

Properties of spontaneous mutations affecting quantitative traits

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Summary

Recent mutation accumulation results from invertebrate species suggest that mild deleterious mutation is far less frequent than previously thought, implying smaller expressed mutational loads. Although the rate (λ) and effect (s) of very slight deleterious mutation remain unknown, most mutational fitness decline would come from moderately deleterious mutation ($s \approx 0.2$, $\lambda \approx 0.03$), and this situation would not qualitatively change in harsh environments. Estimates of the average coefficient of dominance (\bar{h}) of non-severe deleterious mutations are controversial. The typical value of $\bar{h} = 0.4$ can be questioned, and a lower estimate (about 0.1) is suggested. Estimated mutational parameters are remarkably alike for morphological and fitness component traits (excluding lethals), indicating low mutation rates and moderate mutational effects, with a distribution generally showing strong negative asymmetry and little leptokurtosis. New mutations showed considerable genotype–environment interaction. However, the mutational variance of fitness-component traits due to non-severe detrimental mutations did not increase with environmental harshness. For morphological traits, a class of predominantly additive mutations with no detectable effect on fitness and relatively small effect on the trait was identified. This should be close to that responsible for standing variation in natural populations.

1. Introduction

Mutation continuously introduces detrimental variation threatening a population's adaptive level. However, it is the ultimate source of polygenic variation and, thus, the raw material for evolution and for genetic improvement of domestic plants and animals. In this review we summarize the main properties of spontaneous mutations affecting quantitative traits. Some of these properties have already been elucidated, but most are currently being debated.

2. Deleterious mutation

Different approaches have been attempted to estimate the properties of deleterious mutation. Here we will concentrate on direct estimates from mutation accumulation (MA) experiments, in which spontaneous

mutations are allowed to accumulate under relaxed selection in lines derived from the same uniform genetic background. MA experiments allow estimation of the per generation rate of decline for a fitness component due to mutation in the absence of selection, $\Delta M = \lambda E(s)$, and the rate of increase of the between-line variance, $\Delta V = \lambda E(s^2)$ (where λ is the gametic rate of mutation affecting the trait and s is the homozygotic deleterious effect of mutations). Thus, lower and upper bound estimates of λ and $E(s)$ can be computed (Bateman estimates: $\lambda \geq \Delta M^2 / \Delta V$, $E(s) \leq \Delta V / \Delta M$). In parallel, the information contained in the observed distribution of line means (\hat{f}_x) can be more efficiently used by assuming a convenient family distribution of mutant effects (gamma, reflected gamma or mixed normal gamma) and finding the mutational parameters that better account for \hat{f}_x . To do that, two different methodologies have been used: maximum likelihood (ML: Keightley, 1994, 1996, 1998) and minimum distance (MD: García-Dorado, 1997; García-Dorado & Marín, 1998). These estimates

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Table 1. *Properties of deleterious mutation for different species (whole genome results for relative viability or relative fitness). See text for explanation*

Species	Trait	Observed		Bateman bounds		MD (ML) estimates		
		$\Delta M\%$	$\Delta V \times 10^3$	λ	$E(s)$	λ	$E(s)$	$\Delta M\%$
<i>D. melanogaster</i>	Viability ^c	-1.01 ^a	0.23 ^a	0.44 ^a	0.02 ^a	0.011	0.19	-0.21
<i>D. melanogaster</i>	Viability ^d	-0.43 ^a	0.13 ^a	0.14 ^a	0.03 ^a	0.010	0.23	-0.23
<i>D. melanogaster</i>	Viability ^e	-0.57 ^a	0.85 ^a	0.04 ^a	0.15 ^a	0.022	0.16	-0.35
<i>D. melanogaster</i>	Viability ^f	-0.16 ^a	0.16 ^a	0.02 ^a	0.10 ^a	0.016	0.10	-0.17
<i>D. melanogaster</i>	Fitness ^g	-	2.07	-	-	0.030	0.26	-0.80
<i>E. coli</i>	Fitness ^h	-0.0002	24×10^{-6}	0.0002	0.01	-	-	-
<i>C. elegans</i>	Fitness ^{i,k}	-0.043	0.014	0.013	0.05	0.0034 ^b	0.10 ^b	-0.034 ^b
<i>C. elegans</i>	Fitness ^{j,k}	-0.168	0.353	0.008	0.21	0.0084 ^b	0.22 ^b	-0.185 ^b

^a Lethals and severely deleterious excluded. ^b ML estimates. ^c Mukai *et al.* (1972). ^d Ohnishi (1977). ^e Fry *et al.* (1999). ^f Fernández & López-Fanjul (1996). ^g Houle *et al.* (1992). ^h Kibota & Lynch (1996). ⁱ Keightley & Caballero (1997). ^j Vassilieva & Lynch (1999). ^k Measured as relative intrinsic growth rate.

depend on the family distribution assumed for s , but MD estimates are expected to be more robust if the true distribution of s does not belong to the family assumed (Donoho & Liu, 1988). Furthermore, MD estimates unconstrained by the observed change of the mean of the MA lines can be obtained. This is an interesting property as the validity of the observed ΔM has often been questioned (see below).

(i) *Drosophila*

For *D. melanogaster*, Bateman bound estimates obtained in different MA experiments are given in Table 1, together with the corresponding MD estimates (chromosome II, adjusted by a factor of 2.5 for extension to the whole genome). To obtain more informative Bateman bounds, observed ΔM and ΔV were computed excluding lines carrying lethals or severely deleterious (outlier MA lines with relative viability below 2/3).

The first results came from Mukai's experiments (Mukai, 1964; Mukai *et al.*, 1972), in which homozygotic viability was assayed by reference to a *Cy* marked chromosome. Lethal mutations occurred at a rate $\lambda_L = 0.015$ per gamete and generation, and Bateman bounds implied a high rate of mild deleterious mutation, its impact on population survival raising general concern.

