

CHAPTER 3

Rates and effects of deleterious mutations and their evolutionary consequences

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In a highly influential paper, Muller (1950) put forward the idea that deleterious mutations affecting the heterozygote appear at a high rate in populations, thus, imposing a reduction in **fitness (mutational load)** that could become unbearable. As shown by Haldane (1937), this load is mainly determined by the rate of deleterious mutations and is practically independent of their severity, although it is modulated by their degree of dominance. It took a long time until adequate experimental data for *Drosophila* viability were first obtained by Mukai (1964), who estimated a high genomic rate of mutations (**haploid rate** $\lambda > \approx 0.3$, the number of deleterious mutations appearing per gamete and generation) showing very little dominance (average **coefficient of dominance** $E(h) \approx 0.4$, where $h = 0, 0.5$, and 1 denotes recessive, additive and dominant gene action of mutations, respectively) and mild average effects (average **selection coefficient** $E(s) < \sim 0.03$, the relative reduction in fitness of the homozygous mutant). These values were corroborated by subsequent work (Mukai *et al.* 1972; Ohnishi 1977a) and became typical parameters extensively used in evolutionary genetic models, apparently supporting Muller's pessimistic views on the role of mutational load.

In the last decade, however, renewed interest on the matter prompted additional experimental work, the results of which raise questions about the validity of former estimates. The controversy is far from resolved, as shown by the contrasting opinions

maintained in the latest reviews (García-Dorado *et al.* 1999; Keightley and Eyre-Walker 1999; Lynch *et al.* 1999). Here, we review the published estimates of λ , $E(s)$, and $E(h)$ obtained from spontaneous **mutation accumulation (MA)** experiments. We focus on deleterious mutations in eukaryotes (see Drake and Holland 1999, for a review on mutation rates among RNA viruses). We also infer the fraction of mutations that are undetected in MA experiments and examine some of the evolutionary consequences of the estimated rates of deleterious mutation.

Review of estimates of λ and $E(s)$ from MA experiments

Before proceeding, a brief description of the experimental and analytic methods seems appropriate. In MA experiments, deleterious mutations accumulate as a Poisson process under relaxed selection in lines derived from the same uniform genetic background. This allows the estimation of the per generation rate of decline for a **fitness component trait**, $\Delta M = \lambda E(s)$, and the rate of increase in the between-line variance, $\Delta V = \lambda E(s^2)$. Thus, lower and upper bound estimates of λ and $E(s)$ can be computed. Such Bateman–Mukai (BM) estimates are calculated as follows: $\lambda \geq \Delta M^2 / \Delta V$, $E(s) \leq \Delta V / \Delta M$. In parallel, the information contained in the observed distribution of line means can be more efficiently used by assuming a convenient family distribution of mutant effects (usually a

gamma distribution) and finding point estimates, rather than estimates of bounds, of the mutational parameters that better account for the data. Two methodologies have been used: maximum likelihood (ML, see Keightley and Bataillon 2000) and minimum distance (MD, see García-Dorado and Gallego 2003).

Estimates have been obtained for several eukaryotic species: the fruitfly *Drosophila melanogaster*, the crustacean *Daphnia pulex*, the nematode *Caenorhabditis elegans*, the yeast *Sacharomices cerevisiae*, and two plants, the crucifer *Arabidopsis thaliana* and the wheat *Triticum durum*. A summary of λ and $E(s)$ estimates (generally rounded, for clarity, to the second decimal) for relative fitness and fitness component traits is given in Table 3.1. For plant and invertebrate animal species, recent BM estimates of λ are about one order of magnitude smaller than classical Mukai/Ohnishi estimates, the corresponding $E(s)$ values being larger. This discrepancy vanishes when MD estimates are considered. On the whole, the data show remarkable concordance, indicating λ and $E(s)$ values of about 0.01 and 0.1, respectively. We will now discuss the most salient aspects of the estimates.

Drosophila melanogaster

The BM analysis of classical experiments by Mukai and Ohnishi indicated that mutations affecting egg-to-adult viability arise at a high rate ($\lambda > \sim 0.3$) and have small average homozygous effects ($E(s) < \sim 0.03$). However, it has been suggested that the viability decline observed in these experiments could be partially ascribed to causes different from spontaneous mutation (Keightley 1996; García-Dorado 1997). In fact, a more recent MA experiment consistently suggests λ values about tenfold lower ($\lambda \sim 0.02$) and larger $E(s)$ estimates ($E(s) \sim 0.1$) through three repeated assays. The first and second assays correspond to non-competitive viability at generations 104–106 (Fernández and López-Fanjul 1996) and 210 (Caballero *et al.* 2002) while, in the third, viability scores were obtained at generations 250–255 in competitive conditions similar to those of Mukai (Chavarrías *et al.* 2001). The two latest assays used contemporary evaluations of the MA

Table 3.1 Estimates of the haploid genomic rate λ and the average homozygous effect $E(s)$ of deleterious mutations for relative fitness or fitness component traits

Species	Character	BM bound estimates		MD/ML direct estimates	
		λ	$E(s)$	λ	$E(s)$
<i>D. melanogaster</i> (1)	Viability	0.44 ^a	0.02 ^a	0.01	0.19
<i>D. melanogaster</i> (2)	Viability	0.14 ^a	0.03 ^a	0.01	0.23
<i>D. melanogaster</i> (3)	Viability	0.04 ^a	0.15 ^a	0.02	0.16
<i>D. melanogaster</i> (4)	Viability	0.02 ^a	0.10 ^a	0.02	0.10
<i>D. melanogaster</i> (5)	Viability	0.01 ^a	0.08 ^a	—	—
<i>D. melanogaster</i> (6)	Viability	0.003 ^a	0.25 ^a	0.005	0.16
<i>D. melanogaster</i> (7)	Fitness	—	—	0.03	0.26
<i>D. melanogaster</i> (8)	Fitness	0.03	0.14	—	—
<i>A. thaliana</i> (9)	Fitness	0.015	0.36	—	—
<i>T. durum</i> (10)	Productivity	—	—	0.05 ^b	0.20 ^b
<i>C. elegans</i> (11)	Intrinsic growth rate	0.01	0.05	0.003 ^b	0.10 ^b
<i>C. elegans</i> (12)	Intrinsic growth rate	0.007	0.22	0.02 ^b	0.06 ^b
<i>S. cerevisiae</i> (13)	Fitness	—	—	0.0001 ^b	0.22 ^{b,c}
<i>S. cerevisiae</i> (14)	Fitness	—	—	0.0005 ^d	0.09 ^d

