EMS-Induced Polygenic Mutation Rates for Nine Quantitative Characters in *Drosophila melanogaster*

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ABSTRACT

Polygenic mutations were induced by treating *Drosophila melanogaster* adult males with 2.5 mm EMS. The treated second chromosomes, along with untreated controls, were then made homozygous, and five life history, two behavioral, and two morphological traits were measured. EMS mutagenesis led to reduced performance for life history traits. Changes in means and increments in genetic variance were relatively much higher for life history than for morphological traits, implying large differences in mutational target size. Maximum likelihood was used to estimate mutation rates and parameters of distributions of mutation effects, but parameters were strongly confounded with one another. Several traits showed evidence of leptokurtic distributions of effects and mean effects smaller than a few percent of trait means. Distributions of effects for all traits were strongly asymmetrical, and most mutations were deleterious. Correlations between life history mutation effects were positive. Mutation parameters for one generation of spontaneous mutation were predicted by scaling parameter estimates from the EMS experiment, extrapolated to the whole genome. Predicted mutational coefficients of variation were in good agreement with published estimates. Predicted changes in means were up to 0.14% or 0.6% for life history traits, depending on the model of scaling assumed.

KNOWLEDGE of mutation rates and characteristics of distributions of mutation effects is fundamentally important for quantitative genetic models (Barton and Turelli 1989). There is a good deal of information available on rates of increase of genetic variance for quantitative traits from spontaneous mutation (reviewed by Lynch 1988; Houle *et al.* 1996), but information is much more scarce on rates of change of means and mutation-induced changes in genotypic distributions, both of which are required to make specific inferences about rates and characteristics of the underlying mutation events (Keightley 1994). Experiments to measure distributions of effects of transposable element (TE) insertional mutations in Drosophila have provided valuable information on this important class of mutation event (Eanes *et al.* 1988; Mackay *et al.* 1992; Lyman *et al.* 1996), but, until recently, the only information on rates of changes of mean for quantitative traits due to accumulation of spontaneous mutations has come from experiments involving the maintenance of Drosophila second chromosomes protected by balancer chromosomes (Mukai 1964; Mukai *et al.* 1972; Ohnishi 1977b). These experiments showed rapid declines in viability of wild-type chromosomes, relative to the balancer, and provided estimates of spontaneous mutation rates per second chromosome per generation for viability in excess of 0.12 events per generation (summarized by Crow and Simmons 1983). These results have had a major influence in several areas of population genetics.

More recently, experiments not involving balancers in which spontaneous mutations were allowed to randomly accumulate in the whole genome have been carried out with replicated inbred lines of Drosophila (Fernandez and Lopez-Fanjul 1996) and the nematode *Caenorhabditis elegans* (Keightley and Caballero 1997). Both experiments showed small rates of decline of fitness traits, and imply a much lower overall mutation pressure than inferred from the experiments involving balancers (Keightley 1996; Garcia-Dorado 1997; Peck and Eyre-Walker 1997). Similar conclusions have been reached from observations of the long-term stability of viability and fertility in small populations of Drosophila and mice (Caballero and Keightley 1998).

The study of spontaneous mutations affecting quantitative traits in *Drosophila melanogaster* is a laborious undertaking. Thus, in the present experiment, ethyl methanesulfonate- (EMS-) induced mutations were investigated as an approximation to spontaneous mutations. EMS induces a spectrum of mutation events different from spontaneous mutations, most critically because it does not induce TE insertions, a major source of spontaneous mutation events (Green 1988). However, the single base-pair changes which constitute the bulk of EMS mutations (Vogel and Natarajan

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1979; Ashburner 1989; Pastink *et al.* 1991) may be more interesting from an evolutionary standpoint, as TE insertions do not appear to become fixed between populations (Langley *et al.* 1988). Previous studies of the effects of EMS on quantitative trait variation in Drosophila have shown large increases in genetic variation for viability (Mukai 1970) and longevity (Koivisto and Portin 1987), and increased response to selection on sternopleural bristles in EMS-treated lines relative to controls (Grundl and Dempfle 1990). The present experiment was carried out by O.O. in the National Institute of Genetics, Mishima, Japan, in 1975, but only a summary of results has ever been published (Crow and Simmons 1983).

Nine quantitative characters were studied: five were important fitness components—competitive viability, egg productivity, hatchability, development time and longevity; two were morphological—body length and abdominal bristle number; and two were behavioral—phototaxis and mating speed of males. In this paper, increases in variance and changes of mean relative to a contemporary untreated control are compared for the nine traits. Based on maximum likelihood (ML), numbers of mutation events induced by EMS and parameters of the distributions of their effects are estimated. Finally, by using information on rates of accumulation of lethal mutations and the rate of accumulation of variability for bristle number as standards, predicted rates of changes of means and variance due to the accumulation of spontaneous mutations are obtained for all the traits measured.

MATERIALS AND METHODS

Drosophila stocks: The wild-type stock used in the experiment, abbreviated *+/+*, was an inbred line from a natural population of Madison, Wisconsin. A laboratory stock abbreviated *Cy/Pm In(2) SM1 al*^{2} *Cy sp*^{2} */ In(2) Pm dp b Pm ds*^{33k}, having the same inbred genetic background, was used to isolate mutagenized and control second chromosomes, and as a competitor in the viability assay.

EMS mutagenesis: Starting from a single second chromosome of the wild-type stock, 203 *Pm/+* lines were established by crossing a *Cy/Pm* female with a wild-type male, then crossing a *Pm/+* son to *Cy/Pm* (Figure 1). In the next generation, two *Pm/+* males were randomly picked from each line; one was used as a control and the other was treated with EMS. Young adult males were treated with 2.5 × 10^{-3} M EMS ac-

