Copyright © 1999 by the Genetics Society of America

Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Terumi Mukai and the Riddle of Deleterious Mutation Rates

Peter D. Keightley* and Adam Eyre-Walker†

**Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, Scotland and †Centre for the Study of Evolution and School of Biological Sciences, University of Sussex, Brighton BN1 9QG, England*

DURING the 1960s and 1970s Terumi Mukai and colleagues conducted some experiments that have had a major impact in population and evolutionary genetics. Their quest was to estimate the genomic rate and effects of deleterious mutations. However, recent reappraisals of their work have led to doubts about the validity of some of their conclusions. Furthermore, a renewed interest in the problem of deleterious mutations, stemming in part from these doubts and in part from an interest in the perennial problem of the evolution of sex, has led to a series of new experiments.

Most biologists would agree that the majority of mutations that change protein sequences or alter gene expression are harmful, because they perturb highly adapted biochemical and physiological systems. Mutations that generate "visible" phenotypes are usually manifestly deleterious, but the deleterious nature of most amino acid changes can also be inferred from the high degree of conservation of protein-coding sequences relative to noncoding DNA. Deleterious mutations impose a "load" (selective reduction in fitness) on populations; individuals either die or fail to reproduce, because they carry harmful mutations, a process Muller (1950) termed "genetic death." Haldane (1937) showed that the load imposed by a deleterious mutation was independent of its selective effect. This has become known as the Haldane-Muller principle and implies that the mutational load depends largely on the rate at which deleterious mutations occur over the whole genome, *U*. Haldane applied this principle to estimate the mutation load in *Drosophila melanogaster* by assuming that the mutation rate to nonlethal deleterious mutations was twice that to lethals, for which an estimate was available at the time. He concluded that populations would experience an ~4% depression in fitness through the elimination of deleterious mutations, a "loss of fitness," he suggested, which was "the price paid by a species for its capacity for further evolution" (Haldane 1937, p. 349).

In principle, estimation of *U* requires an unbiased way to measure the mutation rate in a random sample of the genes in the genome. The first detailed work was carried out by Muller (1928) in *D. melanogaster* to estimate rates for mutations that are lethal when homozygous. Crow and Temin (1964) reviewed a large body of work on recessive lethal mutation rates; average rates are 0.0026 and 0.0046 per generation for the X and second chromosome, respectively, and imply a lethal rate of ~0.01 for the haploid genome. However, many deleterious mutations are probably not lethal, and the rate for nonlethals could be considerably higher.

One approach to estimating the mutation rate to deleterious, but nonlethal, mutations is to use information on the rate at which visible mutations arise. For loci that generate visible mutations in Drosophila, rates typically run around 10^{-5} (Drake *et al.* 1998). Assuming that *D. melanogaster* contains 15,000 loci, this translates to a genome-wide mutation rate of 0.15 per haploid, some 15-fold higher than the lethal rate. Paradoxically, however, mutations with visible effects occur much less frequently than lethals in genome-wide surveys (Muller 1950). The likely explanation is that genes used in assays for visible phenotypes have mutation rates higher than those of other genes, for a variety of reasons. Furthermore, many deleterious mutations do not have visible effects. We need a method that gives an unbiased, genome-wide estimate of the mutation rate for all mutations that are deleterious.

Measuring rates and effects of viability mutations in Drosophila: The idea of a mutation accumulation (MA) experiment can be traced back to Muller (1928, p. 288): "if a given lot of individuals, known to contain no mutant genes at the start, is bred through a series of *n* generations (that is, to "F_n"), and one of the individuals of this last (*n*th) generation is then tested for mutant genes . . . , this test will reveal all mutant genes that arose in any of the preceding *n* generations." Muller

Corresponding author: Peter D. Keightley, Institute of Cell, Animal and Population Biology, University of Edinburgh, W. Mains Rd., Edinburgh EH9 3JT, Scotland. E-mail: p.keightley@ed.ac.uk

(1928) also suggested the use of the curly (*Cy*) chromosome balancer system to make large-scale mutation accumulation more feasible (see Crow and Abrahamson 1997 for a recent *Perspectives*). The first experiment to measure the fitness effects of a chromosome-wide accumulation of spontaneous mutations was carried out more than 30 years later by Mukai, working with lines of Drosophila at the National Institute of Genetics, Mishima, Japan (Mukai 1964). Mukai's design made use of the *Cy*/*Pm* balancer chromosome system to maintain a wild-type second chromosome protected from selection in the heterozygous state for several tens of generations. The wild-type chromosome (+) was replicated, so the random accumulation of spontaneous mutations leads to divergence between chromosome lines for a measurable phenotype and, if mutational effects are directional, to a change in the mean phenotype. The phenotype measured by Mukai was a viability index, the fraction of +/+ homozygotes relative to *Cy*/+ heterozygotes in progeny of crosses between *Cy*/+ heterozygotes (Wallace 1956) (*Cy* is homozygous lethal). Mukai carried out assays with several vials in each chromosome line and so could partition the variance in viability between and within lines at different generations to obtain an estimate of the rate of increase of genetic variance per generation, V_m. The surprising observation, however, was that mean viability declined at the high rate of ~0.4% per generation. The rate of erosion in viability extrapolated to the haploid genome was in excess of 1% per generation, excluding lethals and severe detrimentals.

Mukai's principal aim was to obtain information on the rates and effects of polygenic mutations that underlie the changes in mean and variance for viability. To do this, he turned to formulae of Bateman (1959) that relate the observed changes of mean and variance to the chromosome-wide mutation rate, U_2, and average deleterious mutation effect, $\bar{s}$, $\Delta M = U_2\bar{s}$, and $V_m = U_2\bar{s}^2(1 + C^2)$, where C is the coefficient of variation among mutational effects (Crow and Simmons 1983). If C is assumed to be zero (*i.e.*, a model of equal mutation effects), an estimate for U_2 is $\Delta M^2/V_m$, and an estimate for $\bar{s}$ is $V_m/\Delta M$. Mukai calculated that a minimum of ~0.14 mutations per generation with viability effects of ≤3% was required to explain the change of mean and variance of the second chromosome lines.