Later results from an analogous experiment (Ohnishi, 1977) gave similar ΔV and a somewhat smaller ΔM , the corresponding Bateman bounds being interpreted as supporting Mukai's previous conclusions. However, most of the viability decline occurred during the first half of the experiment (generations 0–20: $\Delta M = -0.8\%$, generations 20–40: $\Delta M = -0.1\%$; significantly different with $P < 0.001$; Ohnishi, 1974). Therefore, different Bateman bounds are obtained in each period (generations 0–20: $\lambda \geq$

0.37 and $E(s) \leq 0.021$; generations 20–40: $\lambda \geq 0.01$ and $E(s) \leq 0.102$). However, both ΔV and the lethal mutation rate ($\lambda_L = 0.011$) were remarkably constant throughout the experiment, suggesting a temporally uniform mutational process. Thus, a fraction of the viability decline observed during the early generations of Ohnishi's experiment could be non-mutational (Keightley, 1996; García-Dorado, 1997), and could be attributed to an initial increase in the viability of the *Cy* reference chromosome through variation introduced by gene conversion or double crossing-over (Tachida *et al.*, 1989). This phenomenon may also apply to Mukai's experiments. A rank order method was then used to make up for the lack of a control, but this procedure is very sensitive to the number of lines, assumed to be deleterious-free, chosen each generation.

Recently, Mukai's experimental design has been improved by including a parallel control whose viability remained constant (Fry *et al.*, 1999). Thus, any important increase in *Cy* chromosome viability can be ruled out in this case. Although the viability decline of Fry's MA lines was not much smaller than Mukai's, the observed larger increase in variance suggests a lower λ and a greater $E(s)$. Thus, it seems that mild deleterious mutations occurred much more frequently in Mukai's than in Fry's lines. This is at odds with the higher rates of lethal ($\lambda_L = 0.25$) and of moderately deleterious mutations (notice the larger ΔV value) found in Fry's lines. Fry *et al.* suggest that Mukai's ΔM estimates could be biased if the experimenter's ability to detect cryptic *Cy* phenotypes had improved during the experiment. Thus, the results of Fry *et al.* would be consistent with estimates from the second half of Ohnishi's experiment, the somewhat larger λ and λ_L values being ascribable to differences in genetic background, perhaps related to a larger rate of transposition.

A different MA design (Fernández & López-Fanjul, 1996) allows mutations to accumulate in highly inbred lines of *D. melanogaster* derived from a single isogenic stock, which was also maintained as a control with large effective size ($N_e > 100$). Although the ΔV was comparable to Mukai's and Ohnishi's, the relative viability decline, estimated by comparison with the control, was lower (severely deleterious excluded). The latter could be underestimated if the control viability had also declined due to mutation accumulation. However, unpublished diffusion results show that, even with the large rates of mild deleterious mutation estimated by Mukai, no relevant viability decline is expected for the control population. In fact, the absolute viability of the control scarcely showed any decline (Caballero & Keightley, 1998). This experiment gives Bateman estimates in agreement with Fry's and later Ohnishi's results.

A recurrent obstacle in the analysis of MA data is the lack of a suitable control allowing unbiased estimates of ΔM . This impediment has been obviated by computing, for all non-lethal lines, MD estimates of mutational parameters unconstrained by the observed ΔM (García-Dorado, 1997; García-Dorado *et al.*, 1998; reflected gamma assumed for s). These are shown in Table 1 for competitive fitness (Houle *et al.*, 1992 MA data; the control was later shown to be contaminated, Houle *et al.*, 1994) and for viability (Mukai, Ohnishi and López-Fanjul data). We have also obtained MD estimates from Fry's MA data (generation 33 histogram). All estimates (adjusted for the whole genome when necessary) consistently suggest that: (1) deleterious mutation occurred at a low rate ($\lambda = 0.015$, average for viability; $\lambda = 0.03$ for fitness), the larger frequency corresponding to moderately deleterious mutation ($0.05 < s < 0.3$ with rate about 0.02); (2) the distribution of mutant effects had a relatively high mean and a not particularly large kurtosis. Thus, MD estimates predict $\Delta M \approx -0.24\%$ and $\Delta M \approx -0.8\%$ for viability and competitive fitness, respectively (all non-lethal lines).

The method, when unconstrained by the observed ΔM , may not detect mutations with such a low effect that they affect neither the shape nor the variance of the distribution of the means of the MA lines. Thus, additional undetected very slight deleterious mutations (VSD) may also be present, resulting in a non-gamma distribution. The viability decline observed by Fry, somewhat larger than the corresponding MD prediction, might be due to this class of mutations. Nevertheless, this difference was clearly non-significant and can also be ascribed to sampling. For López-Fanjul's data, observed and MD estimates of ΔM (-0.18% and -0.17% , respectively, all lines included) were very close, implying that a discontinuity class of VSD did not induce an appreciable viability decline. The above experiments differ in the level of

competitive harshness at which viability or fitness were assayed (high for Mukai, Houle and Fry data, low for Ohnishi and López-Fanjul data). Thus, MD estimates under harsh conditions do not suggest larger deleterious mutation rates, albeit larger effects were occasionally observed.

Finally, molecular information can be considered. The rate of single base pair mutation (SBPM) in *Drosophila* can be up to 1.5 per gamete and generation (Drake *et al.*, 1998) but the rate of constrained SBPM should be at most 0.1 (Kreitman, 1996; Zeng *et al.*, 1998). Since the evolutionary effective population size of *D. melanogaster* seems to be larger than 10^6 (Kreitman, 1996), the constrained value is equivalent to $s > 10^{-5}$ and, therefore, a distinction between severe, moderate, mild or VSD mutation rates cannot be made. In addition, spontaneous transpositions occur at high rate ($\lambda \approx 0.5$) with unknown deleterious effect. On the whole, molecular data could be made consistent with any of the different mutational estimates discussed above.