(1) Mukai *et al.* (1972), (2) Ohnishi (1977b), (3) Fry *et al.* (1999), (4) Fernández and López-Fanjul (1996), (5) Chavarrías *et al.* (2001), (6) Caballero *et al.* (2001), (7) Houle *et al.* (1992), (8) Ávila and García-Dorado (2002), (9) Schultz *et al.* (1999), (10) Bataillon *et al.* (2000), (11) Keightley and Bataillon (2000), (12) Vassilieva *et al.* (2000), (13) Zeyl and DeVisser (2001), and (14) Wloch *et al.* (2001).

^a Lethal and severely deleterious mutations excluded.

^b ML estimates.

^c Estimate of $E(sh)$.

^d Direct estimate obtained by tetrad analysis.

lines and the control, and the rate of decline in mean and the rate of increase in variance (and, hence, the BM estimates of λ and $E(s)$) were close to those observed earlier.

Natural selection could have acted during the course of the Fernández and López-Fanjul experiment, producing downward biases of λ and $E(s)$. However, computer simulations accounting for selection within and between MA lines, as well as in the control population, suggested that elimination of mutations through selection is not the main cause of the discrepancy between the contrasting

estimates of mutational parameters (Caballero *et al.* 2002). Two other results support the same conclusion. First, there was no indication of a temporal decline of the control average, suggesting that accumulation of mildly deleterious mutations was not important in this case. Second, the number of lines lost after 255 generations was only 9 percent larger than that expected from accidental losses (Chavarrías *et al.* 2001) and this was only concordant with a simulated model with few mutations of large effect (Caballero *et al.* 2002).

The discrepancy between Fernández and López-Fanjul's and Mukai's results can be ascribed to a much larger decline in mean in the latter, as the pertinent increase in variance was about the same in all *Drosophila* experiments. This may be due to the lack of a suitable control in Mukai's experiment that would allow unbiased estimates of ΔM . This impediment has been obviated by computing MD estimates of mutational parameters unconstrained by the observed ΔM , giving similar estimates of λ for both experiments (García-Dorado 1997) (Table 3.1).

Viability estimates of ΔM obtained by an "order method," have been recently reported by Fry (2001) using both Mukai's original data and new data. With this method, ΔM is calculated as the relative difference between the means of "control" MA lines (those with the highest viability in the later assays, inferred to carry few or no mutations) and quasinormal MA lines (those with at least one-half the viability of the "controls", thus, excluding lethal and highly deleterious mutations). The average value of ΔM for the entire genome (1.1 percent) did not modify classical estimates substantially. However, the mutational nature of the viability difference between "control" and "quasinormal" MA lines is not clear, at least for Mukai's 1964–9 data, and the validity of the ΔM estimate relies on the arbitrary choice of an initial MA period, where viability is assumed to decline linearly. For example, using Mukai's (1969) data, the quadratic regression of viability on time suggests that the initial viability decay was five times smaller than previously calculated (García-Dorado and Caballero 2002).

Estimates of mutation rates for fitness have been obtained by Houle *et al.* (1992) and by Ávila

and García-Dorado (2002). In Houle's experiment (Houle *et al.* 1994) the control was later shown to be contaminated, but MD estimates were nevertheless obtained (see García-Dorado *et al.* 1999). Both experiments gave very similar estimates (see Table 3.1).

Arabidopsis thaliana

Schultz *et al.* (1999) and Shaw *et al.* (2000) reported results from 924 or 40 MA lines maintained by single-seed descent and derived from a single inbred founder, after 10 or 17 generations of MA, respectively. A control obtained from generation 0 stored seeds was assayed synchronously to the MA lines. In both experiments, plants were grown in benign conditions and a number of reproductive and quantitative traits were scored. For all traits and experiments, the mutational decay was very weak and generally non significant. Significant between-line variances were detected by Shaw *et al.* (2000), but none of those calculated by Schultz *et al.* (1999) significantly departed from zero.

At face value, the BM estimates obtained by Shaw *et al.* (2000) were $\lambda = 0.002$ and $E(s) = 0.47$ averaging over traits (mean number of seeds per fruit, number of fruits per plant, and dry mass of infructescence). A later analysis using Markov Chain Monte Carlo Maximum Likelihood gave values of $\lambda = 0.06$ and 0.10 for fruit and seed number, respectively (R.H. Shaw *et al.* 2002). However, the distribution of mutational effects indicated an approximate equal number of positive and negative mutational effects. The behavior of these traits, therefore, is not typical of a fitness component. As only deleterious mutations are considered in this review, these estimates are not included in Table 3.1.

For a measure of fitness, Schultz *et al.* (1999) provided BM estimates $\lambda = 0.05$ and $E(s) = 0.23$. However, these values were calculated as the average of estimates obtained in a set of bootstrapped samples. While bootstrapping is very useful to compute errors of estimates, the estimates themselves must be calculated from the original sample. This can be done using Tables 3.1 and 3.2 in Schultz *et al.* (1999), resulting in estimates of $\lambda = 0.015$ and $E(s) = 0.36$.

Triticum durum

Preliminary results have been reported by Bataillon *et al.* (2000). Starting from a homozygous base population, 135 MA lines were derived and subsequently maintained through enforced selfing. After seven generations, an experiment was performed where the total number of seeds per plant was scored both in the MA lines and in 60 control lines (from generation 0 seeds). ML estimates of mutational parameters (Table 3.1) were compatible with the *A. thaliana* and later *D. melanogaster* results mentioned above. However, the **polyploid** condition of *T. durum* casts doubts on the validity of a comparison with the mutation rate of diploid species, as the masking effect of mutations by different alleles in a polyploid species may lead to a larger mutation rate.