Mukai subsequently moved to the University of Wisconsin, where, encouraged by James Crow, he repeated his 1964 experiments (Mukai *et al.* 1972). Distributions of relative viability at four generations in one of three sets of lines studied are shown in Figure 1.

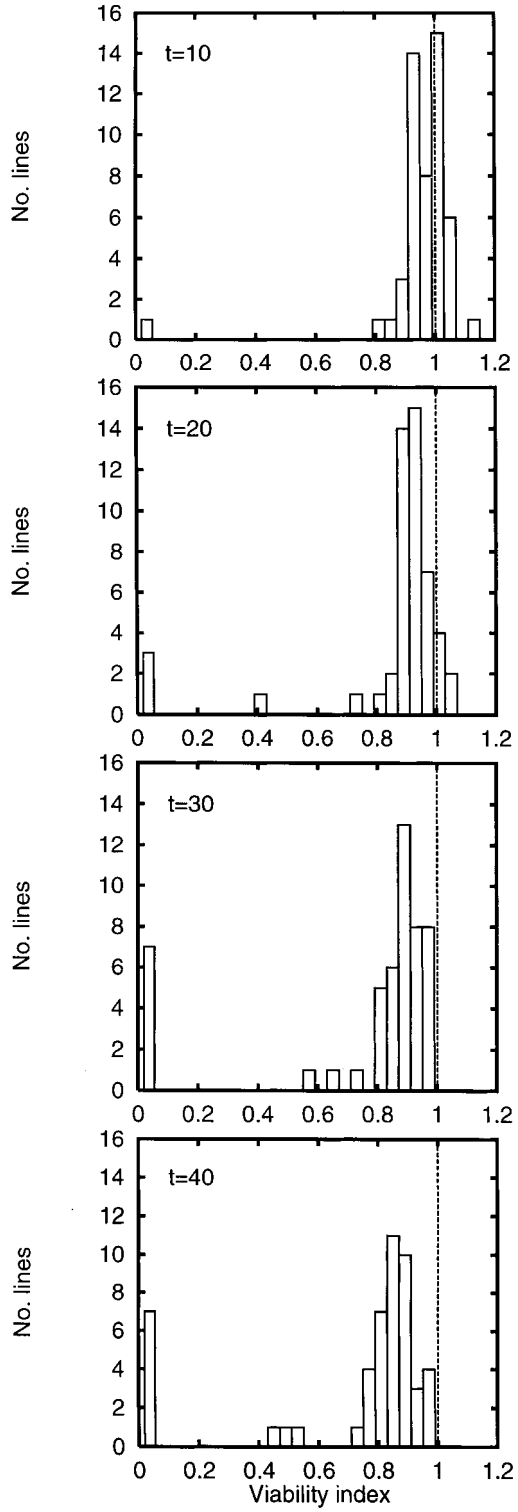

Figure 1.—Distributions of line means for relative viability in *Drosophila melanogaster* at four generations (t) of mutation accumulation, replotted from Mukai *et al.* (1972) (CH lines).

There was a build-up of lethal-bearing chromosomes at a rate similar to the first study (~0.006/second chromosome/generation). Chromosomes with severely reduced viability (detrimentals) accumulated at a frequency similar to that of the lethals. As with Mukai's earlier study, the most striking result was the drop in relative viability of the remaining "quasinormal" chromosomes, at a rate of about 1% per generation, when extrapolated to the whole genome. Also at Wisconsin, Ohmi Ohnishi, as part of a study of the effects of the

TABLE 1

Estimates of rates and effects of deleterious mutations (per haploid genome)

Species	Method	Reference	U	(SE) [95% CI]	$\bar{s}$	(SE)
D. melanogaster	MA (competitive viability)	Mukai (1964)	0.35		0.027	
		Mukai *et al.* (1972)	0.47		0.023	
		Ohnishi (1977)	0.14		0.030	
	MA (noncompetitive viability)	Garcia-Dorado *et al.* (1999)	0.02		0.10	
	MA (competitive viability)	Fry *et al.* (1999)	0.052	(0.017)	0.11	(0.02)
	Band-morph[a]	Harada *et al.* (1993)	0.050	[0.014–0.13]		
	Molecular divergence[b]	This article	0.028	(0.002)		
E. coli	MA (fitness)	Kibota and Lynch (1996)	0.00017		0.012	
	Molecular[c]	This article	0.0016			
C. elegans	MA (intrinsic growth rate)	Keightley and Caballero (1997)	0.0035	(0.001)	0.10	(0.01)
		Vassilieva and Lynch (1999)	0.0080	(0.014)	0.21	(0.18)
H. sapiens	Band-morph[d]	Neel *et al.* (1988)	0.4	[0.09–3.4]		
	Molecular divergence[e]	Eyre-Walker and Keightley (1999)	0.8	(0.4)		

Estimates from MA experiments are all based on models of equal effects. Approximate standard errors of confidence limits are given, where available.

[a] Band-morph amino-acid-altering mutation rate in protein coding sequences.

[b] Amino-acid-altering mutation rate based on divergence between melanogaster and obscura groups.

[c] Assumes an independent estimate for nucleotide mutation rate (see text).

[d] Band-morph amino-acid-altering mutation rate in protein coding sequences, assuming 38% of amino acid mutations are deleterious (see text).

[e] Amino-acid-altering mutation rate based on divergence between humans and chimpanzees.

chemical mutagen ethyl methanesulfonate (EMS), investigated the viability effects of spontaneous mutations in a design similar to Mukai's. The results were qualitatively similar to the two earlier studies, although the rate of mutational decay of the quasinormal chromosomes was somewhat lower (Ohnishi 1977). A summary of the findings from these three studies, along with a series of later experiments, is given in Table 1.