(ii) Other species

Results from MA experiments carried out in species other than *D. melanogaster* are also presented in Table 1 (frozen controls have been used in all cases). A small decline was estimated for *E. coli* non-competitive fitness (Kibota & Lynch, 1996), giving also very low λ and $E(s)$ Bateman bounds.

In *Caenorhabditis elegans*, Keightley & Caballero (1997) found a low rate of productivity decline, giving Bateman estimates $\lambda > 0.0013$ and $E(s) < 0.23$ (P. D. Keightley, personal communication), but no significant decline was detected for longevity. In the same species, Vassilieva & Lynch (1999) reported an increase in productivity (not significant), a considerable decline for longevity (giving $\lambda > 0.06$ and $E(s) < 0.05$) and a small significant decline for viability (giving $\lambda > 0.006$, $E(s) < 0.136$). Finally, the observed decline for non-competitive fitness, measured as the intrinsic growth rate, did not depart significantly from zero, but ML reanalysis of both *Caenorhabditis* experiments suggests $\lambda = 0.006$ and $E(s) = 0.16$, which predicts $\Delta M = -0.1\%$ (P. D. Keightley & T. Bataillon, personal communication; see Table 1).

The effect of MA on several fitness components has recently been studied in *Daphnia pulex* (Lynch *et al.*, 1998), ΔM values ranging from -2.06% (viability) to $+2.9\%$ (third clutch size). Thus, Bateman estimates ranged from $\lambda > 0.8$, for mutations reducing viability, to $\lambda > 0.25$, for mutations increasing third clutch size. The frozen control was evaluated only at generations 7 and 16, but was disregarded later on the basis of its poor performance. However, the lack of significant differences between control means at generations 7 and 16 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) may undergo important changes, and the *Scenodesmus* culture used to feed the MA lines might have evolved. These circumstances could have affected the expression of fitness components of *Daphnia*, which is known to be a very sensitive organism, and could have reduced the final performance of the disregarded control. Therefore, these data should be accepted with caution.

Putting together results from such diverse species is speculative, involving too many unknowns, but the exercise is worthwhile. This can be done by adjusting different fitness results so that they can be compared with those for *D. melanogaster*, using published information from Drake *et al.* (1998). The simplest case is that of *C. elegans*, since its effective genomic size (the size of the genomic fraction where deleterious mutations occur) as well as its rate of base mutation per effective genome and germ cell replication, are comparable to those of *Drosophila*. One generation of *D. melanogaster* or *C. elegans* takes 25 or 8 germ cell divisions, respectively. Thus, adjusting average ML estimates for fitness in *C. elegans* by a factor of 25/8 gives $\lambda = 0.018$ and $\Delta M = -0.3\%$. Considering that the *C. elegans* strain used in these experiments showed no transposition, these extrapolations are in good agreement with MD estimates for *D. melanogaster* fitness ($\lambda = 0.03$ and $\Delta M = -0.8\%$). Adjusting *E. coli* estimates should take account of its mutation rate per effective genome and germ cell division (about half that of *Drosophila*), as well as the number of germ cell divisions. Doubling the adjusted values to account for transposition gives $\lambda > 0.017$, comparable to MD estimates from *Drosophila*, while the rate of fitness decline ($\Delta M = -0.02\%$) remains too small. These adjusted $|\Delta M|$ and λ are smaller than Mukai's estimates.

For the human genome, the rate of constrained mutation (i.e. those with a deleterious effect larger than about $10/N_e$) has been estimated to be $\lambda \approx 1.6$ (Eyre-Walker & Keightley, 1999), raising concern about the effect of relaxed selection in humans or about any increase in the deleterious mutation rate (Crow, 1999). This estimate includes an unknown fraction of VSD mutations, which might contribute to the genetic load (Kondrashov, 1995) but do not necessarily produce a large mutational fitness decline. Considering that the number of germ cell divisions in human males is about 16 times that of *Drosophila* and that the rates of mutation per effective genome and germ cell division are similar, an adventurous adjustment of *Drosophila* estimates can be attempted. For competitive fitness this gives $\lambda \approx 0.5$, an important mutational decline that natural selection should constantly counterbalance (up to $\Delta M \approx -13\%$,

mostly due to moderate to severe deleterious mutation), and also a lethal mutation rate $\lambda_L \approx 0.25$. Since these estimates refer to non-VSD mutation, they are not in disagreement with the quoted rate for constrained mutation. However, the above adjustment is very speculative. For example, it disregards observations that few spontaneous mutations seem to be due to transposable elements or retroviruses in humans, or that increasing effective genomic size might be accompanied by enhanced genomic homeostasis, resulting in a smaller fraction of mutations being deleterious. Obviously, direct estimates of deleterious mutation for vertebrate species are needed.

3. Average dominance coefficient of spontaneous mutations

The coefficient of dominance of mutations ($h = 0, 0.5$ and 1 for recessive, additive and dominant gene action, and $h < 1$ or $h > 1$ for over- or underdominance) is an essential parameter for theoretical predictions in population and quantitative genetics. Yet the difficulties inherent in estimating this parameter, in terms of both statistical analysis and workload, have precluded the availability of clear-cut estimates. Several procedures have been used to estimate the average h of mutations, and these are discussed below.