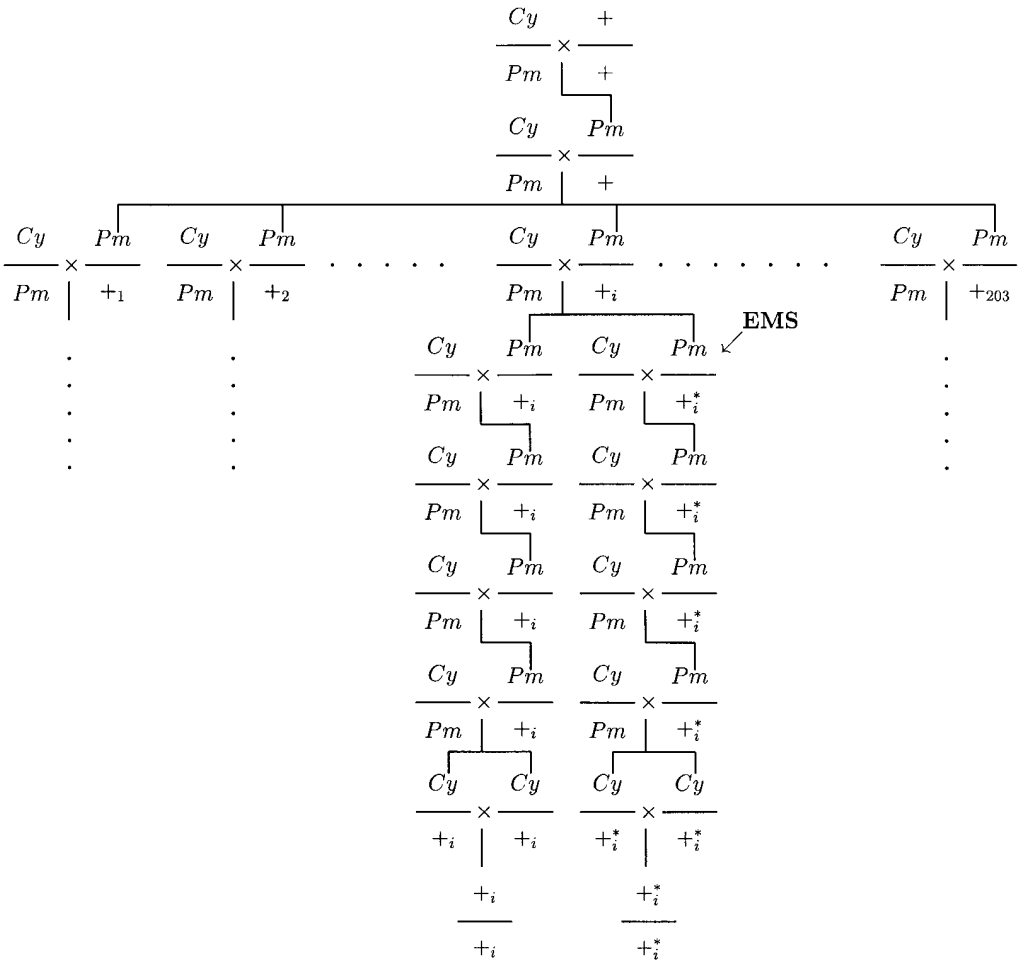

Figure 1.—Crossing scheme to establish EMS-treated and control homozygous chromosome lines from a single second chromosome.

cording to the method of Lewis and Bacher (1968) (see also Ohnishi 1977a). Treated and untreated second chromosomes were then made homozygous by mating *Cy/+* individuals *inter se*, after backcrossing males to *Cy/Pm* females for three generations to purge chromosome *3* of EMS-induced mutations. The number of EMS-treated chromosomes containing a homozygous lethal mutation was 70 out of the total of 203 tested. Assuming that the number of lethal mutations per chromosome is Poisson distributed, the estimated lethal mutation rate per chromosome is 0.42, which is similar to rates measured previously for similar doses of EMS (Alderson 1968; Ohnishi 1977a). No lethal or sterile chromosomes were found in the untreated lines, 75 of which were randomly chosen as controls. Among the 203 EMS-treated chromosomes, 121 lines homozygous for mutagenized second chromosomes were established and maintained for assays of quantitative characters; the remainder were maintained as *Cy/* lethal or *Cy/*sterile. Flies were cultured in 3 × 9.5 cm vials containing cornmeal-ebios medium at 25° unless otherwise stated, and quantitative traits measured for each line in three replicates. Trait assays were performed concurrently for control and EMS-treated lines. For time-consuming traits such as phototaxis, the measurements were carried out over a few days in groups of randomized control and treated lines. Viability was the only trait for which homozygotes were measured relative to *Cy/+*. For the remaining traits, all assays were performed using homozygous flies from the treated or untreated lines.

Preadult viability: The *Cy* method of Wallace (1956) was used to measure viabilities of treated and control chromosomes in the homozygous state relative to heterozygotes for the *Cy* chromosome. A single *Pm/+* male was backcrossed to *Cy/Pm* females as per Figure 1. The parents for the viability test were five *Cy/+* females and five *Cy/+* males, mated in 180 ml milk bottles at 25°. These are essentially the same conditions as used in a previous study of effects of EMS-induced and spontaneous mutations on viability (Ohnishi 1977a,b,c). There were three replicates per line, and progeny were counted four times at two-day intervals starting from the 10th day after the cross. The viability index was the ratio of *+/+* flies to *Cy/+* flies.

Fecundity: For each chromosome line, three replicates of 10 pair matings were set up in milk bottles. The pairs came from the EMS-treated or control homozygous lines maintained in vials. From each bottle, six virgin females and six young males were collected and kept at 25° for 48 hr. These flies were mated and transferred to vials containing fresh medium after 24, 48, and 72 hr, and discarded after 96 hr. Eggs deposited during each of the four 24-hr periods were counted. The trait is expressed as the average daily productivity per female.

Hatchability: The number of offspring emerging from the above four successive cultures in the fecundity assay was counted from day 11 after the first transfer. The trait is the total number of emerged flies divided by the total eggs deposited, expressed as a percentage. It is equivalent to the trait egg-to-adult viability assayed by Fernandez and Lopez-Fanjul (1996) in a spontaneous mutation accumulation experiment.

Development time: For each chromosome line, three replicates of 10 pairs of 2–3-day-old homozygous males and females from the milk bottle cultures mentioned in the fecundity assay were kept in a vial for one day, then transferred to a vial containing fresh medium, and allowed to deposit eggs for 3 hr to achieve good synchronization of larval development. The vials, which contained about 100 eggs, were kept at 25° under constant illumination of 30 lux. They were checked for emerged adults at 180 hr after the cross and at 3-hr intervals thereafter. The time of emergence of the first five adult flies was recorded. The trait is the time of emergence of the fifth fly in hours.

Longevity: Three replicates of 15 virgin females and 15 males were sampled from the bottle cultures mentioned in the fecundity assay. These flies were crossed in a vial, then live flies transferred at 3-day intervals. The number of dead flies was counted every day. The mean survival time of the middle five flies of each sex, that is, the 6th to 10th longest lived flies was used as an index of longevity, expressed in days.

Mating speed of males: For each line, there were three replicates consisting of 10 homozygous males and 12 virgin females of the original wild-type stock in two groups of 6 females and 5 males, mated in vials. Flies were 72 hr old at the start of the assay. The number of copulated pairs at 5 and 10 min after the cross was counted. The trait is the proportion of copulated pairs at 5 or 10 min after the cross.

Phototaxis: Three replicates of 30 24-hr-old males from the milk bottle cultures mentioned in the fecundity assay were kept in the dark until the time of the experiment. These flies were put in the light-neutral central section of a three chamber phototaxis choice box with sliding gates. One side of the box was darkened by covering with a cloth, then the gates on both the light and dark sides were opened. After one minute the gates were closed and the number of flies in each section counted. The trait is the number of flies in the light zone. The intensity of illumination at the surface of the box was about 100 lux.

Body length: Eight males and eight females were sampled from each of three replicates of 10 mated pairs of flies. The trait is the distance from the top of the head to the tip of a wing in millimeters, measured using a micrometer under a microscope.

Abdominal bristle number: The same flies were used as in the body length assay. The trait is the number of bristles on the fourth sternite.

ML estimation of *U* and mutation distribution parameters: The likelihood of data for each trait was computed independently. Likelihood evaluation was based on the method described by Keightley (1994), with several computational improvements. An observed value used in the likelihood evaluation was the mean trait value for a line. By using mean values, rather than values for each of the three individual replicates within a line, there is a potential loss of information. However, the use of within line information would be computationally difficult, as an additional common environmental effect would need to be fitted by an additional integration step (*e.g.*, Haley *et al.* 1993). The method, as presently implemented, assumes that control and EMS lines are subject to the same common environmental influences. For the control lines, it was assumed that the distribution of line means was normal with mean M and variance σ_E^2. The variation among these lines is due to random environmental effects, and, as seen in the results, some background genetic variation. The EMS-treated lines were assumed to be subject to the same sources of variation as the controls, and to carry n independent mutations, where n was assumed to be a random variable from the Poisson distribution, $p(n|U)$, parameter U. The effects of individual mutations, a, were assumed to be from a gamma distribution, $g(a|\alpha,\beta)$, reflected about zero, with parameters α specifying scale, β shape, and a parameter P specifying the proportion of the density positive [see Keightley (1994) for details of the density function]. Changes in β generate distributions with a wide variety of shapes, ranging from equal effects ($\beta \to \infty$) to strongly leptokurtic distributions with the majority of effects close to zero and a long tail ($\beta \to 0$). Mutations were assumed to act additively.