Taken together, Mukai and Ohnishi's experiments imply that most individual flies will contain one new, mildly deleterious mutation with an effect of the order of a few percent, but the mutation rate could be very much higher and the mean mutation effect lower if mutation effects varied. Mukai and Ohnishi's results on the genomic deleterious mutation rate in Drosophila have been the only data available for the past 20 years, have been highly influential in evolutionary genetics, and figure frequently as parameters in population genetic models (see, *e.g.*, Charlesworth and Charlesworth 1998).

Mutation rates in Drosophila protein-coding genes: Mukai's 1977 experiment on spontaneous mutation rates at enzyme loci in Drosophila, carried out at North Carolina State University, clearly presented a challenge to the earlier results on rates of polygenic mutation for viability (Mukai and Cockerham 1977). The experiment involved the maintenance of 1000 $Cy/+$ second chromosome lines for 175 generations, followed by a search for new electrophoretic (band-morph) variants at five enzyme loci located on chromosome 2. Like many of Mukai's experiments, this experiment was carried out on a grand scale. After more than 1.6 million allele generations, in total three new variants were detected, giving a band-morph mutation rate of 1.8×10^{-6}. Assuming 6000 chromosome 2 genes, the chromosomal mutation rate for amino-acid alterations is, therefore, 0.032. However, a follow-up study of the same lines after an additional 36–49 generations with two additional loci (>3.1 million allele generations) revealed no new band-morph mutations (Voelker *et al.* 1980). A later study by Harada *et al.* (1993) with a different set of lines and seven enzyme loci (~1.7 million allele generations) also revealed no band-morph mutations. The pooled band-morph mutation rate estimate for the two surveys together is 7.5×10^{-7} (upper 95% confidence limit of 1.9×10^{-6}; Harada *et al.* 1993). The inferred chromosome 2 amino-acid mutation rate is, therefore, 0.013 (upper limit 0.034). This is an underestimate because the genes surveyed by Mukai and colleagues are rather shorter than Drosophila genes in general: ~400 codons in the band-morph studies *vs.* an average of ~600 codons. A revised estimate for chromosome 2 is, therefore, 0.020 (0.051), and this translates to a haploid genomic rate of 0.05, a figure far below the minimum estimates of mutation rates to viability polygenes (Table 1). The null mutation rate was 17 times higher than the band-morph mutation rate, but it is likely that this was because of hybrid dysgenesis. Mobilization of the *hobo* element occurred in the lines initiated by Mukai and Cockerham (Yamaguchi and Mukai 1974; Harada *et al.* 1990). In the other set of lines, Harada *et al.* (1993) found that five out of six null alleles analyzed were associated with the insertion of a *P* element near a transcription initiation site. We can assume that transposable element (TE)

mobilization would not have inflated the band-morph mutation rates.

Can the band-morph studies be reconciled with high rates for phenotypically detectable mutations? There are several possible reasons for the discrepancy between the MA and band-morph studies. On the basis of their estimate for the band-morph mutation rate, Mukai and Cockerham (1977) concluded that the majority of the viability mutations observed in the earlier studies must have occurred outside coding sequences. Is the target in the noncoding DNA sufficiently large? About 60% of the *Drosophila melanogaster* genome is single copy, and approximately 25% of that is protein coding; *i.e.*, the Drosophila genome is 1.8×10^8 bp in length and contains approximately 15,000 genes (Simmen *et al.* 1998), which are on average 1800 bp in length (E. N. Moriyama, personal communication). Let us assume that all band-morph-altering mutations are deleterious and that deleterious mutations are as common in the single-copy, noncoding DNA as in coding DNA. Under these generous assumptions the genomic deleterious mutation rate would be 0.2, a figure that is just about consistent with the minimum deleterious mutation rate estimates from MA experiments (Table 1). But we have assumed that the single-copy, noncoding DNA is under the same level of constraint as nonsynonymous sites, and we know this to be untrue: 5′ flanking sequences, introns, and synonymous sites evolve much faster than nonsynonymous sites in Drosophila (Kreitman and Hudson 1991; Li 1997), indicating that the proportion of "silent" mutations removed by natural selection is much lower than the proportion of amino-acid-altering mutations. These considerations make it implausible that point mutations at a rate inferred from the band-morph mutation rate studies can explain the minimum estimates for viability mutations. Small insertion-deletion mutations do not occur at a sufficiently high rate to make up the deficit (Petrov *et al.* 1998; Ramos-Onsins and Aguade 1998).

A role for transposable elements? TEs could explain the discrepancy between the estimate of U from MA experiments and band-morph studies, since TEs are unlikely to generate band-morph changes other than nulls, while they can generate deleterious mutations. To do this, TEs need to occur at appreciable frequencies and cause effects of a few percent.

In the case of the second chromosome lines investigated by Mukai *et al.* (1972), cytogenetic analysis ruled out the possibility that *P-M* hybrid dysgenesis had occurred (Yamaguchi and Mukai 1974). However, there are many other types of transposable element in Drosophila, and evidence that crosses involving balancers often lead to the mobilization of several TE families has accumulated over the last decade (Pasyukova *et al.* 1988; Garcia Guerreiro and Biemont 1995; Kozhemiakina and Furman 1995). The element families for which movement is increased are usually *copia* or *copia*-related. The phenomenon may not be connected with the balancer *per se*, but seems to be related to outcrossing, is strain dependent, and persists for many generations (Georgiev *et al.* 1990).

Do TEs cause effects that are large enough? Eanes *et al.* (1988) carried out an experiment to measure the hemizygous fitness effects of *P*-element insertion, without subsequent excision, in the X chromosome of male Drosophila. Their estimate for the mean hemizygous effect of a *P*-element insertion was 0.014 ($\pm$0.006 SE). As noted by Eanes *et al.*, this is close to the estimates for viability effects of spontaneous mutations (Table 1). A second line of evidence suggesting that TEs can generate mildly detrimental mutations comes from work in *Escherichia coli*. The distribution of fitnesses of lines carrying independent single Tn*10* insertions (Elena *et al.* 1998) is remarkably similar to the distribution of viability in the Drosophila balancer studies (Figure 1). The average fitness effect of a Tn*10* insertion was estimated at $\sim$3%.

