of egg laying and identity of counter as effects. Following this, the least squares means for day of laying were added to the residuals, the data back-transformed, and the two adjusted counts added together to obtain the fecundity. This procedure removed most of the effect of counters, as well as differences among blocks. Tables 2 and 3 give the raw means for each block. Because of the counter adjustment procedure, the block means differ from those in the data used for further analyses.

Longevity: Male and female survival was assessed in single sex groups of 20 virgin flies. Flies eclosing on a single target date were used where possible, although variation among lines necessitated the use of flies up to 4 days older in some cases. Longevity statistics were calculated from the mean ages of adults in a vial. Once a week, flies were anesthetized, and surviving MA flies counted. Dead flies were replaced with virgin flies from a stock bearing the eye mutation *white-apricot* (w^a), so that the density of flies in a vial was always close to 20. The 20 flies were then placed on fresh unyeasted food.

Male mating ability: We assayed early male mating ability on day 3 and late mating ability on day 21 post-eclosion. Males used in the early mating assay were always collected from flies eclosing on the same day. For the late mating experiment, most flies could be obtained from the target date, although we occasionally used males up to 2 days older or younger than the target date. Prior to assay, males were held in single sex groups of up to 25 flies. Late flies were transferred weekly to fresh food. To assay mating ability, we placed 10 MA and 10 w^a males in vials with 10 virgin w^a females without anesthesia. The age of w^a males used was chosen so that approximately equal proportions of each type of mating were obtained. After approximately 2 hr, the flies were anesthetized and the males and females separated. Females were then placed individually in vials, and their offspring checked for the expression of w^a. The proportion of mated females producing wild-type offspring was used as the index of male mating success.

Fitness: We have previously reported the results of fitness assays of lines at generation 44 (Houle *et al.* 1992). Briefly, we constructed a population consisting of a test chromosome, or sample of chromosomes, and a balancer chromosome (bw^{V1}) carrying a recessive lethal. The equilibrium gene frequency in adults was then used to obtain a measure of the relative fitness of the test chromosome(s).

Productivity and weight: To control for potential effects of rearing density on other traits, we counted the number of puparia on the side of each vial after we had collected flies for other assays. We refer to this character as productivity. In addition to reflecting larval environment, productivity is also an index of parental fecundity and offspring viability, and so is itself a life history trait. For blocks where two layings were used, two measures of productivity are available. As a direct measure of size, we weighed groups of approximately 10 live males at least 5 days post-eclosion, whenever sufficient flies were available.

Analyses showed that the environmental correlations involving these traits were small, with r^2 values less than 5%, and the signs of the correlations do not correspond well with the expectations for environmental correlations based on previous studies (Robertson 1963; Prout and McChesney 1985). Consequently, all analyses reported are not adjusted for productivity or weight.

Statistical analyses: Standard statistical analyses were carried out with the SAS statistical package, version 6.03 (SAS Institute, 1988a,b). Before further analyses, the data were examined for outliers. For both early and late fecundity the data show two modes, one at zero fecundity, so ANOVA residuals were very far from normal. There was no detectable genetic component to the probability of these very low fecundities. We eliminated the data in this low mode, in order to render the distribution more normal. Early fecundities with less than 10 and 5 eggs were eliminated from generations 33 and 44, respectively; all late fecundities of 0 were dropped in both generations. For the other phenotypes each block was analyzed separately, and the residuals examined for outliers, which were subjected to Grubb's test (Sokal and Rohlf 1981) and discarded if significant at $P < 0.05$. When sample sizes exceeded the maximum tabled value of 150, observations whose residuals were separated from the main body of residuals, and were more than 4 SD from the mean were discarded. This resulted in the removal of three male longevity vial means, two female longevities, two male weights and two vial productivities in generation 33. In generation 44, one fitness cage, two early and one late mating trial, eight male weights and six vial productivities were removed.

