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On the three methods for estimating deleterious genomic mutation parameters

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Summary

Due to the tremendous cost of the traditional mutation-accumulation approach (the Bateman–Mukai technique), data are rare for deleterious mutation parameters such as genomic mutation rate, selection and dominance coefficients. Two alternative approaches have been developed (the Morton–Charlesworth and Deng–Lynch techniques). Except for the Deng–Lynch method, the statistical properties (bias and sampling variance) of these techniques are poorly understood; therefore we investigated them using computer simulation. With constant fitness effects of mutations, the Bateman–Mukai (assuming additive effects) and Deng–Lynch (assuming multiplicative effects) techniques are unbiased; the Morton–Charlesworth technique (assuming multiplicative effects) is very biased if *fitness* is used in the regression to estimate h, but slightly biased if the *logarithm of fitness* is used. With variable fitness effects, all techniques are biased. The Deng–Lynch technique is statistically better than the others except when *fitness* is used to estimate the average degree of dominance in selfing populations with the Morton–Charlesworth technique. If fitness effects are multiplicative but additivity is assumed, the Bateman–Mukai technique is biased under constant fitness effects, and less biased under variable fitness effects relative to when fitness effects are additive (as assumed by the technique). Our study not only quantifies the degree of bias under the biologically plausible situations investigated, thus forming a basis for correct inference of the true parameters by using these techniques, but also provides insights into the relative efficiencies of these techniques when the same number of genotypes are handled experimentally.

1. Introduction

The three essential parameters of deleterious genomic mutations are: (1) the genomic mutation rate (U, measured in units of the number of new mutation occurrences per genome per generation), (2) the mean selection coefficient ($\bar{s}$, measuring the relative reduction of performance of mutant homozygotes relative to the wild-type homozygotes), and (3) the mean dominance coefficient ($\bar{h}$, describing the extent to which heterozygotes express harmful effects of mutant alleles).

Estimation of these parameters in diverse taxa is important for testing many modern evolutionary and population genetics theories, and is also of considerable practical value. For example, U estimates are crucial in testing hypotheses on the evolution of sex and recombination (Muller, 1964; Kondrashov, 1985, 1988; Charlesworth, 1990), mate choice (Kirkpatrick & Ryan, 1991), diploidy (Kondrashov & Crow, 1991) and outbreeding mechanisms (Charlesworth & Charlesworth, 1987). They also determine the magnitude of mutation load in populations at equilibrium (Haldane, 1937; Kimura *et al.*, 1963; Burger & Hofbauer, 1994). Estimates of h and s are important for testing the hypothesis of transition from haploidy to diploidy (Perrot *et al.*, 1991). Joint estimates of U and s are critical in determining the role of deleterious mutations in the extinction of small populations through the accumulation of mutations (Lande, 1994; Lynch *et al.*, 1995, 1996). Estimates of

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U, h and s determine the rate of input of genetic variance from mutation per generation (Deng & Lynch, 1996) and the extent to which neutral molecular variation is reduced due to background selection (Charlesworth *et al*., 1993, 1995; Hudson & Kaplan, 1995).

Despite their importance, estimates are rare. There are now three approaches to estimation:

1. The mutation-accumulation (M-A) approach. This was proposed by Bateman (1959) and first employed by Mukai (1964) and Mukai *et al*. (1972) (referred to as the Bateman–Mukai technique hereafter). This technique estimates U and s. Most estimates have come from this approach applied to *Drosophila melanogaster* (Mukai, 1979; Crow & Simmons, 1983; Keightley, 1994), and have been very hard to acquire, requiring large and long-term mutation-accumulation (M-A) and special chromosomal constructs or inbred lines. The data from M-A can also be analysed by the maximum likelihood method of Keightley (1994); however, due to its large computational requirement, investigation of its statistical properties by large-scale simulations remains a challenge.

2. The inbreeding depression approach. This was implied by Morton *et al*. (1956) in outcrossing populations, and explicitly proposed by Charlesworth *et al*. (1990) for use with highly selfing populations (both collectively referred to as the Morton–Charlesworth technique hereafter). This technique *per se* estimates U only. In the highly selfing annual plants *Leavenworthia* (Charlesworth *et al*., 1994) and *Amsinckia* (Johnston & Schoen, 1995), U estimates from this approach are in line with earlier ones from M-A in *Drosophila*.

3. The fitness moments approach. This is an extension of the Morton–Charlesworth technique by Deng & Lynch (1996) (referred to as the Deng–Lynch technique hereafter). It estimates U, h and s. For two outcrossing species of cyclical parthenogenetic *Daphnia* (a freshwater microcrustacean), the preliminary data estimated by this approach generally agree with earlier ones (Deng & Lynch, 1997).

Except for the Deng–Lynch technique, statistical properties such as bias and sampling variances have not been investigated. Such investigations, especially those under realistic biological situations, are important, since they will provide a basis for a correct interpretation of the estimates obtained under some necessary but unrealistic assumptions. Although Charlesworth's technique was investigated for its robustness under synergistic mutations and partial selfing (Charlesworth *et al*., 1990), it assumed constant fitness effects of mutations with a known h. However, h estimation is not trivial and fitness effects are most likely variable (Mackay *et al*., 1992; Keightley, 1994). While the traditional M-A analysis assumes additive fitness effects, there is good evidence that genes for fitness or its components may act multiplicatively (Morton *et al*., 1956; Crow, 1986; Fu & Ritland, 1996). In order to infer the parameters correctly from experimental data, investigating the estimation properties under realistic mutational effects is important. Additionally, the statistical properties of these techniques need to be investigated under the same number of genotypes that are handled experimentally, so that their relative efficiencies can be compared.

In this study, in order to provide necessary background information, we will first outline the principles of the techniques. Next, we will investigate their statistical properties under constant mutational effects, which will serve as a starting point for the comparison with the more realistic situations later in this study. We will then investigate their robustness under variable fitness effects. Finally, we will study the robustness of the Bateman–Mukai technique under multiplicative fitness effects.

2. Principles

(i) *The Bateman–Mukai technique*

If data on the rates of change of mean fitness (M) and genetic variance (V) due to new mutations are available, bounds on U and $\bar{s}$ may be estimated: Assuming that fitness effects are additive, the mutation probability per generation at each locus is small, and mutations on all loci are independent, then (Mukai *et al*., 1972):

$$U \geqslant \frac{M^2}{V}, \quad \bar{s} \leqslant \frac{V}{M}. \tag{1}$$

In M-A experiments involving special chromosomal constructs in *Drosophila*, U is typically the haploid mutation rate for the chromosome isolated for M-A (Mukai *et al*., 1972).

(ii) *The inbreeding depression approach*

This approach applies to fitness (or related traits) in large populations at mutation–selection (M–S) equilibrium, in which the number of mutations per genome is Poisson distributed. It assumes that the fitness effects of mutations are constant and multiplicative, and loci are unlinked.

In such outcrossing populations (Morton *et al*., 1956), selfing (or any other form of inbreeding; here we concentrate on selfing) of random genotypes results in inbreeding depression

$$\delta_o = \frac{\bar{w}_s}{\bar{w}_o},$$

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where $\bar{w}_o$ and $\bar{w}_s$ are the mean fitnesses of the outcrossed parental and selfed offspring generations respectively. Given h:

$$U = \frac{4h \ln \delta_o}{2h-1}. \quad (2)$$

U in the inbreeding depression approach and in the Deng–Lynch technique is usually for the whole diploid genome.

In highly selfing populations (Charlesworth *et al.*, 1990), crossing random genotypes results in outbreeding enhancement $\delta_i = \bar{w}_s/\bar{w}_o$, where $\bar{w}_s$ and $\bar{w}_o$ are the mean fitnesses of the selfed parental and outcrossed offspring generations respectively. Given h:

$$U = \frac{2 \ln \delta_i}{2h-1}. \quad (3)$$

An advantage of estimating U in selfing populations is that overdominance or lethals are unlikely to bias U estimation (Charlesworth *et al.*, 1990).