Crow and Simmons (1983) also noted that in dysgenic hybrids, a form of meiotic drive can lead to an increased frequency of recovery of the balancer chromosome relative to the wild type (Kidwell *et al.* 1977). Since viability is measured as relative numbers of wild types to balancers, this could be important if the strength of meiotic drive increased with time. There is no direct evidence that such a phenomenon occurred in MA experiments.

Arguments about the controls: It has been suggested that some of the apparent decline in fitness of the quasinormal lines in Mukai and Ohnishi's experiments might be nonmutational in origin (Keightley 1996; Garcia-Dorado 1997). Two observations prompted this suggestion. First, there was a relatively large decrease in the fitness of the quasinormal lines without a correspondingly large increase in the variance. Statistical analyses suggested that this was inconsistent with a model in which the effects of new mutations come from a continuous (gamma) distribution, unless a nonmutational effect is included in the model. Second, the decline in viability of the quasinormal lines in an EMS mutagenesis experiment of Ohnishi, and in a more recent spontaneous MA experiment (Fernandez and Lopez-Fanjul 1996), was not nearly as dramatic as in Mukai and Ohnishi's MA experiments. The most plausible nonmutational explanation for Mukai and Ohnishi's results comes from a later Drosophila MA experiment involving *Cy*/*Pm*. Fry *et al.* (1999) observed that *Cy* expression is variable, and heterozygotes may be distinguished from wild types only if an additional chromosome 2 marker is present. If the ability of an experimentalist to recognize weak *Cy* expression improved over time, the relative viability of wild-type chromosomes would appear to decline over time.

However, there is some evidence that the decline in fitness of the MA lines analyzed by Mukai is genuine. Mukai (1964) and Mukai *et al.* (1972) used an "order method" to obtain control viability values. High viability

lines in generation t_2 were used to select lines at generation t_1 for use as the controls, the latter assumed to be free from new mutations. Although Mukai's calculation in his 1964 paper for the last generation was probably inappropriate (Keightley 1996), the remaining data imply values similar to those originally inferred (J. D. Fry, personal communication).

How, then, could there be a large decrease in the mean viability of Mukai's lines, but not a large increase in the variance? It is possible that the distribution of mutation effects is multimodal (Keightley 1996); in addition to a class of mutations with quite large effects, there might be a much larger class of mutations with very small effects. The mutations with small effects could lead to a decrease in mean viability without a large increase in the variance. TEs could be implicated in such a decline (see above) and therefore could explain the qualitatively different distribution of line means for viability observed by Ohnishi in his EMS study (EMS generates mostly point mutations).

Recent mutation accumulation experiments: There has recently been renewed interest in inferring rates and effects of deleterious mutations, and we briefly review the published experiments below.

Drosophila melanogaster: The longest-running published MA experiment in a eukaryote has been reported by Fernandez and Lopez-Fanjul (1996). Starting from a marked isogenic strain, 200 lines were maintained by brother–sister matings for more than 100 generations. Although selection against deleterious mutations acts with higher efficiency in full-sib lines than chromosome balancer lines, mildly deleterious mutations should fix randomly in full-sib lines, at least during the initial phase of the experiment, when fertility and viability are relatively high. Strongly deleterious mutations are expected to be selectively eliminated, however. As a control, a population of large effective size of the same strain was maintained, the idea being that deleterious mutations would be eliminated by natural selection acting at a higher efficiency in a large population. Mean egg-to-adult viability (measured under noncompetitive conditions) declined relative to the control at a rate of only about 0.1% per generation (Garcia-Dorado 1997). Bateman estimates of U (per haploid genome) and $\bar{s}$ are $\sim$0.02 and $\sim$0.10, respectively (Garcia-Dorado *et al.* 1999). The estimate for U is, therefore, more than 10-fold lower than those obtained by Mukai and Ohnishi (Table 1).

Fry *et al.* (1999) have reported the results from a chromosome 2 MA experiment in Drosophila carried out over 27–33 generations with a design similar to Mukai and Ohnishi's, but in addition they performed parallel assays of three control populations maintained at large effective size. Viability of second chromosome homozygotes (relative to the *Cy* heterozygotes) declined at a rate intermediate to that observed by Mukai and Ohnishi. However, genetic variance for viability increased three to five times faster, and the rate of lethal mutations was twofold higher. Resulting Bateman estimates of U (extrapolated to the whole haploid genome) and $\bar{s}$ are 0.05 and 0.11, respectively.

The controls in these experiments are not entirely satisfactory, since they do not preclude the possibility of adaptation to the laboratory environment from the fixation of beneficial mutations or a decline in mean fitness from a build-up of deleterious mutations that will remain at low frequency, although Fry *et al.* did not observe significant changes in the viability of control populations or between-control population genetic variance. It is notable that actively transposing *copia* elements were present in Fry *et al.*'s lines (S. V. Nuzhdin, personal communication).

A different design of MA experiment in Drosophila ("middle class neighborhood") employing outbred lines has provided estimates for the rate of loss in fitness from mutation accumulation (Shabalina *et al.* 1997), although there has been debate about whether changes in fitness can be wholly attributed to mutation (Keightley *et al.* 1998; Lynch *et al.* 1999b).

An MA experiment in a different arthropod species, *Daphnia pulex*, has been reported by Lynch *et al.* (1999a), but problems were encountered with low fitness of a frozen control population.

E. coli: Kibota and Lynch (1996) carried out an MA experiment in which 50 lines were assayed for fitness contemporaneously with a cryopreserved ancestral population, so possible problems connected with an evolving control should not occur. Cells grew exponentially for $\sim$25 rounds of cell division between bottlenecks of one cell per line. In simulations, Kibota and Lynch showed that the majority of mutations with effects exceeding $\sim$6% would be selectively lost during the exponential growth phase, while mutations with effects approaching 1% or less would behave as selectively neutral and be retained. Mean fitness of the lines declined linearly by $\sim$2% in $\sim$7500 generations, and between-line variance also increased linearly. With Bateman's (1959) method, the lower limit for U was 0.00017 and the upper limit for $\bar{s}$ was 0.012.