Bootstrap analyses were carried out in several steps. First, data were corrected for counter effects and outliers were removed, as indicated by the above analyses. Second, the corrected data were resampled at all levels of the design, except for the extraction level. Since there were only two replicate extractions, resampling can only reduce the variance at this level. At each level, sampling was always carried out with replacement. To resample the data from each generation, a line was first chosen at random, then from within each block-extraction combination individual observations for each trait were again drawn, up to the actual sample size of that cell. If

TABLE 1

Trait means by block for generations 33 and 44

Phenotype		Generation 33 at block:			Generation 44 at block:	
		1	2	3	1	2
Early fecundity	Mean	61.56	81.10	100.29	90.56	80.65
	SE	0.75	0.98	0.72	0.94	0.79
	N	652	700	764	731	741
Late fecundity	Mean	35.98	47.56	66.02	45.88	61.60
	SE	0.70	0.81	0.75	0.76	0.95
	N	604	759	722	618	671
Male longevity (days)	Mean	31.07	29.07	39.70	31.47	31.57
	SE	0.63	0.64	0.69	0.56	0.70
	N	73	84	79	63	74
Female longevity (days)	Mean	56.70	52.09	61.06	53.95	58.60
	SE	0.57	0.85	0.68	0.73	1.09
	N	69	85	79	65	67
Early male mating	Mean				0.570	0.668
	SE				0.064	0.053
	N				71	60
Late male mating	Mean				0.461	0.301
	SE				0.082	0.088
	N				76	56
Fitness	Mean				0.462	0.313
	SE				0.021	0.018
	N				77	80

the line drawn was not assayed in a particular block, no data were included for that block-line combination. Similarly, if a given extraction was missing for that block-line combination, this generated a missing cell in the resampled data. This process was repeated until the same number of lines were resampled as were assayed in the set of experiments for that generation. The data were always somewhat unbalanced, and the resampling scheme preserved this lack of balance.

Each trait necessitated a slightly different resampling scheme. Fecundities were resampled at the level of 2-day egg counts, longevities as individual dates of death, and productivities at the vial level. For the male matings, samples were drawn from the binomial distribution with the estimated proportion and sample size as parameters. For fitness and weight, only a single observation was made in all (fitness) or many (weight) block-line-extraction combinations, so observations were sampled by adding a normal deviate with variance equal to the error variance to the observation. Since fitness is constrained to be between 0 and 1, observations were arc-sin transformed, resampled on this scale, then back-transformed. To yield a uniform type of analysis, block-line-extraction means from the resamples were analyzed. The results of each analysis were saved, and after 1,000 bootstrap resamples, the quantiles of the desired statistics were examined to determine the parameter estimates and confidence intervals.

RESULTS

Lethals: A random sample of MA lines were extracted and assayed for lethality in generations 22, 33 and 44. With random sampling of chromosomes, many chromosomes were tested more than once, so lethality tests were made after variable numbers of generations of mutation. We identified a total of 38 lethals: 9 in 101 chromosomes tested 11 generations after the last assay; 17/113 tested after 22 generations; 7/56 tested after 33 generations; and 5/13 tested only after 44 generations. After correcting for chromosomes with more than one lethal (MUKAI *et al.* 1972), lethals were estimated to occur at a rate of 41.6/6017 = 0.0069 per generation. Although this is slightly higher than many previous estimates (SIMMONS and CROW 1977), it is not significantly different from the rate of 36.0/6000 estimated by MUKAI *et al.* (1972) ($G = 0.39$, 1 d.f., $P > 0.5$).

Distribution of MA line means: The block means for all traits are given in Tables 1 and 2. Forty-two lines were assayed for life history traits at generation 33, and 43 at generation 44. The generation 44 lines were among the 47 lines whose fitness was the subject of a previous report (HOULE *et al.* 1992). The lines were sampled at random from the non-lethal lines remaining at each generation, so 14 of the lines were studied at both generations 33 and 44. The distribution of MA line means was always unimodal, and generally close to normal, although a few lines usually showed markedly lower performance. This is consistent with data on viability in previous mutation-accumulation experiments (MUKAI 1964; MUKAI *et al.* 1972). In these previous experiments, an arbitrary cutoff point was chosen, and lines with viabilities below the cutoff were assumed to carry a severely deleterious mutation. We tested atypical lines using Grubb's test (SOKAL and ROHLF 1981), and lines were identified as probable carriers of severely deleterious mutations if they were significant outliers at the $P < 0.05$ level. In generation 33,

TABLE 2

Block means of productivity and male weight

Generation	Block	Productivity			Male weight (mg)		
		Mean	SE	*N*	Mean	SE	*N*
33	1	136.70	2.43	177	0.722	0.012	88
33	2L	52.09	1.71	240			
33	2F	103.83	2.29	321	0.773	0.012	170
33	3	131.02	1.73	612	0.763	0.026	56
44	1L	30.90	0.83	337	0.813	0.037	39
44	1F	77.36	1.81	405	0.732	0.010	186
44	1L	60.22	1.39	396	0.817	0.015	83
44	1F	80.50	1.66	391	0.758	0.013	71

two lines were significant outliers for both male and female longevity. In generation 44, one of these same lines was again an outlier for longevity and fitness, and another line was also an outlier for fitness. All of these severely deleterious lines also showed below average performance for other life-history traits. The remaining lines were considered to have "quasi-normal" performance. Subsequent analyses were carried out both including and excluding the severely deleterious lines for all traits, to determine what influence these few large mutations have on the results.