Daphnia pulex

In the MA experiment by Lynch *et al.* (1998), BM estimates ranged from $\lambda > 0.8$, for mutations reducing viability, to $\lambda > 0.25$, for mutations increasing 3rd clutch size. However, the frozen control was only evaluated at generations 7 and 16 (it was later disregarded on the basis of its poor performance), and the lack of significant differences between control means at these generations does not imply environmental stability over the whole experiment (32 generations). Furthermore, the originally collected water (recycled in a diatomaceous earth filter and used throughout the experiment) could have undergone important changes, and the *Scenedesmus* culture used to feed the MA lines might have evolved in the laboratory. These circumstances could have affected the expression of fitness components in *Daphnia*, which is known to be a very sensitive organism, and could have reduced the final performance of the MA lines and the disregarded control. Therefore, these data should be taken with extreme caution and are not included in Table 3.1.

Caenorhabditis elegans

Results from two MA experiments have been reported (Keightley and Caballero 1997; Vassilieva

and Lynch 1999) and they have been reanalyzed by Keightley and Bataillon (2000), who also included estimates for intrinsic growth rate newly obtained from Keightley and Caballero's data. Across traits (lifetime productivity, longevity, and intrinsic growth rate), both experiments provided similar estimates of λ (BM: mean = 0.013, range = 0.001–0.031; ML: mean = 0.005, range = 0.003–0.011). Average estimates of $E(s)$ were more divergent (BM: mean = 0.10, range = 0.05–0.23; ML: mean = 0.32, range = 0.07–0.68). However, the experiments agree in showing low mutation rates and large average mutational effects.

More recently, results covering 214 generations of MA for the Vassilieva and Lynch experiment were reported (Vassilieva *et al.* 2000). Estimates of λ and $E(s)$ averaged over productivity and growth rate were comparable to the earlier ones (BM: $\lambda = 0.03$, $E(s) = 0.15$; ML: $\lambda = 0.02$, $E(s) = 0.16$). These results refer to an optimal temperature of 20 °C. In addition, a parallel assay at a stressful temperature (12 °C) was performed, resulting in BM λ estimates sevenfold lower and $E(s)$ estimates fivefold higher.

Saccharomyces cerevisiae

Zeyl and DeVisser (2001) have studied the accumulation of spontaneous nuclear mutations affecting competitive fitness. Data pertain to 50 MA lines, all derived from a homogeneous wild type base, propagated by the transfer of colonies established from single cells, and maintained in a rich medium. Competitive fitness relative to a marked strain, otherwise identical to the base strain, was obtained after 36 transfers (*ca* 600 generations). After excluding those MA lines carrying mitochondrial deleterious mutations, no overall fitness decline was detected, thus precluding BM estimates. However, ML estimates were computed, resulting in a very low λ value (*ca* 10^{-4}) and a large heterozygous mutational effect $E(sh)$ (*ca* 0.22). The experimental procedure used casts some doubts on the validity of those estimates, as transfers were performed every two days (*ca* 16 generations) and, thus, selection could have acted during these periods of colony growth, eliminating detrimental mutations.

Wloch *et al.* (2001) obtained direct estimates of mutational rates and effects by analyzing **tetrads** in a large number of yeast colonies. Basically, deleterious mutations were identified when the four meiotic products show a segregation pattern 2:2 between wild type haploids and those with a reduced growth rate. The estimates are in agreement with the previous ones, and the extrapolation to *Drosophila*, correcting by the number of genes and that of cell divisions, gives $\lambda = 0.04$ (Wloch *et al.* 2001), in agreement with the indirect fitness estimates for this species (Table 3.1).

Indirect estimates from artificial mutagenesis

Artificial mutagenesis has been used as a shortcut to long spontaneous MA experiments. The method requires calibrating the number of MA generations equivalent to some mutagenic treatment, under the assumption that the distribution of effects of induced and spontaneous mutations are similar. For example, in *D. melanogaster* the number of MA generations “equivalent” to a given dosage of the mutagenic agent ethyl methanesulfonate (EMS) can be calibrated as that giving the same frequency of accumulated recessive lethals, or the same increase in variance for some quantitative trait. However, the EMS treatment imposed by Ohnishi (1977a,b) produced as many recessive lethal mutations as 84 generations of spontaneous MA, and as much increase in variance for abdominal bristle number as 364 spontaneous MA generations. These contrasting figures show that EMS and spontaneous mutations are different regarding their relative effects on lethals and quantitative variation. This is not surprising, as EMS mostly induces C/G → A/T transitions, while an important amount of spontaneous mutation in *D. melanogaster* is due to **transposition** events (see Fontdevila, Chapter 16). Even if EMS and spontaneous mutations were similar regarding deleterious effects, it is unclear which of the above calibrations apply, if any. Thus, the EMS-induced viability decline in Ohnishi’s data suggests a spontaneous $\Delta M = 0.21$ or 0.05 percent, depending on the calibration chosen. Similarly, the viability decline in the EMS *D. melanogaster* experiment by Yang *et al.* (2001a) gives $\Delta M = 1.3$ percent

using the lethal frequency to calibrate the number of MA generations equivalent to the EMS dosage used, and $\Delta M = 0.13$ or 0.3 percent using the rate of increase in variance for bristle traits (0.13 percent using the average increase in bristle variance found by Yang *et al.* (2001a) in the EMS experiment and the MA estimates reported by Houle *et al.* (1996); 0.3 percent using the calibration obtained from Ohnishi’s experiment). Interestingly, these EMS induced deleterious mutations did not show detectable **epistasis** or increased effect under harsh environmental conditions.

Coefficient of dominance of spontaneous mutations

As each new mutation appears only in heterozygous condition for some time, and natural selection generally maintains deleterious mutations at low frequencies, the mutational impact on fitness depends on the fraction of their effects that is expressed in heterozygosis, that is, on their degree of dominance. If the estimation of the homozygous effects of mutations is very demanding, that of heterozygous effects is even harder, mainly due to statistical biases and the low resolution of experimental designs.

