Spontaneous Mutation Accumulation Studies in Evolutionary Genetics

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Abstract

Mutation accumulation (MA) experiments, in which mutations are allowed to drift to fixation in inbred lines, have been a principal way of studying the rates and properties of new spontaneous mutations. Phenotypic assays of MA lines inform us about the nature of new mutational variation for quantitative traits and provide estimates of the genomic rate and the distribution of effects of new mutations. Parameter estimates compared for a range of species suggest that the genomic mutation rate varies by several orders of magnitude and that the distribution of effects tends to be dominated by large-effect mutations. Some experiments suggest synergistic interactions between the effects of spontaneous deleterious mutations, whereas others do not. There is little reliable information on the distribution of dominance effects of new mutations. Most evidence does not suggest strong dependency of the effects of new mutations on the environment. Information from phenotypic assays has recently been augmented by direct molecular estimates of the mutation rate.

INTRODUCTION

MA: mutation accumulation

ΔM: change in M per generation

V_m: mutational variance, the variance introduced into a population each generation as a result of mutation per generation

As the ultimate source of genetic variation, new mutations have a central place in genetics. The rate and fitness effects of new mutations are important for understanding the nature of genetic variation within populations and genetic differences among them. Quantitative genetic variation originates from new mutations and underpins the response to artificial selection and much evolutionary adaptation, and is the basis for variation in susceptibility to many common genetic diseases in humans. In evolutionary genetics, the mutation rate and the distribution of effects of new mutations feature in evolutionary and population genetics theory, including such questions as the evolution of sex and recombination (Otto & Lenormand 2002), the maintenance of variation (Bürger 2000), and the persistence of small populations (Lande 1994, Lynch et al. 1995).

Much of what we know about the nature of new genetic variation originating from spontaneous mutations has come from mutation accumulation (MA) experiments. In a typical MA experiment (**Figure 1**), new mutations are allowed to accumulate over the whole genome (or whole chromosomes) for many generations in replicated inbred lines. Selection is expected to be dramatically reduced in inbred lines so the majority of mutations (all but the most deleterious) are expected to become fixed/removed at random. To study the phenotypic effects of the accumulated mutations, MA lines are typically assayed contemporaneously along with controls for quantitative or life-history traits after multiple generations. From these data, it is possible to estimate the rate of change of the mean for a quantitative trait (ΔM) and the new mutational variance (V_m) per generation. MA line phenotypic data also allow the inference of the genomic rate of new mutations affecting quantitative traits (Mukai 1964) and the distributions of their effects (García-Dorado 1997, Keightley 1994, Shaw et al. 2002). In this review, we summarize the information that has been obtained on these parameters from diverse traits and species in a range of environmental conditions. The analysis of MA lines has also allowed the study of interactions between different mutations, their degrees of dominance, and the extent by which their effects depend on the environment. Recently, the analysis of the genomes of MA lines has provided information on rates of mutation at the nucleotide level, and in this review we discuss these new experiments.

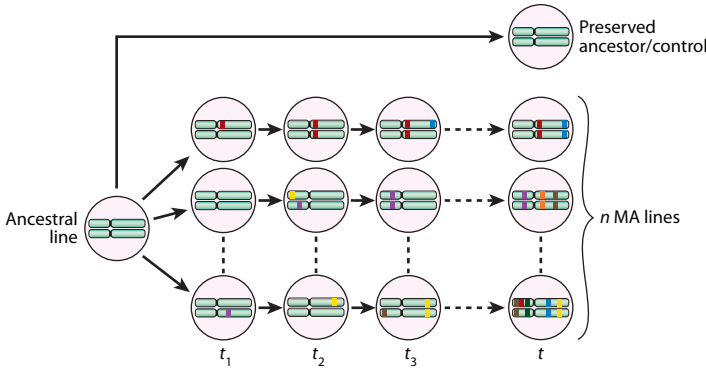

Figure 1

A typical mutation accumulation (MA) experiment in a selfing diploid organism. An ancestral line is split into n MA lines, which are then allowed to accumulate mutations for t generations. Mutations (represented as colored blocks within chromosomes) are heterozygous when they first appear but can become fixed or lost in subsequent generations. After t generations, lines are expected to have accumulated different sets of mutations. Assuming that mutations are deleterious, this leads to a decrease in mean fitness relative to a control line (which is assumed to be free of mutations) and an increase in among-line variation.

There have been several previous reviews of MA experiments and the evolutionary impact of new mutations (García-Dorado et al. 1999, Houle et al. 1996, Keightley & Eyre-Walker 1999, Lynch 1988, Lynch et al. 1999), but since their publication many new experiments and new statistical analysis methods have arisen. We limit this review to spontaneous MA experiments of the kind outlined in **Figure 1** and largely exclude discussion of the many experiments that have studied induced mutations. We have not included discussion of experiments that aim to study new mutational variation in outbred populations, as exemplified by the "middle-class neighborhood" design (Shabalina et al. 1997), or experiments in which the fitness effects of new mutations are inferred by comparing life-history traits in outbred and inbred populations (Charlesworth et al. 1990, Deng & Lynch 1996).

THE ORIGIN OF MUTATION ACCUMULATION EXPERIMENTS

As Muller pointed out in 1927, mutations are intrinsically hard to study because the spontaneous mutation rate is so low. One potential solution to this problem is to elevate the rate of mutation, which Muller succeeded in doing, and reported the first "artificial transmutation" of a gene using X-rays (Muller 1927). However, Muller also realized that mutations could be investigated by studying their effects not just in a single gene, but over a whole chromosome by allowing mutations to "accumulate" over several generations in multiple lines (Muller 1928). Muller employed a balancer chromosome system in *Drosophila* that included dominant markers and multiple inversions to suppress recombination in order to maintain mutations in the heterozygous state, thus preventing selection from operating on recessive mutations. He was then able to estimate the number of detectable mutations (both visible and lethal) that had occurred along the chromosome, and even infer their approximate locations. Estimates of the rate of recessive lethal mutations from this and other experiments have been summarized by Crow & Temin (1964), and imply an average rate of ~0.01 per haploid genome per generation in *Drosophila*.

Although the lethal mutation rate is important, the impact of mutations on the fitness of a population (termed the genetic load; Crow & Simmons 1983) is largely determined by the total deleterious mutation rate and is relatively insensitive to the effects of individual mutations (Haldane 1937). Using the mutation rate to lethals estimated for *Drosophila*, Haldane (1937) conjectured that the total loss of fitness to the species owing to selection against lethals and nonlethals combined (assuming that nonlethals are twice as frequent as lethals) would be about 4% per generation. At that time, however, there was no information on rates of mildly deleterious mutations, and inferring this parameter has therefore been a central theme of subsequent MA studies.

MUKAI'S MUTATION ACCUMULATION EXPERIMENTS IN DROSOPHILA

Mukai (1964) continued the pioneering work of Muller by carrying out experiments to estimate the rate of mildly deleterious mutations in *Drosophila*. This work has been reviewed previously (Crow & Simmons 1983, Keightley & Eyre-Walker 1999, Simmons & Crow 1977), so we provide only a brief summary here.