(i) Direct estimates from MA experiments

The most direct way of investigating the dominance of mutations is through MA experiments. Most results refer to viability in *D. melanogaster*, and derive from the MA chromosome lines of Mukai (1964) and Ohnishi (1974). The main interest is in the dominance of mutations with small effect on viability, because there seems to be general agreement about the dominance coefficient of lethal mutants ($h = 0.01-0.03$; Simmons & Crow, 1977). Relevant results are therefore for chromosomes with viability greater than about 60%, called quasinormals. In one type of experiment the MA chromosome was paired with a chromosome supposedly carrying very few or no mutations. These so-called coupling heterozygotes are, therefore, assumed to carry in the same chromosome all mutations that arose during the experiment. The estimated average coefficient of dominance, \bar{h} , for the coupling heterozygotes in the studies of Mukai and co-workers was generally negative (about -0.2), indicating overdominance, or positive but close to zero (see Simmons & Crow, 1977). On the contrary, the results from Ohnishi's experiments were radically different, with an estimated value of 0.49.

In another type of experiment, 'repulsion heterozygotes' were made by pairing different MA chromosomes, the assumption being that mutations that

arose during the experiment were distributed along both chromosomes. For repulsion heterozygotes, Mukai's and Ohnishi's results were coincident, with an estimate around 0.4. Given the discrepancy between Mukai's and Ohnishi's results for the coupling heterozygotes, overdominance was taken as spurious, and the typical value which is generally admitted for the dominance of minor viability mutations is around 0.4. However, some arguments cast doubts on the validity of this widely accepted figure. The discrepant results for coupling and repulsion heterozygotes obtained by Mukai are still unresolved. Further, the estimate of about 0.4 from Mukai's repulsion heterozygotes was obtained after removing from the analysis about one-fifth of the heterozygotes on the basis that these showed overdominance (Mukai & Yamazaki, 1968; Mukai, 1969). This puts a question mark over Mukai's results.

The results from Ohnishi's lines seem to be consistent for coupling and repulsion heterozygotes. The basic equation used was

$$\bar{h} = (v_o - v_{ij}) / [(v_o - v_{ii}) + (v_o - v_{jj})], \quad (1)$$

where v_o is the homozygote viability of the control chromosome, v_{ij} is the mean viability of the heterozygote, and v_{ii} and v_{jj} are mean viabilities of the homozygotes. The average value obtained by (1) estimates the arithmetic mean, \bar{h} , weighted by the selection coefficient, s (Mukai, 1969). However, if part of the change in homozygous and heterozygous viabilities were due to environmental causes, as has recently been suggested (see Section 2(i)), this estimate would be biased towards 0.5.

This source of bias can be avoided by using the regression of heterozygous on homozygous viabilities,

$$\bar{h} = \text{cov}(v_{ii} + v_{jj}, v_{ij}) / \text{var}(v_{ii} + v_{jj}), \quad (2)$$

(Mukai & Yamazaki, 1968), where the denominator refers to the genotypic component of variance. Apparently, no estimates using (2) were computed by Ohnishi. We have reanalysed Ohnishi's results for quasinormal chromosomes by means of (2), and the estimated \bar{h} is around 0.1 for both coupling and repulsion heterozygotes (A. García-Dorado & A. Caballero, unpublished). Equation (2) applied to MA chromosomes gives the arithmetic mean of h , weighted by the square selection coefficient, s^2 . In this respect, if s and h are negatively correlated, estimates of \bar{h} from (2) are expected to be lower than those from (1) (which gives averages weighted by s). A negative correlation between s and h is very likely (see review by Caballero & Keightley, 1994). However, the bias of an estimate from (2) is not necessarily large in the analysis of Ohnishi's lines because most severe mutations were surely excluded from the analysis. After 40 generations, 78 of 80 chromosomes with

homozygous viability larger than 0.6 had viabilities larger than 0.85. Therefore, it is possible that the above estimate of 0.1 may be a little biased downwards, and the generally assumed estimate of about 0.4 for the dominance coefficient of mildly deleterious mutations affecting viability in *D. melanogaster* can be, at least, questioned.

More recently, direct estimates of \bar{h} for spontaneous mutations in *D. melanogaster* have been obtained by Houle *et al.* (1997). Estimates of \bar{h} were obtained by means of (2) for several life-history traits. The pooled estimate is 0.12, with confidence limits -0.17 and 0.41. In this analysis, all chromosomes (except those carrying lethals) were considered, so the estimate is expected to be lower than that from Ohnishi's lines. However, the large confidence limits preclude any valid comparison.

In summary, we can conclude that the direct estimates of \bar{h} from Mukai's experiments are controversial. Those from Ohnishi's results using (1) can be highly biased if environmental changes have occurred in the marker chromosome during the MA experiment. The most reliable and consistent estimates, therefore, are those obtained from Ohnishi's lines by regression, being of the order of 0.1.

(ii) Indirect estimates from segregating populations

Indirect estimates of h come from analysis of chromosomes extracted from natural populations, and are always based on the assumption of mutation-selection balance. The method used more extensively is the regression of heterozygous viabilities on the sum of the viabilities of the corresponding homozygotes, as from (2). Assume that, for a given locus, the genotypes of the random heterozygotes are AA , Aa , aa , with frequencies p^2 , $2pq$, q^2 , and viabilities (y) are 1 , $1 - sh$, $1 - s$, respectively; the sums of the viabilities of the corresponding homozygotes (x) are 2 , $2 - s$, $2 - 2s$. Thus, the regression of the viabilities of heterozygotes on the sum of the viabilities of the two homozygotes, assuming locus mutation rate u and equilibrium frequency $q \approx u/sh$, is

$$b_{y,x} \approx \sum pq s^2 h / \sum pq s^2 \approx \sum us / \sum \frac{us}{h} = \bar{h}_m, \quad (3)$$

(Mukai *et al.*, 1972), which is the harmonic mean of h weighted by s and u .