This MA estimate for U can be compared with a molecular estimate based on the rate of spontaneous mutation per nucleotide. By measuring rates of nonsense mutation for *lacI* or histidine auxotrophs, Drake (1991) estimated that the spontaneous mutation rate per base pair in *E. coli* is $\sim 6 \times 10^{-10}$, a figure consistent with estimates from reversion experiments (Hall 1991). The *E. coli* K12 genome is 4.6×10^6 bp, of which 87.8% is protein-coding, 0.8% RNA encoding, and the remainder noncoding or repetitive DNA (Blattner *et al.* 1997). The relative divergences of synonymous and nonsynonymous sites between related coliformes imply that >95% of amino-acid-altering mutations are deleterious under natural conditions (A. Eyre-Walker, unpublished results). The fraction of nucleotides that change an amino

acid if mutated is ~0.7, so an estimate of U in *E. coli* is $(6 \times 10^{-10}) \times (4.6 \times 10^{6}) \times 0.878 \times 0.95 \times 0.7 = 0.0016$. The MA estimate is, therefore, about 10 times lower than the molecular estimate, presumably owing to variability among effects of new mutations. Deleterious mutations in noncoding DNA and insertion–deletion mutations are not included in the molecular estimate.

Caenorhabditis elegans: In two MA experiments carried out over 60 generations (Keightley and Caballero 1997, KC97) and 50 generations (Vassilieva and Lynch 1999, VL99), individual self-fertilizing hermaphrodite worms of the wild-type N2 strain were transferred in replicated sublines each generation, and frozen ancestral populations were used as controls. The results from the two experiments are qualitatively similar. For example, in the case of intrinsic growth rate (r), estimates for U are 0.008 (VL99) and 0.003 (KC97) and for $\bar{s}$ are 0.21 (VL99) and 0.10 (KC97). For r, Bateman estimates are similar to maximum likelihood (ML) estimates, if equal mutational effects are assumed. For other life history traits, Bateman and ML estimates are more divergent, but ML estimates agree reasonably well with each other between experiments and also have smaller standard errors, with mean estimates among life history traits for U of ~0.005 in both experiments (P. Keightley and T. Bataillon, unpublished results). The estimates of U ($\bar{s}$) are one to two orders of magnitude lower (one order of magnitude higher) than Mukai and Ohnishi's corresponding estimates for Drosophila. Only a small part of the difference between the rates can be explained by the difference in the number of cell divisions per generation, about three times lower in *C. elegans* than in Drosophila. However, the most striking difference between the Drosophila and *C. elegans* MA experiments is the much smaller drop in mean of the *C. elegans* quasinormal lines over a comparable number of generations, while the numbers of detrimental lines were similar (compare Figures 1 and 2). This difference between the Caenorhabditis and Drosophila experiments cannot be explained by natural selection, which operates with greater efficiency in selfing lines than in chromosome balancer lines, because selection removes a higher fraction of strongly deleterious mutations than mildly deleterious mutations. Changes of mean and variance in *C. elegans* were, therefore, dominated by lines containing mutations with strongly deleterious effects, hence the larger $\bar{s}$ estimates, while mutations with small effects have had a much smaller impact. *C. elegans* N2 strain does not have significant TE activity (Eide and Anderson 1985), and this could explain the qualitative difference in behavior between the Drosophila and *C. elegans* MA lines.

Limitations of mutation accumulation experiments and molecular approaches: Although MA experiments have yielded important information about the rate and nature of deleterious mutations, their major drawback is that they give us little or no information about mutations

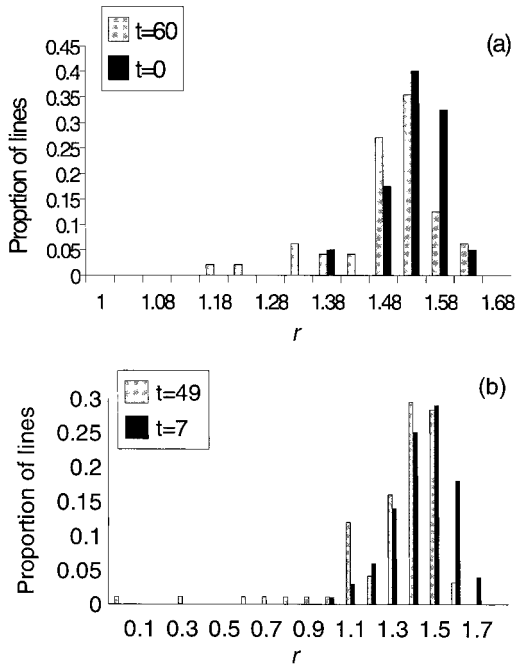

Figure 2.—Distributions of line means for intrinsic growth rate r (day^{-1}) in two *C. elegans* MA experiments. (a) Calculated from data on age-specific reproduction from Keightley and Caballero (1997) by methods of Charlesworth (1994). The r measure is the mean for lines with four replicate plates each containing a pair of worms. (b) Replotted from Vassilieva and Lynch (1999). The r measure is the mean for lines with five replicate plates each containing one worm; this generates more variance than a measure based on pairs of worms.

with very small effects. Yet mutations of small effect are often as important as mutations of large effect in evolution; for example, the mutation load exerted by a mutation is independent of the strength of selection under multiplicative selection, and weakly selected mutations can actually have larger effects on genetic variation through background selection than strongly selected mutations (Nordborg *et al.* 1996). Mutations of small effect can go undetected in an MA experiment because fitness assays in laboratory experiments are crude. While natural selection influences the fate of any mutation with an effect greater than $1/N_e$, the best chemostat experiments can detect fitness differences of only 0.5% (Dykhuizen 1990). Since most organisms probably have effective population sizes greater than 10,000 (Nei and Graur 1984), the majority of deleterious mutations could be missed in an MA experiment.