Mutational variances: Conventional variance component analyses within generations were carried out using the SAS GLM procedure (SAS Institute, 1988b) with lines, extractions nested within lines, and block by line interactions as random effects, and block as a fixed effect. For longevity, flies were handled in trays, and tray effects were also included in the analyses for these traits. Every phenotype except the male mating abilities showed significant genetic effects, either as line effects or block by line interactions (at $P < 0.05$; results not shown) in one generation or the other. Block by line interactions were significant, or nearly so for all of the fecundity and longevity traits in generation 33, making it difficult to interpret variance components from the full analyses. Instead, we assume that the genetic variances in each block are drawn from the same distribution, even though the relative performance of lines may vary among blocks. Analysis of each block separately also facilitates bootstrap resampling of the data, as such analyses are much simpler in structure and more similar across phenotypes than the full analyses.

The variance components from these single block analyses are shown in Table 3. The variables were not transformed before analyses, so the variance components are given on the original measurement scale. The ANOVA variance components were obtained by the type I method in the SAS Varcomp procedure (SAS Institute, 1988b). Type I estimates were chosen because they are not constrained to be greater than or equal to 0, which would bias the regression analyses below. The significance levels shown are for the line effect from the corresponding GLM analysis. Bootstrap resampling was carried out as described in MATERIALS AND METHODS, yielding a sample of line-extraction means for each phenotype. These were analyzed as a two-way ANOVA with tray (where appropriate) and lines as main effects, and the line effect variance component estimated by a method similar to the SAS type I method. The Table shows medians of 100 bootstrap samples, with the significance level calculated from the proportion of bootstrap estimates greater than 0. With few exceptions, the bootstrap and ANOVA results are rather close, indicating little bias. However, the significance levels of the bootstrap samples are usually more conservative than those from ANOVAs, taking into account that the highest significance level from the bootstrap sample is 0.01.

A slightly different structure was used for analyses of productivity and male weight because of the availability of data from two layings in some blocks. In generation 33, flies were only weighed from the fecundity assay vials, while flies were weighed from both layings in generation 44. These laying effects are highly significant for productivity, so that each laying was analyzed separately, as shown in Table 3. Analyses of weights from block 1 generation 33, and from laying L, block 1, generation 44 are not included, as the samples were small and highly unbalanced.

Severely deleterious lines were detected for longevity phenotypes, so removing these lines has a relatively large impact on the results for these traits. Male and female longevity data were also analyzed using the Gompertz regression model, where log mortality is partitioned into "intrinsic" component which is assumed constant throughout life, and a "senescent" component which is assumed to increase linearly with age (FINCH *et al.* 1990; HUGHES and CHARLESWORTH 1994). Neither component showed significant genetic variance for either sex (results not shown).

To combine variances into a single estimate of the per generation mutational variance (V_M) we used the slopes of regressions of genetic variance on generation. The regressions were forced to go through the origin, since it is reasonable to assume that no genetic differences