Mukai allowed mutations to accumulate in a wild-type *Drosophila melanogaster* second chromosome by using the *Cy*/*Pm* balancer chromosome system. Only one wild-type (+) chromosome is involved in each cross during each generation, so mutations (except those with large fitness effects when heterozygous) should fix essentially at random. Mukai (1964) found that the viability index of the MA lines (viability of +/+ individuals, relative to +/*Cy*) decreased rapidly—by ~0.4% per generation—which, scaled up to the whole genome, implies a decline in viability of ~1%

per generation owing to nonlethal mutations alone. Subsequent studies in *Drosophila* measuring viability (Mukai et al. 1972, Ohnishi 1977a) and development time (Mukai & Yamazaki 1971) qualitatively agreed with the original findings. As well as a reduction in mean fitness, Mukai and Ohnishi also observed an increase in among-line variance, presumably owing to the fixation of different mutations in different MA lines.

***U*:** deleterious mutation rate per genome (per haploid genome in the case of diploids)

Assuming that mutations have equal unidirectional effects on viability, it is possible to estimate the chromosomal rate and the average effects of deleterious mutations from the rate of increase in among-line variance and decrease in mean viability (see below). Mukai (1964) estimated a deleterious mutation rate, U, of 0.34 per haploid genome per generation, and an average selection coefficient, E(a), of 0.027. Mukai et al.'s (1972) results were also consistent with a high rate of mutation [$U = 0.47$ and E(a) $= 0.023$], as were the results of Ohnishi (1977a), although the estimate of $U = 0.14$ [with E(a) $= 0.030$] was somewhat smaller.

In all of these experiments (Mukai 1964, Mukai et al. 1972, Ohnishi 1977a), an accumulation of lethal-bearing chromosomes at a rate of ~0.006 per chromosome per generation and chromosomes with severely reduced fitness (at a similar rate) were observed, consistent with previous experiments (Crow & Temin 1964). However, the substantial declines in mean fitness for the remaining chromosomes (labeled quasinormal) were accompanied by little apparent increase in the variance among them. This has led to suggestions that the decline in fitness of the quasinormal chromosomes may have been nonmutational (García-Dorado 1997, Keightley 1996). This hypothesis is supported by the observation that similar declines in fitness were not observed in a later *Drosophila* MA experiment that principally used matings between full sibs (Fernández & López-Fanjul 1996), although the controls for this experiment have been criticized (Lynch et al. 1999). There are several possible nonmutational explanations for the apparent viability decline of the quasinormal chromosomes. First, *Cy* expression is variable, so misidentification of *Cy*/*Cy* individuals could lead to an apparent decline in relative viability of +/+ individuals if, for example, the ability to identify *Cy*/*Cy* individuals improved over the course of the experiment (Fry et al. 1999). Second, the *Cy* balancer control chromosome may have adapted to the laboratory environment over time (Keightley 1996, García-Dorado 1997), although this would require controls to evolve in a similar manner in multiple independent experiments (Lynch et al. 1999). Finally, Fry (2004) argued that Mukai's (1964) MA lines may have become genetically contaminated early on in the MA experiment, for example, as a result of crosses with another stock containing another unrelated second chromosome. The lack of a good control has been the bugbear of many MA experiments in *Drosophila*. Better controls involving cryopreserved cells (e.g., Kibota & Lynch 1996, Zeyl & DeVisser 2001), larvae (e.g., Baer et al. 2005, Keightley & Caballero 1997, Vassilieva et al. 2000), or dried seeds (e.g., Schoen 2005, Shaw et al. 2000) from ancestral populations have led to more convincing estimates of the rates of change of mean for fitness and other quantitative traits in other species.

Reanalyses of Mukai and Ohnishi's data (García-Dorado & Caballero 2002, Fry 2004) suggest that viability declined nonlinearly in both sets of experiments, implying that there is synergistic epistasis between deleterious mutations or that the mutation rate accelerated (see below). Estimates of U then strongly depend on whether a linear or nonlinear regression is fitted to estimate the rate of decline of viability or whether a regression line is forced through the origin (Fry 2004, García-Dorado & Caballero 2002). Furthermore, Mukai's estimates of the deleterious mutation rate are unexpectedly high in the light of molecular-based estimates of the per-nucleotide per-generation mutation rate in *Drosophila* (Haag-Liautard et al. 2007).

Since Mukai's first experiments, many MA studies have been carried out in a variety of organisms, and lines have been assayed for diverse traits. Here we summarize the findings from these studies in terms of estimates of changes of means and variances per generation, genomic

mutation rates, and distributions of effects. First, we describe the methods available to analyze MA experiments.

BM: Bateman-Mukai

***a*:** homozygous effect of a mutation

ML: maximum likelihood

***M*:** mean trait value

ESTIMATING MUTATION PARAMETERS

Using a method proposed by Bateman (1959), Mukai (1964) estimated U and the mean mutational effect, E(a), from the per-generation changes in mean and among-line variance of viability. To illustrate the Bateman-Mukai (BM) method, consider a MA experiment in a diploid organism consisting of n lines each of population size N. If U is the haploid genomic mutation rate, the expected number of mutations is $2NU$ per line per generation. Mutations are assumed to become fixed in the population at random, so each has a probability of fixation of $1/(2N)$ and, at equilibrium, we therefore expect $2NU/(2N) = U$ fixations per line per generation. Assuming that mutations have independent directional effects on the mean of the trait, the expected change in the mean viability of the lines per generation is

$$\Delta M = U\mathrm{E}(a), \qquad 1.$$

where a refers to the homozygous effect. The expected change in among-line variance per generation (ΔV) can be derived by considering the expected increase in among-line variance as a result of a single fixation in one line, that is, $\mathrm{E}\{[a^2 + (n-1).0^2]/n\} = \mathrm{E}(a^2)/n$. Multiplying by the expected number of fixations in n lines per generation (nU) gives

$$\Delta V = U\mathrm{E}(a^2) = U[\mathrm{E}(a)^2 + \mathrm{Var}(a)]. \qquad 2.$$

If we assume that mutations have equal effects, such that $\mathrm{Var}(a) = 0$, then $\Delta V = U\mathrm{E}(a)^2$. U and E(a) can then be easily calculated from estimates of ΔM and ΔV using a method of moments, that is, $U = \Delta M^2/\Delta V$ and $\mathrm{E}(a) = \Delta V/\Delta M$. If a is not constant, implying that Var(a) is positive, using these estimators will give lower and upper bound estimates of U and E(a), respectively (Lynch & Walsh 1998). ΔM and ΔV can both be estimated by regression from data collected across several generations or more simply from changes in mean trait value and between line variance up to generation t. The change in mean trait value can also be estimated from comparison to a contemporaneous control line assumed to be free from mutations, or alternatively using an "order method" where MA lines that have high fitness in later generations (implying that they are free from mutations) are used as a proxy for control lines (Fry 2001, Mukai 1964).