For the unreflected gamma distribution, the likelihood of an observed value Z_i can be written:

$$L(Z_i|\alpha, \beta, U, M, \sigma_E^2) = p(0|U)f(Z_i|M, \sigma_E^2) + p(1|U)\int f(Z_i - a|M, \sigma_E^2)g(a|\alpha,\beta)da + p(2|U)\iint f(Z_i - a_1 - a_2|M, \sigma_E^2)g(a_1|\alpha,\beta) g(a_2|\alpha,\beta)da_1 da_2 + \ldots, \quad (1)$$

where $f(x|M, \sigma_E^2)$ is the normal density function (Keightley 1994). Due to the additivity property of the gamma distribution, sums of n gamma deviates parameters α, β are gamma distributed with parameters α, $n\beta$, so (1) simplifies to:

$$L(Z_i|\alpha, \beta, U, M, \sigma_E^2) = p(0|U)f(Z_i|M, \sigma_E^2) + p(1|U)\int f(Z_i - a|M, \sigma_E^2)g(a|\alpha,\beta)da + p(2|U)\int f(Z_i - a|M, \sigma_E^2)g(a|\alpha,2\beta)da + \ldots \quad (2)$$

Note that (1) contains a series of terms with multidimensional integrals, which have become single integrals in (2). with a reflected gamma distribution of mutation effects, in which there are fractions P and $1 - P$ of mutations with effects greater and less than zero, respectively, terms in (2) need to be expanded to account for the binomial probabilities of different numbers of positive or negative effects:

$$L(Z_i|\alpha,\beta,P,U,M,\sigma_E^2) = p(0|U)f(Z_i|M,\sigma_E^2) + p(1|U)[P\int f(Z_i - a|M, \sigma_E^2)g(a|\alpha,\beta)da + (1-P)\int f(Z_i + a|M, \sigma_E^2)g(a|\alpha,\beta)da] + p(2|U)[P^2\int f(Z_i - a|M, \sigma_E^2)g(a|\alpha,2\beta)da + 2P(1-P)\iint f(Z_i - a_1 + a_2|M, \sigma_E^2) g(a_1|\alpha,\beta)g(a_2|\alpha,\beta)da_1 da_2 + (1-P)^2\int f(Z_i + a|M, \sigma_E^2)g(a|\alpha, 2\beta)da] + \ldots. \quad (3)$$

Algorithm for computation of likelihood: In principle, it is possible to evaluate (2) or (3), which contain series of single or double integrals, by standard numerical integration procedures (*e.g.*, Press *et al.* 1992). However, for cases with strongly leptokurtic gamma distributions (say, $\beta < 1$), numerical integration by standard procedures was found to become increasingly slow, and was not feasible at all for double integrals. The following integration method was found, however, to give satisfactory accuracy. A list of tables of standardized gamma distributions, each with 325 elements, was precomputed for 224 values of β, and stored. The likelihood maximization procedure, however, required arbitrary values of β. If the procedure "demanded" a value of β^* not in the list, a new temporary table was generated by linear interpolation using the precomputed values of β on either side of β^*. Such tables could then be used in a numerical integration procedure to evaluate (3). The procedure gave essentially the same answers as a Monte Carlo procedure previously described (Keightley 1994). The main advantage is that the likelihood is evaluated without sampling error, more than 100 times faster than by Monte Carlo methods in many cases.

Maximization of likelihood: The overall likelihood of the data was the product of results of evaluation of (3) with Z_i values from the independent EMS-treated lines and the control lines (U was set to zero for the latter lines). For the traits with data in two sexes, Z_i was the average over sexes. As with the previous version of the procedure (Keightley 1994), the natural log likelihood of the data as a function of the parameters (U, α, β, P, M, σ_E^2) was maximized using the downhill simplex method (Nelder and Mead 1965). Following the suggestion of Press *et al.* (1992, Chapter 10) the maximization routine was automatically restarted after convergence was claimed until no further increase in likelihood was observed, as a guard against spurious convergence. For each parameter of interest, profile likelihoods (the likelihood of data as a function of one parameter with likelihood maximized with respect to all the others) were computed. Support limits for the parameter estimates were obtained on the basis of a drop in natural log likelihood of two from the maxima using the profile likelihood. Calculation of profile likelihoods builds up a picture of the multi-dimensional likelihood surface, and doing so helps guard against failure to find global maxima. As a further check, however, convergence to maxima was tested by restarting the procedure with different sets of starting parameter values. It was found that the mutation rate and distribution parameters are always very strongly confounded with one another, and likelihood maximization was often difficult for all these parameters fitted simultaneously, a problem overcome by producing profile likelihoods.

RESULTS

Effects of EMS on trait means and variance: EMS mutagenesis led to major changes in the frequency distributions of line means for the nine quantitative traits (Figure 2). To compare the effects of EMS on the overall trait means (M), differences in overall means between EMS-treated and control lines were scaled by control means, $\Delta M/M = 100 \times (M_{EMS} - M_C)/M_C$ (Table 1). EMS mutagenesis, as expected, led to reduced performance for life history traits. In all cases, $\Delta M/M$ is significantly different from zero, including the behavioral and morphological traits, indicating a prevalence of directional mutation effects. Note, for example, abdominal bristle number, a trait for which stabilizing selection has been detected (Nuzhdin *et al.* 1995; Garcia-Dorado and Gonzalez 1996), is mostly subject to downwardly acting mutations, as mutagenized lines tend to have fewer abdominal bristles than controls. The scaled changes of mean were precisely measured, as SE are relatively small. It is clear that directional mutation pressure is much higher for life history traits than for the morphological traits.

Analysis of variance (ANOVA) was used to estimate components of variance attributable to differences between lines, and, where data on two sexes were available, to sex $\times$ line interaction effects (Table 2). In the latter case, the "split plot" method was used to estimate the interaction component, with the two sexes split within each vial, and error variances between vials (error 1) and within vials (error 2) estimated [Snedecor and Cochran (1989), Chapter 16]. Because the control lines generally showed a significant component of variance between lines, presumably due to background and accumulated spontaneous mutational variation, the genetic variances induced by EMS treatment were estimated as differences between the between line variance component estimates for the treated and control lines, $V_g = V_{g,EMS} - V_{g,C}$. One way to compare the different V_g estimates is by scaling by the overall mean squared, that is, by