TABLE 3

Among line variance components from single block analyses for all phenotypes

Phenotype	Generation	Block	All lines		Quasi-normals	
			VARCOMP	Bootstrap	VARCOMP	Bootstrap
Early fecundity	33	1	10.384	13.196	10.384	7.966
		2	67.295*	80.608*	51.137*	62.843*
		3	2.469	0.624	2.810	2.180
	44	1	-4.663	-9.669	-9.791	-11.921
		2	53.649**	56.692*	51.519**	52.090**
Late fecundity	33	1	0.802	20.645	0.802	14.009
		2	32.772*	45.655*	28.004	25.949†
		3	-0.616	16.104	-1.471	12.902
	44	1	60.785**	60.452*	60.239**	58.675*
		2	91.932****	103.089**	77.628***	79.313*
Male longevity	33	1	9.606**	3.977	9.606**	4.952
		2	21.448****	20.291*	7.900**	7.136†
		3	16.316***	7.971	16.716***	8.958
	44	1	-4.387	0.257	-4.387	0.039
		2	0.365	2.864	-1.849	2.583
Female longevity	33	1	2.831	6.622	2.831	5.350
		2	33.079****	29.345**	7.654*	7.181
		3	0.538	-4.408	0.599	-6.113
	44	1	1.029	1.239	1.029	4.183
		2	24.987*	27.735†	-1.395	5.005
Early male mating $\times 10^3$	44	1	-1.94	-8.395	-2.795	-8.434
		2	5.671†	6.037	5.853†	7.027
Late male mating $\times 10^3$	44	1	16.05†	11.743	15.534†	11.391
		2	11.96	27.816†	13.14	22.921
Male weight $\times 10^3$	33	2	-0.085	0.096	-0.088	0.220
		3	0.730	1.458	0.796	1.385
	44	1F	-0.231	-0.250	-0.205	-0.358
		2L	0.259	0.350	0.272	0.240
		2F	-0.288	-0.186	-0.323	-0.258
Productivity	33	1	297.989**	325.484**	297.989**	274.542*
		2L	27.097	44.774	-4.755	-133.083
		2F	245.439†	176.028	117.726	95.492
		3	314.479†	302.092*	260.378	240.028*
	44	1L	22.419	24.002	23.037	30.444†
		1F	-22.026	6.881	-49.419	-25.456
		2L	115.554†	160.875†	113.177†	146.539*
		2F	97.705	157.655†	93.658	114.389

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Significance levels not adjusted for multiple comparisons. Values are given on the measurement scale.

existed in generation 0. Median slopes for regressions of trait variances on generation from 1,000 bootstrap samples are shown in Table 4. To place the estimates for different blocks and traits on the same scale, the data within each block were standardized by the mean for that block. Significance levels were again obtained from the quantiles of the bootstrap sample of estimates. The significance levels indicated following the median estimates are for one-sided tests for differences from 0, with upper and lower two-sided 95% confidence limits given below. V_M is significant for all traits except for male mating and weight when all lines are analyzed. Only the fecundity traits and productivity are significant in the quasi-normal sample.

Mutational covariances: The bootstrap resampling procedure used in the last section was also used to obtain estimates and confidence limits for the mutational covariances and correlations among traits. Covariances were calculated from the variance of sums (KEMPTHORNE 1957, p. 265). A single best estimate of the increase in each covariance was calculated from the slope of covariance *vs.* generation. Corresponding V_M values were calculated using only data from block-line-extraction combinations where both traits had been measured. Table 5 gives the genetic correlations for traits with significant increases in V_M in Table 4. Since correlations cannot be calculated when a variance component is negative, the confidence limits on the valid correlations do not necessarily agree with the significance levels from the covariance test. In addition the confidence limits are two-sided, while the covariance has been subjected to a one-sided test.

The overall pattern of these correlations is quite striking; every correlation is positive. They range from 0.41

TABLE 4

Rate of increase in line variance ($V_M \times 10^4$) per generation due to mutation, estimated from combined data, standardized to a block mean of 1.0

Trait		All lines	Quasi-normals
Early fecundity	Med.	1.192*	1.025*
	L	0.069	-0.190
	U	2.574	2.304
Late fecundity	Med.	5.252**	4.312**
	L	1.832	1.199
	U	9.100	8.537
Male longevity	Med.	1.798*	0.927
	L	0.135	-0.556
	U	4.137	2.588
Female longevity	Med.	1.141*	0.311
	L	-0.013	-0.425
	U	2.710	1.057
Early mating	Med.	-1.629	-1.697
	L	-12.488	-13.670
	U	8.810	9.170
Late mating	Med.	33.311†	33.995†
	L	-11.708	-17.605
	U	96.024	93.288
Productivity	Med.	5.001*	4.141*
	L	0.565	-0.315
	U	9.551	8.942
Male weight	Med.	-0.018	-0.027
	L	-0.221	-0.248
	U	0.194	0.166

Data are quantiles from 1,000 bootstrap resamplings of the entire data set. For each character, the median estimate is presented in the first row. The second and third lines give the 2.5 and 97.5% percentiles.

to 0.93 for all lines, and from 0.238 to over 1 for the quasi-normal sample. While many of the covariances and correlations are significantly different from 0, none of the correlations are significantly different from 1.0. This suggests that most new mutations affect all components of these life-history traits in similar ways. This pattern is not caused by the severely deleterious lines, as the mean correlation is 0.68 for all lines, and 0.62 for the quasi-normals.