Since the inbreeding depression approach depends on prior knowledge of h, it must be evaluated with an h-estimating technique. The h-estimating technique suggested (Charlesworth *et al.*, 1990) was the one proposed by Mukai *et al.* (1972). It estimates the average h at individual loci weighted by the genetic variance of the homozygotes ($\bar{h}$), from the regression slope of the outcrossed progeny fitness (x) on the fitness sum (y) of the two corresponding homozygous parents (Mukai *et al.*, 1972; Simmons & Crow, 1977; Crow & Simmons, 1983):

$$\bar{h} = \frac{\mathrm{Cov}(x, y)}{\mathrm{Var}(y)}. \quad (4)$$

In the derivation of this expression, additive fitness effects were implicitly assumed (Mukai *et al.*, 1972). A key assumption is that the rare allele frequency at any locus is very low (Charlesworth & Hughes, 1997; Deng, 1998), which is usually met given M–S equilibrium (Crow & Kimura, 1970). In (4), the regression is for the genotypic values x and y. Thus genetic variance and covariance should be used to estimate $\bar{h}$ (Mukai *et al.*, 1972). Assuming additivity, $\bar{h}$ can also be estimated by the genetic variances among the homozygous and heterozygous lines derived from populations at M–S equilibrium (Hughes, 1995).

(iii) *The Deng–Lynch technique*

Under the same assumptions as the Morton–Charlesworth technique, this method requires that genetic variances of fitness before and after selfing/outcrossing be measured (Deng, 1995; Deng & Lynch 1996, 1997).

In outcrossing populations. I. A sample of genotypes are selfed to obtain a number of selfed progeny from each parent to form selfed families. II. Parental genotypes are cloned. Genotypes from both generations are assayed together in one environment to estimate $\bar{w}_o$ and $\bar{w}_s$. III. One-way ANOVA are performed. In the outcrossed parental generation, parental genotypes are treated as main effects and clonal replicates as random effects, so that we obtain estimates of the genetic variance (σ_0^2). In the selfed offspring generation, selfed families are treated as main effects and selfed progeny genotypes within each family as random effects so that we can obtain the genetic variance among the mean of selfed families (σ_s^2). Then:

$$h = \frac{1}{4 \quad \frac{z}{x} - 2} \qquad U = \frac{4hy}{2h-1} \qquad s = \frac{z}{Uh}, \quad (5)$$

where

$$x = \ln \frac{\sigma_o^2}{\bar{w}_o^2} + 1 \ , \quad y = \ln \frac{\bar{w}_s}{\bar{w}_o} \ , \quad z = \ln \frac{\sigma_s^2}{\bar{w}_s^2} + 1 \ . \quad (6)$$

In highly selfing populations. I. Random pairs of genotypes are sampled and outcrossed. II. The selfed parent and outcrossed progeny genotypes are cloned and assayed in one common environment. III. One-way ANOVAs are performed with genotypes as main effects and clonal replicates as random effects, to estimate the genetic variances in the outcrossed progeny (σ_o^2) and selfed parental (σ_s^2) generations, together with $\bar{w}_s$ and $\bar{w}_o$. Let x, y, z be defined in (6), then:

$$h = \frac{x}{2z} \qquad U = \frac{2y}{2h-1} \qquad s = \frac{2z}{U}. \quad (7)$$

3. Statistical properties under constant fitness effects

The investigation of the methods under their respective assumptions with constant fitness effects can serve as a starting point for the comparison with the more realistic situations investigated later in this study. In order to focus on comparing the estimation power of the three approaches (M-A and change of fitness moments upon outbreeding/selfing in populations at M–S equilibrium), all genotypic values are assumed to be measured accurately in this study. In reality, this would require that each genotype be clonally replicated and assayed a very large number of times. Ignoring measurement error for genotypic values will

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Table 1. *Estimation with the Bateman–Mukai technique under constant fitness effects*

U	s	$\hat{U}$	$\hat{s}$
0·1	0·01	0·10 (0·02)	0·010 (0·001)
	0·03	0·10 (0·02)	0·030 (0·004)
	0·05	0·10 (0·02)	0·050 (0·007)
0·5	0·01	0·53 (0·09)	0·010 (0·003)
	0·03	0·53 (0·09)	0·029 (0·004)
	0·05	0·53 (0·09)	0·048 (0·007)
1·0	0·01	1·03 (0·15)	0·010 (0·001)
	0·03	1·04 (0·15)	0·029 (0·004)
	0·05	1·04 (0·15)	0·049 (0·007)
1·5	0·01	1·53 (0·24)	0·010 (0·002)
	0·03	1·53 (0·24)	0·030 (0·005)
	0·05	1·53 (0·24)	0·050 (0·008)

The numbers in this and all of the following tables are based on 100 simulations, in which genotypic values are assumed to be measured accurately. Throughout, the number within each set of parentheses indicates the associated standard deviation of the estimate over the repeated simulations. Each simulation is based on 100 independent M-A lines. ˆ denotes an estimated value throughout. Mutations are allowed to accumulate for 40 generations and the fitnesses of the lines are assayed every 10 generations, which is similar to the actual experiment (Mukai *et al*., 1972). The total sample size of genotypes used in the M-A is 4000.

probably reduce the sampling error of estimates, but is unlikely to bias either the estimation or the comparison of the techniques, assuming the same number of genotypes that would be handled experimentally. This is partly supported by the investigation of measurement error on mutation parameter estimation for the Deng–Lynch technique (Deng & Lynch, 1996).

(i) *The Bateman–Mukai technique*

This simulation simplifies some complex features of the actual experiments (e.g. the need for raising large controls for temporal environmental changes and backups in case of line losses):

1. Fitnesses for L identical lines are set to 1·0 at the onset of the M-A.
2. As in the *Drosophila* experiments (Mukai *et al*., 1972), mutations are allowed to accumulate in the heterozygous state, but are measured in the homozygous state. Mutations, with constant s and h, occur in each line as a Poisson process at a rate of U per generation, and are allowed to accumulate independently for T generations. The fitness (W) equals $1-s\times n$, where n is the number of mutations accumulated. All lines are assayed at time intervals of I generations. $L\times T$ is the total number (S) of genotypes handled, which includes those strenuously maintained as M-A lines but not in the measurement generations.
3. As in Mukai *et al*. (1972), the means and variances among the lines measured at different times are used in simple regression analyses to estimate M and V, and then U and s (1).

The simulation is performed for a range of parameter sets (e.g. $U = 0{\cdot}1$–$1{\cdot}5$, $s = 0{\cdot}01$–$0{\cdot}05$). There is no estimation bias and the sampling variance is very small regardless of the parameters simulated (Table 1). Therefore, for the parameters $U = 1{\cdot}0$ and $s = 0{\cdot}03$, we investigate the estimation under different experimental designs (Table 2). Employing this parameter set in simulations may help facilitate later comparison with the other techniques under the same parameter set (Tables 4, 7). It can be seen that, for the simulated parameters, *if genotypic values are measured with high accuracy*, 10 M-A generations and two assays (at the onset and the end of M-A) are enough to obtain unbiased estimates with small sampling variance, if many M-A lines are employed ($\geqslant 100$). This conclusion should hold regardless of the parameters simulated, as supported by the results in Table 1 and those not shown.

(ii) *The Morton–Charlesworth technique*

It is assumed that the number of mutations per individual (n) is Poisson distributed and all are at the heterozygous/homozygous stage in outcrossing/selfing populations at M–S equilibrium (Charlesworth *et al*., 1990; Deng & Lynch, 1996).