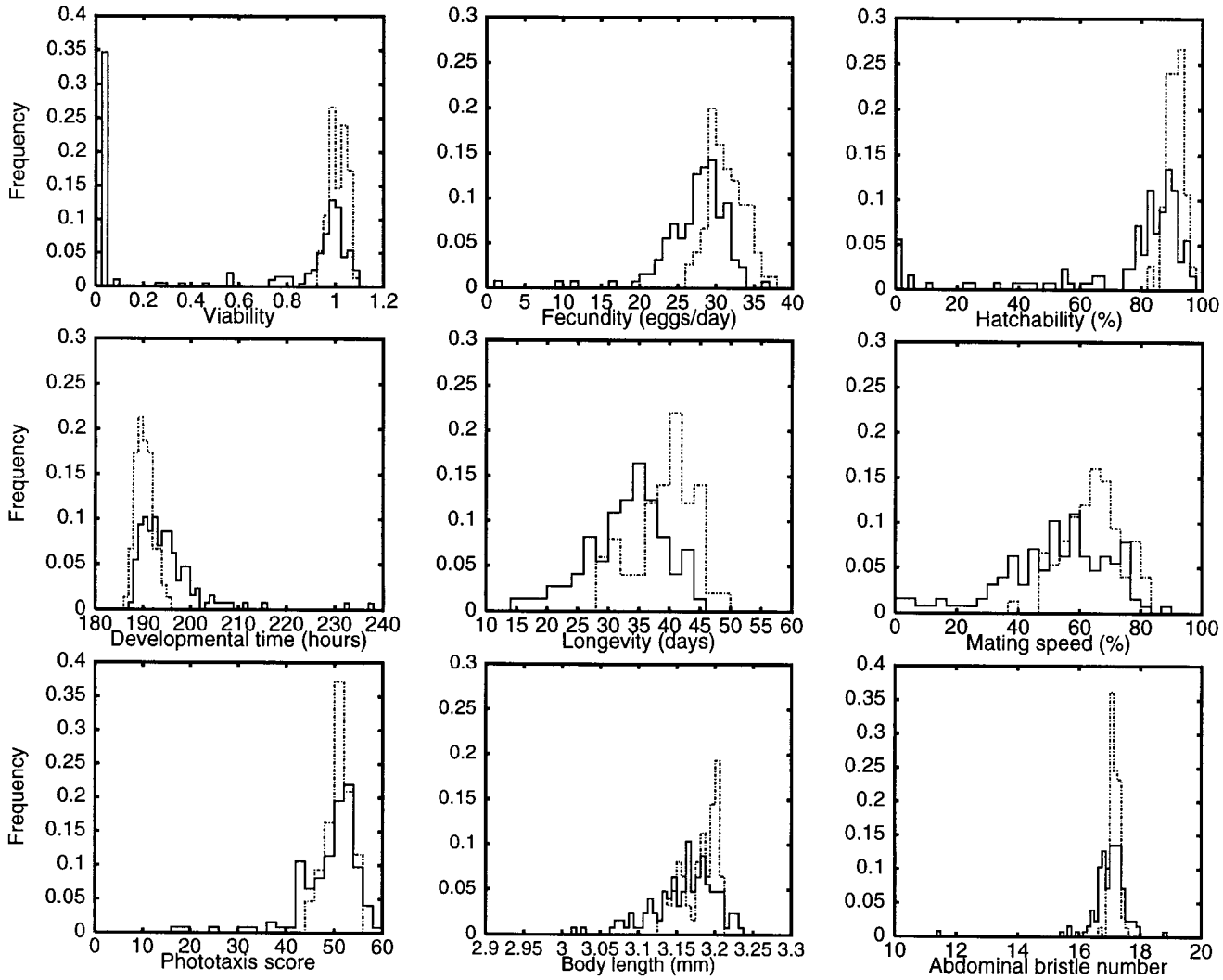

Figure 2.—Frequency distributions of mean trait values for control (·····) and EMS-treated (——) second chromosome lines.

expressing as the mutational coefficient of variation = $CV_g = 100 \times \sqrt{V_g}/M$, which is appropriate for fitness-related traits (Houle 1992; Houle *et al.* 1994). (Another possible scaling is by the environmental variance, but estimates for the traits are generally not available.) The CV_g estimates (Table 1) reveal a similar pattern to the $\Delta M/M$ estimated, with the life history traits having substantially higher values than morphological traits.

TABLE 1

Changes of trait means and between line variances from EMS mutagenesis for nonlethal lines

Trait	Trait type	% $\Delta M/M$	(SE)	% CV_g	(SE)[a]
Viability	Life history	−7.0	(1.4)	14	(2.3)
Fecundity	Life history	−13	(1.4)	12	(2.6)
Hatchability	Life history	−14	(2.0)	20	(2.9)
Development time	Life history	2.5	(0.35)	3.5	(0.70)
Longevity	Life history	−17	(2.5)	9.1	(3.4)
Mating speed	Behavioral	−20	(2.8)	24	(2.6)
Phototaxis	Behavioral	−3.6	(1.5)	12	(2.2)
Body length	Morphological	−0.62	(0.14)	1.1	(0.12)
Abdominal bristle number	Morphological	−1.0	(0.38)	3.8	(1.0)

[a] Obtained by bootstrapping.

TABLE 2

Results from ANOVAs to estimate between line and sex × line interaction variance components

Trait	Treatment	Source of variation	d.f.	MS	F	M	V_g
Viability	Control	Lines	74	4.20	1.58[b]	31.77	0.52
		Error	150	2.64			
	EMS	Lines	129	67.4	20.6[c]	29.54	21.4
		Error	260	3.27			
Fecundity	Control	Lines	74	17.2	3.16[c]	30.82	3.91
		Error	150	5.45			
	EMS	Lines	125	61.5	10.6[c]	26.77	18.6
		Error	252	5.78			
Hatchability	Control	Lines	74	22.8	1.77[b]	72.04	3.3
		Error	150	12.9			
	EMS	Lines	120	683	26.7[c]	61.99	219
		Error	242	25.6			
Development time	Control	Lines	74	10.6	2.05[c]	189.9	1.82
		Error	150	5.20			
	EMS	Lines	127	150.6	13.1[c]	194.7	46.4
		Error	256	11.5			
Longevity	Control	Lines	49	143	6.66[c]	38.18	19.9
		Error 1	100	21.4	2.33		
		Sex × Lines	49	10.8	1.18		0.54
		Error 2	100	9.20			
	EMS	Lines	72	251	5.08[c]	31.84	32.1
		Error 1	146	49.4	5.50		
		Sex × Lines	72	18.4	2.04[c]		3.13
		Error 2	146	8.99			
Mating speed	Control	Lines	74	255	2.05[c]	61.67	43.47
		Error	150	125			
	EMS	Lines	126	962	6.60[c]	49.46	272
		Error	254	146			
Phototaxis	Control	Lines	42	18.2	1.55[a]	49.64	2.17
		Error	86	11.7			
	EMS	Lines	122	134	9.03[c]	47.76	39.6
		Error	246	14.8			
Body length	Control	Lines	61	3.36×10^{-3}	7.57[c]	3.177	4.8×10^{-4}
		Error 1	124	4.44×10^{-4}	2.76		
		Sex × Lines	61	2.18×10^{-4}	1.35		1.9×10^{-5}
		Error 2	124	1.61×10^{-4}			
	EMS	Lines	125	1.04×10^{-2}	25.4[c]	3.157	1.6×10^{-3}
		Error 1	252	4.10×10^{-5}	1.99		
		Sex × Lines	125	4.16×10^{-4}	2.02[c]		7.0×10^{-5}
		Error 2	252	2.06×10^{-4}			
Bristle number	Control	Lines	68	0.125	1.05	17.08	0
		Error 1	138	0.119	1.13		
		Sex × Lines	68	0.164	1.55[a]		0.019
		Error 2	138	0.106			
	EMS	Lines	125	2.75	20.6[c]	16.91	0.43
		Error 1	252	0.134	1.05		
		Sex × Lines	125	0.182	1.42[b]		0.018
		Error 2	252	0.128			

The hatchability data were square-root arcsine transformed.
[a] $0.01 < P < 0.05$.
[b] $0.001 < P < 0.01$.
[c] $P < 0.001$.