Most estimates obtained with (3) involve only quasinormal chromosomes, and the pooled estimate over all experiments is around 0.2-0.3 (Simmons & Crow, 1977; Eanes *et al.*, 1985; Johnston & Schoen, 1995; Hughes, 1995), with some exceptions. This is larger than the direct estimate of 0.1 from Ohnishi's MA lines obtained by the same regression method. The comparison is very tricky, though. The first

estimate is a harmonic mean weighted by s , while the second is an arithmetic mean weighted by s^2 . The harmonic mean is expected to be lower than the arithmetic mean, but the s^2 -weighting is expected to give a reduced estimate, so it is not evident which one should be lower. It is also possible that, because all these estimates refer only to quasinormal chromosomes, neither of those two factors are very important, i.e. for mutations of small effect the harmonic and arithmetic mean can be similar, and the different weights can also have similar effects.

The regression estimate obtained with (3) (0.2–0.3) is also considered the arithmetic mean of h for genes segregating in natural populations, in which the h values are weighted by pqs^2 , the variance of homozygotes. Thus it is generally assumed that the arithmetic mean of h for segregating genes equals the harmonic mean of h for mutations (e.g. Lynch & Walsh, 1997, p. 285). Because it is intuitive that the mean h for mutations must be larger than that for segregating genes, we would expect that the mean h for mutations is larger than 0.2–0.3. However, the relation between the estimate obtained from (3) and the true arithmetic mean of h for segregating genes is not clear. The values averaged are weighted by the genetic variance that would be contributed by each locus if the population consisted only of the two homozygous types at frequencies p and q . This weighting has little biological justification (Caballero *et al.*, 1997). A more obvious arithmetic mean of h for segregating genes would occur if h values are weighted by the frequency of heterozygotes or the allele frequency, which requires constant s in (3).

If the estimates of the arithmetic mean of h for mutations were really larger than the harmonic mean (as the above results seem to indicate), this may suggest that the mutation–selection balance model does not hold as a complete explanation of genetic variability (Charlesworth & Hughes, 1996). This latter situation is indirectly supported by the following. An estimate of the inverse of the arithmetic mean of h weighted by s values for mutations can also be obtained from the regression reciprocal to that in (3), i.e. the regression of the sum of viabilities of the two homozygotes on the viability of the heterozygote ($b_{x,y}$). Mukai & Yamaguchi (1974) showed that if there were overdominance or other sources of balancing selection, the estimate of \bar{h} from $b_{x,y}$ would be highly biased upwards. The results obtained with this method give very large values of \bar{h} (of the order of one or more; see Mukai & Yamaguchi, 1974).

Other indirect methods that have been used for estimating h are based on the changes of the mean and the variance of fitness under inbreeding (see, e.g. Lynch & Walsh, 1997, chapters 10 and 12). Let us consider one of these methods and the available estimates (Lynch & Walsh, 1997, pp. 284–287). As-

sume the one-locus model of fitnesses given above. Neglecting terms in q^2 , the mean fitness of an outbred population is $W_0 = 1 - \sum 2pqsh$, and the mean fitness of a completely inbred population is $W_1 = 1 - \sum qs$. Thus, we note that assuming mutation–selection balance,

$$(1 - W_0)/2(1 - W_1) = \sum pqsh/\sum qs \approx \sum u/\sum (u/h) = \bar{h}_m$$

gives an estimate of the harmonic mean of mutations weighted by their mutation rate. Values of \bar{h}_m obtained for a range of vertebrates have an average of 0.08 ± 0.01 . For *Drosophila* the average is 0.14. To compare this value with the above estimates we should consider the following. First, this estimate again assumes mutation–selection balance, neglecting frequencies of homozygous mutants. Secondly, it assumes that all sources of mortality are genetic. Thirdly, it includes lethal and semilethal mutants. The first and second assumptions can be sources of overestimation, particularly the second, if mortality due to environmental reasons is large. The third, however, would produce a downward bias of the estimation of \bar{h} for mildly deleterious mutations. In conclusion, because of the counteracting factors of bias, these indirect estimates are difficult to interpret.

Finally, consider another indirect method reported by Lynch *et al.* (1995). The average number of mutations per gamete in a mutation–selection equilibrium population is $\sum q = \sum 2u/sh = 2\lambda/sh$, assuming all mutations have the same s and h . Thus, the expected mean fitness of an outbred population is $W_0 = (1 - sh)^{2\lambda/sh} \approx e^{-2\lambda}$, and that of an inbred population is $W_1 = (1 - s)^{\lambda/sh} \approx e^{-\lambda/h}$. Therefore, $\ln(W_1/W_0) = -\lambda/h + 2\lambda$, and $h = \lambda/[2\lambda - \ln(W_1/W_0)]$ is an estimate of the dominance coefficient provided an estimate of λ is available. Using values of (W_1/W_0) from experimental data for viability in *Drosophila* species, and assuming $\lambda = 0.36$, Lynch *et al.* (1995) deduced an estimated $h = 0.39$, in agreement with the typical value. However, this estimate is strongly dependent on the mutation rate and assumes constant s and h . Taking the value of $\lambda = 0.01$ (viability, Table 1), the estimate is about $h = 0.08$.

In summary, we can conclude that the estimates of \bar{h} for mutations arising from indirect methods are generally very difficult to interpret, the assumption of mutation–selection balance being an arguable starting point.

4. Joint inferences for morphological and fitness component traits

(i) Mutational parameters

The most commonly reported parameter is the amount of variation due to mutation per gamete and generation (mutational variance $\sigma_m^2 = \Delta V/2$). To com-

Table 2. Summary of MD mutational parameter estimates for sternopleural (ST) and abdominal (AB) bristle number, wing length (WL), viability and fitness in *Drosophila*.^a See text for explanation

Trait	λ	$E(a)^b$	P^+	g_2	$10^3 \times (\sigma_m^2)^c$	% σ_m^2 ($ a > \delta$) ^d
ST ^e	0.043	-0.01	0.398	45.4	0.61	72
AB ^e	0.009	-0.24	0.094	9.3	0.49	61
WL ^e	0.011	-0.31	0.068	4.9	0.85	57
Viability ^f	0.015	-0.17	0.050	6.7	0.36	99
Fitness ^g	0.030	-0.26	0.000	6.7	1.65	99

^a Conditional to ANOVA $\lambda E(a^2)$ estimates for all traits excepting fitness.