Estimates of $E(h)$ can be obtained from analyses of natural populations (see García-Dorado *et al.* 1999), but these estimates assume that allele frequencies are at mutation–selection balance and, therefore, are highly prone to several sources of bias. For this reason, this type of estimates will not be considered in the present review. Direct estimates from MA experiments are more reliable, but they are not free of shortcomings, as will be shown below. In the following, we will confine ourselves to estimates for mutations with small effects on fitness (i.e. excluding lethal or highly detrimental mutations) because they are the most interesting from an evolutionary viewpoint. Moreover, the class of mild to severe mutations corresponds to that detected in MA experiments (see below).

There are several procedures to estimate the average coefficient of dominance from the homozygous and heterozygous effects of MA lines. In the early analyses made in the 1960s and 1970s, the ratio of

the observed reductions in mean viability in heterozygotes and homozygotes was used (Mukai and Yamazaki 1968). This provides an estimate of the average of h values of mutations weighted by their corresponding selection coefficients. The main disadvantage of this method is that, if the reductions in viability are not only due to mutation but also to other genetic or environmental factors, the estimates obtained are biased upwards. Thus, the available evidence suggests that the estimates of $E(h)$ obtained by Ohnishi (1977c) by this method are biased (see García-Dorado and Caballero 2000). An alternative way is based on the regression of heterozygous on homozygous viabilities. This method is not affected by the bias mentioned, but gives estimates of the average h values of mutations weighted by their squared selection coefficients. Although mutations with larger effects tend to have lower values of h , the method gives roughly unbiased estimates of $E(h)$ for subsets of mutations with small deleterious effects. Therefore, only if MA lines with slight reductions in fitness (quasinormal lines) are used, the bias incurred can be assumed to be small. In what follows, only regression estimates for quasinormal MA lines are considered, unless otherwise indicated.

Estimates of $E(h)$ obtained from MA experiments are summarized in Table 3.2. In the initial experiments carried out by Mukai and coworkers in the 1960s, the viability of homozygotes and heterozygotes for chromosomes that had accumulated mutations during some period of time were compared. Mukai made a distinction between two classes of heterozygotes: (1) “**repulsion heterozygotes**” (derived from crosses of two different MA lines, implying that different mutations accumulate on each homologous chromosome), and (2) “**coupling heterozygotes**” (derived from crosses between MA lines and either the “original” chromosome line or an independently sampled one, assumed to be free of mutations).

The results from these experiments were conflicting. Most estimates of $E(h)$ using coupling heterozygotes were negative or very low, implying overdominance at some loci or nearly complete recessive gene action (see Table 3.2). For the repulsion heterozygotes, however, the estimated $E(h)$

Table 3.2 Summary of regression estimates of the average coefficient of dominance $E(h)$ of deleterious mutations from MA experiments

References		$E(h) \pm \text{S.E.}$
<i>Viability in D. melanogaster</i>		
Mukai and coworkers ^a	Coupling heterozygotes (with “original” chromosome)	-0.17 ^b
Mukai and coworkers ^a	Coupling heterozygotes (with non-isogenic chromosomes)	0.11 ^c
Mukai and Yamazaki (1968)	Repulsion heterozygotes	0.40 ^d
Ohnishi (1977c)	Coupling heterozygotes (with “original” chromosomes)	0.10 ^e \pm 0.08
	Repulsion heterozygotes	0.03 ^e \pm 0.08
Chavarrías <i>et al.</i> (2001)	Coupling heterozygotes (with “control” chromosomes)	0.32 \pm 0.36
<i>Other life-history traits in D. melanogaster</i>		
Houle <i>et al.</i> (1997)	Early fecundity	-0.03 \pm 0.16
	Late fecundity	0.12 \pm 0.32
	Male longevity	0.37 \pm 0.60
	Female longevity	0.26 \pm 0.18
	Male mating ability	-0.07 \pm 0.68
<i>C. elegans</i>		
Vassilieva <i>et al.</i> (2000)	Productivity	0.64 \pm 0.18
	Survival to maturity	0.05 \pm 0.14
	Longevity	-0.10 \pm 0.28
	Intrinsic rate of increase	0.55 \pm 0.18
	Rate of convergence	0.48 \pm 0.19
	Mean age at reproduction	0.69 \pm 0.29

^a See references in Simmons and Crow (1977).

^b Average of five negative estimates.

^c Average of two estimates, 0.09 and 0.13, obtained in different genetic backgrounds.

^d Estimate obtained after excluding overdominant heterozygotes.

^e Regression estimates obtained by García-Dorado and Caballero (2000).

was 0.40. An additional complication was that a fraction of the repulsion heterozygotes (about one-fifth of the total) also indicated overdominance. For this fraction, one or both parental MA lines presented a high viability. This prompted Mukai and Yamazaki (1968) to interpret that these

heterozygotes were, in fact, coupling heterozygotes, and excluded them from the analysis (the estimate of 0.40 was obtained after that exclusion). The reason for the discrepancy between both types of heterozygotes is not evident, although García-Dorado and Caballero (2000) have discussed some plausible explanations. A possibility is that the same recurrent recessive mutations could have occurred in different chromosome lines. Thus, repulsion heterozygotes would be, in fact, homozygous for those mutations shared by both parental lines, while this would not occur in coupling heterozygotes. If this was the case, the large estimate obtained from the repulsion heterozygotes would be biased upwards.

In contrast to Mukai, Ohnishi (1977c) found similar results for coupling and repulsion heterozygotes. Both estimates were also close to 0.40, but there are reasons to believe that they are biased upwards. The estimation method used by Ohnishi was the ratio of viabilities, and a putative nonmutational decline in viability could have inflated the estimates to a large extent. The regression estimates obtained by García-Dorado and Caballero (2000) from the reanalysis of Ohnishi's data are free from such a bias, and indicate a low coefficient of dominance (Table 3.2). Out of 80 chromosomes assayed by Ohnishi (1977c) in heterozygous condition, 78 had homozygous viabilities larger than 0.85. Therefore, the bias incurred in these estimates due to weighting by the squared selection coefficient should not be very large.