DISCUSSION

We have studied the homozygous effects of mutations accumulated on the second chromosome of *D. melanogaster*, in the virtual absence of natural selection. We obtained strong evidence for the importance of mutation for fecundity, longevity, productivity (a measure of fecundity times viability) and a measure of fitness, and somewhat weaker evidence for male mating ability late in life. We found no evidence for mutation-accumulation in male mating ability early in life, and very weak evidence for weight. The most striking results of this study are the high, positive, mutational correlations among traits.

Several factors must be borne in mind when examining our results. First of all, we studied effects accumulated only on the second chromosome, about 40% of the *D. melanogaster* genome. Therefore, our estimates of V_M in Table 4 should be multiplied by 2.5 to extrapolate them to the whole genome. On the other hand, we have estimated the homozygous effects of new mutations. Previous experiments have shown that mutations affecting life history traits are overwhelmingly deleterious in their effects (CROW and SIMMONS 1983; LYNCH 1988), so that the homozygous effects themselves are of little interest in random mating populations. Spontaneous mutations affecting viability have been shown to be nearly additive in their effects. Using a model where the relative phenotypes of the original homozygote, mutant heterozygote and mutant homozygote are 1, $1 - hs$ and $1 - s$, respectively, experiments suggest that for new mutations affecting viability h, the dominance coefficient, is approximately 0.4 (MUKAI *et al.* 1965; MUKAI and YAMAZAKI 1968; OHNISHI 1977a; SIMMONS and CROW 1977). Assuming that these estimates are applicable to our life history traits, the increment in additive variance due to mutation over the whole genome should be $2(1/0.4)h^2 = 0.8$ times the values in Table 4.

Our estimates of the genetic variances and covariances are biased slightly, as the replicate extractions have an average coancestry of 0.042 for third and fourth chromosomes and 0.021 for the X chromosome. The shared segments in all cases are derived from the balancer stock used during mutation-accumulation. Using standard estimates of relative chromosome sizes we estimate that the among line variance would be biased upwards by an amount between 0.021 times the additive genetic variance in the balancer stock and 0.021 times the homozygous genetic variance. Since our V_M estimates are calculated on a per generation basis, the expected bias is the average of 0.021/33 and 0.021/44, or about 0.0006, times the appropriate genetic variance.

Several factors argue that the biases are unlikely to be large in our experiments. Following the initial generations of extraction, each extraction was maintained under crowded conditions in single vials, slowing further inbreeding. This suggests that the bias will be much closer to the additive variance than the homozygous variance. Our mean-standardized estimates of V_M, scaled to additive effects on the whole genome, as above, are comparable to I_A (the ratio of additive genetic variance to the square of the trait mean) values for fecundity and longevity reviewed by HOULE (1992). For these traits the ratio V_M/V_A for all lines ranges from 0.03 to 0.006, at least an order of magnitude larger than the 0.0006 possible from background additive variance. Furthermore, the population size following extraction would also allow some selection, thereby reducing the frequencies of any shared segments with large effects on fitness, which would be likely to contribute a large proportion of the background variance. The balancer stock is also unlikely to have levels of genetic variance as high as those in an

TABLE 5

Genetic correlations between traits with significant increases in variance

Trait		Fitness		Early fecundity		Late fecundity		Male longevity		Fem. longevity		Productivity	
Fitness	Med.			**0.690†**	85	**0.691****	99	**0.664†**	61	**0.875***	90	**0.605***	81
	L				-1.434		0.116		-0.350		-0.033		0.046
	U				2.953		1.265		2.984		3.540		2.137
Early fecundity	Med.	**0.571†**	77			**0.666****	97	**0.680†**	95	**0.636**	97	**0.756†**	94
	L		-0.446				0.203		-0.409		-0.285		-0.105
	U		2.280				1.682		2.288		1.820		1.961
Late fecundity	Med.	**0.736***	100	**0.591***	92			**0.562†**	95	**0.817****	99	**0.448†**	94
	L		0.010		0.085				-0.200		0.155		-0.172
	U		1.758		2.523				1.305		1.673		1.240
Male longevity	Med.	**0.818**	57	**0.555†**	88	**0.399**	89			**0.780***	97	**0.607†**	93
	L		-0.400		-0.245		-0.320				0.232		-0.324
	U		2.964		3.880		1.715				1.877		1.813
Female longevity	Med.	**1.022†**	73	**0.854**	67	**1.192****	77	**0.666**	66			**0.606***	93
	L		-0.787		-1.007		0.378		-5.680				0.028
	U		3.53		26.503		4.949		3.093				1.698
Productivity	Med.	**0.641***	76	**0.588**	84	**0.334**	81	**0.367**	78	**0.697**	66		
	L		-0.090		-0.517		-0.574		-1.670		-1.151		
	U		1.642		4.113		1.478		3.181		3.748		