In outcrossing populations. 1. *Estimating h*: Assume H random pairs of homozygotes are established from natural populations, such as with special chromosomal constructs (Mukai *et al*., 1972). The fitness of a line is $W = (1-s)^n$, where n is the number of mutations randomly determined from the Poisson distribution with mean $U/(2hs)$. At M–S, the frequency of the mutant allele at a locus is usually very low (Crow & Kimura, 1970); it is thus unlikely that these homozygotes have mutations at the same loci. For an outcrossed progeny, the number of its heterozygous mutations is the sum of those homozygous mutations in its parents (n_m and n_f), with its fitness being $W = (1-hs)^{n_m+n_f}$. h is estimated by (4).

2. *Estimating U*: N individuals are sampled, each having fitness $W = (1-hs)^n$, where n is determined as above. From each of them, O selfed progeny are produced. The genotype of each selfed offspring is obtained by allowing the n parental heterozygous loci to segregate randomly into **AA**, **Aa**, and **aa** classes with respective probabilities of 0·25, 0·5 and 0·25. Letting n_1 and n_2 (both resulting from random

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Table 2. *Different experimental designs with the Bateman–Mukai technique* ($U = 1{\cdot}0$ *and* $\bar{s} = 0{\cdot}03$)

				Constant fitness effects		Variable fitness effects	
T	I	L	S	$\hat{U}$	$\hat{s}$	$\hat{U}$	$\hat{\bar{s}}$
40	10	50	2000	1·039 (0·232)	0·0300 (0·0059)	0·526 (0·135)	0·0603 (0·0133)
		100	4000	1·039 (0·149)	0·0294 (0·0040)	0·516 (0·084)	0·0597 (0·0093)
		200	8000	1·021 (0·112)	0·0297 (0·0032)	0·510 (0·053)	0·0597 (0·0065)
	40	50	2000	1·039 (0·236)	0·0303 (0·0066)	0·517 (0·109)	0·0608 (0·0124)
		100	4000	1·038 (0·154)	0·0295 (0·0042)	0·508 (0·071)	0·0602 (0·0083)
		200	8000	1·020 (0·104)	0·0297 (0·0031)	0·502 (0·049)	0·0603 (0·0056)
10	2	50	500	1·075 (0·227)	0·0290 (0·0058)	0·519 (0·124)	0·0605 (0·0146)
		100	1000	1·049 (0·164)	0·0292 (0·0044)	0·516 (0·082)	0·0597 (0·0095)
		200	2000	1·012 (0·115)	0·0300 (0·0031)	0·507 (0·056)	0·0599 (0·0068)
	10	50	500	1·043 (0·225)	0·0300 (0·0063)	0·501 (0·096)	0·0621 (0·0111)
		100	1000	1·016 (0·133)	0·0299 (0·0038)	0·498 (0·074)	0·0615 (0·0094)
		200	2000	1·011 (0·093)	0·0298 (0·0027)	0·504 (0·054)	0·0602 (0·0066)

T denotes the total number of M-A generations, I the number of interval generations between each assay, L the number of M-A lines, and S ($= T \times L$) the total number of genotypes handled in M-A.

Table 3. *Estimating h by Mukai's regression method and U under constant fitness effects*

			Regression of fitness to estimate h		Regression of ln(fitness) to estimate h	
U	h	s	$\hat{U}$	$\hat{h}$	$\hat{U}$	$\hat{h}$
(a) *Morton's technique in outcrossing populations*						
0·5	0·2	0·01	−1·732 (0·092)	0·886 (0·033)	0·497 (0·005)	0·199 (0·000)
		0·05	−1·818 (0·369)	0·876 (0·090)	0·483 (0·016)	0·196 (0·000)
	0·4	0·01	−12·822 (98·715)	0·511 (0·013)	0·493 (0·014)	0·399 (0·000)
		0·05	0·074 (31·090)	0·507 (0·027)	0·464 (0·058)	0·394 (0·000)
1·5	0·2	0·01	−2·316 (0·013)	17·804 (1·169)	1·490 (0·007)	0·199 (0·000)
		0·05	−2·322 (0·041)	16·843 (3·494)	1·453 (0·020)	0·196 (0·000)
	0·4	0·01	−0·944 (0·078)	0·834 (0·035)	1·482 (0·025)	0·399 (0·000)
		0·05	−1·041 (0·333)	0·813 (0·081)	1·391 (0·070)	0·394 (0·000)
(b) *Charlesworth's technique in selfing populations*						
0·5	0·2	0·01	0·561 (0·018)	0·232 (0·003)	0·499 (0·014)	0·199 (0·000)
		0·05	0·555 (0·035)	0·231 (0·006)	0·491 (0·029)	0·196 (0·000)
	0·4	0·01	0·628 (0·061)	0·420 (0·003)	0·495 (0·039)	0·399 (0·000)
		0·05	0·611 (0·112)	4·18 (0·008)	0·466 (0·074)	0·394 (0·000)
1·5	0·2	0·01	2·416 (0·109)	0·313 (0·008)	1·497 (0·024)	0·199 (0·000)
		0·05	2·379 (0·205)	0·309 (0·014)	1·485 (0·058)	0·196 (0·000)
	0·4	0·01	4·445 (1·143)	0·464 (0·008)	1·483 (0·059)	0·399 (0·000)
		0·05	4·412 (6·249)	0·458 (0·018)	1·424 (0·143)	0·399 (0·000)

segregation) be the numbers of heterozygous and homozygous loci containing mutations in a selfed offspring, then its fitness is $W(n_1, n_2) = (1-hs)^{n_1}(1-s)^{n_2}$. U is then estimated by (2).

In each simulation, $H = 10$, $N = 100$ and $O = 1$. $\hat{U}$ and $\hat{h}$ are summarized in columns 4 and 5 of Table 3*a*. $\hat{h}$ is significantly biased and sometimes much larger than 1·0, and $\hat{U}$ is almost always negative. This is because the fitness function is assumed to be multiplicative in the Morton–Charlesworth technique, but additive in Mukai's h-estimating technique. For the multiplicative fitness function, the logarithm of fitness approximates the fitness reduction due to mutation in the additive fitness function:

$$\ln W = \ln(1-s)^n \approx -ns \quad \text{(for small } s\text{)}. \tag{8}$$

Thus $\ln(W)$ should be used in the regression to estimate h, and U should be estimated by this $\hat{h}$ and a change of fitness on the original scale. The results of simulations using this procedure are summarized in columns 6 and 7 of Table 3*a*. h and U can now be estimated with small bias and sampling variance. The bias of $\hat{h}$ decreases with a decreasing s, due to a better approximation of (8).

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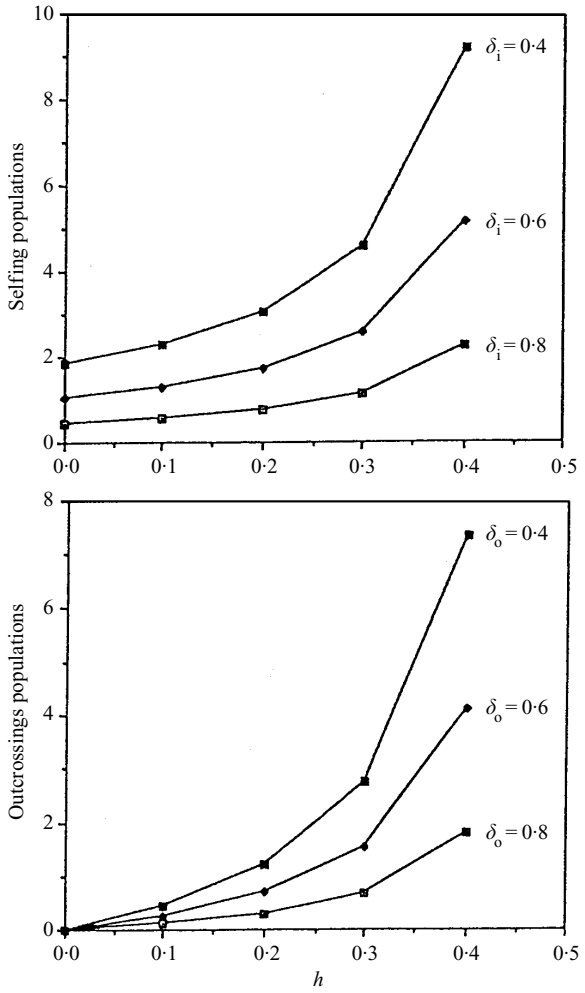

Fig. 1. Estimation of U(Y-axis) as functions of h, and inbreeding depression (δ_o) in outcrossing populations or outbreeding enhancement (δ_i) in selfing populations. The upper plot is for selfing populations (equation 3), the lower one is for outcrossing populations (equation 2).

