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Analysis of the biases in the estimation of deleterious mutation parameters from natural populations at mutation–selection balance

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Summary

Indirect estimates of the genomic rate of deleterious mutations (λ), their average homozygous effect (s) and their degree of dominance (h) can be obtained from genetic parameters of natural populations, assuming that the frequencies of the loci controlling a given fitness trait are at mutation–selection equilibrium. In 1996, H.-W. Deng and M. Lynch developed a general methodology for obtaining these estimates from inbreeding/outbreeding experiments. The prediction of the sign and magnitude of the biases incurred by these estimators is essential for a correct interpretation of the empirical results. However, the assessment of these biases has been tested so far under a rather limited model of the distribution of dominance effects. In this paper, the application of this method to outbred populations is evaluated, focusing on the level of variation in h values (σ_h^2) and the magnitude of the negative correlation ($r_{s,h}$) between s and h. It is shown that the method produces upwardly biased estimates of λ and downwardly biased estimates of the average s in the reference situation where $r_{s,h}=0$, particularly for large values of σ_h^2, and biases of different sign depending on the magnitude of the correlation. A modification of the method, substituting the estimates of the average h for alternative ones, allows estimates to be obtained with little or no bias for the case of $r_{s,h}=0$, but is otherwise biased. Information on $r_{s,h}$ and σ_h^2, gathered from mutation-accumulation experiments, suggests that σ_h^2 may be rather large and $r_{s,h}$ is usually negative but not higher than about $-0{\cdot}2$, although the data are scarce and noisy, and should be used with caution.

1. Introduction

A crucial task for evolutionary genetics is to understand the genetic architecture of fitness-related traits and how their genetic variation is maintained in natural populations (Charlesworth & Hughes, 2000). Because most mutations affecting fitness are deleterious (Keightley & Lynch, 2003; but see Shaw *et al.*, 2003), the rate of occurrence of deleterious mutations and their homozygous and heterozygous effects critically affect predictions of models, not only in the case of evolution (e.g. Keightley & Eyre-Walker, 2000; Kondrashov, 2001; García-Dorado *et al.*, 2004), but also for conservation (e.g. García-Dorado, 2003; Rodríguez-Ramilo *et al.*, 2006) and genetic improvement of plants and animals (e.g. Keightley, 2004). Direct estimates of the mutation rate (λ), the average homozygous effect ($\bar{s}$) and the average coefficient of dominance ($\bar{h}$) of newly arisen mutations can be obtained from mutation-accumulation experiments where natural selection is partially avoided (for a recent review see García-Dorado *et al.*, 2004). However, indirect estimates can also be inferred with less effort from segregating natural populations (Morton *et al.*, 1956; Charlesworth *et al.*, 1990, 1994; Johnston & Schoen, 1995; Deng & Lynch, 1996, 1997). These latter methods heavily rely on the assumption that deleterious allele frequencies are maintained in the population at the mutation–selection balance (MSB). Nevertheless, the availability of methods for estimating mutational parameters from natural populations by means of simple inbreeding or outbreeding experiments can be

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useful, particularly if these estimates can be compared with others obtained directly from mutation-accumulation experiments.

The method described by Deng & Lynch (1996), based on the original developments of Morton *et al.* (1956) and Charlesworth *et al.* (1990), allows for the simultaneous estimation of λ, $\bar{s}$ and $\bar{h}$ for deleterious mutations from experiments involving outcrossing of naturally self-fertilizing populations or self-fertilization of naturally outbred populations. This procedure makes use of the expected mean fitness and genetic variance of fitness of infinite populations at MSB. Because, even in the absence of balancing selection, estimates of mutational parameters by the method of Deng & Lynch (1996) may be biased, it is important to investigate the sign and magnitude of these biases for a range of possible scenarios. Deng & Lynch (1996) and subsequent analytical and simulation studies carried out by Deng and co-workers (Deng & Lynch, 1997; Deng, 1998*a*, *b*; Deng & Fu, 1998; Li *et al.*, 1999; Deng *et al.*, 1999, 2002; Li & Deng, 2000, 2005) analysed a number of situations, including infinite populations at MSB, large populations that are not at equilibrium, populations with variable selfing rates, finite population size, linkage, epistasis and overdominance. As the method was derived assuming mutations with constant s and h, the above analyses revealed biases when mutations have variable effects. In general, the results for fully outbred or inbred populations under MSB pointed towards an underestimation of the average dominance coefficient, an underestimation of the rate of mutation, and an overestimation of the average mutational effect. However, all the above analyses assumed the same distribution of dominance values: an exponential distribution such that the coefficient of dominance for a given mutation with selection coefficient s is $h=(1/2)\exp(-13\,s)$. This distribution generates a very high negative correlation between s and h values (close to -1) and a relatively low variation in h values (Fernández *et al.*, 2004). Although a negative correlation between s and h values is suggested by the empirical data (Greeenberg & Crow, 1960; Simmons & Crow (1977); Kacser & Burns, 1981; Caballero & Keightley, 1994; Keightley, 1996; Phadnis & Fry, 2005), the magnitude of this correlation is unknown. In addition, information on the variance of h values is also very scarce. As it will be shown, these two parameters have remarkable implications for the sign and magnitude of the biases incurred by the method.

The objective of the present paper is threefold. First, to investigate the sign and magnitude of the biases incurred by the Deng & Lynch (1996) estimators when applied to outbred populations, focusing on the effect of different levels of variation in h and different values of the correlation between s and h. The main focus will be on the biases in the estimation of λ and $\bar{s}$, arising, in turn, from the biases incurred by the estimates of $\bar{h}$, as no available estimator can predict $\bar{h}$ without bias (see, for example, Fernández *et al.*, 2004). Second, to propose a modification of the method such that unbiased estimates of λ and $\bar{s}$ can be obtained, at least for the reference case where there is no correlation between s and h values. And third, to gather estimates of the variance of h and of the correlation between s and h from several mutation-accumulation experiments.