Three methods to infer U and E(a) from MA data have been developed that also include information about the observed distribution of fitness among MA lines, and thus allow inferences to be made about the distribution of mutational effects (García-Dorado 1997, Keightley 1994, Shaw et al. 2002). For example, a maximum likelihood (ML) approach (Keightley 1994) analyzes data consisting of phenotypic measurements of mutation-free control lines and MA lines after t generations of mutation accumulation. The model assumes that the phenotypic measurements of the control data are normally distributed with mean M and variance V_e, although the normality assumption is not necessary (Halligan et al. 2003). The MA lines have values that are the sum of an environmental deviate (from the same distribution as the control lines) and a mutational deviate, which is the sum of n mutational effects, where n is Poisson distributed with parameter Ut, and each effect is an independent draw from a distribution of effects, $f(a)$. In principle, any distribution for $f(a)$ can be assumed, although this has usually been a gamma distribution, which has two parameters: (*a*) scale, α; and (*b*) shape, β. The likelihood of each MA line phenotypic value is calculated by summing over possible numbers of mutations (only considering the possibility of zero mutations for the control data), and fitting a distribution of effects for each mutation

number by numerical integration. The overall likelihood of the data is maximized with respect to the parameters M, V_e, U, α, and β. Unlike the BM approach, the method can allow mutations to have positive or negative effects on the trait. An additional parameter, P, the fraction of positive mutations, is then included by reflecting the gamma distribution about zero, such that a fraction P of the density is greater than 0, and $1 - P$ is less than 0. This method has, for example, been used recently to estimate mutational parameters for mutations affecting sex ratio in *Nasonia*, where mutations could potentially have both positive and negative effects on the trait (Pannebakker et al. 2008).

The minimum distance (MD) (García-Dorado 1997) and Markov Chain Monte Carlo (MCMC) (Shaw et al. 2002) approaches also jointly infer U and $f(a)$ and can also allow bidirectional mutational effects. They also find parameters that best fit the observed MA line data under the assumption that mutation numbers are Poisson distributed. The MD analysis method examines a single generation of MA line data with optional control line data. The MCMC method can utilize data from multiple generations and models covariances that are generated by different lines accumulating different mutations at different times, so in principle extra information can be extracted from the MCMC data when compared to the ML and MD approaches. Rather than a reflected gamma distribution, the MCMC method has been implemented while assuming that the distribution of mutational effects is a "shifted" gamma distribution, such that a constant is added to each mutational effect. This has the effect of displacing the mode of the gamma distribution (which is at zero if the shape parameter $\beta < 1$). In principle, however, any distribution could be modeled, and the merits of alternative distributions have been debated (Keightley & Lynch 2003, Shaw et al. 2003). In practice, however, there may be little information in the MA line data to distinguish different distributions.

MD: minimum distance

MCMC: Markov Chain Monte Carlo

CV_m: coefficient of mutational variation, expressed as a percentage = $\sqrt{V_m}/M \times 100$

h^2_m: mutational heritability, the mutational variance scaled by the environmental variance for the trait = V_m/V_e

V_e: environmental variance

PARAMETER ESTIMATES FROM MUTATION ACCUMULATION EXPERIMENTS

The increase in among-line variance in a MA experiment can be used to calculate V_m. For example, under a simple model for a diploid population of size N, $2NU$ mutations are introduced into the population each generation. Assuming mutations act additively (that is, the heterozygote is exactly intermediate between the homozygotes), each mutation has an effect of $a/2$, and so each increases the variance by $E(a/2)^2/N$. Therefore, $V_m = UE(a^2)/2$, and so, from Equation 2, $V_m = \Delta V/2$ (see Lynch & Hill 1986). To allow comparison of V_m among traits, estimates are often standardized by the trait mean to give the coefficient of mutational variation ($CV_m = \sqrt{V_m}/M$ expressed as a percentage) or by the environmental variance to give the mutational heritability ($h^2_m = V_m/V_e$). It has been argued that the mutational coefficient of variation is the more appropriate measure for fitness-related traits (Houle et al. 1996).

Most MA experiments have focused on changes in variance and mean for life-history traits that are believed to be closely related to fitness, and in this section we review estimates of mutational parameters from these studies. New mutations influencing fitness-related traits are frequently assumed to lead to a directional change in the mean and, as discussed above, under this assumption it becomes possible to obtain estimates of the genomic mutation rate and the mean effect size of a mutation. We have chosen not to review studies that have measured effects of mutations on morphological traits for three reasons. First, these have previously been well reviewed (Houle et al. 1996, Lynch 1988), although it should be noted that some new studies measuring effects on morphological traits have been reported more recently (Ajie et al. 2005, Azevedo et al. 2002, Estes et al. 2005, García-Dorado & Marín 1998, García-Dorado et al. 2000, Wayne & Mackay 1998). Second, because different traits are considered, these studies are hard to compare between

species. Finally, although estimates of CV_m and h_m^2 are often reliably estimated, estimation of the rate of mutation and of the average mutation effect is problematic because mutations can have both positive and negative effects on the trait (but see Pannebakker et al. 2008).

In **Table 1**, we present estimates of ΔM, ΔV, CV_m, and h_m^2 along with BM estimates of U and the effect of a mutation (the exact definition of which varies depending on experimental design and organism). We have attempted to compile all available estimates for fitness-related traits and scale estimates of U and E(a) so that they are comparable. Across all taxa (excluding estimates from Gong et al. 2005 in *Drosophila*, which seem abnormally high in relation to other *Drosophila* estimates), mean ΔM is estimated to be $\sim 3.6 \times 10^{-3}$, mean CV_m is ~1.7%, and mean h_m^2 is $\sim 1.3 \times 10^{-3}$. These are consistent with results for other traits reported by Lynch (1988, see his table 1) and Houle et al. (1996, see their table 1).

In **Table 2**, we present estimates of: (*a*) U, (*b*) the effect of a mutation, and (*c*) the shape parameter β; where possible, we present estimates obtained using ML, MD, and MCMC methods, which all assume that effects follow a gamma distribution. As β tends to zero, the distribution of effects becomes increasingly L-shaped (that is, leptokurtic), and as β tends to infinity the distribution tends toward a model with equal effects. In cases where estimates of β are large (**Table 2**), estimates of U and E(a) generally agree well with BM estimates. This is expected because the models for the distribution of mutational effects are effectively the same. In all cases, estimates of β are noisy (that is, they have wide confidence intervals). However, one surprising pattern emerges: Of the 18 estimates, 13 are greater than one (5 significantly so), whereas only 4 are less than one. It has long been assumed that the distribution of mutational effects for fitness is strongly leptokurtic (Kimura 1983, Robertson 1967), and this has been corroborated by analysis of the frequency distribution of polymorphisms segregating within populations (Boyko et al. 2008, Eyre-Walker & Keightley 2007). That many MA-based estimates of β exceed one implies that the distribution is more platykurtic than an exponential distribution and that the mode of the distribution is not at zero. This may indicate that a gamma distribution does not adequately capture the shape of the true distribution of effects. For example, the distribution may be complex and multimodal. In other words, there may be many sites with small effects on fitness, as well as classes of large-effect mutations that are observed in MA experiments, but that are rarely observed segregating in natural populations (Keightley & Halligan 2008).

Clearly the distribution of mutational effects is difficult to estimate based on measurements of the fitness of MA lines alone. However, there is now some evidence, based on other sources of information, supporting the hypothesis that there are many mildly deleterious mutations. For example, induced mutations whose effects are too small to detect in the laboratory can be inferred to have deleterious fitness effects in nature (Davies et al. 1999). When these induced mutations are caused to segregate, fitness variation is largely caused by a few large-effect mutations (Halligan et al. 2003). Further evidence supporting the hypothesis that fitness declines are dominated by large-effect deleterious mutations comes from the analysis of MA experiments with populations of different sizes. Estes et al. (2004) found that MA lines kept at even very small population sizes (as low as three) maintained relatively high levels of mean fitness compared to a population of size one, and Ávila & García-Dorado (2002) found that MA line fitness quickly recovered when population size was increased to the order of a few tens.