An alternative approach to estimate U is to use DNA sequence data. The general method is implicit in the neutral theory of molecular evolution, proposed some 30 years ago. Under the neutral theory, mutations are either neutral, *i.e.*, they have no fitness effects, or they are deleterious. Neutral DNA evolves at a rate u, the nucleotide mutation rate, while DNA under selection

evolves at a rate uf, where f is the proportion of mutations that are neutral, so $1 - f$ is the proportion that are deleterious. An estimate of the proportion of mutations that are deleterious in a section of DNA can therefore be obtained by comparing the rate of evolution in some sequence to that of a completely neutral sequence. This is an underestimate if there have been advantageous mutations. The idea of using DNA sequence data lay dormant until Alexey Kondrashov and James Crow resurrected it earlier in this decade (Kondrashov and Crow 1993). They mapped out a scheme for estimating U by sequencing random orthologous sections of the genome in two closely related species. By comparing the rates of evolution in these random clones to that found in a neutral sequence, they proposed that it would be possible to estimate the average proportion of mutations that are deleterious over the whole genome, and hence U. At the time, data required for this approach were not available. However, Shabalina and Kondrashov (1999) have been able to perform part of the calculation recently with *C. elegans* and its relative *C. briggsae*. They aligned random sections of the *C. briggsae* genome to the *C. elegans* genome; where they found an alignment above that expected in a randomized sequence of DNA, they inferred constraint. They estimated that about 17% of the nucleotides in introns and intergenic regions were under constraint compared with 72% of the nucleotides in exons. The results imply that about one-third of all mutations in the Caenorhabditis genome are deleterious. Unfortunately, there are no reliable estimates of the per nucleotide mutation rate in Caenorhabditis, so they could not estimate U.

A simplification of Kondrashov and Crow's idea is to estimate U for protein-coding sequences alone by assuming that synonymous mutations are neutral; the synonymous substitution rate (K_s) is, therefore, an estimate of the nucleotide mutation rate. The proportion of amino-acid-changing mutations that are deleterious can be estimated from the ratio of the nonsynonymous (K_a) to the synonymous substitution rate; *i.e.*, $1 - K_a/K_s = 1 - f$. Thus, a simple formula, $K_s(1 - K_a/K_s)$, yields an estimate of the deleterious mutation rate per nucleotide site over the period of time the substitution rate is estimated (usually the divergence time of the species being considered). This can be converted to a genomic estimate, per generation, if the length and number of genes in the genome are known, and estimates are available for the generation time and the divergence time. We recently performed this calculation for humans and estimated that on average there had been 2.1 amino-acid-changing mutations each generation in the haploid genome since the split from chimpanzees and that 0.8 of those were deleterious (Eyre-Walker and Keightley 1999). These are likely to be underestimates for several reasons, principally because deleterious mutations that occur outside protein-coding sequences are not included. The DNA sequence approach to estimating U is not without its problems. If an independent estimate of the nucleotide mutation rate is not available, as is generally the case, then we must estimate the nucleotide mutation rate from DNA sequence data, and this means that we need an estimate of the generation time and the time of divergence of the species being considered. Furthermore, many divergence times have been estimated from DNA sequence data and the molecular clock, so there is a danger of circularity.

In humans, an independent estimate of U can be obtained from studies to assess the effect of exposure to radiation from the Hiroshima and Nagasaki atomic bomb explosions on rates of point mutation (Neel *et al.* 1988). In children of an unexposed (control) cohort, three band-morph mutations were detected in $\sim$470,000 allele tests, giving a band-morph mutation rate of 6.4×10^{-6} (95% confidence interval 1.3×10^{-6} to 19×10^{-6}). About one-third of amino acid mutations change mobility, which implies a haploid amino-acid mutation rate of 1.1. This is fairly close to the value obtained from DNA sequence data. In the DNA sequence study we estimated that the proportion of amino-acid mutations that were deleterious and removed by natural selection was only 38% in humans, so the electromorph-based estimate for U is 0.4.

How does a DNA sequence-based estimate of U in Drosophila compare with values obtained from MA and band-morph experiments? Applying the DNA sequence method to Drosophila is complicated by selection on synonymous codon bias (Akashi *et al.* 1998), since selection can depress the rate of synonymous substitution and hence lead to an underestimate of the nucleotide mutation rate. Li (1997) lists substitution rates for 32 Drosophila genes, estimated from the divergence between the melanogaster and obscura groups, which are thought to have split 30 million years ago. The mean synonymous substitution rate is 1.56×10^{-8}/site/year, and the mean nonsynonymous substitution rate is 1.9×10^{-9}. If we assume that the Drosophila genome contains 15,000 genes of average length 1800 bp (E. N. Moriyama, personal communication) and that Drosophila undergoes 10 generations a year, we estimate that the amino-acid mutation rate for Drosophila is 0.030 per haploid per generation, of which 0.028 are deleterious. The estimate of the number of amino-acid-changing mutations is slightly lower than the value obtained from the band-morph studies (0.05 per haploid). If we estimate the synonymous substitution rate from the 25% of genes with the highest synonymous substitution rates, assumed to be under the weakest selection, the discrepancy disappears.

Table 1 summarizes the estimates of U, in Drosophila and other organisms, that have been obtained since Mukai's groundbreaking experiments. In Drosophila the estimates vary by over an order of magnitude, with the estimates given by Mukai and colleagues being con-

siderably larger than all other estimates. Are the estimates of U given by Mukai and colleagues correct? It is possible that they are, but for the wrong reasons. The experiments performed by Fernandez and Lopez-Fanjul (1996) and Fry *et al.* (1999) suggest that Mukai and colleagues probably did overestimate the mutation rate to mutations of moderate effect (*i.e.*, the mutations detected in an MA experiment), or there was exceptional TE activity in Mukai's lines. However, the band-morph and molecular divergence estimates are underestimates because they do not include mutations outside coding regions or mutations caused by TEs. The analysis of constraint in noncoding regions of the Caenorhabditis genome suggests that half of the deleterious mutations occur in noncoding regions; this means that the deleterious mutation rate caused by point mutations in Drosophila is likely to be $\sim$0.1. TE activity might elevate the rate to levels that approach those estimated by Mukai and Ohnishi.