In selfing populations. 1. *Estimating h*: Homozygotes are readily obtainable with fitness $W = (1-s)^n$, where n is randomly determined from the Poisson distribution with mean $U/(2s)$.

2. *Estimating U*: N pairs of individuals are sampled. For each individual, $W = (1-s)^n$, where n is determined as above. From each pair, an outcrossed progeny is produced with fitness $W(n_m, n_n) = (1-hs)^{n_m+n_n}$. U is estimated by (3).

In each simulation, $H = 10$ and $N = 100$, $\hat{h}$ and $\hat{U}$ are summarized in columns 4 and 5 of Table 3*b*. $\hat{h}$ and $\hat{U}$ are always significantly upwardly biased, although the bias is not as dramatic as in outcrossing populations. The bias is for the reason outlined for outcrossing populations. The degree of bias of $\hat{h}$ and $\hat{U}$ depends largely upon U, with smaller bias under smaller U. This is because $W = (1-s)^n \approx 1-ns$ holds better with smaller n, and $E(n) = U/(2s)$ is smaller with smaller U. The ridiculous $\hat{U}$ values in outcrossing populations are partly due to its larger $E(n) = U/(hs)$ than $E(n) = U/(2s)$ in selfing populations, for the same U, h and s. Additionally, $\hat{U}$ is more sensitive to $\hat{h}$ in outcrossing populations than in selfing populations (Fig. 1). Negative estimates of $\hat{U}$ and their larger sampling variance in outcrossing than in selfing populations are due to $\hat{h}$ values being over 0·5 (2) and having larger sampling variance. Using $\ln W$ in the regression, h and U can be estimated satisfactorily (columns 6 and 7 of Table 3*b*). The bias of $\hat{h}$ and $\hat{U}$ is small and decreases with a decreasing s. Therefore, with the Morton–Charlesworth technique, the procedure for estimating h is extremely important. Under constant fitness effects, only with an appropriate procedure can U and h be estimated accurately.

(iii) *The Deng–Lynch technique*

Simulations similar to the Morton–Charlesworth technique are performed. Besides $\bar{w}_s$ and $\bar{w}_o$, σ_o^2 and σ_s^2 are computed as described in Section 2. Additionally:

In outcrossing populations. The number of selfed progeny (O) from each of N outcrossed parents has to be at least two to form selfed families. $\bar{H}$, $\hat{h}$ and $\hat{s}$ are computed by (5) and (6).

In selfing populations. $\hat{U}$, $\hat{h}$ and $\hat{s}$ are estimated by (6) and (7).

For a range of parameters simulated ($U = 0{\cdot}5$–$1{\cdot}5$, $h = 0{\cdot}2$–$0{\cdot}4$, $s = 0{\cdot}01$–$0{\cdot}5$) in both outcrossing and selfing populations, data not shown here and those in Deng & Lynch (1996) indicate that estimates are unbiased and have small sampling errors.

(iv) *Comparison of the techniques*

The most frequently cited data suggest that $U \approx 1{\cdot}0$, $h \approx 0{\cdot}36$ and $s \approx 0{\cdot}03$ (Mukai *et al.*, 1972; Lynch *et al.*, 1995). We compare the techniques with these parameters under approximately 1000 genotypes handled in the experiments (Table 4). For the Bateman–Mukai and Deng–Lynch techniques, the estimates are unbiased, the sampling variance for $\hat{U}$ is the smallest in selfing populations with the Deng–Lynch technique, and that for $\hat{s}$ by the Bateman–Mukai technique is only slightly smaller than by the Deng–Lynch technique in selfing populations. For outcrossing populations studied using the Deng–Lynch technique, the results show that sampling variance changes little with the three experimental designs simulated. The sampling variance of the Morton–Charlesworth technique is smaller than that of the other two, but the estimates are slightly biased.

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Table 4. *Comparison of methods under constant fitness effects* ($U = 1{\cdot}0$, $h = 0{\cdot}36$ *and* $s = 0{\cdot}03$)

Techniques employed and the experiment design	$\hat{U}$	$\hat{h}$	$\hat{s}$
B–M ($L = 100$, $T = 10$, $I = 10$)	1·016	—	0·0299
	(0·133)		(0·0038)
M–C (outcrossing populations)	0·964	0·357	—
$H = 10$, $N = 485$, $O = 1$	(0·012)	(0·000)	—
M–C (selfing populations)	0·970	0·357	—
$H = 10$, $N = 323$	(0·034)	(0·000)	—
D–L (outcrossing populations)			
1. $N = 50$, $O = 19$	1·015	0·359	0·0298
	(0·187)	(0·017)	(0·0094)
2. $N = 40$, $O = 24$	0·987	0·357	0·0293
	(0·180)	(0·016)	(0·0094)
3. $N = 25$, $O = 39$	0·941	0·352	0·0332
	(0·146)	(0·014)	(0·0113)
D–L (selfing populations)	0·995	0·358	0·0309
$N = 333$	(0·115)	(0·014)	(0·0050)

B–M, M–C and D–L, respectively, denote the techniques of Bateman–Mukai, Morton–Charlesworth and Deng–Lynch. L, T, I are denoted as in the note to Table 2. H is the number of pairs of homozygotes used to estimate h. N is the number of genotypes sampled in outcrossing/selfing populations. O is the number of selfed offspring obtained from each outcrossed parent. For the Morton–Charlesworth technique, ln(fitness) is used to estimate h.

4. Statistical properties with variable fitness effects

It seems clear that s and h are not constant across loci, and the few available data suggest that s has a roughly leptokurtic distribution (Gregory, 1965; Mackay *et al.*, 1992; Keightley, 1994). As in Deng & Lynch (1996), we model the mutation rate for different $s(f(s))$ as:

$$f(s) = \frac{1}{\bar{s}}\exp(-s/\bar{s}), \quad (1 > s > 0), \tag{9a}$$

where $\bar{s}^2 = V_s$. This is an exponential distribution, which is a special form of the gamma distribution modelled by Keightley (1994). We adopt it as a special alternative to the constant effects. It is used for modelling the non-lethal mutations, since lethal mutations appear to represent a true discontinuity in the distribution of mutant effects (Crow & Simmons, 1983). The effect of lethals on the estimation can be minimized by eliminating extreme lines as practised/suggested with the Bateman–Mukai (Mukai *et al.*, 1972) and Deng–Lynch (Deng & Lynch, 1996) techniques. Little information exists on the distribution of h, but biochemical arguments suggest an inverse relationship between s and h, mutant alleles with larger effects tending to be more recessive (Kacser & Burns, 1981). Therefore, we approximate this relationship as

$$h = \frac{1}{2}\exp(-13s). \tag{9b}$$

This function yields $h = 0{\cdot}36$ when $s = 0{\cdot}03$, $h \to 0{\cdot}5$ as $s \to 0{\cdot}0$, and $h \to 0{\cdot}0$ as $s \to 1{\cdot}0$ – all in rough accordance with the few available data (Crow & Simmons, 1983).

With variable fitness effects and $U = 1{\cdot}0$, $\bar{h} = 0{\cdot}36$ and $\bar{s} = 0{\cdot}03$, we studied the respective techniques, then compared them with approximately 1000 genotypes handled in the experiments. Simulations are the same as before, except that s is variable.