To help visualize the differences in estimates obtained from the studies performed to date, we have plotted estimates of ΔM against CV_m and h_m^2 (both scaled up to the whole genome; **Figure 2*a*,*b***) and have grouped species into different taxonomic groups (that is, viruses, microbes, plants, nematodes, and *Drosophila*). All plotted estimates are from independent MA experiments, although some were reported in the same study and conducted concurrently. In particular, we have used results from five such MA experiments in nematodes (Baer et al. 2005), two in *Amsinckia*

Table 1 Standardized estimates of mutational parameters (ΔM, h_m^2, and CV_m) and Bateman-Mukai estimates of U and the mutational effect for life-history traits[a]

Species	Reference	Generations	Trait	ΔM ($\times 10^{-3}$)	h_m^2 ($\times 10^{-3}$)	CV_m (%)	U	Effect
VSV	Clarke et al. (1993), Duarte et al. (1992)	80–120	w[b]	−3.1 (0.20)	–	0.64	1.6 (0.57)	−0.0022 (0.0008)[c]
			w[b]	−2.1 (0.04)	–	0.30	0.88 (0.054)	−0.0024 (0.0001)[c]
	Clarke et al. (1993)		w[b]	−2.5 (0.04)	–	0.34	1.2 (0.052)	−0.0022 (0.0001)[c]
ϕ6 virus	Burch et al. (2007)	~200	w	−0.92	–	0.65	0.035	−0.027[c]
E. coli	Kibota & Lynch (1996)	~7500	Exponential growth rate	−0.0025	–	0.020	0.00017	−0.012[c]
S. cerevisiae	Zeyl & DeVisser (2001)	~600	w	0.005 [−0.022/0.032]	0.48	–	–	–
	Joseph & Hall (2004)	~1012	w	−0.0059	1.1	0.087	2.4e−5	−0.13[d]
A. gloriosa	Schoen (2005)	11	Number of flowers	−4.6	0.63	1.2	0.055	−0.085
A. douglasiana	Schoen (2005)	11	Number of flowers	−9.7	3.7	3.2	0.059	−0.16
			Dry weight	−2.4	0.65	0.58	0.090	−0.027
A. thaliana	Schultz et al. (1999)	10	w	−8.9 [−30/−1]	3.1 [−6/10]	3.2 [−5/6.3]	0.050 [0.002/0.4]	−0.23 [−0.9/−0.02]
	Shaw et al. (2000)	17	Number of fruits	−2	1.7	5.1	4e−3	−0.54
			Seeds/fruit	−0.1	1.3	0.64	1.5e−4	−0.92
C. elegans	Keightley & Caballero (1997)	60	Productivity	−0.30 (0.3)	1.2	1.2	0.00065	−0.46
		60	r	−0.35	–	0.42	0.0035 (0.001)	−0.10 (0.01)
	Vassilieva et al. (1999, 2000)	214	Productivity	−2.1	0.97	0.8 (0.3)	0.024 (0.012)	−0.088 (0.032)
			r	−1.5	1.3	3.3 (0.8)	0.0068 (0.0029)	−0.22 (0.060)
	Baer et al. (2005)	200	w	−0.94 (0.37)	1.2	1.3	0.0042 (0.0034)	−0.25
			w	−1.2 (0.25)	1.3	1.9	0.0033 (0.0026)	−0.36
C. briggsae	Baer et al. (2005)	200	w	−3.1 (0.26)	0.52	3.0	0.037 (0.020)	−0.10
			w	−2.4 (0.19)	2.2	2.6	0.012 (0.0055)	−0.19
O. myriophila	Baer et al. (2005)	200	w	−1.2 (0.26)	2.7	2.1	0.0028 (0.0022)	−0.44

D. melanogaster	Mukai (1964), Mukai & Yamazaki (1968)[e]	25	Viability[b]	−9.6[f]	0.22	1.1	0.35	−0.027 (≤0.013)[g]
	Mukai et al. (1972)[e]	40	Viability[b]	−11 (0.75)	0.13	2.0	0.47	−0.023 (≤0.012)[g]
	Ohnishi (1977a)[e]	40	Viability[b]	−4.3 [2.6/6.1]	0.30	0.87	0.14	−0.030[g]
	Houle et al. (1992)[e]	44	Fitness	–	–	4.1	–	–
	Fernández & López-Fanjul (1996), Caballero et al. (2002), Ávila & García-Dorado (2002)	104–106	Viability	−1.6	0.60 (0.12)	0.91	0.02	−0.10
		210	Viability	−0.81 (0.41)	–	1.5	0.0031	−0.25
		288	w[b]	−0.30 (0.03)	–	1.1 (0.67)	0.037 (0.0018)	−0.083 (0.031)[g]
	García-Dorado et al. (1998)	177–183	w[b]	−0.82 (0.43)		1.5 (0.95)	0.0015	−0.55
	Fry et al. (1999)[e]	27–33	Viability[b]	−6.0 [−7.8/−4.0]	–	1.2 [0.89/1.3]	0.053 [0.028/0.095]	−0.11 [0.073/0.16][g]
				−8.3 [−13/−1.3][f]	–	2.0 [1.2/2.6]	0.082 [0.0038/0.23]	−0.10[g]
	Fry (2001)[e]	31–35	Viability[b]	−8.0 [2.6/12.6][f]	–	2.2 [1.5/2.6]	0.068 [0.0085/0.19]	−0.12[g]
	Charlesworth et al. (2004)[e]	35	Viability[b]	−3.9 [−8.7/0.80][f]	–	2.4 [1.7/3.0]	0.012 [0.0/0.051]	−0.23 [−1.1/0.89][g]
		41	Viability[b]	−3.7 [−4.9/−2.5][f]	–	1.5 [1.1/1.7]	0.030 [0.020/0.039]	−0.11 [−0.12/−0.095][g]
		31	Viability[b]	−5.0 [−6.4/−3.4][f]	–	1.7 [1.4/2.0]	0.039 [0.028/0.049]	−0.11 [−0.12/−0.099][g]
	Gong et al. (2005)[e]	16	Viability[b]	−86 [−109/−65]	–	11 [9/13]	0.34 [0.20/0.55]	−0.29 [−0.40/−0.18][h]
		25	Viability[b]	−32 [−42/−23]	–	7 [5/8]	0.10 [0.065/0.19]	−0.33 [−0.49/−0.22][h]
		25	Viability[b]	−100 [−117/−87]	–	12 [10/14]	0.40 [0.26/0.66]	−0.29 [−0.40/−0.20][h]

[a]"Effect" refers to E(a), except where noted. Estimates of ΔM are given per generation. ΔM, CV_m, h_m^2, and U are scaled up to the whole (haploid) genome where necessary. Standard errors (round brackets) or 95% confidence limits (square brackets) are given where available. References and details of individual estimates are provided in the **Supplementary Material**. Follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**.

[b]Assay used competitive conditions.

[c]Mutational effect reported is in a haploid organism.

[d]Mutational effect reported is E(ah) instead of E(a).