The original estimates of $U \geq 0.5$ and $\bar{s} \leq 3\%$ given by Mukai have been extensively cited and used by geneticists. However, it is evident from Table 1 that they may have only very limited application. For all but *C. elegans* we have estimates of U for protein-coding sequences, and they vary by several orders of magnitude, from *E. coli* at 0.0016 to humans at 0.8. TE activity also appears to vary considerably across taxa, with humans and nematodes having few TE mutations compared with Drosophila and *E. coli* (Kazazian 1999; Eide and Anderson 1985). Unless there is a strong negative correlation between TE activity and the point mutation rate, TE activity will generate even greater variation in the deleterious mutation rate.

Muller was one of the first scientists to take an interest in deleterious mutations. His principal interest was the mutation load in human populations, a topic that has received renewed interest. Crow (1997) has argued that we need to be aware that modern medicine and improved sanitation may have important impacts on our genetic legacy. As natural selection is relaxed, some populations will accumulate deleterious mutations, leading to a greater dependence on medicine, ultimately putting our population at risk if the ability to sustain high-level health care and sanitation is reduced. We know that humans have a high deleterious mutation rate, but the consequences of relaxing natural selection in contemporary populations will depend on the distribution of fitness effects of new mutations, and we currently lack information from an appropriate model. By assuming Mukai's estimate of the average selective effect of deleterious mutations in Drosophila, Crow (1997) and Lynch *et al.* (1999b) have argued that human populations may suffer significant genetic degradation within a short period of time. However, if there is variation among selective effects, as seems likely given the contrasting MA and molecular estimates of U in *E. coli*, then the average selective effect is a gross overestimate, as is our likely genetic degradation. How humans and related species evade the effects of mutation load on an evolutionary time scale is also an open question.

LITERATURE CITED

Akashi, H., R. M. Kliman and A. Eyre-Walker, 1998 Mutation pressure, natural selection, and the evolution of base composition in Drosophila. Genetica **103:** 49–60.

Bateman, A. J., 1959 The viability of near-normal irradiated chromosomes. Int. J. Radiat. Biol. **1:** 170–180.

Blattner, F. R., G. Plunkett, C. A. Bloch, N. T. Perna, V. Burland *et al.*, 1997 The complete genome sequence of *Escherichia coli* K-12. Science **277:** 1453–1469.

Charlesworth, B., 1994 *Evolution in Age Structured Populations*, Ed. 2. Cambridge University Press, Cambridge.

Charlesworth, B., and D. Charlesworth, 1998 Some evolutionary consequences of deleterious mutations. Genetica **102/103:** 3–19.

Crow, J. F., 1997 The high spontaneous mutation rate: Is it a health risk? Proc. Natl. Acad. Sci. USA **94:** 8380–8386.

Crow, J. F., and S. Abrahamson, 1997 Seventy years ago: mutation becomes experimental. Genetics **147:** 1491–1496.

Crow, J. F., and M. J. Simmons, 1983 The mutation load in Drosophila, pp. 1–35 in *The Genetics and Biology of Drosophila*, Vol. 3C, edited by M. Ashburner, H. L. Carson and J. N. Thompson. Academic Press, London.

Crow, J. F., and R. G. Temin, 1964 Evidence for the partial dominance of recessive lethal genes in natural populations of Drosophila. Am. Nat. **98:** 21–33.

Drake, J. W., 1991 A constant rate of spontaneous mutation in DNA-based microbes. Proc. Natl. Acad. Sci. USA **88:** 7160–7164.

Drake, J. W., B. Charlesworth, D. Charlesworth and J. F. Crow, 1998 Rates of spontaneous mutation. Genetics **148:** 1667–1686.

Dykhuizen, D. E., 1990 Experimental studies of natural selection in bacteria. Annu. Rev. Ecol. Syst. **21:** 373–398.

Eanes, W. F., C. Wesley, J. Hey, D. Houle and J. W. Ajioka, 1988 The fitness consequences of *P* element insertion in *Drosophila melanogaster*. Genet. Res. **52:** 17–26.

Eide, D., and P. Anderson, 1985 The gene structures of spontaneous mutations affecting a *Caenorhabditis elegans* myosin heavy-chain gene. Genetics **109:** 67–79.

Elena, S. F., L. Ekunwe, N. Hajela, S. A. Oden and R. E. Lenski, 1998 Distribution of fitness effects caused by random insertion mutations in *Escherichia coli*. Genetica **103:** 349–358.

Eyre-Walker, A., and P. D. Keightley, 1999 High genomic deleterious mutation rates in hominids. Nature **397:** 344–347.

Fernandez, J., and C. Lopez-Fanjul, 1996 Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. Genetics **143:** 829–837.

Fry, J. D., P. D. Keightley, S. L. Heinsohn and S. V. Nuzhdin, 1999 New estimates of rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **96:** 574–579.

Garcia-Dorado, A., 1997 The rate and effects distribution of viability mutation in *Drosophila*: minimum distance estimation. Evolution **51:** 1130–1139.

Garcia-Dorado, A., C. Lopez-Fanjul and A. Caballero, 1999 Properties of spontaneous mutations affecting quantitative traits. Genet. Res. (in press).

Garcia Guerreiro, M. P., and C. Biemont, 1995 Changes in the chromosomal insertion pattern of the copia element during the process of making chromosomes homozygous in *Drosophila melanogaster*. Mol. Gen. Genet. **246:** 206–211.

Georgiev, P. G., S. L. Kiselev, O. B. Simonova and T. I. Gerasimova, 1990 A novel transposition system in *Drosophila melanogaster* depending on the *Stalker* mobile genetic element. EMBO J. **9:** 2037–2044.