^b Estimates scaled by σ (morphological traits) or by \bar{X} (viability and fitness).

^c ANOVA estimates scaled by σ^2 (morphological traits) or by \bar{X}^2 (viability and fitness).

^d Percentage of σ_m^2 due to mutations with $|a| > \delta$ ($\delta = \sigma/2$ for morphological traits, $\delta = E(|a|)/2$ for viability and fitness).

^e García-Dorado & Marín (1998).

^f Average estimates from MD analysis of the four data sets in Table 1 (mutational effect $a = -s$).

^g MD analysis of Houle *et al.* (1992) data (mutational effect $a = -s$).

pare the σ_m^2 values of different traits, estimates are scaled by the environmental variance σ^2 (mutational heritability, $h_m^2 = \sigma_m^2/\sigma^2$) or by the mean \bar{X} (mutational coefficient of variation, $CV_m = \sigma_m/\bar{X}$). For spontaneous mutations in *D. melanogaster*, a recent review by Houle *et al.* (1996) indicates higher CV_m values for life-history traits (average: 0.021, range: 0.009–0.045) than for morphological traits (average: 0.004, range: 0.001–0.012). However, the mean of life-history traits is expected to decline due to accumulation of deleterious mutations, and this factor may increase the corresponding CV_m values. On the other hand, no clear difference in h_m^2 between classes of traits was detected (average: 5×10^{-3} , range: 10^{-4} – 10^{-2}).

The mutational variance, however, is a composite parameter ($\sigma_m^2 = \lambda E(a^2)/2$, where a is the homozygous effect of mutations on the trait). Therefore, a separate evaluation of λ and the shape of the distribution of mutant effects on the trait is needed. MD mutational parameters for morphological and life-history traits are shown in Table 2. Across traits, a remarkable similarity of parameters was found and only sternopleural bristle number (ST) consistently departed from the general pattern.

Summarizing:

- Mutation rates were small for all traits ($\lambda < 0.05$).
- The average mutational effect was not too small and always negative.
- Most mutations had negative effects, the proportion of mutations with positive effects (P^+) ranging from practically zero to 0.1. Therefore, for those morphological traits showing spatio-temporal constancy of the mean, some direct stabilizing selection must be acting.
- The kurtosis coefficient of the distribution of mutant effects (g_2 , normal distribution $g_2 = 3$) was not large. Thus, an important fraction of σ_m^2 will be due to mutations with absolute effects

smaller than $\sigma/2$ for morphological traits or $E(a)/2$ for viability and fitness (see last column in Table 2). For morphological traits undergoing weak selection, this implies that a large amount of the genetic variance at equilibrium can be due to loci segregating at intermediate frequencies.

- The distribution of mutant effects for ST differs from that of the remaining traits considered in being leptokurtic and practically symmetrical, this implying a smaller average effect of mutations.

(ii) Individual mutations

Information has been obtained from MA lines and artificially selected lines, all derived from a genetically homogeneous population. For any trait, the mean of a line undergoes periods of temporal change punctuated by stasis, and each of the former can be ascribed to a single major mutation reaching its maximum possible frequency. These putative mutations can be studied individually and estimates of their homozygous and heterozygous effects on metric traits, as well as their pleiotropic effects on fitness, can be computed. Data are restricted to abdominal (AB) and sternopleural (ST) bristle number and wing length (WL) and width in *D. melanogaster* (Caballero *et al.*, 1991; Santiago *et al.*, 1992; López & López-Fanjul, 1993a, b; Merchante *et al.*, 1995). For AB, 45 mutations were detected and the results can be summarized as follows:

- Both experimental methods identify a class of mutations that were predominantly additive and had effects ranging from 0.2σ to 0.7σ . No indication of directional dominance or epistasis was found and mutations with an effect smaller than 0.5σ behaved quasineutrally. This class should be close to that responsible for the observed variation in natural populations (see Section 4(i)).

- (b) A few (5/45) non-lethal mutations had large effect ($a > \sigma$) and were totally or partially recessive and deleterious.
- (c) About one-half of the individual mutations detected in artificially selected lines (12/33) were lethals with an effect on the heterozygote ranging from 0.1σ to 2σ . Lethals contributing to response are a common feature of selection experiments starting from natural populations, and are generally considered to be mutations occurring during the selective process (for a review see Merchante *et al.*, 1995).

For AB and ST, MA lines and artificially selected lines derived from a highly inbred base population have been studied by Mackay and co-workers (Mackay *et al.*, 1992, 1994; Fry *et al.*, 1995; Nuzdihin *et al.*, 1995). Individual characterization of non-lethal mutations has not been attempted. Significant departures from additivity were not, however, detected from analysis of the lines for both bristle traits, and suggested recessive action for fitness of the mutations involved. On the other hand, only three lethal mutations at relatively high frequencies were found in 12 selected lines after 125 generations of selection, and just one of those lethals had a significant effect on the selected trait (ST). This result is at odds with those mentioned above and could perhaps be ascribed to differences in the base stocks used.

(iii) Mutational genotype–environment interaction

In *D. melanogaster*, genotype–environment ($G \times E$) interaction of new mutations in a highly inbred background has been evaluated by scoring MA lines for traits of interest in different environmental conditions. Kondrashov & Houle (1994) reported that the difference in fitness between two MA lines dramatically increased under harsh conditions. Based on this result, they pointed out that the genome deleterious mutation rate would be underestimated if quasi-neutral mutations under benign standard laboratory conditions become detrimental in stressful environments. In this situation, an increase in σ_m^2 for fitness and fitness-related traits should be observed with enhanced environmental harshness. On the other hand, if the fitness ranking of mutations alters with environment, causing reaction norms to cross, changes in σ_m^2 across environments will be unpredictable.