Estimation of sex × line interaction effects was possible for three traits, longevity, body length, and bristle number, for which measurements were made in both sexes. ANOVA by the split plot method detected significant sex × line interactions for all three traits (Table 2), but the variance components are small, less than one-tenth of the between line variance component. This result contrasts with the large sexual dimorphism

TABLE 3

ML estimates of mutation parameters with minimum and maximum support limits obtained from profile likelihoods

	U			Mean effect = $\beta/(\alpha M_C)$			β			*P*		
Trait	Min	ML	Max	Min	ML	Max	Min	ML	Max	Min	ML	Max
Viability	0.21	$\to\infty$	$\to\infty$	$\to 0$	$\to 0$	0.28	$\to 0$	$\to 0$	3.1	0	0	0.095
Fecundity	2.8	$\to\infty$	$\to\infty$	$\to 0$	$\to 0$	0.043	$\to 0$	$\to 0$	0.60	0	0	0.11
Hatchability	1.6	$\to\infty$	$\to\infty$	$\to 0$	$\to 0$	0.11	$\to 0$	$\to 0$	0.45	0	0	0.055
Dev. time	1.4	$\to\infty$	$\to\infty$	$\to 0$	$\to 0$	0.019	$\to 0$	$\to 0$	0.90	0.94	1	1
Longevity	0.78	$\to\infty$	$\to\infty$	$\to 0$	$\to 0$	0.16	$\to 0$	$\to 0$	$\to\infty$	0	0	—
Mating speed	0.39	0.75	$\to\infty$	$\to 0$	0.26	0.41	$\to 0$	$\to\infty$	$\to\infty$	0	0	0.18
Phototaxis	0.21	0.37	$\to\infty$	$\to 0$	0.15	0.22	$\to 0$	2.5	$\to\infty$	0	0	0.19
Body length	0.17	0.41	2.8	0.0031	0.016	0.024	0.27	11	$\to\infty$	0	0	0.18
Bristle no.	1.8	$\to\infty$	$\to\infty$	$\to 0$	$\to 0$	0.015	$\to 0$	$\to 0$	0.68	0.25	0.36	0.45

—, Unknown support limit.

effects for abdominal bristle number observed in studies of *P* element insertional mutagenesis (Mackay *et al.* 1992; Lyman *et al.* 1996) and quantitative trait loci (Long *et al.* 1995).

Numbers of mutations and the distributions of their effects: To estimate mutation rates and parameters of distributions of mutation effects, data from each trait were independently analyzed by ML, under the assumptions that mutation numbers are Poisson distributed and their effects are gamma distributed (Table 3). For the viability data, lethal-bearing lines were excluded from the analysis. For all traits analyzed, the important parameters in the model are heavily confounded with one another, as illustrated in Figure 3, where likelihood for viability data is plotted as a function of *U* and *E(a)*, with fixed values of β and *P*. There is a ridge in the two-dimensional likelihood surface due to negative correlation between *U* and *E(a)*, so an increase in mutation rate can be compensated for by a decrease in the mean effect. The three-dimensional likelihood as a function of α, β, and *U* is subject to even more serious confounding effects. For example, increasing *U* can be compensated for by simultaneously decreasing β (making the distribution of effects more leptokurtic), while decreasing the ratio $E(a) = \beta/\alpha$ to keep the product $U\,E(a)$ roughly constant. The confounding between the parameters explains, in part, why estimates of *U*, mean mutant effect and β are usually unbounded, and has been noted previously (Keightley 1994). For most traits the likelihood surface becomes flat as the mutation rate increases, or β decreases, or the mean mutation effect decreases, and the ML seems to be at the limit for these parameters. With the exception of body length, it is possible only to estimate a lower limit for *U*. Minimum *U* estimates vary by more than a factor of 10, but there is no clear pattern across trait types. For example, the highest minimum estimates are for fecundity, a major component of fitness, and for bristle number. For the majority of traits, it is possible only to obtain maximum estimates for mean mutation effect. As noted previously (Keightley 1996), the maximum estimate of the mean EMS-induced mutation effect for viability is an order of magnitude higher than the estimated mean effect for spontaneous mutations, obtained from mutation accumulation experiments with balancer chromosomes (Mukai 1964; Mukai *et al.* 1972; Ohnishi 1977b), a conclusion also drawn by Mukai (1970) in a separate investigation of the effects of EMS on viability. Fecundity, development time, body length, and bristle number, however, show maximum mean mutant effect estimates of the order of a few percent. Not surprisingly, it is most difficult to obtain information on β, the shape parameter of the distribution of mutation effects, estimates for which are always unbounded. In two cases, longevity and mating speed, for which environmental effects are particularly

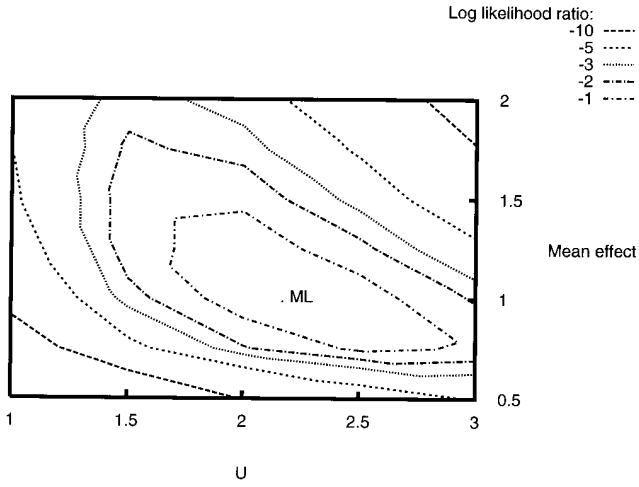

Figure 3.—Contour plot showing the log likelihood ratio of viability data as a function of mutation rate (U) and mean mutation effect, with the shape parameter of the gamma distribution fixed at 0.1, and all mutation effects negative ($P = 0$).

TABLE 4

ML estimates of mutation rates and mean mutation effects with minimum and maximum support limits assuming a gamma distribution with shape parameter β = 0.5

	U			Mean effect = $\beta/(\alpha M_C)$		
Trait	Min	ML	Max	Min	ML	Max
Viability	0.36	0.58	0.97	0.071	0.11	0.19
Fecundity	1.96	3.34	5.75	0.024	0.037	0.057
Hatchability	0.99	1.42	1.93	0.096	0.13	0.19
Dev. time	1.30	1.86	2.81	0.0093	0.013	0.019
Longevity	2.24	5.53	17.6	—[a]	0.030	0.063
Mating speed	1.22	2.15	7.43	0.050	0.097	0.16
Phototaxis	0.36	0.71	2.33	0.051	0.083	0.15
Body length	0.65	1.36	4.95	0.0025	0.0052	0.0094
Bristle no.	1.35	2.34	4.18	0.0071	0.012	0.017

[a] Dash indicates unknown support limit because of computational limitation.

strong, there is almost no information available on the shape parameter at all. The general pattern of β estimates suggests, however, that distributions of mutation effects tend to be leptokurtic with a substantial contribution from mutations with small effects. (For example, $\beta < 1$ implies a distribution more leptokurtic than an exponential distribution.) Estimates of *P*, the proportion of positive mutation effects, were found, in general, to be much less confounded with the other parameters in the model. With two exceptions, bristle number and development time, ML estimates of *P* are zero, implying that a model with no advantageous mutations at all gives the best fit to the data. Similarly, the ML estimate of *P* for development time is one, so the best fit is a model with all mutations increasing development time (presumably such mutations decrease fitness). The data do not rule out the possibility of a rather large fraction of beneficial mutations for major components of fitness (5.5% is the upper limit for hatchability; nearly 20% is the upper limit for several other traits). The larger number of deleterious mutations induced by EMS tends to hide any beneficial mutation effects, hence the model is unable to exclude the presence of a fairly high fraction of these. Only one trait, abdominal bristle number, shows clear evidence of mutations with both positive and negative effects (see also Figure 2), although, interestingly, effects are mostly downwards, and the support limits for *P* exclude 0.5. For the longevity data, increasing *P* led to a very slow drop in likelihood, but a correlated increase in *U* until a computational limit was reached, so a limiting value for *P* could not be given.