[e]Mutations are accumulated using a balancer chromosome system.

[f]"Order method" used to estimate ΔM.

[g]Mutational effect reported is E[$a(1 - h)$] instead of E(a) (see Crow & Simmons 1983, p. 13 for derivation).

[h]Mutational effect reported is for a hemizygous mutation on the X chromosome.

Table 2 ML, MD, and MCML estimates of *U*, mutational effect, and the gamma distribution shape parameter, β, obtained for fitness-related life-history traits[a]

Species	Reference	Generations	Trait	Method	*U*	Effect	β
ϕ6 virus	Burch et al. (2007)	~200	*w*	ML	0.045	−0.021[b]	→∞
S. cerevisiae	Zeyl & DeVisser (2001)	~600	*w*	ML	4.7e–5	−0.22[c]	[0/→∞]
	Joseph & Hall (2004)	~1012	*w*	ML	6.3e–5 [4.6e–5/∞]	−0.061 [−0.077/0.00][c]	2
A. gloriosa	Schoen (2005)	11	Number of flowers	ML	0.099	−0.050	2 [>0/→∞]
A. douglasiana	Schoen (2005)	11	Number of flowers	ML	0.073	−0.18	→∞ [>1/→∞]
			Dry weight	ML	0.088	−0.030	→∞ [<1/→∞]
A. thaliana	Shaw et al. (2000)	17	Number of fruits	MCML	0.060 [0.035/0.085]	−0.020 [−0.17/0.11]	3.53 [0.00/7.75]
			Seeds/fruit	MCML	0.10 [0.085/0.12]	0.0012 [−0.0092/0.0098]	1.91 [1.09/2.71]
C. elegans	Keightley & Caballero (1997)	60	Productivity	ML	0.0026 [0.0001/0.01]	−0.21 [0.06/0.29]	→∞ [>1/→∞]
	Vassilieva et al. (1999, 2000)	214	Productivity	ML	0.0075 [0.004/0.013]	−0.22 [−0.34/−0.15]	→∞
			r	ML	0.018 [0.0085/0.18]	−0.058 [−0.12/−0.0007]	1.2 [1.0/2.0]
D. melanogaster	Mukai et al. (1972)[d]	40	Viability[e]	MD	0.011	−0.19[f]	3.1
				ML	→∞ [0.35/→∞]	→0 [−0.034/→0][f]	→0 [→0/1.69]
	Ohnishi (1977a)[d]	40	Viability[e]	MD	0.010	−0.23[f]	0.9
				ML	→∞ [0.17/→∞]	→0 [−0.020/→0][f]	→0 [→0/0.25]
	Houle et al. (1992)[d]	44	Fitness	MD	0.030	−0.26	1.6
	Caballero et al. (2002), Fernández & López-Fanjul (1996)	104–106	Viability	MD	0.016 (0.006)	−0.10 (0.031)	3.4 [2.0/12]
		210	Viability	MD	0.0050 (0.0047)	−0.16 (0.12)	–
	Fry et al. (1999)[d]	27–33	Viability[e]	ML	→∞ [0.024/→∞]	→0 [−0.14/→0][f]	→0 [→0/<∞]
				MD	0.022	−0.16[f]	–

[a]"Effect" refers to E(*a*), except where noted. Estimates of *U* are scaled up to the whole (haploid) genome where necessary. Standard errors (round brackets) or 95% confidence limits (square brackets) are given where available. References and details of individual estimates are provided in the **Supplementary Material**. Follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**.

[b]Mutational effect reported is in a haploid organism.

[c]Mutational effect reported is E(*ah*) instead of E(*a*).

[d]Mutations are accumulated using a balancer chromosome system.

[e]Assay used competitive conditions.

[f]Mutational effect reported is E[*a*(1 − *h*)] instead of E(*a*) (see Crow & Simmons 1983, p. 13 for derivation).

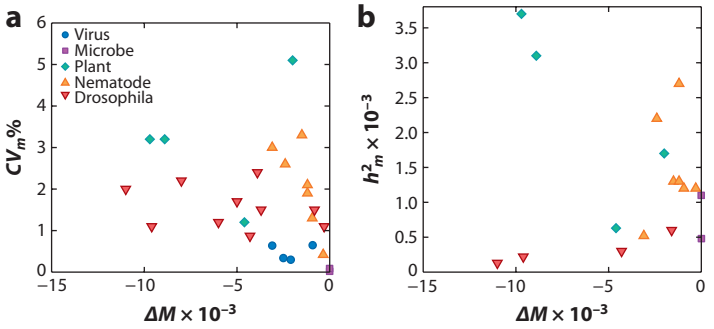

Figure 2

Estimates of ΔM plotted against CV_m and h_m^2. Estimates of ΔM plotted against (*a*) CV_m (%) and (*b*) h_m^2 ($\times 10^{-3}$) from a variety of species. We have excluded results from the study by Gong et al. (2005) from panel *a*, because estimates of ΔM are one order of magnitude higher than others from the same species. Panel *b* contains results from fewer studies than panel *a* because, in many cases, unavailability of V_e estimates prevented the calculation of h_m^2.

(Schoen 2005) and three in *Drosophila* (Charlesworth et al. 2004). In cases where there are multiple estimates from the same MA experiment at different generations, we used either estimates based on a regression across generations or estimates from the latest generation. We have also plotted estimates of U against the estimated effect of a mutation for independent MA experiments in **Figure 3**, and to allow consistent comparison, we report only estimates that have used the BM method.

It is clear from **Figures 2** and **3** that estimates from the same study tend to cluster together. One possible explanation for this pattern is that experiments performed at the same time are subject to similar environmental conditions and are likely to use similar assays for fitness, and the populations are likely to have experienced similar levels of natural selection, which would tend to reduce the number of fixations below that expected under neutrality. This is of particular concern in MA experiments in microbes and viruses, where there is the opportunity for selection during a growth phase between MA line transfers and could cause a downward bias in estimates of ΔM and U. One exception to this conclusion is that the results obtained for plants (*Amsinckia* and *Arabidopsis*), which, although consistent with results from the other multicellular eukaryotes, are

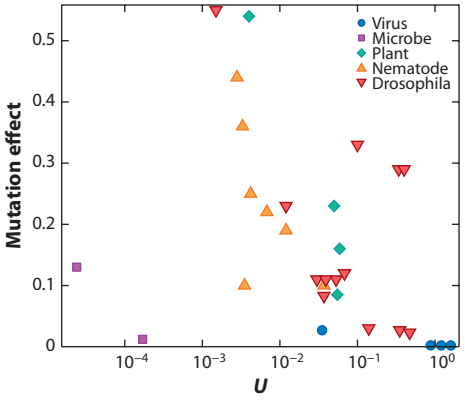

Figure 3

Bateman-Mukai (BM) estimates of the effect of a mutation plotted against U from a variety of taxa.

quite scattered. In these cases, the discrepancies likely represent larger estimation errors for these values (see confidence intervals/standard errors reported in **Table 1**), possibly owing to the low number of generations in the MA experiments.