Correlations among all lines above the diagonal, quasi-normal lines only below. For each trait, the median correlation is in bold type, and followed by the significance level for a 1-tailed test that the covariance is greater than 0, and by the number of estimates of the correlation where both variances were greater than 0. The second and third lines give the lower and upper 95% confidence limits, respectively, for the correlation. Significance values were not adjusted for multiple comparisons.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

outbred population. The balancer stock's genetic background was initially derived from the *IV* population, but during the course of these experiments the stock was expanded from small numbers of flies on several occasions, probably reducing the background variation in the process.

Aside from these factors which are expected to reduce the background variance, the data themselves do not suggest an important role for bias from this source. Our variance estimates for cage fitnesses are not affected by background variation, as fitnesses are calculated relative to the performance of a genotype within the same cage, and therefore with the same genetic background. If the background effect was large, we would expect to see a discrepancy between the variances and correlations involving fitness, and those which do not involve fitness. In particular the variance of the non-fitness components would be biased upward, while the covariance would not, leading to low estimates of the genetic correlations. In fact all of the estimates seem consistent, as well as consistent with previous studies of variation in viability (see below).

Our estimates of genetic correlations may be biased downward, as the environments flies experienced during each assay were quite different. Our fecundity and longevity assays were carried out in benign environments with little competition, but the fitness cages constitute an extremely competitive, if physically benign, environment. Flies used in the longevity assays were unmated. Rather different estimates might have been obtained if, for example, longevity could be assayed within the crowded cages where fitness was assayed. Consistent with these arguments, A. S. KONDRASHOV and D. HOULE (submitted for publication) have shown that the differences in relative fitness between mutation-accumulation lines drawn from generation 62 of this experiment and a line subjected to natural selection are greatly magnified under more competitive conditions.

Our relative lack of success in detecting mutational variance in male mating ability and weight is not unexpected. Our assay of male mating ability is the proportion of matings obtained by test males in competition for 10 females, and only about 130 such tests were performed with MA lines at each age. Since the error variance for each assay is approximately binomial, it is clear that our power to detect mutational effects was limited. We measured weight as an indicator of density effects on other traits, rather than as a trait of primary interest in this study. We expect that careful control of larval densities might reveal genetic variation in size.

Comparisons to previous mutation studies: Our mean standardized results are consistent with measures of egg-to-adult viability and development rate from previous mutation-accumulation experiments on the sec-

ond chromosome of *D. melanogaster*. In a large series of experiments, MUKAI *et al.* (1972) estimated a standardized mutational variance of 3.3×10^{-4} for non-lethal chromosomes. Very similar estimates were also obtained for development rate (MUKAI and YAMAZAKI 1971; YOSHIMARU and MUKAI 1985). OHNISHI (1977b) also obtained estimates of mutational parameters for viability in similar experiments, obtaining an estimate for non-lethals of $V_M = 2.1 \times 10^{-4}$. For our study, comparable values for the five fitness components with significant variance among all lines average $V_M = 2.88 \times 10^{-4}$. For cage fitnesses, the comparably standardized estimate is 17.2×10^{-4}, with a lower 95% confidence limit of 6.1×10^{-4} (HOULE *et al.* 1992). MUKAI's viability estimates differ slightly in that they are standardized to a premutation mean of 1.0, while we have standardized to a mutant mean of 1.0.

The high, positive mutational correlations in our data have precedents in other mutation-accumulation experiments. Development rate and viability are strongly positively correlated for unselected mutations, with most estimates being 0.9 or greater (MUKAI and YAMAZAKI 1971; YOSHIMARU and MUKAI 1985). Unpublished observations of O. OHNISHI on EMS-induced mutations also show positive correlations (CROW and SIMMONS 1983). SIMMONS *et al.* (1980) compared viability and fitness of heterozygotes from four different sets of mutation-accumulation lines, and found weaker evidence for positive correlations. Experimental difficulties may have affected the results of this study, as the genetic variances among groups of lines do not correspond to the amounts of mutation-accumulation they had undergone, and one set of lines gave strikingly different results than the others. The authors noted a high frequency of hybrid dysgenesis in crosses similar to those used to estimate viability and fitness. A small mutation-accumulation experiment in Daphnia showed a variety of correlations among fitness components, although the only correlations which were significantly different from 0 were consistent with positive correlations near 1 (LYNCH 1985).