An alternative way to compare mutational target sizes is to estimate *U* and *E(a)* under the assumption that different traits have the same shaped distribution of mutation effects. The Mukai-Bateman method assumes, for example, that mutation effects are equal (*i.e.*, $\beta \to \infty$), in which case an estimate of *U* is obtained from $\Delta M^2/V_g$, and an estimate of *E(a)* obtained from $V_g/\Delta M$ (Bateman 1959; Mukai 1964). However, data on many of the traits are incompatible with a model assuming equal mutation effects (Table 3). To investigate a similar kind of model as used by Mukai-Bateman, ML estimates of *U* and *E(a)* were obtained under the assumption of a gamma distribution with $\beta = 1/2$, a model compatible with all traits except hatchability (Table 3). Estimates of mutation rates and mean effects appear to reveal large differences in mutational target sizes (Table 4). For example, the estimated mutation rate for longevity is 10 times higher than that for viability. Several life history traits have estimated mean mutational effects of the order of a few percent. The estimates in Table 4 should be treated with caution, however, as a fixed β is assumed, and estimates become unbounded if β is allowed to vary (Table 3).

Mutational correlations: The experiment was not set up with the aim of measuring genetic correlations between pairs of traits, as measurements were mostly performed at different times on different samples from the lines. However, a comparison of phenotypic correlations between line means (r_P) can provide some information on the overall pattern (Table 5). With the exception of development time, r_P for the EMS lines are mostly positive. Phenotypic correlations involving development time are mostly negative, so would be positive for the reciprocal trait "development speed," which is positively related to fitness. Although there are rather more significant r_P values than expected by chance in the controls, presumably reflecting genetic variance in these lines (see Table 2), there is no clear pattern in the sign of r_P. There are many more significant r_P values in the EMS lines, and some of the largest ones are associated with major components of fitness. Examples of bivariate plots for EMS-treated and control lines are shown in Figure 4. It would be desirable to produce estimates of parameters of bivariate distributions of mutation effects, including correlations between mutation effects for pairs of traits [see Hill and Keightley (1988) and Keightley and Hill (1990) for examples of possible parameterization]. Although this might be possible in principle, the number of parameters that would require to be estimated may well become too large.

DISCUSSION

Variation among traits in mutational target size: A major motivation for this study was to compare susceptibilities of different quantitative traits to EMS mutagenesis. Scaled changes in mean ($\Delta M/M$) and mutational coefficients of variance (CV_g) are calculated directly from the data in a model-free manner, whereas estimates of *U* and mutation distribution parameters depend on the assumption of some distribution of mutation effects,

TABLE 5

Correlation coefficients between line means for nine quantitative traits in EMS lines (above diagonal) and control lines (below diagonal)

	1	2	3	4	5	6	7	8	9
1. Viability		0.22*	0.32**	−0.19*	0.18	0.20*	0.00	0.11	0.10
2. Fecundity	−0.12		0.49**	−0.22*	0.09	0.34**	0.08	0.19*	0.23**
3. Hatchability	−0.26*	0.16		−0.13	0.24*	0.29**	0.21*	0.19*	0.25**
4. Dev. time	0.21	−0.02	−0.06		−0.02	0.01	−0.23*	−0.03	−0.20*
5. Longevity	−0.10	0.03	0.30*	0.08		0.16	0.15	0.19	0.15
6. Mating speed	0.06	−0.05	0.09	0.14	0.03		0.10	0.14	0.12
7. Phototaxis	−0.16	−0.13	−0.11	−0.26	−0.22	−0.27		0.17	0.15
8. Body length	−0.37**	0.05	0.18	−0.02	0.48**	−0.13	0.13		0.24**
9. Abdominal bristles	0.27*	−0.24*	−0.12	0.06	0.26	0.11	−0.11	0.04	

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

and are therefore potentially model-sensitive. Both $\Delta M/M$ and CV_g estimates range over more than one order of magnitude among traits. The two morphological traits, body length and abdominal bristle number, have by far the lowest figures, a pattern consistent with a previous comparison of spontaneous mutational variability for quantitative traits (Houle *et al.* 1996). Presumably, a far higher proportion of genes in the genome can have mutations with appreciable effects on life history traits than morphological traits. Viability shows scaled change of mean and variance one-third to one-half smaller than other major fitness components (*e.g.*, fecundity, hatchability), but was the only trait for which homozygous effects were measured relative to a *Cy*/+ heterozygote. Polygenic mutations affecting viability appear to have close to intermediate degrees of domi-

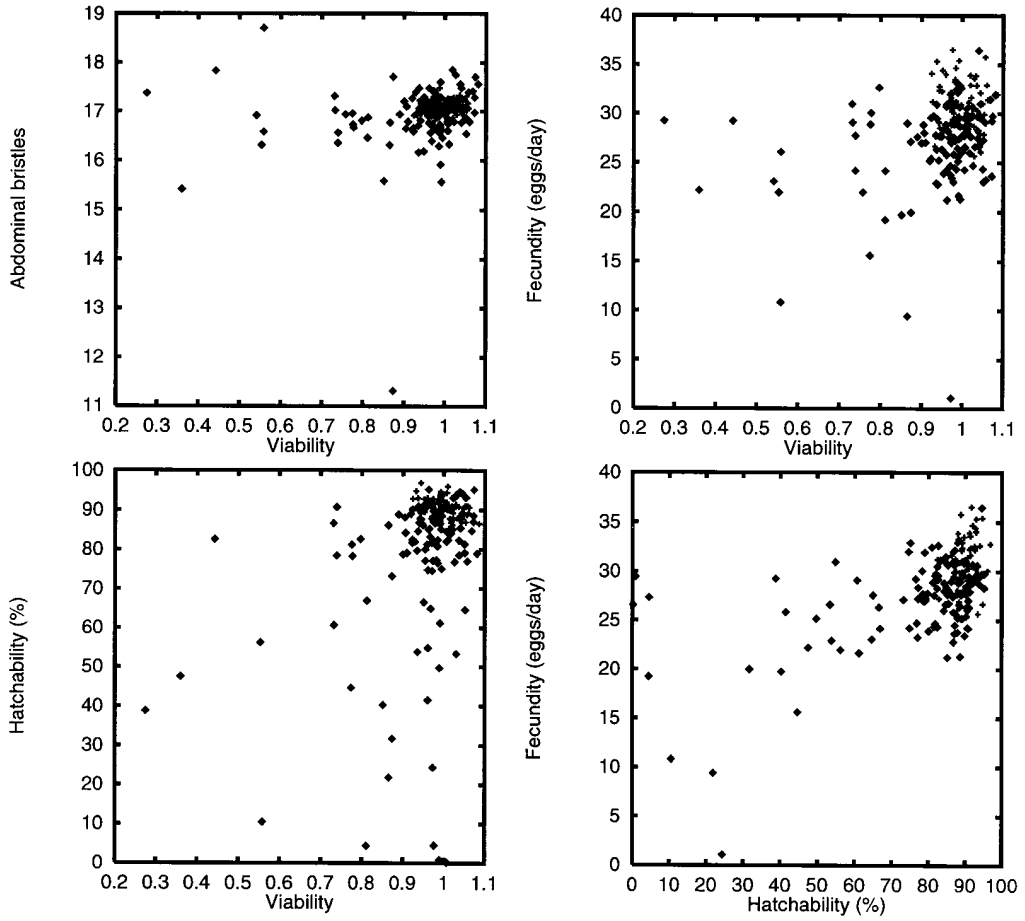

Figure 4.—Examples of bivariate plots of trait values for control lines (+) and EMS treated lines (◆).

nance (Crow and Simmons 1983), so it is likely that competitive viability is subject to mutation pressure similar to other major fitness components.