Studies have been carried out for: productivity in a benign environment (low competition) and in four other media including the standard one (reducing productivity by 33–80%) (Fry *et al.*, 1998), and three fitness components (fecundity and egg-to-pupa and pupa-to-adult viabilities) in the standard medium and three harsh media (reducing productivity by 25–80%) (Fernández & López-Fanjul, 1997). In both instances, an increase in σ_m^2 with intensified environmental stress

was not observed. For productivity, the mutational correlations between character states in different media were large and positive (average value 0.75), suggesting unconditionally deleterious mutations with environment-dependent effects. For fecundity and viability, however, those mutational correlations were usually small (average absolute value 0.3), revealing a high degree of environmental specificity of the mutations involved. In this case, highly inbred MA lines were used and, therefore, the results refer mostly to non-severely detrimental mutations, those that are mainly responsible for the long-term erosion of fitness leading to the eventual extinction of populations, but do not necessarily apply to highly deleterious mutations. These are likely to affect basic organic functions and, therefore, their effects may be intensified with increasing environmental stress, but they will not contribute significantly to the mutational load or the equilibrium genetic variance in natural populations.

The behaviour of four morphological traits (ovariole number, thorax length, AB and ST) in three different temperatures has also been examined in MA lines (Wayne & Mackay, 1998; Mackay & Lyman, 1998). On the whole, line \times temperature interaction variance components were not large (average value 28% of the between-line variance), but a greater $G \times E$ variance component, attributable to fluctuation of uncontrolled environmental agents, was also found (average value 65% of the between-line variance). For AB and WL, mutational genotype–generation interaction effects were also detected by García-Dorado & Marín (1998). Mutational correlations between character states across environments were only reported for bristle traits and they were generally large (average value 0.75). For all morphological traits, no association was found between the magnitude of σ_m^2 and a specific temperature.

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References

- Caballero, A. & Keightley, P. D. (1994). A pleiotropic nonadditive model of variation in quantitative traits. *Genetics* **138**, 883–900.
- Caballero, A. & Keightley, P. D. (1998). Inferences on genome-wide deleterious mutation rates in inbred populations of *Drosophila* and mice. *Genetica* **102/103**, 229–239.
- Caballero, A., Toro, M. A. & López-Fanjul, C. (1991). The response to artificial selection from new mutations in *Drosophila melanogaster*. *Genetics* **127**, 89–102.
- Caballero, A., Keightley, P. D. & Turelli, M. (1997). Average dominance for polygenes: drawbacks of regression estimates. *Genetics* **147**, 1487–1490.
- Charlesworth, B. & Hughes, K. A. (1996). The quantitative