In a more recent experiment, chromosomes extracted from full-sib lines after 250 generations of MA were analyzed by a method similar to that of Mukai and Ohnishi (Chavarrías *et al.* 2001). The regression estimate of $E(h)$ was 0.32. Because only 93 MA lines survived out of the initial 200, it is possible that some selection occurred during the experiment, eliminating mutations of moderately large effect. Thus, considering the negative correlation between s and h , the above value is expected to overestimate $E(h)$ for new unselected mutations. Nevertheless, its large standard error precludes a clear-cut conclusion.

Houle *et al.* (1997) obtained regression estimates of $E(h)$ for different life-history traits in *D. melanogaster*,

indicating varying degrees of partial recessivity. Weighting the estimates of the different traits by their corresponding squared mutational coefficients of variation, the average estimate across traits is 0.05 (García-Dorado and Caballero 2000). However, this analysis differs from all others reviewed in that all nonlethal lines—not just quasinormals—were considered. The possibility of chromosomes carrying mutations of very substantial effect being involved in the analysis implies that the estimates of $E(h)$ for mild mutations can be biased downwards.

To date, the only set of $E(h)$ estimates from MA experiments in a species different from *D. melanogaster* is that recently obtained by Vassilieva *et al.* (2000) in *C. elegans* (Table 3.2). Those estimates were generally compatible with additive gene action ($E(h) = 0.5$), but their large standard errors do not allow rejecting partial recessivity.

Incorporating molecular information

The previous review of spontaneous mutational parameters for fitness (MD/ML/direct estimates in Table 3.1) indicates that the rate of mutation observed in MA experiments ranges from 0.0001 in *Saccharomyces* to 0.05 in *Triticum*. Likewise, the detected homozygous mutational effects range from 0.06 to 0.26. Despite the variation among species and traits, the classical view that there is a large rate of mutations ($\lambda > \sim 0.15$) with mild effect ($s < \sim 0.03$) is obviously questionable.

MA experiments have limited power. Regarding ΔM , the more precise estimates should be those obtained from the longer MA experiments which, for eukaryotic organisms, are those of Vassilieva *et al.* (2000) in *C. elegans* and Chavarrías *et al.* (2001) in *D. melanogaster*, run for 214 and 255 MA generations, respectively. BM, ML, and MD analyses indicate that the pertinent mutational decays ($\Delta M = 0.0015 \pm 0.0003$ for intrinsic fitness and $\Delta M = 0.0023 \pm 0.0004$ for viability) should be mainly ascribed to moderate to severe deleterious mutations, although mutations with tiny deleterious effects making no significant contribution to the fitness decline could pass undetected. From the standard errors involved in the above experiments, an additional undetected rate of decline should be

smaller than $ca 5 \times 10^{-4}$. If we assume a high rate for those "tiny" mutations (say $\lambda_{\text{tiny}} > 0.5$), the corresponding deleterious effects should be very low ($E(s) < 10^{-3}$). The contribution of such mutations to the mutational load can be relevant in the very long term, and it has been argued that they could be responsible for the evolution of sex (see below).

The available experimental evidence concerning the rate and distribution of deleterious mutations is inconclusive, particularly when "tiny" effects are considered. However, given the evolutionary relevance of this issue, we will try to draw some inferences from the joint consideration of published molecular and MA evidences in *C. elegans*, and we will tentatively extrapolate the conclusions to other organisms.

Deleterious mutations undetected in *Caenorhabditis* MA experiments

For *C. elegans*, 32 percent of all point mutations are **constrained mutations**, that is, they are subject to selection pressure (Shabalina and Kondrashov 1999). Furthermore, about 19 percent of all point mutations cause amino acid substitutions, and 90 percent of these changes are evolutionary constrained (Davies *et al.* 1999). Thus, $0.19 \times 0.90 = 17$ percent of all point mutations cause amino acid constrained changes, so that about half of the constrained mutations occur in noncoding DNA. In addition, for this species, the product of the haploid genomic size (8×10^7 bp) by the nucleotide mutation rate per generation (2×10^{-9}) gives a total rate of point mutation per haploid genome and generation equal to 0.16 (Drake *et al.* 1998). Therefore, the rate of amino acid mutation is $\mu_a \approx 0.16 \times 0.19 \approx 0.0304$, that of constrained amino acid mutation $\lambda_a \approx 0.16 \times 0.17 = 0.027$, and that of constrained point mutation $\lambda_p \approx 0.16 \times 0.32 = 0.051$.

Since ΔM in MA experiments seems to be due to moderate or severe deleterious effects, we assume that in *C. elegans* it is mostly due to amino acidic mutations. Considering that the rate of deleterious mutation detected in *C. elegans* MA experiments is 0.0066, we can infer the fraction of amino acid mutations for the following three classes (Table 3.3): (1) "mild to severe," the only ones detected in

Table 3.3 Classification of amino acid mutations (μ_a) in *Caenorhabditis elegans*

Class	Evolutionary effect	MA experiments	% of μ_a	Range of effects
"Neutral"	Unconstrained	Undetected	10	$s < 5 \times 10^{-7}$
"Tiny"	Constrained	Undetected	67	$5 \times 10^{-7} < s < 5 \times 10^{-4}$
"Mild to severe"	Constrained	Detected	23	$5 \times 10^{-4} < s$

MA experiments, representing the $0.0066/0.027 = 25$ percent of the constrained amino acid mutations and, therefore, the $0.25 \times 0.9 = 23$ percent of the total rate of amino acid mutation; (2) "tiny," representing the remaining 75 percent of the constrained amino acid mutations and, therefore, a $0.9 \times 0.75 \approx 67$ percent of the total rate of amino acid mutation; (3) "neutral," embracing the remaining 10 percent of amino acid mutations.