(i) *The Bateman–Mukai technique*

s is randomly sampled from the exponential distribution of (9*a*). $\hat{U}$ is $\sim 0{\cdot}5\,U$, $\hat{\bar{s}} \sim 2\bar{s}$ (Table 2), as expected theoretically (Mukai *et al.*, 1972). Again, if the genotypes can be measured accurately, increasing the total number of M-A generations (T) from 10 to 40 gives little improvement in the estimates, nor does increasing the number of assays (Table 2).

(ii) *The Morton–Charlesworth technique*

We use a discrete version of the exponential distribution of (9*a*) by dividing the entire range of s (0, 1) into 200 classes of width 0·005 (Deng & Lynch, 1996). Each parent is randomly assigned a number of mutations from each of these classes by drawing from a Poisson distribution with an expectation of $Up_i/(h_i s_i)$ in outcrossing and $Up_i/(2s_i)$ in selfing populations, where p_i is the density of the mutational

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Table 5. *Estimating $\bar{h}$ by Mukai's regression method and U under variable fitness effects*

			Regression of fitness for estimating *h*		Regression of ln(fitness) for estimating *h*	
U	$\bar{h}$	$\bar{s}$	$\hat{U}$	$\hat{\bar{h}}$	$\hat{U}$	$\hat{\bar{h}}$
Outcrossing populations (*Morton's technique*)						
1·5	0·44	0·01	−1·492	0·592	0·697	0·377
			(0·214)	(0·013)	(0·067)	(0·008)
	0·30	0·05	−2·949	9·661	0·273	0·045
			(0·120)	(2·824)	(0·052)	(0·008)
0·5	0·44	0·01	0·570	0·440	0·233	0·376
			(0·109)	(0·008)	(0·029)	(0·008)
	0·30	0·05	0·581	0·322	0·093	0·045
			(13·078)	(0·050)	(0·018)	(0·007)
Selfing populations (*Charlesworth's technique*)						
1·5	0·44	0·01	1·222	0·427	0·796	0·389
			(0·177)	(0·008)	(0·089)	(0·007)
	0·30	0·05	1·168	0·244	0·909	0·172
			(0·097)	(0·015)	(0·063)	(0·012)
0·5	0·44	0·01	0·306	0·405	0·265	0·390
			(0·053)	(0·007)	(0·044)	(0·008)
	0·30	0·05	0·348	0·204	0·315	0·174
			(0·036)	(0·015)	(0·032)	(0·015)

The simulation conditions are the same as those for Table 3 (see text), except that 100 random pairs of homozygotes and their crossed progeny are used to estimate $\bar{h}$.

Table 6. *Estimation by the Deng–Lynch technique under variable fitness effects*

U	$\bar{h}$	$\bar{s}$	$\hat{U}$	$\hat{\bar{h}}$	$\hat{\bar{s}}$
Outcrossing populations					
1·5	0·44	0·01	0·784 (0·054)	0·387 (0·006)	0·0202 (0·0026)
	0·30	0·05	0·543 (0·040)	0·122 (0·004)	0·1356 (0·0159)
0·5	0·44	0·01	0·262 (0·025)	0·386 (0·009)	0·0200 (0·0027)
	0·30	0·05	0·281 (0·020)	0·117 (0·007)	0·1411 (0·0182)
Selfing populations					
1·5	0·44	0·01	0·943 (0·238)	0·402 (0·019)	0·0169 (0·0044)
	0·30	0·05	1·045 (0·055)	0·216 (0·013)	0·0728 (0·0088)
0·5	0·44	0·01	0·308 (0·087)	0·400 (0·023)	0·0175 (0·0051)
	0·30	0·05	0·358 (0·022)	0·217 (0·012)	0·0700 (0·0085)

For outcrossing populations, each simulation is based on 200 random parents, from each of which 40 random selfed offspring are obtained as described in the text. For selfing populations, each simulation is based on 200 random pairs of parents and their outcrossed progeny.

distribution in the *i*th class. The results are summarized in Table 5.

In outcrossing populations. Using fitness in the regression to estimate $\bar{h}$ results in ridiculous values of $\hat{U}$ and $\hat{\bar{h}}$ when $U = 1{\cdot}5$, and reasonable estimates when $U = 0{\cdot}5$ and $\bar{s} = 0{\cdot}01$ (when $\bar{s} = 0{\cdot}05$, the sampling error for $\hat{U}$ is very high due to the larger V_s). When $\bar{s} = 0{\cdot}01$, using ln(fitness) to estimate $\bar{h}$ results in $\hat{U}$ of $\sim 0{\cdot}5\,U$ and $\hat{\bar{h}}$ of $\sim 0{\cdot}86\,\bar{h}$. When $\bar{s}$ gets larger ($\bar{s} = 0{\cdot}05$), so does V_s, the bias gets much larger, $\hat{U}$ and $\hat{\bar{h}}$ are $\sim 0{\cdot}18\,U$ and $0{\cdot}15\,\bar{h}$ respectively.

In selfing populations. Using fitness to estimate $\bar{h}$ results in $\hat{U}$ of $\sim 0{\cdot}6\,U$ to $0{\cdot}8\,U$ and $\hat{\bar{h}}$ of $\sim 0{\cdot}65\,\bar{h}$ to $0{\cdot}95\,\bar{h}$. Using ln(fitness) to estimate $\bar{h}$ results in $\hat{U}$ of $\sim 0{\cdot}55\,U$ to $0{\cdot}60\,U$ and $\hat{\bar{h}}$ of $\sim 0{\cdot}58\,\bar{h}$ to $0{\cdot}89\,\bar{h}$. The sampling variances are relatively small.

Therefore, under variable fitness effects of mutations, applying the Morton–Charlesworth tech-

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Table 7. *Comparison under variable fitness effects* ($U = 1{\cdot}0$, $\bar{h} = 0{\cdot}36$, $\bar{s} = 0{\cdot}03$)

Techniques employed and the experimental design	$\hat{U}$	$\hat{\bar{h}}$	$\hat{\bar{s}}$
B–M ($L = 100$, $T = 10$, $I = 10$)	0·519 [0·238]	—	0·0594 [0·9·5E-4]
M–C (outcrossing populations):			
$H = 100$, $N = 350$, $O = 1$	0·340 [0·437]	0·173 [0·035]	—
	−3·273 [18·79]	0·624 [0·072]	
$H = 200$, $N = 200$, $O = 1$	0·345 [0·430]	0·175 [0·034]	
	−3·257 [18·790]	0·632 [0·075]	
M–C (selfing populations):			
$H = 100$, $N = 233$	0·569 [0·187]	0·253 [0·012]	—
	0·703 [0·092]	0·300 [0·004]	
$H = 200$, $N = 133$	0·569 [0·187]	0·253 [0·012]	—
	0·704 [0·091]	0·300 [0·004]	
D–L (outcrossing populations):			
1. $N = 50$, $O = 19$	0·574 [0·197]	0·234 [0·017]	0·0597 [0·0012]
2. $N = 40$, $O = 24$	0·583 [0·195]	0·236 [0·016]	0·0606 [0·0013]
3. $N = 25$, $O = 39$	0·557 [0·212]	0·231 [0·017]	0·0609 [0·0015]
D–L (selfing populations)			
$N = 333$	0·650 [0·123]	0·284 [0·006]	0·047 [0·0004]

Numbers within square brackets are the associated *MSE*s. B–M, M–C, D–L, H, N and O are defined in the note to Table 4, and L, T, I in the note to Table 2. For populations applied with the Morton–Charlesworth technique, results are given for two experimental designs, with each given two results (the upper one is for the estimate obtained by using ln(fitness) to estimate $\bar{h}$, and the lower one for that obtained by using fitness). For outcrossing populations studied using the Deng–Lynch technique, results are given for three experimental designs.