Mutation parameter estimates are strongly confounded: Unfortunately, a model to estimate numbers of mutation events and the distribution of their effects tends to be overparameterized, so resulting parameter estimates are often unbounded (see Table 3). Most frequently, the set of parameters which gives the best fit to the data is a very high mutation rate in combination with a very small average mutation effect, and a highly leptokurtic distribution of effects. Consequently it is only possible to use likelihood to estimate lower or upper limiting values for the mutation parameters. For several traits, however, there is strong evidence for a leptokurtic distribution of mutation effects, and for mean mutation effects of at most a few percent. Part of the problem in disentangling the parameters is that the dose of EMS generated rather a large amount of variability; each line presumably contained several mutations with appreciable effects on each trait. Significantly, body length is the only trait with support limits for U and $E(a)$ within bounds, and this trait also had the lowest CV_g estimate. Assuming that all DNA affects all traits, the number of mutation events is logically the same for each trait. If this number were known, or could be guessed, it would be possible to fit U as a fixed parameter in the model (Keightley 1994). The resulting parameter estimates would almost certainly involve a highly leptokurtic distribution with a continuously increasing proportion of effects close to zero, but could be misleading. The true situation may be that mutations at a high proportion of sites in the genome have negligible effects (*i.e.*, they occur at third codon positions, introns, and intergenic regions), and that these mutations represent a discontinuity in the distribution of mutation effects. Some of these sites may be under very weak selection in Drosophila (Akashi 1996).

Distributions of mutation effects are strongly asymmetrical: This study has provided the first opportunity to compare proportions of positive and negative mutation effects for a wide range of quantitative traits. As expected, the majority of mutations affecting major fitness components and other life history traits had deleterious effects. While best estimates for proportions of beneficial mutations for life history traits were zero, the possibility of a rather large fraction of positive effects was not excluded. Presumably the much larger number of deleterious mutations hides possible effects of beneficials. To keep the number of parameters manageable, a critical assumption of the analysis was identical distributions for positive and negative effects. It is likely, however, that characteristics of the distributions of positive and negative effects differ. For example, the ratio of numbers of mutations with large to small effects may be smaller for beneficial mutations than deleterious mutations.

In the present experiment, the distribution of mutation effects for abdominal bristle number is strongly skewed downwards, and this has generally, but not always, been seen in previous experiments involving selected or unselected accumulation of mutations in initially homozygous populations. The most striking asymmetric selection responses to selection on abdominal bristle number have been seen in very large scale selection experiments with an inbred base population (Mackay *et al.* 1994; Mackay 1995). Asymmetrical responses have also been noted in one other experiment involving selection for spontaneous mutations affecting abdominal bristle number (Frankham 1980), and in a selection experiment for X-ray-induced mutations (Kitagawa 1967). *P*-element insertional mutations have also been observed to generate negatively skewed distributions of effects (Mackay *et al.* 1992; Lyman *et al.* 1996). Although, more or less symmetrical distributions of mutation effects for abdominal bristles have also been seen (Lopez and Lopez-Fanjul 1993; Merchante *et al.* 1995), it appears that there is a general tendency for spontaneous and induced mutations to decrease abdominal bristle number. Intriguingly, the reverse seems to be true for sternopleural bristle number (Santiago *et al.* 1992; Mackay *et al.* 1994).

Responses to artificial selection on reproductive fitness traits from standing variation are usually higher in the downwards direction (Frankham 1990), which is consistent with the majority of mutations having deleterious effects. However, initial responses to selection on abdominal bristle number from outbred populations are usually slightly higher in the upward direction (Mackay *et al.* 1994), an observation apparently at odds with the mostly downward effects of spontaneous and induced mutations on abdominal bristle number noted above. Similarly, responses to artificial selection on body size traits in outbred populations of Drosophila are usually fairly symmetrical (Robertson and Reeve 1952; Partridge and Fowler 1993; Reeve and Fairbairn 1996), while the majority of mutations for body length had downward effects in the present study. There are at least three possible explanations for these apparently conflicting observations. First, alleles affecting the traits in outbred populations may be at intermediate frequencies (Lai *et al.* 1995; Mackay 1995). Second, standing variation in quantitative traits may be mostly due to alleles with very small effects, originating from minor mutations whose effects are symmetrically distributed about zero (Fisher 1930; Peck *et al.* 1997), a subclass of mutations not detected by the present analysis. Finally, if directional dominance is prevalent, symmetrical short term selection responses are possible with an asymmetrical distribution of mutation effects.

Consequence of among line variation in mutagen uptake: It is usually assumed that the number of spontaneous mutation events per genome per generation is Poisson distributed. With induced mutagenesis, how-

ever, individuals may vary in their intake of mutagen or susceptibility to a given intake, so the distribution of mutation numbers may not be Poisson. For example, a subset of individuals could take up no mutagen whatsoever, while the remainder could take up a larger than average dose. This would obviously have implications for the present analysis and a previous analysis of the viability data from this experiment (Keightley 1996). Two experiments give data on the variation in uptake of sucrose solution by individual Drosophila. Ayaki *et al.* (1984) employed the standard feeding technique of Lewis and Bacher (1968) as was used here (*i.e.*, mutagen dissolved in 1% sucrose fed to males for 24 hr). The coefficient of variation of uptake (CV_D) of solution containing 0–1 mm ethylnitrosourea was about 18%. Thompson *et al.* (1991) observed a somewhat higher variability of uptake ($CV_D \sim 30\%$) for flies of unspecified sex fed 5% sucrose solution. A factor that would tend to reduce the effect of variability of uptake is that 50% of EMS-induced mutations can be attributed to vapor inhalation rather than feeding (Munoz 1987). Assume that the mutation rate is linearly related to mutagen intake, and the number of mutations generated by a given intake of mutagen is Poisson distributed. If intake is normally distributed among individuals, expected numbers of mutations among individuals are also normally distributed (variance = σ_U^2), and the variance of mutation numbers among individuals is $U + \sigma_U^2$. With a gamma distribution of mutation effects, it can be shown that the among line variance of genotypic values is

$$V_g = E(a^2)[U + \sigma_U^2\beta/(\beta + 1)],$$

(S. P. Otto, personal communication). In terms of the coefficient of variation of mutagen intake, the genetic variance is

$$V_g = UE(a^2)[1 + U(CV_D)^2\beta/(\beta + 1)].$$

Thus, between line variation in dosage inflates the genetic variance by a factor $1 + U(CV_D)^2\beta/(\beta + 1)$. The greatest inflation in the among line variance occurs for the case of equal mutation effects ($\beta \to \infty$), and declines to zero for increasingly leptokurtic distributions of effects. For example, with a gamma distribution of mutation effects with shape parameter 0.5, a coefficient of variation in mutagen dose of 25%, and $U = 10$ (*cf.* Table 4), the among line variance would be inflated by about 21% compared to uniform dosage.

Comparison of the spectra of EMS-induced and spontaneous mutation events: A major difference between the spectra of spontaneous and EMS mutations is that a high fraction of spontaneous events are associated with TE insertion (Green 1988). It is an open question as to whether spontaneous TE insertion generates more or less severe phenotypic effects, on average, than spontaneous non-TE insertion events. Most information on the fitness effects on TE relates to the *P* element of Drosophila. Effects of homozygous *P*-element insertion events on viability have been measured to be of the order of 1% for the *X* chromosome (Eanes *et al.* 1988) and 12% for chromosome 3 (Mackay *et al.* 1992), but much larger effects may be associated with *P* element-mediated rearrangements (reviewed by Ajioka and Hartl 1989). There is some evidence that *P* elements insert preferentially near transcription start sites (Engels 1989).