Using EMS, Davies *et al.* (1999) induced an average of 45 constrained amino acid mutations per line, equivalent to about 1700 generations of spontaneous MA, but only 3.6 (8 percent) of those mutations were detected in fitness laboratory assays (Keightley *et al.* 2000). This fraction is about one-third of that deduced above (23 percent; Table 3.3), and the difference cannot be ascribed to transposition, which was not active in the MA lines. A possible explanation for the discrepancy could be the loss of a fraction of moderate to severe deleterious mutations in the EMS lines, due to selection. The authors provide simulation results showing that at most three mutations could have been lost per line during the 10 selfing generations preceding the fitness assay, due to average plate mortality. However, using Vassilieva and coworkers' (2000) mutational estimates, the EMS treatment of Davies *et al.* (1999) (presumably equivalent to 1700 MA generations with multiplicative effects on fitness) is expected to reduce fitness average to only 5 percent of its original value. The proportion of plates surviving after the first generation of selfing was not given, but after the second generation it was about 60 percent that of the control, and raised afterwards. This suggests that **purifying selection** may

have been important immediately after the EMS treatment, causing a reduction in the number of fitness mutations to be detected in the laboratory assay.

Table 3.3 also shows a tentative range of selection coefficients for each class of mutations. Davies *et al.* (1999) showed that the deleterious effect of mutations undetected in MA experiments should be very small (about $s < 5 \times 10^{-4}$), so we use this lower bound to indicate the minimum effect of the detected mutations ("mild to severe") in *C. elegans*. From **diffusion theory**, the probability of **fixation** of an additive deleterious mutation with an effect $s = 5/N_e$ (where N_e is the long-term evolutionary size of the species) is 0.04 percent that of a neutral mutation. Thus, we take this arbitrary but conservative value as the lower bound for severely constrained mutations. Assuming that the N_e of *C. elegans* may be as large as 10^7 , we consider that mutations with $s < 5 \times 10^{-7}$ are effectively "neutral." Therefore, the effects of constrained mutations undetected in fitness assays ("tiny" mutations) may be in the range $5 \times 10^{-7} < s < 5 \times 10^{-4}$.

Inferences on the rate of mutations with "tiny" or "mild to severe" deleterious effects to other species

As explained above, there are grounds to consider a class of "mild to severe" deleterious mutations detected in *C. elegans* MA experiments, representing between 8 and 23 percent of amino acid mutations, and a class of undetected "tiny" deleterious mutations comprising at least 67 percent of amino acid mutations. We can tentatively extend this classification to other species, keeping in mind that the fraction of each class that would be selectively constrained in any given species will depend upon the corresponding effective population size.

As stated before, about half of the constrained point mutations in *C. elegans* are amino acidic ones and the proportion of amino acid mutations that are constrained is 90 percent. Thus, if μ_a is the total rate of amino acid mutations, $0.9 \times \mu_a$ are constrained amino acidic mutations and about the same amount are constrained non-amino acidic ones. Higher organisms often have larger proportions of noncoding DNA, but

we will assume that this is mostly due to an excess of nonfunctional DNA, so that the ratio of noncoding to coding deleterious mutations remains roughly similar. We will also assume that non-amino acidic point mutations usually have "tiny" deleterious effects and, therefore, we will include all of them into this class. Therefore, the inferred rate of point mutations with "mild to severe" deleterious effects is $\lambda_{p\text{m-s}} \approx 0.23 \mu_a$ (from Table 3.3), and that for "tiny" deleterious effects is about $\lambda_{p\text{tiny}} \approx (0.67 + 0.9) \times \mu_a \approx 1.57 \mu_a$ (the first term within brackets refers to the amino acidic mutations and the second one to non-amino acidic mutations).

The total (μ_a) and the constrained (λ_a) amino acidic mutation rates can be inferred from molecular data, and have been recently reviewed for different species by Keightley and Eyre-Walker (2000). The mutation rates for vertebrates should be corrected, however, as they were computed assuming 80 000 genes, instead of the currently accepted number (about 35 000). The corrected haploid rates are given in Table 3.4, averaged for different groups of organisms. The values for *C. elegans* are also included for completeness. As expected, the proportion of amino acid mutations that are constrained (λ_a/μ_a) increases as we go downward in the table, since the effective population size presumably also increases. Table 3.4 also gives the rates of point deleterious mutation for the "tiny" and "mild to severe" classes, obtained using the above inferences.

For *D. melanogaster* $\lambda_{p\text{m-s}} = 0.009$, a value slightly lower than that obtained from recent MA experiments and MD estimates discussed in the previous sections (see Table 3.1). The difference can be ascribed to transposition, which is very active in this organism, up to 0.1 insertions occurring per gamete and generation (Maside *et al.* 2000). Insertions in coding sequences, which represent 10 percent of the *Drosophila* genome, could account for an important fraction of the rate of recessive lethals (about 0.015, Ohnishi 1977b) and other mutations with severe effects. Thus, most loss-of-function mutations detected for eye color loci have been found to be due to insertion or **deletion** events (Yang *et al.* 2001b). However, transposition events at noncoding sequences often have only "tiny"

Table 3.4 Total (μ_a) and constrained (λ_a) haploid rate of amino acid mutation estimated for different species, computed from Keightley and Eyre-Walker (2000) and Shabalina and Kondrashov (1999) (see text for explanation)

Species	μ_a	λ_a	λ_a/μ_a	$\lambda_{p \text{ tiny}}^a$ ($5 \times 10^{-7} < s < 5 \times 10^{-4}$)	$\lambda_{p \text{ m-s}}^a$ ($s > 5 \times 10^{-4}$)
Primates	1.061	0.536	0.52	1.67	0.244
Other mammals ^b	0.394	0.273	0.68	0.62	0.091
Mouse/Rat	0.131	0.107	0.82	0.21	0.030
Chicken/ Old world quail	0.129	0.109	0.85	0.20	0.030
<i>D. melanogaster</i>	0.040	0.033	0.84	0.06	0.009
<i>C. elegans</i>	0.030	0.027	0.90	0.05	0.007

^a Speculative inference of the haploid rate of point mutation (λ_p) for different ranges of deleterious effects using *Caenorhabditis* estimates.

^b Sheep, cow, cat, and dog.

deleterious effects, and the frequency of those with “mild to severe” effects is unknown.