Haldane, J. B. S., 1937 The effect of variation on fitness. Am. Nat. **71:** 337–349.

Hall, B. G., 1991 Spectrum of mutations that occur under selective and non-selective conditions in *E. coli*. Genetica **84:** 73–76.

Harada, K., K. Yukuhiro and T. Mukai, 1990 Transposition rates

of movable genetic elements in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **87:** 3248–3252.

Harada, K., S. Kusakabe, T. Yamazaki and T. Mukai, 1993 Spontaneous mutation rates in null and band-morph mutations of enzyme loci in *Drosophila melanogaster*. Jpn. J. Genet. **68:** 605–616.

Kazazian, H. H., 1999 An estimated frequency of endogenous insertional mutations in humans. Nat. Genet. **22:** 130.

Keightley, P. D., 1996 Nature of deleterious mutation load in Drosophila. Genetics **144:** 1993–1999.

Keightley, P. D., and A. Caballero, 1997 Genomic mutation rates for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **94:** 3823–3827.

Keightley, P. D., A. Caballero and A. Garcia-Dorado, 1998 Surviving under mutation pressure. Curr. Biol. **8:** R235–R237.

Kibota, T. T., and M. Lynch, 1996 Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. Nature **381:** 694–696.

Kidwell, M. G., J. F. Kidwell and J. A. Sved, 1977 Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility, and male recombination. Genetics **86:** 813–833.

Kondrashov, A. S., and J. F. Crow, 1993 A molecular approach to estimating the human deleterious mutation rate. Hum. Mutat. **2:** 229–234.

Kozhemiakina, T. A., and D. P. Furman, 1995 Increased transposition rates of copia-like TEs while deriving isogenic lines of *Drosophila melanogaster*. Dros. Inf. Serv. **76:** 100–103.

Kreitman, M., and R. R. Hudson, 1991 Inferring the evolutionary histories of the *Adh* and *Adh-dup* loci in *Drosophila melanogaster* from patterns of polymorphism and divergence. Genetics **127:** 565–582.

Li, W.-H., 1997 *Molecular Evolution*. Sinauer, Sunderland, MA.

Lynch, M., M. Pfrender, K. Spitze, N. Lehman, J. Hicks *et al.*, 1999a The quantitative and molecular genetic architecture of a subdivided species. Evolution **53:** 100–110.

Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz *et al.*, 1999b Perspective: spontaneous deleterious mutation. Evolution **53:** 645–663.

Mukai, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics **50:** 1–19.

Mukai, T., and C. C. Cockerham, 1977 Spontaneous mutation rates at allozyme loci in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **74:** 2514–2517.

Mukai, T., S. I. Chigusa, L. E. Mettler and J. F. Crow, 1972 Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics **72:** 333–355.

Muller, H. J., 1928 The measurement of gene mutation rate in Drosophila, its high variability, and its dependence upon temperature. Genetics **13:** 279–357.

Muller, H. J., 1950 Our load of mutations. Am. J. Hum. Genet. **2:** 111–176.

Neel, J. V., C. Satoh, K. Goriki, J. Asakawa, M. Fujita *et al.*, 1988 Search for mutations altering protein charge and/or function in children of atomic bomb survivors: final report. Am. J. Hum. Genet. **42:** 663–676.

Nei, M., and D. Graur, 1984 Extent of protein polymorphism and the neutral mutation theory, pp. 73–118 in *Evolutionary Biology*, Vol. 17, edited by M. K. Hecht, B. Wallace and G. T. Prance. Plenum, New York.

Nordborg, M., B. Charlesworth and D. Charlesworth, 1996 The effect of recombination on background selection. Genet. Res. **67:** 159–174.

Ohnishi, O., 1977 Spontaneous and ethyl methanesulfonate-induced mutations controlling viability in *Drosophila melanogaster*. II. Homozygous effect of polygenic mutations. Genetics **87:** 529–545.

Pasyukova, E. G, E. S. Belyaeva, L. E. Ilyinskaya and V. A. Gvozdev, 1988 Outcross-dependent transpositions of *copia*-like mobile genetic elements in chromosomes of an inbred *Drosophila melanogaster* stock. Mol. Gen. Genet. **212:** 281–286.

Petrov, D. A, Y. C. Chao, E. C. Stephenson and D. L. Hartl, 1998 Pseudogene evolution in Drosophila suggests a high rate of DNA loss. Mol. Biol. Evol. **15:** 1562–1567.

Ramos-Onsins, S., and M. Aguade, 1998 Molecular evolution of the Cecropin multigene family in Drosophila: functional genes *vs.* pseudogenes. Genetics **150:** 157–171.

Shabalina, S. A., and A. S. Kondrashov, 1999 Pattern of selective constraint in *C. elegans* and *C. briggsae* genomes. Genet. Res. (in press).

Shabalina, S. A., L. Y. Yampolsky and A. S. Kondrashov, 1997 Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed natural selection. Proc. Natl. Acad. Sci. USA **94:** 13034–13039.

Simmen, M. W., S. Leitgeb, V. H. Clark, S. J. M. Jones and A. Bird, 1998 Gene number in an invertebrate chordate, *Ciona intestinalis*. Proc. Natl. Acad. Sci. USA **95:** 4437–4440.

Vassilieva, L., and M. Lynch, 1999 The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. Genetics **151:** 119–129.

Voelker, R. A., H. E. Schaffer and T. Mukai, 1980 Spontaneous allozyme mutations in *Drosophila melanogaster*: rate of occurrence and nature of the mutants. Genetics **94:** 961–968.

Wallace, B., 1956 Studies of irradiated populations of *Drosophila melanogaster*. J. Genet. **56:** 280–293.

Yamaguchi, O., and T. Mukai, 1974 Variation of spontaneous occurrence rates of chromosomal aberrations in the second chromosomes of *Drosophila melanogaster*. Genetics **78:** 1209–1221.