EMS mutagenesis, under standard conditions, generates mostly G/C → A/T transitions and a small fraction of large scale aberrations detectable by Southern blotting (reviewed by Ashburner 1989). A detailed analysis by sequencing of 28 EMS-induced mutant alleles at the *vermilion* locus showed 26 to be single base-pair changes and 2 to be small deletions (Pastink *et al.* 1991), a fairly typical result. A higher frequency of large scale aberrations in Drosophila occurs if sperm storage is allowed to occur in females. Storage occurs if, for example, females are maintained with food lacking protein (Ashburner 1989). The mutagenesis conditions used in the present experiment were standard, so sperm storage effects would not be expected. It is possible to compare the spectra of EMS-induced and naturally occurring single base pair mutational changes in protein coding sequences by combining data on rates of codon usage in Drosophila (Shields *et al.* 1988) and frequencies of different kinds of transitions and transversions derived from population surveys of Drosophila gene sequences (*e.g.*, Kreitman and Hudson 1991). The latter data suggest that about 30% of single base pair spontaneous mutations in Drosophila are G/C → A/T transitions, the type of mutation event usually generated by EMS, and that spontaneous transitions and transversions occur at approximately equal frequencies, a conclusion also drawn by Moriyama and Powell (1996). By calculating the expected frequencies of amino acid, synonymous, and nonsense substitutions for each possible type of transition and transversion from the codon usage data, and weighting these by the observed transition and transversion frequencies from the population survey data, it can be inferred that EMS induces silent substitutions in coding sequences nearly twice as frequently as spontaneous mutations (42 *vs.* 24%), but nonsense mutations occur at a slightly higher frequency (6.1 *vs.* 5.4%). The similar predicted frequencies for spontaneous and EMS-induced nonsense mutations is slightly surprising: stop codons are A/T rich, so are generated relatively frequently by G/C → A/T transitions, but A/T → G/C transitions generate no nonsense mutations at all. However, the population survey data show that spontaneous base pair changes also include A/T → T/A and G/C → T/A transversions, both of which generate a higher fraction of nonsense mutations than G/C → A/T transitions. It can tentatively be concluded that EMS tends to generate somewhat milder mutagenic effects than non-TE spontaneous mutation events in Drosophila.

Prediction of spontaneous mutation parameters from the EMS data: It is possible to predict spontaneous mutation parameters such as the rate of change of trait mean per generation by scaling the parameter estimates from the EMS experiment using two different sources of information: (1) *Lethal frequency model*. Rates for spontaneous lethal mutation are well known in Drosophila, and are typically about 0.005 for the second chromosome per generation (Simmons and Crow 1977). The observed frequency of lethal mutations in the present experiment was 0.42, so the 2.5 mm dose of EMS can be equated to 0.42/0.005 = 84 generations of spontaneous mutation accumulation. Such a method of scaling was suggested by Mukai (1970). (2) *Bristle variance model*. The rate of appearance of spontaneous mutational variation for abdominal bristle number has been measured experimentally on several occasions, and for M strains of *D. melanogaster* averages about 0.0014 per chromosome *2* when expressed as a proportion of the environmental variance for the trait. For the purpose of making comparisons, this figure is corrected for potential downwards bias from natural selection and the tendency for mutations to be partially recessive (Keightley *et al.* 1993). In the present experiment, the increase in variance for abdominal bristle number due to EMS scaled by the estimated environmental variance was (from Table 2) $0.43/(0.106 \times 8) = 0.51$, so the dose of EMS is equivalent to approximately $0.51/0.0014 = 364$ generations of spontaneous mutational accumulation, about four times greater than predicted by comparison of the number of lethals. Predicted changes of mean for the nine quantitative traits from one generation of spontaneous mutation ($\Delta M^*/M$) and predicted spontaneous mutational coefficients of variation (CV_M) are shown in Table 6. Predictions were derived using conversion factors from the above calculations, that is, for $\Delta M^*/M$, values of $\Delta M/M$ from Table 1 were divided by 84 for the lethal frequency model, or by 364 for the bristle variance model; for CV_M, values of CV_g from Table 1 were divided by either $\sqrt{84}$ or $\sqrt{364}$, since mutational variance accumulates linearly with generation number (Lynch and Hill 1986). Also, in order to express $\Delta M^*/M$ and CV_M on a per genome basis, values were multiplied by 2.5 to account for the fact that chromosome *2* in Drosophila represents about two-fifths of the genome.

Predicted $\Delta M^*/M$ and CV_M may now be compared with values observed in other spontaneous mutation accumulation experiments, for which there are data for several quantitative traits (Houle *et al.* 1996; Fernandez and Lopez-Fanjul 1996). Estimates relevant to a comparison with the predicted values are summarized in Table 6. The average CV_M figure for abdominal bristles excludes data from two experiments involving strong P strains of Drosophila, which seemed to show unusually high rates of accumulation of variation (Keightley *et al.* 1993). In Table 6, the trait wing dimensions has been assumed to be comparable with body length from Houle *et al.* (1996). Table 6 shows that there is good agreement between the observed CV_M and the predicted CV_M under the bristle variance model. Furthermore, the predicted $\Delta M/M$ figure for hatchability agrees almost perfectly with the observed figure of −0.093% from the spontaneous mutation accumulation experiment of Fernandez and Lopez-Fanjul (1996) (Garcia-Dorado 1997). The predicted CV_M values under the lethal frequency model are always too high. Under the lethal frequency and bristle variance

TABLE 6

Predicted rates of change of mean or scaled increments of variance for one generation of spontaneous mutation expressed on a per genome basis, and observed CV_M estimates from spontaneous mutation accumulation experiments

	Predicted				Observed
	Lethal rate model		Bristle variance model		
Trait	% $\Delta M^*/M$	% CV_M	% $\Delta M^*/M$	% CV_M	% CV_M
Viability	−0.21	3.8	−0.048	1.8	2.1
Fecundity	−0.39	3.3	−0.089	1.6	1.9
Hatchability	−0.42	5.5	−0.096	2.6	1.1[a]
Development time	0.074	0.95	0.017	0.46	—
Longevity	−0.51	2.5	−0.12	1.2	1.3
Mating speed	−0.60	6.5	−0.14	3.1	—
Phototaxis	−0.11	3.3	−0.025	1.6	—
Body length	−0.018	0.30	−0.0043	0.14	0.14
Abdominal bristles	−0.030	1.0	−0.0069	0.50	0.25

[a] Data from the spontaneous mutation accumulation experiment of Fernandez and Lopez-Fanjul (1996) (A. Garcia-Dorado, personal communication). All other observed figures are derived from Houle *et al.* (1996).

models, predicted rates of change of mean for life history traits are less than 0.60 or 0.14%, respectively. Mutation accumulation experiments involving balancers have provided estimates for genome-wide $\Delta M/M$ for viability in the range -1 to -2% (summarized by Crow and Simmons 1983), while the predictions for viability from the EMS mutagenesis experiment are much lower, -0.21 or -0.048%, depending on the model of scaling assumed. This discrepancy can be interpreted in a number of different ways. First, the effect of variability in mutagen dose among flies could have been larger than suggested by the available data on variability of mutagen uptake, and this would tend to bias the predicted $\Delta M/M$ values downwards. Second, spontaneous mutations could be dominated by a class of event which does not occur under EMS mutagenesis, for example, TE insertion. If these events generate directional effects with approximately equivalent values, the rate of change of mean viability could be large relative to the rate of change of variance (Keightley 1996). Finally, it has been suggested that the apparent changes in viability observed in the experiments with balancers could have occurred if the balancer system had adapted (Keightley 1996; Garcia-Dorado 1997), although arguments against this interpretation have also been made (Crow 1997). The correct interpretation of these results is important, as they have been central for models to predict rates of fitness loss in small populations from deleterious mutation pressure and consequent rates of extinction (Lande 1995; Lynch *et al.* 1995), and to predict the fate of the human species, for it has been proposed that we are currently suffering an appreciable loss of fitness and increase in associated health problems from an accumulation of mildly deleterious mutant alleles (Crow 1997).

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