For humans, the effective evolutionary size is in the order of 10^4 (Zhao *et al.* 2000), so that the “mild to severe” class ($s > 5 \times 10^{-4}$) will be included into the class of evolutionarily constrained mutations (conservatively, those with $s > 5/N_e = 5 \times 10^{-4}$). Accordingly, the fraction of amino acid mutations with mild to severe effects inferred for *C. elegans* (23 percent, Table 3.3) is somewhat smaller than the fraction of amino acidic mutations that are constrained (48 percent averaged for the human/chimpanzee genomes; Keightley and Eyre-Walker 2000). This suggests that the distribution of mutational effects could be roughly similar across taxa, and gives some support to the extrapolation of *C. elegans* results to other organisms. Thus, assuming $\mu_a = 1.38$ (obtained from Keightley and Eyre-Walker 2000 considering 35 000 genes) we obtain $\lambda_a = 1.38 \times 0.48 = 0.66$, and $\lambda_{p \text{ m-s}} = 1.38 \times 0.23 = 0.32$.

Evolutionary inferences

Previous sections have provided a description of the rate and effects of deleterious mutations, characterized by an increase of the haploid rate of the species with generation length (or the number of germ cell divisions). The values range from about

0.05 in *C. elegans* to about 1.8 in primates, with most mutations (c.85 percent) having only “tiny” deleterious effects ($s < 5 \times 10^{-4}$) (see Table 3.4). Although mildly deleterious mutations could have been frequent in some *Drosophila* experiments (possibly due to transposition events), they do not seem to be particularly common in most cases. The scenario for *Drosophila* and *Caenorhabditis* appears to be that of a low rate of deleterious mutations with “mild to severe” effects, whose average is about 0.2. The rate for higher organisms is unknown, but could be relatively high for primates (up to about 0.25) and other large mammals (up to about 0.1). The rate of deleterious mutation due to insertions and deletions should be added in order to compute mutational loads in sexual species. That rate is supposed to be small for most taxa, but may amount to 0.1 per gamete in *Drosophila*. Although the homozygous deleterious effects of “mild to severe” mutations is on the average high, the degree of dominance seems to be inversely related to the magnitude of the effect, so that their impact will generally be low in large populations.

In what follows we will discuss some of the more direct evolutionary consequences of this rough description. Unpublished diffusion predictions will be mentioned; these are based on a mean coefficient of dominance of $E(h) = 0.2$ and the distribution of deleterious effects (MD estimates) for *Drosophila*

quoted above (García-Dorado 2003), adjusted for the λ_{m-s} inferred for the species considered (Table 3.4).

Evolution of sex

The role of deleterious mutations on the evolution of sex has prompted scores of printed pages (and continues to do so). Here, we discuss the more direct implications of our inferences for one popular hypothesis for the evolution of sex.

It has been shown that if, on average, more than one deleterious mutation occurs per genome and generation ($\lambda > 0.5$) and there is **synergistic epistasis**, sex could increase the rate at which the population gets rid of the mutational load, up to the point of compensating, in the long-term, for the twofold cost of sexual anisogamic reproduction (see Serra *et al.*, Chapter 12). This has been called the **mutational deterministic hypothesis** of sex evolution. However, Table 3.4 shows that the total rate of point mutation ($\lambda_{p \text{ tiny}} + \lambda_{p \text{ m-s}}$) is below 0.25 for groups of organisms with anisogamic sexual reproduction. Thus, the overall rate of deleterious mutation per gamete is below 0.5, even after allowing a comfortable margin for non-point deleterious mutations. Thus, the deterministic mutational hypothesis cannot be invoked as a general explanation for the evolutionary advantage of anisogamic sex (Keightley and Eyre-Walker 2000; Yang *et al.* 2001b).

Furthermore, even for large mammals, the rate of “mild to severe” deleterious mutation is below 0.5. Thus, the possible advantage of sex should be ascribed to “tiny” mutations. Theoretically, in sexual populations, these “tiny” mutations could put asexual **clones** to disadvantage, but it would take an extremely long time before such disadvantage is worse than the twofold cost of sexual reproduction, and it is likely that the asexual clones will invade the sexual population before this happens.

The conditions for the evolution of obligate versus facultative sex are even more restrictive, because sexual and asexual gene pools are mixed and there is individual within-population selection. If this is the case, the advantage of obligate sex requires large rates of genome degradation that, in turn, depend both on mutation rates and on deleterious

effects. Therefore, the available data on deleterious mutation suggest that the deterministic mutation hypothesis cannot be held as a general cause of the evolution of anisogamy and obligate sexual reproduction.

Mutational load in large populations

Another question of interest, both from the evolutionary and the conservationist point of view, is the impact of deleterious mutations on population fitness. The mutational load of an infinite equilibrium population due to segregating deleterious mutations is defined as $L_s = [W_{\max} - E(W)]/W_{\max}$ (where W_{\max} is the expected fitness of a genotype carrying none of the deleterious mutations segregating in the population, and $E(W)$ is the mean population fitness). It is well known that L_s depends on the deleterious mutation rate (λ). Assuming additive gene action within loci, $L_s = 1 - \exp[-2\lambda]$ under a between loci multiplicative fitness model, and $L_s = 2\lambda/(1 + 2\lambda)$ under an additive one.

Since the mutational load does not depend on the magnitude of the deleterious effects, the high rate of constrained mutations found in some organisms (like humans, where L_s could be as high as 0.97), raises concern about their future survival. However, this measure of load represents the population fitness loss relative to a hypothetical individual that, in populations with large deleterious mutation rates, does not exist. This assertion qualitatively holds for large finite populations. For example, with $N_e = 10^4$ and mutations with effect 5×10^{-4} occurring at a rate $\lambda = 1$, the mutational load is $L_s \approx 0.7$, but the probability of the optimum genotype (G_{opt} with W_{\max}) is virtually zero ($P[G_{\text{opt}}] = \exp[-9.000]$). Thus, the relevance of this measurement of load is obscure. It has a clearer meaning for bottlenecked populations that have attained a new equilibrium after subsequent expansion, so that G_{opt} is a genotype actually present after the bottleneck. However, if the population expanded to a large N_e , the new equilibrium would only be attained after a very long time, so that positive selection on new favorable mutations cannot be ignored. On the contrary, if the population expanded to a relatively low N_e , the threat from continuous random fixation of deleterious mutations,

due to **drift**, would, in the long term, exceed that ascribable to the segregating ones.