Table 8. *Estimation by the Bateman–Mukai technique with multiplicative fitness effects*

		Constant fitness effects ($V_s = 0$)		Variable fitness effects ($V_s = s^{(-2)}$)	
U	$\bar{s}$	$\hat{U}$	$\hat{s}$	$\hat{U}$	$\hat{\bar{s}}$
0·1	0·01	0·105	0·0097	0·053	0·0190
		(0·017)	(0·0013)	(0·011)	(0·0040)
	0·03	0·106	0·0283	0·054	0·0555
		(0·017)	(0·004)	(0·011)	(0·0110)
	0·05	0·108	0·0458	0·055	0·0901
		(0·018)	(0·0058)	(0·011)	(0·0169)
0·5	0·01	0·536	0·0093	0·274	0·0181
		(0·087)	(0·0013)	(0·043)	(0·0027)
	0·03	0·592	0·0240	0·301	0·0471
		(0·097)	(0·0033)	(0·045)	(0·0063)
	0·05	0·653	0·0345	0·330	0·0681
		(0·109)	(0·0047)	(0·049)	(0·0085)
1·0	0·01	1·124	0·0086	0·550	0·0177
		(0·147)	(0·0011)	(0·080)	(0·0026)
	0·03	1·377	0·0191	0·669	0·0394
		(0·183)	(0·0024)	(0·094)	(0·0053)
	0·05	1·694	0·0239	0·815	0·0491
		(0·233)	(0·0030)	(0·116)	(0·0064)

For each simulation, 100 M-A lines are accumulated for 10 generations and assayed at the outset and the end of the M-A.

nique to outcrossing populations results in ridiculous estimates if ln(fitness) is used to estimate $\bar{h}$, and estimates that are sensitive to the parameters if fitness is used. In selfing populations, using fitness to estimate $\bar{h}$ results in better estimates of $\bar{h}$ and U than using ln(fitness). Note that this is quite different from the situation with constant fitness effects. This is because, under variable fitness effects, $\bar{h}$ and U are always underestimated, and $\hat{U}$ and $\hat{\bar{h}}$ estimated by using fitness in the regression are always larger than those

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using ln(fitness) (Tables 3, 5). Hence, whether to use fitness or ln(fitness) to estimate $\bar{h}$ largely depends on whether fitness effects are constant or variable, and probably on how variable they are.

(iii) *The Deng–Lynch technique*

All of the values of $\hat{U}$ and $\hat{\bar{h}}$ are underestimated and those of $\hat{\bar{s}}$ overestimated; the degree of bias depends on the parameters of U, $\bar{h}$ and $\bar{s}$ (Table 6). In outcrossing populations, $\hat{U}$ is $\sim 0{\cdot}36\ U$ to $0{\cdot}56\ U$, $\hat{\bar{h}} \sim 0{\cdot}41\ \bar{h}$ to $0{\cdot}88\ \bar{h}$, and $\hat{\bar{s}} \sim 2{\cdot}0\ \bar{s}$ to $2{\cdot}8\ \bar{s}$. In selfing populations, $\hat{U}$ is $\sim 0{\cdot}63\ U$ to $0{\cdot}73\ U$, $\hat{\bar{h}} \sim 0{\cdot}72\ \bar{h}$ to $0{\cdot}91\ \bar{h}$ and $\hat{\bar{s}} \sim 1{\cdot}40\ \bar{s}$ to $1{\cdot}75\ \bar{s}$. The sampling variances for all the estimates are reasonably small.

(iv) Comparison of the techniques

Since all the estimates are biased, we compute their *MSE* (mean square error) for comparison:

$$MSE = E(\hat{x} - E(x))^2 = \mathrm{Var}(\hat{x}) + (\hat{\bar{x}} - E(x))^2,$$

where $\hat{\bar{x}}$ stands for the estimated mean. Note that when $\hat{\bar{x}}$ is unbiased, *MSE* is simply the variance of $\hat{x}$.

By comparing *MSE*s, several conclusions emerge (Table 7): (1) For the Morton–Charlesworth and Deng–Lynch techniques, the different experimental designs simulated have negligible effects on estimation. (2) For selfing populations, better estimates of U and $\bar{h}$ will be achieved by the Deng–Lynch than by the Morton–Charlesworth technique if ln(fitness) is used in the regression to estimate $\bar{h}$. The opposite is true if fitness is used. (3) For outcrossing populations, better estimates of U and $\bar{h}$ are obtained by the Deng–Lynch than by the Morton–Charlesworth technique. (4) For estimating U, the Deng–Lynch and Morton–Charlesworth (for selfing populations) techniques are better than the Bateman–Mukai technique, which is better than the Morton–Charlesworth technique for outcrossing populations. (5) For estimating $\bar{s}$, the Deng–Lynch technique applied to selfing populations is better than the Bateman–Mukai technique, which is slightly better than the Deng–Lynch technique applied to outcrossing populations.

5. The Bateman–Mukai technique with multiplicative fitness effects

We lack the knowledge of the mode of gene action underlying fitness. However, there is good evidence that genes for fitness or its components may act multiplicatively, at least as a first approximation (Morton *et al*., 1956; Crow, 1986; Fu & Ritland, 1996). If this is indeed so, a logarithmic transformation of data will solve the problem in employing the Bateman–Mukai technique, as is supported by simulation data (not shown). Unfortunately, *a priori* knowledge of the real gene action is generally lacking, and previous M-A experiments (Mukai *et al*., 1972) assumed additive fitness effects. Therefore, there is a need to study the Bateman–Mukai technique assuming additive fitness effects, but with real effects being multiplicative.

With multiplicative fitness effects, for a homozygous M-A line $W = (1-s)^n \approx 1-ns$ with small s and n, where n is the number of mutations accumulated. Thus, estimation by the Bateman–Mukai technique should be relatively robust to different fitness effects, if n and s are small. We perform simulations for both constant and variable effects where s is exponentially distributed (Table 8).

For constant effects, when U and s are small (e.g. $U = 0{\cdot}1$ and $s = 0{\cdot}01$), the bias of $\hat{U}$ and $\hat{s}$ is very small. When U and s get larger, so does the bias, due to the poorer approximation of $W = (1-s)^n \approx 1-ns$, where n is smaller with smaller U. With $U = 1{\cdot}0$ and $s = 0{\cdot}05$, $\hat{U}$ is $\sim 1{\cdot}7\ U$ and $\hat{s} \sim 0{\cdot}48\ s$.

For the variable effects, when U and $\bar{s}$ are small (e.g. $U = 0{\cdot}1$ and $\bar{s} = 0{\cdot}01$), $\hat{U}$ is $\sim 0{\cdot}5\ U$ and $\hat{\bar{s}} \sim 2\ \bar{s}$, similar to that with additive effects (Table 2). Interestingly, when U and $\bar{s}$ get larger, the bias of $\hat{U}$ and $\hat{\bar{s}}$ get *smaller*. With $U = 1{\cdot}0$ and $\bar{s} = 0{\cdot}05$, $\hat{U}$ is $\sim 0{\cdot}82\ U$ and $\hat{\bar{s}} \sim 0{\cdot}96\ \bar{s}$. This is because, under variable fitness effects alone, U is underestimated and s overestimated (Table 2); while under multiplicative fitness effects alone, U is overestimated and s underestimated. The two sources of bias act in different directions and decrease the overall degree of bias when acting simultaneously.

In summary, when fitness effects are multiplicative, but additivity is assumed, U will generally be overestimated and s underestimated with constant effects. With variable effects, U will generally be underestimated and $\bar{s}$ overestimated, but interestingly the bias is smaller than with the assumed additive effects by the technique.