Although apparent differences between taxa may be magnified by these difficulties, genuine differences between the organisms in their deleterious mutation rates and spectrum of mutational effects are also highly likely to be present (Baer et al. 2007). In particular, three estimates from vesicular stomatitis virus (VSV) show rapid declines in fitness and increases in variance per generation as a result of MA and very high mutation rates, consistent with high per-nucleotide mutation rates in this virus (although the estimate of U for $\phi 6$ is at least an order of magnitude lower than that for VSV, it is still high relative to other estimates). Microbes, on the other hand, experience the lowest input of mutational variation, measured on any scale, because they have both the lowest decrease in mean trait value per generation, the lowest estimates of CV_m, and some of the lowest estimates for h^2_m. They also have very low estimates of U, and all of these are consistent with their low mutation rates per nucleotide per generation (Drake et al. 1998).

Interestingly, results for *Drosophila* and nematodes reveal somewhat different patterns. *Drosophila* show a lower increase in variance as a result of mutation for a given decline in mean fitness. This result has been noted before (Keightley 1996). Estimates of U tend to be lower, and estimates of E(a) higher, in nematodes than *Drosophila*. This can be intuitively understood considering that the nematode experiments show greater increases in variance for a given decrease in mean fitness (**Figure 2**) and that estimates of U and E(a) depend on the relative magnitude of these quantities (see above). In particular, the results from *Drosophila* experiments by Mukai (1964), Mukai et al. (1972), Ohnishi (1977a), Fry (1999, 2001), and especially Gong et al. (2005) stand out as showing particularly rapid declines in fitness and therefore have high estimates of U. The majority of MA experiments performed in *Drosophila* used balancer chromosomes and competitive fitness assays, and these factors may be partially responsible for the differences observed. Alternatively, it is possible that there is a real difference in mutation rate and the spectrum of mutational effects between nematodes and *Drosophila*. It has been speculated that the apparent mutation rate difference could be an adaptation to self-fertilization, via selection on a mutation rate modifier (Baer et al. 2006, Drake et al. 1998), although this remains to be tested.

A final observation from **Figure 3** is that U and the estimated mutational effect are negatively correlated. Although this pattern may have a partial biological explanation, it is also expected because the sampling covariance between these parameters is negative. In other words, it is difficult to tease apart a high mutation rate with mutations of small effect from a low mutation rate with mutations of large effect.

FURTHER INFERENCES FROM MUTATION ACCUMULATION EXPERIMENTS

Estimates of the Fraction of Beneficial Mutations

In the majority of MA experiments, mean fitness measures are observed to decline (see **Table 1**), and few individual MA lines have shown significantly higher values than control MA lines. In experiments in *A. thaliana* (Shaw et al. 2003) and *Saccharomyces cerevisiae* (Dickinson 2008, Joseph & Hall 2004), however, a substantial proportion of lines with significantly increased values for fitness-related life-history traits have been found. This conflicts with the generally held idea that most beneficial mutations have been previously fixed by natural selection, implying that deleterious mutations should greatly outnumber advantageous mutations. There are at least three alternative explanations for these findings: (*a*) Some mutations that are deleterious in nature are

beneficial in the novel laboratory assay environments. (*b*) The life-history traits assayed in many MA experiments are not closely related to fitness in nature. For example, *A. thaliana* is a colonizer of disturbed soil, and growth rate and time to first reproduction may be better correlates of fitness in nature than the trait measured (that is, total reproductive output). (*c*) In the cases of yeast and other microbes, single-generation transfers during MA have not always been possible. As a result, it is possible that some adaptive mutations may have been selected to fixation during the cellular growth phase that inevitably occurs between transfers, where, owing to the larger population size, selection may be sufficiently effective.

h: coefficient of dominance, defined such that the effect of a heterozygous mutation is *ah*

Dominance

We assign the genotypic values for a quantitative trait as 1, 1–*a*, and 1–*ha* for the wild-type homozygote, mutant homozygote, and heterozygote, respectively, where *h* is the dominance coefficient. The average dominance coefficient of mutations affecting fitness, E(*h*), is important for several issues concerning the maintenance of genetic variability (Charlesworth & Hughes 1999). For example, under a simple deterministic model of a balance between mutation and recurrent deleterious mutations, the additive genetic variance for fitness at a locus is equal to twice the product of the mutation rate and the average heterozygous effect for fitness, *ah* (that is, $V_A = 2\mu ah$) (Mukai et al. 1974). The distribution of *h* is also an important parameter in determining the amount of inbreeding depression (the reduction in fitness or fitness components observed on inbreeding), because the number of segregating mutations maintained in a population at mutation-selection balance decreases as *h* increases. The decline in fitness experienced when such a population undergoes inbreeding increases as *h* decreases, so recessive deleterious mutations lead to more severe inbreeding depression.

Several experiments to estimate the dominance effects of viability mutations have been carried out in *Drosophila* by Mukai and coworkers (summarized by Simmons & Crow 1977) and involve comparing the fitness of individuals that are heterozygous and homozygous for accumulated spontaneous mutations. Ideally, we would like to know the joint distribution of homozygous and heterozygous effects of new mutations; however, most work has focused on estimating the mean dominance effect, E(*h*), of a mutation. Estimates of E(*h*) have been obtained using two methods. The first calculates E(*h*) as the ratio of the wild type minus heterozygous fitness to the wild type minus homozygous fitness, giving an average weighted by the mutation effect, *a* (Mukai 1969a). The second method calculates the regression coefficient of the heterozygous effect on the sum of two homozygous effects of the parental chromosomes, yielding an estimate weighted by the square of the mutation effect (Mukai et al. 1972, Mukai & Yamaguchi 1974). There are reasons to believe that the ratio estimator is more useful than the regression estimator (Caballero et al. 1997, García-Dorado & Caballero 2000).

Mukai and Ohnishi estimated E(*h*) for mildly deleterious mutations accumulated on "quasinormal" chromosomes (that is, chromosomes that had not experienced drastic reductions in viability) after several tens of generations of MA. Viabilities of these chromosomes were assayed in the homozygous and heterozygous states, using control chromosomes that were assumed to be genetically similar to the ancestral chromosome. The results obtained depended on whether coupling (where a mutagenized chromosome is paired with a wild-type) or repulsion (where two mutagenized chromosomes are paired) heterozygotes were used, and also on whether the ratio or regression method was used to estimate *E*(*h*). Estimates of *E*(*h*) vary considerably, ranging from −0.32 to 0.49 (see reviews in Simmons & Crow 1977 and García-Dorado & Caballero 2000). Repulsion heterozygotes yielded estimates that were nearly additive when either method of calculation was used [E(*h*) = 0.36–0.47; Mukai & Yamazaki 1968]. However, results for coupling heterozygotes were

inconsistent and depended on whether the heterozygotes were formed by pairing the mutagenized chromosome with a nonisogenic wild type or control wild-type-like chromosome 2. Results using a wild-type-like chromosome gave consistently negative estimates of E(h) with either method of calculation [E(h) = −0.32 – −0.09; Simmons & Crow 1977], suggesting that mildly deleterious mutations are overdominant, on average. On the other hand, when heterozygotes were formed by pairing the test chromosome with a nonisogenic wild-type chromosome, they yielded estimates indicating that mutations are partially recessive when calculated using the ratio approach [E(h) = 0.09–0.13; Simmons & Crow 1977], or nearly additive when using the regression approach [E(h) = 0.27–0.56; Mukai et al. 1965]. Ohnishi's estimates of E(h) suggest that mutations act nearly additively, when E(h) is calculated via the ratio approach [E(h) = 0.40–0.47 for either coupling or repulsion heterozygotes; Ohnishi 1977b], but suggest they are partially recessive when E(h) is calculated by regression [E(h) = 0.12–0.15; García-Dorado & Caballero 2000].