- genetics of life history traits. In *Evolutionary Genetics from Molecules to Morphology* (ed. R. S. Singh & C. B. Krimbas). Cambridge: Cambridge University Press.
- Crow, J. F. (1999). The odds of losing at genetic roulette. *Nature* **397**, 293–294.
- Donoho, D. L. & Liu, R. C. (1988). The automatic robustness of minimum distance functionals. *Annals of Statistics* **16**, 552–586.
- Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. (1998). Rates of spontaneous mutation. *Genetics* **148**, 1667–1686.
- Eanes, W. F., Hey, J. & Houle, D. (1985). Homozygous and hemizygous viability variation on the X chromosome of *Drosophila melanogaster*. *Genetics* **111**, 831–844.
- Eyre-Walker, A. & Keightley, P. D. (1999). High genomic deleterious mutation rates in hominids. *Nature* **397**, 344–347.
- Fernández, J. & López-Fanjul, C. (1996). Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. *Genetics* **143**, 829–837.
- Fernández, J. & López-Fanjul, C. (1997). Spontaneous mutational genotype–environment interaction for fitness-related traits in *Drosophila melanogaster*. *Evolution* **51**, 856–864.
- Fry, J. D., deRonde, K. A. & Mackay, T. F. C. (1995). Polygenic mutation in *Drosophila melanogaster*: genetic analysis of selection lines. *Genetics* **139**, 1293–1307.
- Fry, J. D., Heinsohn, S. L. & Mackay, T. F. C. (1998). Heterosis for viability, fecundity, and male fertility in *Drosophila melanogaster*: comparison of mutational and standing variation. *Genetics* **148**, 1171–1188.
- Fry, J. D., Keightley, P. D., Heinsohn, S. L. & Nuzhdin, S. V. (1999). New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **96**, 574–579.
- García-Dorado, A. (1997). The rate and effects distribution of viability mutation in *Drosophila*: minimum distance estimation. *Evolution* **51**, 1130–1139.
- García-Dorado, A. & Marín, J. M. (1998). Minimum distance estimation of mutational parameters for quantitative traits. *Biometrics* **54**, 1097–1114.
- García-Dorado, A., Monedero, J. L. & López-Fanjul, C. (1998). The mutation rate and the distribution of mutational effects of viability and fitness in *Drosophila melanogaster*. *Genetica* **102/103**, 255–265.
- Houle, D., Hoffmaster, D. K., Assimacopoulos, S. & Charlesworth, B. (1992). The genomic mutation rate for fitness in *Drosophila*. *Nature* **369**, 58–60.
- Houle, D., Hoffmaster, D. K., Assimacopoulos, S. & Charlesworth, B. (1994). Correction: the genomic mutation rate for fitness in *Drosophila*. *Nature* **371**, 358.
- Houle, D., Morikawa, B. & Lynch, M. (1996). Comparing mutational variabilities. *Genetics* **143**, 1467–1483.
- Houle, D., Hughes, K. A., Assimacopoulos, S. & Charlesworth, B. (1997). The effects of spontaneous mutation on quantitative traits. II. Dominance of mutations with effects on life-history traits. *Genetical Research* **70**, 27–34.
- Hughes, K. A. (1995). The inbreeding decline and average dominance of genes affecting male life-history characters in *Drosophila melanogaster*. *Genetical Research* **65**, 41–52.
- Johnston, M. O. & Schoen, D. J. (1995). Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* **267**, 226–229.
- Keightley, P. D. (1994). The distribution of mutation effects on viability in *Drosophila melanogaster*. *Genetics* **138**, 1–8.
- Keightley, P. D. (1996). Nature of deleterious mutation load in *Drosophila*. *Genetics* **144**, 1993–1999.
- Keightley, P. D. (1998). Inference of genome-wide mutation rates and distributions of mutation effects for fitness traits: a simulation study. *Genetics* **150**, 1283–1293.
- Keightley, P. D. & Caballero, A. (1997). Genomic mutation rate for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **94**, 3823–3827.
- Kibota, T. T. & Lynch, M. (1996). Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. *Nature* **381**, 694–696.
- Kondrashov, A. (1995). Contamination of the genome by very slight deleterious mutation: Why have we not died 100 times over? *Journal of Theoretical Biology* **175**, 583–594.
- Kondrashov, A. S. & Houle, D. (1994). Genotype–environment interaction and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proceedings of the Royal Society of London, Series B* **258**, 221–227.
- Kreitman, M. (1996). The neutral theory is dead. Long live the neutral theory. *BioEssays* **18**, 678–683.
- López, M. A. & López-Fanjul, C. (1993a). Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. I. Response to artificial selection. *Genetical Research* **61**, 107–116.
- López, M. A. & López-Fanjul, C. (1993b). Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. II. Distribution of mutant effects on the trait and fitness. *Genetical Research* **61**, 117–126.
- Lynch, M. & Walsh, B. (1997). *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer.
- Lynch, M., Conery, J. & Bürger, R. (1995). Mutation accumulation and the extinction of small populations. *American Naturalist* **146**, 489–518.
- Lynch, M., Latta, L., Hicks, J. & Giorgianni, M. (1998). Mutation, selection and the maintenance of life-history variation in a natural population. *Evolution* **52**, 727–733.
- Mackay, T. F. C. & Lyman, R. F. (1998). Polygenic mutation in *Drosophila melanogaster*: genotype × environment interaction for spontaneous mutations affecting bristle number. *Genetica* **102/103**, 199–215.
- Mackay, T. F. C., Lyman, R. F., Jackson, M. S., Terzian, C. & Hill, W. G. (1992). Polygenic mutation in *Drosophila melanogaster*: estimates from divergence among inbred strains. *Evolution* **46**, 300–316.
- Mackay, T. F. C., Fry, J. D., Lyman, R. F. & Nuzhdin, S. V. (1994). Polygenic mutation in *Drosophila melanogaster*: estimates from response to selection of inbred strains. *Genetics* **136**, 937–951.
- Merchante, M., Caballero, A. & López-Fanjul, C. (1995). Response to selection from new mutation and effective size of partially inbred populations. II. Experiments with *Drosophila melanogaster*. *Genetical Research* **66**, 227–240.
- Mukai, T. (1964). The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics* **50**, 1–19.
- Mukai, T. (1969). The genetic structure of natural populations of *Drosophila melanogaster*. VIII. Natural selection on the degree of dominance of viability polygenes. *Genetics* **63**, 467–478.
- Mukai, T. & Yamaguchi, O. (1974). The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a large local population. *Genetics* **76**, 339–366.
- Mukai, T. & Yamazaki, T. (1968). The genetic structure of natural populations of *Drosophila melanogaster*. V. Coupling–repulsion effects of spontaneous mutant polygenes controlling viability. *Genetics* **59**, 513–535.

- Mukai, T., Chigusa, S. I., Mettler, L. E. & Crow, J. F. (1972). Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* **72**, 333–355.
- Nuzhdin, S. V., Fry, J. D. & Mackay, T. F. C. (1995). Polygenic mutation in *Drosophila melanogaster*: the causal relationship of bristle number to fitness. *Genetics* **139**, 861–872.
- Ohnishi, O. (1974). Spontaneous and ethyl methane-sulfonate-induced polygenic mutations controlling viability in *Drosophila melanogaster*. PhD thesis, University of Wisconsin.
- Ohnishi, O. (1977). Spontaneous and ethyl methane-sulfonate-induced mutations controlling viability in *Drosophila melanogaster*. II. Homozygous effects to polygenic mutations. *Genetics* **87**, 529–545.
- Santiago, E., Albornoz, J., Domínguez, A., Toro, M. A. & López-Fanjul, C. (1992). The distribution of effects of spontaneous mutations on quantitative traits and fitness in *Drosophila melanogaster*. *Genetics* **132**, 771–781.
- Simmons, M. J. & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11**, 49–78.
- Tachida, H., Harada, K., Langley, C. H., Aquadro, C. F., Yamazaki, T., Cockerham, C. C. & Mukai, T. (1989). Restriction map and α -amylase activity variation among *Drosophila* mutation accumulation lines. *Genetical Research* **54**, 197–203.
- Vassilieva, L. L. & Lynch, M. (1999). The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. *Genetics* **151**, 119–129.
- Wayne, M. L. & Mackay, T. F. C. (1998). Quantitative genetics of ovariole number in *Drosophila melanogaster*. II. Mutational variation and genotype–environment interaction. *Genetics* **148**, 201–210.
- Zeng, L., Comeron, J. M., Chen, B. & Kreitman, M. (1998). The molecular clock revisited: the rate of synonymous vs replacement change in *Drosophila*. *Genetica* **102–103**, 369–382.