6. Discussion

Our results show that, under constant effects, the Bateman–Mukai and Deng–Lynch techniques are unbiased; the Morton–Charlesworth technique is very biased if fitness is used to estimate h, and slightly biased if ln(fitness) is used. Under variable effects, all three techniques are biased. The Deng–Lynch technique is statistically better than the others, except when *fitness* is used in the regression to estimate $\bar{h}$ in selfing populations by the Morton–Charlesworth technique. When fitness effects are multiplicative but additivity is assumed, the Bateman–Mukai technique is biased under constant effects and less biased under variable effects relative to when fitness effects are

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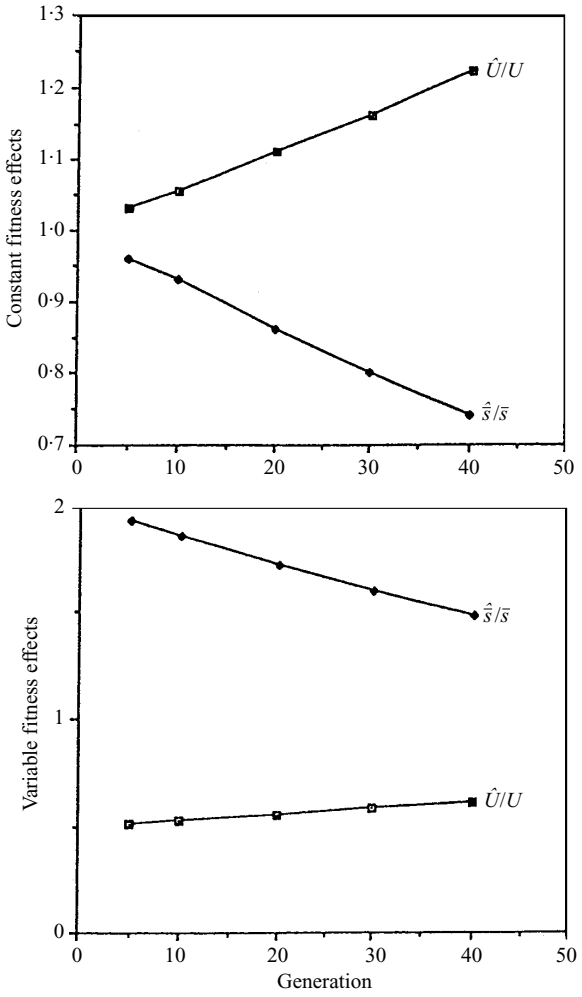

Fig. 2. Change of the estimation bias with M-A generations by the Bateman–Mukai technique when fitness effects are multiplicative but additivity is assumed. Data for each point are obtained by 100 simulations, with each using 10000 M-A lines. The upper plot is for constant fitness effects, and the lower one for variable fitness effects (according to the exponential distribution).

additive as assumed by the technique. The reasons underlying the bias for each technique are detailed in the previous sections. The observed differences in statistical performance among the approaches are largely due to the different genetic processes utilized (M-A, change of fitness moments at M–S balance, etc.) and the different complex estimation methods developed.

Compared with the others, the Bateman–Mukai technique does not need to assume M–S balance. However, it requires a much longer time and also much more labour, even with the same number of genotypes involved in the experiments. This is partly because of the need to raise large controls for temporal environmental changes, and to raise backups in case of accidental line losses (Mukai *et al.*, 1972). Lines need to be replicated in order to estimate genetic variance. Thus, increasing the number of assays greatly increases the cost but does not necessarily improve the estimation (Tables 2, 5), although it may shed some light on the mode of gene action (Mukai, 1964). Without knowing the fitness effects of mutations, increasing M-A generations may result in estimation of larger deviations from analytical predictions. This is because, for example, if fitness effects are multiplicative but additivity is assumed, increasing M-A generations results in progressively larger bias under constant effects and progressively smaller bias than predicted under variable effects (Fig. 2). An important message from this is that, if fitness effects are variable and do act multiplicatively, the common practice of inferring U to be twice the value of $\hat{U}$ from M-A by assuming constant and additive effects (Mukai *et al.*, 1972; Lynch *et al.*, 1995) *overestimates* U. The degree of overestimation depends on the number of M-A generations (Fig. 2). Again, this is because the approximation $W = (1-s)^n \approx 1-ns$ holds better for smaller n; and n is, on average, smaller with a smaller number of M-A generations. In our simulations, 100 M-A lines, 10 M-A generations and two assays may yield reasonable estimations (Table 2). This is interesting. However, it assumes that genotypic values can be measured accurately with many replicates for each genotype. Since there is a growing interest in performing M-A, extensive research is needed to concentrate on studying optimal M-A designs by considering the number of replicates for each genotype and the number of M-A generations under different cost schemes and with different mutation parameters.

The Deng–Lynch technique estimates all three essential parameters; additionally, it may provide other useful information, such as the input of genetic variation introduced by mutation per generation (V_m), and the mean number of mutations per genome in the population (Deng & Lynch, 1996, 1997). The Bateman–Mukai technique *per se* can estimate V_m *for homozygous mutation lines*, but it cannot estimate V_m in outcrossing populations (such as *Drosophila*) in nature, unless h is also estimated by additional crossing experiments. Then our formulae linking V_m with U, h and s (Deng & Lynch, 1996) can be employed to estimate V_m for outcrossing populations. Nevertheless, the larger amount of information that the Deng–Lynch technique generates involves no cost in accuracy as reflected by the comparison of the variances and *MSE*s (Tables 4, 7). Although the Morton–Charlesworth technique sometimes results in slightly better estimation in selfing populations, it is compromised by its dependency on whether fitness or ln(fitness) should be used to estimate h.

We concentrate on studying the most plausible case of multiplicative fitness effects. If the fitness effects of mutations are additive, the techniques developed for natural populations are not recommended, as a large bias will result as revealed by our simulation (not

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shown here). Although epistatic fitness effects may be possible, little convincing information exists on the subject. We therefore do not study its effects here. The effects of synergistic epistasis on estimation have been investigated and it was found that U will be overestimated by the Charlesworth technique (assuming a known h; Charlesworth *et al*., 1990), and U and h will be underestimated and s overestimated by the Deng–Lynch technique (Deng & Lynch, 1996). However, the effects of even strong synergism are slight (Charlesworth *et al*., 1990; Deng & Lynch, 1996).

The techniques of Morton–Charlesworth and Deng–Lynch makes the same assumptions – M–S equilibrium, large population size, unlinked loci, etc., – whose limitation, validity and effects on estimation have been discussed (Charlesworth *et al*., 1990; Deng & Lynch, 1996). Deng & Lynch (1996) also discussed the effects on estimates of a pool of very mildly deleterious but effectively neutral mutations (those with $s < 1/(4N_e)$, where N_e is the effective population size), and concluded that they are unlikely to be significant. Some practical issues (experimental design, elimination of maternal effects, estimation of sampling variance, etc.) have also been discussed for the Deng–Lynch technique (Deng & Lynch, 1996, 1997). The effects of a small proportion of lethal mutations on estimation have been simulated for the Deng–Lynch technique (Deng & Lynch, 1996, 1997), and discussed for data analyses using the Bateman–Mukai technique (Mukai *et al*., 1972). For the techniques of Morton–Charlesworth and Deng–Lynch, it is assumed that the net fitness can be measured or that the fitness component under study is largely independent of the other fitness components. Net fitness is usually difficult to measure, if not entirely impossible. Pleiotropic effects underlying fitness components can be a source of bias (Charlesworth & Hughes, 1997) if only one of the correlated fitness components is studied. For the Deng–Lynch technique, cloning of genotypes is currently required in order to estimate the total genetic variance in the parental and offspring generations. In highly selfing plant populations, this can be easily achieved. Multiple seeds resulting from each homozygous parental line will, upon further selfing, be genetically identical clones of their parental line. Multiple seeds resulting from crossing two homozygous parental lines will also be genetically identical clones, which form the outcrossed offspring generation. In outcrossing populations such as those of cyclical parthenogenetic populations, cloning of genotypes can be easily achieved by asexual reproduction (Deng, 1995; Deng & Lynch, 1997). Additionally, for outcrossing populations, we have been developing techniques in which cloning of genotypes in parent/inbred offspring generations may not be necessary.