The difference between Mukai's and Ohnishi's results has been interpreted in various ways. First, the two methods of analysis produce estimates that are weighted differently (see above) and are expected to be different because they are weighted differently (Caballero et al. 1997). Second, Fry (2004) hypothesized that a subset of Mukai's lines had become contaminated by an external source. Third, the wide variation in estimates of mutational parameters might reflect real genetic differences among strains, perhaps caused by transposable element activity (Fry 2004). Finally, García-Dorado & Caballero (2000) have suggested that a nonmutational decline in fitness in Ohnishi's experiment could have inflated his estimate of E(h). Other investigations of dominance have not greatly clarified the issue of the dominance coefficients of mildly deleterious mutations. In a MA experiment using a balancer chromosome system, Houle et al. (1997) estimated the mean dominance coefficient for five life-history traits (other than viability) and found mutations to be partially recessive on average [using a regression approach, E(h) = 0.12]. However, it should be noted that nonquasinormal chromosomes were included in the analysis, which might be expected to have lower dominance coefficients, because strongly deleterious mutations tend to be highly recessive (Caballero & Keightley 1994). Vassilieva et al. (2000) reported several estimates of E(h) from 73 MA lines in *Caenorhabditis elegans*, ranging from −0.10 to 0.69 (0.38 on average) for the six traits measured (using the regression approach). This suggests that new mutations tend to be partially recessive, although standard errors for individual traits were relatively large (0.21 on average). Clearly this question remains unresolved and merits more work in the future.

Epistasis

Almost all of the work on the epistatic effects of new spontaneous mutations has concerned epistasis for fitness or fitness-related traits such as viability. In particular, many experiments have tested for evidence of synergistic epistasis, meaning that the effects of deleterious mutations tend to increase on a genetic background already loaded with mutations. This is important because synergistic epistasis could be a mechanism for limiting the load of mutations (Crow 2000), and it is a key feature in some models for the evolution of sexual reproduction (Kondrashov 1988). In a spontaneous MA experiment, synergistic epistasis would manifest itself as a greater than linear decline in log fitness as a function of generation number. In order to detect such a second-order phenomenon, experiments therefore need to be run for many generations, be highly replicated, and have good controls. In experiments that meet these criteria, evidence for net synergistic epistasis is mixed. Mukai (1969b) reported a strongly nonlinear decline in viability in *D. melanogaster*, indicating synergistic epistasis. Subsequent reanalysis that attempted to account for possible changes in the control chromosome (Fry 2004) corroborated the nonlinear decline in these lines and suggested that Ohnishi's (1977b) lines also showed a nonlinear decline. A recent MA experiment in *S. cerevisiae*

(Dickinson 2008) also shows clear evidence of an accelerating decline in fitness. However, *C. elegans* (Baer et al. 2005, Vassilieva et al. 2000) and *Escherichia coli* (Kibota & Lynch 1996) MA experiments show nearly linear or even concave upward declines in fitness or fitness-related traits. Furthermore, experiments involving induced mutations that are combined in different numbers or by combining doses of a mutagen have generally not yielded strong evidence of net synergistic epistasis (Elena & Lenski 1997, Korona 2004, Peters & Keightley 2000, Wloch et al. 2001).

There are two factors that complicate the interpretation of synergistic epistasis in spontaneous MA experiments. First, an acceleration in the rate of change of fitness could be caused by an increase in the mutation rate (Ávila et al. 2006). Of particular concern is the mutagenic effect of transposable elements (TEs): Unusually high rates of transposition of *copia* and *roo* TEs have been detected in *Drosophila* MA experiments (Houle & Nuzhdin 2004, Papaceit et al. 2007), and it is conceivable that transposition of unidentified TEs, leading to an acceleration in the genomic rate of transposition, could be misattributed to an effect of synergistic epistasis. Second, natural selection against new deleterious mutations is impossible to completely eliminate from MA experiments, and this tends to make it harder to detect synergistic epistasis.

Effect of Environmental Conditions

The distribution of effects of new mutations could depend on the assay environment, and this in turn could affect V_m and ΔM. There has therefore been interest in knowing whether the overall effect of MA is environment dependent, particularly if there is a "hidden" genetic load of mutations that reveal their effects in harsh environments, the premise being that natural environments are harsh and competitive. There have now been several studies examining the effect of accumulated spontaneous mutations in different environments, either by testing for differences in V_m or ΔM or for genotype-environment interactions. In the first study of this kind, two MA lines showed substantially larger declines in fitness (compared to a relatively mutation-free control line) in environments perceived to be harsh (Kondrashov & Houle 1994). Subsequent studies in a variety of organisms (Baer et al. 2006, Chang & Shaw 2003, Fernández & López-Fanjul 1997, Fry & Heinsohn 2002, García-Dorado et al. 1999, Kavanaugh & Shaw 2005, Vassilieva et al. 2000, Xu 2004), however, have presented a less clear picture (reviewed by Martin & Lenormand 2006). On average, the difference in ΔM between harsh and benign environments is close to zero. However, V_m tends to be higher in harsh environments. This pattern therefore implies that BM estimates of the genomic mutation rate would tend to be smaller in harsh environments, whereas the average effect of a mutation would tend to be larger. However, the differences in estimates between environments really reflect changes in the distribution of effects of mutations rather than differences in the numbers of accumulated mutations, which, of course, remain constant.

Effects of Mutation Accumulation on Expression

Variation in gene expression underlies much phenotypic variation, and therefore understanding effects of mutation on gene expression is important for understanding how organisms evolve at the phenotypic level. Three recent studies have examined the effects of spontaneous MA on global gene transcription divergence (Denver et al. 2005, Landry et al. 2007, Rifkin et al. 2005). Denver et al. (2005) used microarrays to compare expression divergence for thousands of unique sequences among four *C. elegans* MA lines (Vassilieva et al. 2000) and their progenitor line (strain N2), as well as among five natural isolate (NI) lines. Strikingly, the fraction of differentially expressed genes was about six times higher in the MA lines, separated by 280 generations of MA, than the NI lines, separated by many thousands of generations. This result suggests that stabilizing

selection very strongly constrains gene expression in the wild. Consistent with this, the variation in expression among the NI lines is much lower than predicted by a neutral model parameterized by the observed mutational variance for expression. This new mutational expression variation may largely represent expression mutations with multiple *trans*-acting effects (Denver et al. 2005), which are likely to be highly deleterious in natural populations.

Rifkin et al. (2005) analyzed expression in 12 MA lines from a 200-generation MA experiment in *D. melanogaster* (Houle & Nuzhdin 2004) and arrived at broadly similar conclusions to Denver et al. (2005). Although estimates of h_m^2 on a gene-by-gene basis were low compared to many morphological traits, they found lower than expected variation between species (*D. melanogaster*, *D. simulans*, and *D. yakuba*) based on their estimates of V_m and a neutral model. This supports the conclusions from *C. elegans* that expression is under strong stabilizing selection.