Probably, the main source of potential danger for the survival of large populations is the load concealed in the heterozygous condition, due to the partially recessive effects of segregating deleterious genes. Paradoxically, this concealed load is greater in large populations, where many deleterious recessives can segregate at low frequency. When homozygosis increases, after a bottleneck or a sudden subdivision of the population, that threat will appear under the form of inbreeding depression. It has often been argued that the high rate of inbreeding depression (d) observed in *Drosophila* could be explained only if deleterious mutations were very common. However, this prediction is based on estimates of the degree of dominance obtained under the assumption of high rates of mildly deleterious mutation, which produces $E(h) \approx 0.4$. Thus, the argument is flawed by circularity. On the contrary, high rates of inbreeding depression are also predicted with low deleterious mutation rates and appropriate levels of recessivity for “moderate to severe” effects ($E(h) \approx 0.2$; see Table 3.2). For example, using *D. melanogaster* parameter values ($\lambda = 0.03$, with $E(s) = 0.22$ and $E(h) = 0.2$), the expected rate of inbreeding depression due to non-lethal mutations in an equilibrium population with $N_e = 10^5$, is about $d = 0.8$ percent decline in fitness per 1 percent increase in inbreeding.

Using the above rates of “mild to severe” mutation, inferred for different species by extrapolating the *C. elegans* estimates (Table 3.4) and the *D. melanogaster* distribution of deleterious effects, we can obtain diffusion predictions for the rate of inbreeding depression. For humans, assuming $N_e = 10^4$ and $\lambda_{p-m-s} = 0.32$, we obtain $d = 5.9$. For other large mammals $d = 2.4$, and for rodents and birds $d = 0.8$ (assuming $N_e = 10^5$ in both cases). Thus, in the offspring of full sibs, multiplicative fitness would be reduced to about 23, 55, or 82 percent that of their parents, respectively. These estimates of inbreeding depression are in rough agreement with estimates of the number of **lethal equivalents** (a measure of the rate of inbreeding depression for viability or other fitness components), which is in the range of 1–4 per gamete (see table 10.4 in Lynch and Walsh 1997). Nevertheless, the actual inbreeding

depression can be larger than the diffusion prediction if nonadditive variance for fitness is being maintained by mechanisms different from the mutation–selection balance.

Mutational meltdown in small populations

In small populations, the most dangerous mutation load is that arising from fixation of deleterious mutations through drift. Essentially, this refers to mutations with $s < 1/N_e$ and, therefore, “moderate to severe” deleterious mutations rarely contribute to the fixation load, except in very small populations, where nonmutational causes of extinction can be more pressing. Mildly deleterious mutations are potentially dangerous for $N_e < 100$, inducing the so-called **mutational meltdown** (Lynch *et al.* 1995), that is, an accumulation of fitness-reducing mutations that lowers the population size, thereby further increasing their probability of fixation. This synergistic effect of mutation and drift may lead to population extinction. However, the process will only be effective in the very long term, unless the rate of mutations with mild effect is extremely large. For example, the fixation of mildly deleterious mutations occurring at a rate $\lambda = 0.5$, with $E(s) = 0.05$, would produce a rate of fitness decline of 5×10^{-4} in populations of $N_e = 100$, but the decline would be two orders of magnitude lower using the rates given in Table 3.1.

It should be noted that many “tiny” deleterious mutations, constrained in very large populations, could eventually become fixed after an apparently irrelevant reduction of N_e . The habitat of many species has been recently restricted and fragmented, so that their N_e could have been reduced by two orders of magnitude, say from 10^5 to 10^3 . Thus, many mutations with $5 \times 10^{-5} < s < 5 \times 10^{-3}$ are no longer constrained and will accumulate, resulting in some fitness decline. At first, the frequency of segregating mutations increases but, after a very long time, a new equilibrium will be reached, characterized by a higher rate of deleterious fixation. The expected fitness decline cannot be predicted, since the rate for those mutations is unknown. For higher organisms, with long

generation intervals, it could be of the order of 10^{-4} , but it could also be much smaller. In the short term, the relevance of this decline is negligible. From an evolutionary viewpoint, however, those mutations make up a new "very slight deleterious" class (Kondrashov 1995), that could reduce the expected evolutionary life of species with moderate N_e . Thus, it is important to know the actual rate and effect distribution of this class of mutations, and whether selection on new favorable mutations (Poon and Otto 2000) or synergistic effects between deleterious ones (Kondrashov 1995) could compensate for the slow rate of decline it causes. A clue can be found in the human genome. Since the human long-term N_e is only of the order of 10^4 and the fraction of constrained mutations is considerably smaller than in other taxa, an important fraction of the load could have already accumulated. However, humans still show a high fitness level, supporting important demographic expansion in the harsh environments of third-world countries.

A different question is the future mutational impact on human populations (Crow 1999). Large rates of moderately deleterious mutation could have been efficiently purged in the past. However, mutation rates may have raised due to mutagenic agents and to increased parental age, and the selection coefficient against many deleterious mutations has recently been dramatically reduced due to beneficial environmental changes. Although even "tiny" deleterious effects can be constrained due to the large current population size, "tiny to mild" deleterious mutations could segregate for some time before being lost, causing fitness impairment.

Summary

Current estimates of genomic rates of deleterious mutation, mean mutational effects and dominance coefficients are reviewed for a variety of fitness traits and species. The experimental evidence suggests that the rate of appearance of deleterious mutations detected in experiments is in the order of up to 0.1 per zygote per generation and, therefore, an order of magnitude smaller than classical *Drosophila* estimates obtained in the 1960s and 1970s. The average effect of detected deleterious mutations is in the order of 0.1 or greater, and it is concluded that the class of mutations with effects in the range 0.01–0.05 is not as common as previously indicated. Recent evidence also suggests that the coefficient of dominance of new mutations (*ca* 0.2 on average) is smaller than previously thought. Combining several pieces of information from *Caenorhabditis* experiments, we tentatively infer the rate of mutation for different deleterious classes and discuss the main evolutionary consequences of these inferences.

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