For the two techniques applicable to natural outcrossing populations, the crucial assumption that the variation of fitness is maintained by M–S balance needs to be examined. Alternatives to M–S balance, such as functional overdominance or overdominance induced by fluctuating selection can, in principle, maintain polymorphisms, although no strong case has emerged for their generality. The evidence for functional overdominance does not seem to be very convincing, most cited examples being compatible with associated overdominance, an artefact of linked deleterious recessive genes (Houle, 1989, 1994). Data bearing directly on the issue of the maintenance of variation by fluctuating selection are essentially nonexistent. By reviewing and comparing all the available data on the mutational variabilities from seven distinct species, Houle *et al*. (1996) concluded that the standing pattern of genetic variability is consistent with an M–S balance model. Charlesworth & Hughes (1997) reviewed the results of over 25 years of investigations of quantitative genetics of *D. melanogaster* life-history traits, concluding that there is probably a substantial contribution from deleterious alleles maintained by mutation to the standing genetic variance for fitness or related traits, and to the genetic load revealed by inbreeding. The well-corroborated data from studies of molecular population genetics in *Drosophila* and emerging data in mice and humans suggest that polymorphism is maintained/operated upon under dominance (rather than overdominance) selection in most genomic regions of these organisms (Deng *et al*., 1998). Briefly, this is because a positive correlation between molecular heterozygosity and the regional recombination rate across the genome is expected under dominance selection and is usually observed (Deng *et al*., 1998). Nevertheless, mechanisms responsible for the maintenance of genetic variance are complex and may differ among populations. A critical question is how robust the techniques are with different degrees of violation of the M–S balance assumption. Extensive studies are needed and are being pursued. Some progress has been made. An algorithm has been developed (Deng, 1998) that can be employed to investigate the effects of overdominance (including balancing selection) on estimation. The violation of M–S balance may not be as substantial as envisioned. This is suggested by our results that the estimation of h with Mukai's regression method (Mukai *et al*., 1972) is not greatly biased by the violation of the M–S balance assumption: even if half the inbreeding depression is due to loci under overdominance or balancing selection, h estimated for the loci under dominance is about 70% of the true value (Deng, 1998).

Besides the statistical properties investigated in this paper, different approaches have different advantages and drawbacks in practice, and are best applicable to

different organisms and in different situations. The estimates obtained by different approaches in different organisms will be able to crosscheck each other and, it is hoped, eventually resolve the issues concerning the genomic mutations.

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References

Bateman, A. J. (1959). The viability of near-normal irradiated chromosomes. *International Journal of Radiation Biology* **1**, 170–180.

Burger, R. & Hofbauer, J. (1994). Mutation load and quantitative genetic traits. *Journal of Mathematical Biology* **32**, 193–218.

Charlesworth, B. (1990). Mutation–selection balance and the evolutionary advantage of sex and recombination. *Genetical Research* **55**, 199–221.

Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.

Charlesworth, B. & Hughes, K. A. (1997). The maintenance of genetic variation in life-history traits. In *Evolutionary Genetics from Molecules to Morphology* (ed. R. S. Singh & C. B. Krimbas. Cambridge: Cambridge University Press (in press).

Charlesworth, B., Charlesworth, D. & Morgan, M. T. (1990). Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* **347**, 380–382.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. (1993). The effects of deleterious mutations on neutral molecular variation. *Genetics* **134**, 1289–1303.

Charlesworth, D., Lyons, E. E. & Litchfield, L. B. (1994). Inbreeding depression in two highly inbreeding populations of *Leavenworthia*. *Proceedings of the Royal Society of London*, *Series B* **258**, 209–214.

Charlesworth, D., Charlesworth, B. & Morgan, M. T. (1995). The pattern of neutral molecular variation under the background selection model. *Genetics* **141**, 1619–1632.

Crow, J. F. (1986). *Basic Concepts in Population*, *Quantitative and Evolutionary Genetics*. New York: W. H. Freeman.

Crow, J. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. New York: Harper & Row.

Crow, J. F. & Simmons, M. J. (1983). The mutation load in *Drosophila*. In *The Genetics and Biology of* Drosophila (ed. M. Ashburner, H. L. Carson & J. N. Thompson), vol. 3c, pp. 1–35. New York: Academic Press.

Deng, H.-W. (1995). Sexual reproduction in *Daphnia*: its control and genetic consequences. PhD thesis, University of Oregon.

Deng, H.-W. (1998). Estimating (over)dominance coefficient and discriminating dominance vs. overdominance as the genetic cause of heterosis. *Genetics* **148**: 2003–2014.

Deng, H.-W. & Lynch, M. (1996). Estimation of the genomic mutation parameters in natural populations. *Genetics* **144**, 349–360.

Deng, H.-W. & Lynch, M. (1997). Inbreeding depression and inferred deleterious mutation parameters in *Daphnia*. *Genetics* **147**, 147–155.

Deng, H.-W., Fu, Y.-X. & Lynch, M. (1998). Inferring the major genomic mode of dominance and overdominance. *Genetica* (in the Press).

Fu, Y.-B. & Ritland, K. (1996). Marker-based inference about epistasis for gene influencing inbreeding depression. *Genetics* **144**, 339–348.

Gregory, W. C. (1965). Mutation frequency, magnitude of change and the probability of improvement in adaptation. *Radiation Botany* **5** (Suppl.), 429–441.

Haldane, J. B. S. (1937). The effects of variation on fitness. *American Naturalist* **71**, 337–349.

Houle, D. (1989). Allozyme-associated heterosis in *Drosophila melanogaster*. *Genetics* **123**, 789–801.

Houle, D. (1994). Adaptive distance and the genetic basis of heterosis. *Evolution* **48**, 1410–1417.

Houle, D., Morikawa, B. & Lynch, M. (1996). Comparing mutational variabilities. *Genetics* **143**, 1467–1483.

Hudson, R. & Kaplan, N. L. (1995). Deleterious background selection with recombination. *Genetics* **141**, 1605–1617.

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. & Schoen, D. J. (1995). Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* **267**, 226–229.

Kacser, H. & Burns, J. A. (1981). The molecular basis of dominance. *Genetics* **97**, 639–666.

Keightley, P. D. (1994). The distribution of mutation effects on viability in *Drosophila melanogaster*. *Genetics* **138**, 1315–1322.

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

Kimura, M., Maruyama, T. & Crow, J. F. (1963). The mutation load in small populations. *Genetics* **48**, 1303–1312.

Kirkpatrick, M. & Ryan, M. J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature* **350**, 33–38.

Kondrashov, A. S. (1985). Deleterious mutations as an evolutionary factor. II. Facultative apomixis and selfing. *Genetics* **111**, 635–653.

Kondrashov, A. S. (1988). Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–440.

Kondrashov, A. S. & Crow, J. F. (1991). Haploidy or diploidy: which is better? *Nature* **351**, 314–315.

Lande, R. (1994). Risk of population extinction from new deleterious mutations. *Evolution* **48**, 1460–1469.

Lynch, M., Conery, J. & Burger, R. (1995). Mutation accumulation and the extinction of small populations. *American Naturalist* **146**, 489–518.

Lynch, M., Conery, J. & Burger, R. (1996). Mutation meltdowns in sexual populations. *Evolution* **49**, 1067–1080.

Mackay, T. F. C., Lyman, R. F. & Jackson, M. S. (1992). Effects of *P* element insertions on quantitative traits in *Drosophila melanogaster*. *Genetics* **130**, 315–322.

Morton, N. E., Crow, J. F. & Muller, H. J. (1956). An estimate of the mutational damage in man from data on consanguineous marriages. *Proceedings of the National Academy of Sciences of the USA* **42**, 855–863.

Mukai, T. (1964). Spontaneous mutation rate of polygenes controlling viability. *Genetics* **50**, 1–19.

Mukai, T. (1979). Polygenic mutations. In *Quantitative Genetic Variation* (ed. J. N. Thompson, Jr & J. M. Thoday), pp. 177–196. New York: Academic press.

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**, 335–355.
Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research* **1**, 1–9.
Perrot, V., Richerd, S. & Valero, M. (1991). Transition from haploidy to diploid. *Nature* **351**, 315–317.
Simmons, M. J. & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11**, 49–78.