The many high positive correlations among life history traits suggest that most unselected mutations will have deleterious pleiotropic effects on all components of fitness. The fact that mutational variance for fitness is higher than those for fitness components is consistent with this interpretation. We have previously shown that mutation load can have a significant impact on genetic covariances of life-history traits under the assumption that mutation-selection balance maintains genetic variance (CHARLESWORTH 1990b; HOULE 1991). This is particularly so when the mutational covariance is very different from that predicted by optimality models (HOULE 1991), as we have shown here.

Comparisons of these mutation studies with the correlations found in outbred populations suggests that selection does tend to purge those alleles contributing positive covariance between life history traits. The result is that correlations due to standing variation are considerably lower than those among unselected mutants, although they do not necessarily become negative. Early and late female fecundity have been shown to have a small negative additive genetic correlation in the same *IV* base population used in our experiments (ROSE and CHARLESWORTH 1981a). YOSHIMARU and MUKAI (1985) found that the correlation of development rate and viability of chromosomes from a natural population in both homozygous and heterozygous condition was about 0.25, as compared to the correlation of 0.9 among new mutants. SIMMONS *et al.* (1980) found evidence for a slight negative correlation between viability and fitness of heterozygotes from a natural population. On the other hand, when genotypes from outbred populations are inbred, they show strong positive correlations between fitness components (HIRAIZUMI 1961; ROSE 1984a; MACKAY 1986), consistent with the inefficiency of selection in removing recessive deleterious variation.

The similarity of our estimates of V_M to those for viability, (see above) and the strong positive mutational correlations found in this and other studies suggest that the genomic mutation rate of our life history traits is similar to that for viability as well. Previous minimum estimates of total mutation rate for viability on the second chromosome are between 0.07 and 0.15 (CROW and SIMMONS 1983). The consistent estimates by MUKAI favor the higher figure (MUKAI 1964; MUKAI *et al.* 1972). Extrapolating to the entire haploid genome a minimal estimate of the haploid genomic mutation rate is about 0.38/generation. This estimate is derived by assuming that there is no variation in the effects of new mutations, which is clearly not true. Assuming reasonable levels of variation in allelic effects, would require increasing his estimate by a factor of two or more (CROW and SIMMONS 1983).

Implications for the evolution of senescence: The mutation-accumulation hypothesis for the evolution of senescence depends directly on two assumptions about new mutations (CHARLESWORTH 1984; PARTRIDGE and BARTON 1993). The first of these is that mutation pressure decreasing mean performance must be large. A series of experiments selecting for late life performance show that early fecundity declines at about 2.5% in such experiments (ROSE and CHARLESWORTH 1981b; LUCKINBILL *et al.* 1984; ROSE 1984b; CLARE and LUCKINBILL 1985), while male mating ability declines at nearly 1%/generation (SERVICE 1993). The second assumption is that mutations must be substantially independent in their effects on early and late life performance. If this were not the case, then, in the experiments cited above, changing the age where selection acts most strongly would have little effect on early life performance, as alleles normally kept at low frequencies by selection early

in life would still be kept at low frequencies by selection acting only late in life. The relatively high mutational correlation of 0.6 we found between early and late fecundity suggests that mutation-accumulation is unlikely to explain the experimental results. In order to rescue the mutation-accumulation hypothesis with such a high correlation, one would have to assume that the mutational pressure decreasing early fecundity is many times larger than that affecting viability in previous mutation-accumulation experiments. Even assuming age-independent effects, the rate of viability decline due to mutation is not as high as the decline in early fecundity noted above (CHARLESWORTH 1984).