Finally, Landry et al. (2007) measured gene expression levels using DNA microarrays in four randomly selected lines from a 4000-generation MA experiment (where N_e was estimated to be ~10) in *S. cerevisiae*. Median V_m for expression was found to be 4.7×10^{-5} (similar to *Drosophila* and also lower than that for morphological traits). Interestingly, Landry et al. (2007) found that V_m was correlated with gene expression variation in natural isolates, suggesting that natural variation is shaped partly by sensitivity to mutation.

Direct Estimation of the Genomic Mutation Rate

The analysis of phenotypic values of MA lines to infer U and $f(a)$ is limited by experimental resolution and by the simplifying assumptions that are required by the analysis methods. Better estimates of mutation rates are likely to come from molecular-based approaches. The first attempt to directly estimate the mutation rate in MA lines used electrophoresis to scan enzyme loci in *D. melanogaster* (Mukai & Cockerham 1977). Although carried out on a large scale, the estimate of the per-nucleotide mutation rate from these data is imprecise, because few events were detected (a total of three), and because there is uncertainty about the relationship between electrophoretic and per-nucleotide mutation rates (Keightley & Eyre-Walker 1999). More recently, the comparison of DNA sequences of MA lines has provided direct estimates of nuclear genome-wide nucleotide mutation rate for *C. elegans* (Denver et al. 2004), *D. melanogaster* (Haag-Liautard et al. 2007, Keightley et al. 2009), and *S. cerevisiae* (Lynch et al. 2008). A notable finding from the *C. elegans* experiment was a relatively high frequency of insertion-deletion (indel) mutations, whereas indels do not seem to be unusually frequent within populations of Caenorhabditids (Denver et al. 2004). The *D. melanogaster* experiment by Haag-Liautard et al. (2007) was carried out with three different progenitor genotypes, and there was evidence for significant mutation rate variation between MA lines from the three genotypes. The mean per-nucleotide mutation rate estimate is about five times higher than estimates based on rates of substitution at synonymous sites between closely related *Drosophila* species (Haag-Liautard et al. 2007, Keightley et al. 2009). These data allow U to be estimated, following a suggestion from Kondrashov & Crow (1993), by equating U to μCG, where μ is the per-nucleotide mutation rate, C is the fraction of strongly deleterious mutations that have a negligible chance of fixation, and G is the number of nucleotide sites in the haploid genome. In *Drosophila*, an estimate of C is 0.58, based on a genome-wide comparison of rates of substitution at selectively constrained and putatively neutrally evolving sites between *D. melanogaster* and *D. simulans* (Halligan & Keightley 2006). This yields an estimate of U for *Drosophila* of about 0.6 per haploid genome (Haag-Liautard et al. 2007). If we assume that C in *C. elegans* and yeast is similar to that estimated for *Drosophila*, estimates of U are about 1 and 0.2, respectively, although most of the yeast mutations were changes in homopolymer length (Lynch et al. 2008), whereas those in *Drosophila* were mostly single nucleotide events. It is notable that these estimates are

2–3 orders of magnitude higher than estimates based on BM analysis of phenotypic data on fitness traits from MA lines of *C. elegans* (mean = 0.010; Baer et al. 2005, Keightley & Caballero 1997, Vassilieva et al. 2000) and yeast (Dickinson 2008), whereas the molecular estimate for U in *Drosophila* is only slightly higher than the estimates obtained by Mukai (Mukai 1964, Mukai et al. 1972). This, therefore, lends further support to the idea that Mukai's lines were subject to unusually high mutation rates.

CONCLUSIONS: LIMITATIONS OF MUTATION ACCUMULATION EXPERIMENTS

The small amount of new mutational variation introduced each generation limits the power of many spontaneous MA experiments. Some experiments have gone on insufficiently long or been assayed with insufficient replication to provide much information on parameters such as ΔM or V_m. Even very large MA experiments are limited in what they can tell us about the nature of variation from new mutations. The BM method of analysis assumes equal, unidirectional mutational effects and can only give lower- and upper-limit estimates for U and $E(a)$, respectively. Newer inference methods (García-Dorado 1997, Keightley 1994, Shaw et al. 2002) are more flexible in that they can assume a distribution of mutational effects and can allow bidirectional effects. However, parameter estimates tend to be strongly confounded in the analysis, such that a low mutation rate and equal mutational effects often fit the data nearly as well as a very much higher mutation rate and an L-shaped (that is, leptokurtic) distribution of mutational effects (García-Dorado & Gallego 2004, Keightley 1998). Furthermore, specific models for the distribution of mutational effects need to be assumed, and this can lead to bias if the true distribution departs substantially from the distribution assumed. For example, the true distribution of mutational effects may be complex and not well modeled by a unimodal distribution, such as the gamma distribution. If true, this may explain the apparent discrepancy in the inferred shape of the distribution of effects inferred from MA experiments (which imply a platykurtic distribution; see **Table 1**) and from analyses of the frequency distribution of segregating alleles within populations (which imply a strongly leptokurtic distribution; reviewed by Eyre-Walker & Keightley 2007).

With these limitations in mind, do MA experiments have a future? With whole-genome sequencing, interest in MA experiments will be catalyzed by the possibility of direct molecular estimation of the mutation rate (Bentley et al. 2008, Lynch et al. 2008, Margulies et al. 2005). Combined with estimates of the number of sites in the genome that are subject to selection, these direct estimates of rates should at last provide reliable estimates of the genomic deleterious mutation rate, U, in a range of taxonomic groups, and these will improve parameter estimation in evolutionary models. As accurate genome sequencing decreases in cost, sequencing of parent-offspring trios sampled from natural populations will also become possible, leading to direct estimates of the nucleotide site mutation rate and its variability in natural populations (Kondrashov 2008). This will circumvent the concern that accumulating mutations on an inbred background is atypical of many natural situations. For inferring the effects of mutations on fitness-related traits, the analysis of nucleotide variation within populations has arguably provided more realistic information (Boyko et al. 2008, Eyre-Walker & Keightley 2007) than MA experiments. However, for other quantitative traits, analysis of MA experiments seems to be the only viable means of inferring the distribution of effects of new mutations. One possible advance in this respect that has not been adequately explored is to use assays that employ automated measurement of quantitative traits (Weber 1990, Weber & Diggins 1990). Combined with analyses that make better use of the information in the data for multiple generations of MA (Shaw et al. 2002), this may provide better information on parameters of the distribution of mutational effects.

FUTURE ISSUES

1. Whole-genome sequencing technology should be used not only to directly estimate the mutation rate, but also to measure the extent of mutation rate variation within and between populations and between species. Crucially, direct estimates will overcome some problems associated with phenotypic estimates of the mutation rate.
2. Genome sequencing of parent-offspring trios should be done to measure the mutation rate in wild-caught individuals, thereby circumventing the possibility that mutation rates in inbred lines are atypical (Kondrashov 2008).
3. There should be more widespread use of transcriptomics and proteomics to infer the effects of mutations on gene expression.
4. Researchers should use automated assays for fitness, perhaps including competitive assays, leading to more accurate estimates of the genomic mutation rate and distribution of effects, potentially revealing subtle effects of mutations otherwise missed in standard assays.
5. There needs to be more exploration of mutation accumulation in populations of different sizes and what this can reveal about the distribution of fitness effects of new mutations.
6. Dominance and interaction effects of new mutants are still poorly understood, and more work seems merited.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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