A number of other lines of evidence have suggested that mutation-accumulation may play a role in determining *D. melanogaster* life span, and our data do not rule this out. SERVICE *et al.* (1988) showed that reversal of selection for late life performance did not reverse gains in ethanol and desiccation resistance, although early fecundity and starvation resistance did return to their original levels. This suggests that alleles which lead to ethanol and dessication resistance do have late-life specific effects, HUGHES and CHARLESWORTH (1994) found that additive genetic variance for survival probability and male mating ability increase markedly with age in males in the *IV* population, as predicted under the mutation-accumulation hypothesis (CHARLESWORTH 1994). Alternative explanations of these data are possible. For example, the average effects of alleles could increase with age, without the presence of mutants with age specific effects. Our mutational data provide some weak evidence that either the number or the effects of alleles are magnified late in life, as both fecundity and male mating have higher V_M values than their early life counterparts. In neither case is this difference significant. A recent series of selection experiments shows weak evidence for tradeoffs between early and late fecundity, and has also been interpreted as providing some evidence for the mutation-accumulation hypothesis (PARTRIDGE and FOWLER 1992; ROPER *et al.* 1993). As the authors point out, many of their results can also be ascribed to unintended selection, due to the fact that the base populations for these experiments is maintained on a continuous schedule, while both selection regimes are carried out on a discrete generation schedule. On the other hand these experiments suggest that tradeoffs did take place between larval and adult fitness. Under the antagonistic pleiotropy hypothesis, it would not be surprising if different pairs of traits are involved in tradeoffs in different populations.

The high mutational correlations among traits may seem to pose a challenge to the antagonistic pleiotropy theory of senescence as well; however, the pleiotropy theory does not depend on continual input of mutations with antagonistic effects. Since mutations which increase early-life fitness at the expense of late-life fitness can be favored by selection, their occurrence at any time in the evolutionary history of a lineage can increase its rate of senescence (PARTRIDGE and BARTON 1993). To explain the correlated decreases in early fecundity under late-life selection outlined above, it is necessary that alleles with antagonistic effects favoring late-life fitness and depressing early-life fitness be segregating in the selected populations. As outlined above, there is substantial evidence that selection decreases the covariance, consistent with the existence of such alleles. In the extreme, alleles with antagonistic effects may form protected polymorphisms, and so maintain polymorphism indefinitely in large outbreeding populations (ROSE 1982). Thus, mutational data cannot reject the antagonistic pleiotropy hypothesis.

Implications for the maintenance of genetic variance: V_M is an essential parameter in mutation-selection balance models for the maintenance of genetic variance (BARTON and TURELLI 1989), and the adequacy of the mutation-selection balance model is also important to models of inbreeding depression, mate choice, and the evolution of outbreeding devices (see *Introduction*). One important indication of the strength of mutation in promoting variation is the ratio V_M/V_A with V_M adjusted to heterozygous effects on the whole genome, as outlined above. Under the mutation-selection balance model V_M/V_A is the inverse of the average time that a deleterious allele would have to persist in the population to explain the observed level of V_A (CROW 1993b). Our values of V_M/V_A (above) suggest short persistence times of 33–167 generations, which are consistent with the expected persistence times for spontaneous mutations affecting viability (CROW 1993b). Therefore, it appears likely that mutation-selection balance can explain a substantial proportion of the genetic variance in fecundity and longevity. V_M is also likely to be a useful predictor of the rate at which populations can respond to long term selection pressures (HILL 1982).

Comparing mutational variances: Previous authors summarizing V_M data have standardized their estimates by the environmental variance (V_E), yielding the increment in heritability due to a single generation of mutation (LYNCH 1988). For fitness components, the environmental variance is irrelevant to the response to selection, making it unlikely that V_M/V_E is an appropriate quantity with which to compare levels of variation (HOULE 1992). However, for comparative purposes we have calculated V_M/V_E from our data by averaging estimates of V_E from the residual variance from the single block ANOVAs of mean standardized data. To make our estimates comparable to those in LYNCH (1988), we adjusted our estimates of V_M to heterozygous effects on the whole genome as described above. Our estimates of V_M/V_E, calculated from all lines, are 1.8×10^{-3} for early fecundity, 3.7×10^{-3} for late fecundity, 0.8×10^{-3} for male longevity, and 1.2×10^{-3} for female longevity.

These estimates cluster around the value of 1×10^{-3} usually assumed to be typical of quantitative traits, based on extensive studies of variation in bristle numbers in Drosophila. In fact there is substantial variation around this figure, although the median over all traits is approximately 1×10^{-3} (LYNCH 1988). This similarity of V_M/V_E estimates probably is an artifact of the choice of V_E to standardize the estimates. Data from outbred populations shows a positive correlation between V_A and V_E, when standardized by trait means (HOULE 1992). Thus, a likely explanation for the clustering of V_M/V_E values is a similar positive relationship between V_M and V_E. On a mean standardized scale, V_E tends to be higher for life history traits than for other traits in outbred populations, even though heritabilities for life history traits are lower, implying that life history traits have disproportionately large environmental variances. Similar V_M/V_E values may conceal considerable variation in mutational parameters.

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