2. Mutational models and parameters, and estimation procedure

(i) *Population scenario*

Let us consider one of the practical scenarios to which the method of Deng & Lynch (1996) can be applied. Assume that a naturally outbred population is sampled and individuals can be cloned and self-fertilized, supplying estimates of fitness (or a fitness trait) for outbred individuals and the average of their selfed progenies. Under this situation, consider the frequencies and genotypic values for a single biallelic locus given in Table 1*A*. The outbred (O) row gives the genotypic values of the three genotypes, and allows the calculation of the mean and variance of fitness for a large random mating population. The selfed (S) row gives the genotypic values for the selfed progeny of individuals from each corresponding genotype, and allows the calculation of the mean and variance of selfed progeny from individuals taken randomly from the outbred population. The last row gives a compound genotypic value equal to 4 times the mean genotypic value of the selfed progeny minus twice the genotypic value of their parent. This compound genotypic value allows us to obtain an estimate of the average coefficient of dominance (Deng, 1998*a*).

For simplicity, consider an additive model of gene action for mutations. In addition, let us assume that there are no sources of decline in fitness other than genetic ones, so that the fitness of an individual free of mutations is 1 (this assumption will have no consequences for most estimators of mutation parameters unless otherwise stated). Under MSB, the approximated mean fitness for outbred ($\overline{W_O}$), selfed ($\overline{W_S}$) and compound genotypes ($\overline{W_T}$), the corresponding variances and covariances, and the expected inbreeding depression (δ) from selfing are shown in Table 1*B*.

(ii) *Estimators of mutational parameters*

The principle behind Deng & Lynch's (1996) method is to obtain an estimate of the average coefficient

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Table 1. *Model, population parameters and estimators used. See text for explanations*

(*A*) Model

Genotype	*AA*	*Aa*	*aa*
Frequency	p^2	$2pq$	q^2
Outbred (O)	1	$1-sh$	$1-s$
Selfed (S)	1	$\frac{1}{4}+\frac{1-sh}{2}+\frac{1-s}{4}$	$1-s$
4S−2O (T)	2	$2-s$	$2-2s$

(*B*) Population parameters

$\overline{W_O} \approx 1-2\lambda$	$\sigma^2_{WO} \approx 2\lambda(\overline{sh})$
$\overline{W_S} \approx 1-\lambda[1+\frac{1}{2}(\overline{1/h})]$	$\sigma^2_{WS} \approx (\lambda/2)[(\overline{s/4h})+(\overline{sh})+\bar{s}]$
$\overline{W_T} \approx 2-2\lambda(\overline{1/h})$	$\sigma^2_{WT} \approx 2\lambda(\overline{s/h})$
$\delta = \overline{W_S}-\overline{W_O} \approx \lambda[1-\frac{1}{2}(\overline{1/h})]$	$\sigma_{WO,S} \approx \lambda[(\overline{sh})+\bar{s}/2]$
	$\sigma_{WO,T} \approx 2\lambda\bar{s}$

(*C*) Estimators for: $\bar{h}$	λ	$\bar{s}$
$\hat{h}_{DL}=\frac{\sigma_{WO}}{4\sigma_{WS}-2\sigma_{WO}} \approx \sqrt{\frac{\overline{sh}}{\overline{s/h}}}$	$\hat{\lambda}_{DL}=\frac{2\hat{h}_{DL}\delta}{2\hat{h}_{DL}-1}$	$\hat{s}_{DL}=\frac{\sigma^2_{WO}}{2\hat{\lambda}_{DL}\hat{h}_{DL}}$
$\hat{h}_R=\frac{\overline{W_O}}{4\overline{W_S}-2\overline{W_O}} \approx \frac{1}{\overline{1/h}}$	$\hat{\lambda}_R=\frac{2\hat{h}_R\delta}{2\hat{h}_R-1}$	$\hat{s}_R=\frac{\sigma^2_{WO}}{2\hat{\lambda}_R\hat{h}_{1/b}}$
$\hat{h}_b=\frac{\sigma_{WO,T}}{\sigma^2_{WT}} \approx \frac{\bar{s}}{\overline{s/h}}$	$\hat{\lambda}_b=\frac{2\hat{h}_b\delta}{2\hat{h}_b-1}$	$\hat{s}_b=\frac{\sigma^2_{WO}}{2\hat{\lambda}_b\hat{h}_{1/b}}$
$\hat{h}_{1/b}=\frac{\sigma^2_{WO}}{4\sigma_{WO,S}-2\sigma^2_{WO}} \approx \frac{\overline{sh}}{\bar{s}}$		

of dominance of mutations, $\hat{h}$, to be included in the following expression for the estimation of the rate of deleterious mutations:

$$\hat{\lambda}=\frac{2\hat{h}\delta}{2\hat{h}-1}. \quad (1)$$

Then, both estimates ($\hat{h}$ and $\hat{\lambda}$) are used to estimate the average coefficient of selection of mutations by means of

$$\hat{s}=\frac{\sigma^2_{WO}}{2\hat{\lambda}\hat{h}}. \quad (2)$$

Deng & Lynch (1996) used an estimator for the average h ($\hat{h}_{DL}$; see Table 1*C*) which, under MSB, provides estimates of $\sqrt{\overline{sh}/\overline{(s/h)}}$ (Fernández *et al.*, 2005). Note that this estimator provides estimates for neither the arithmetic mean of h values, $\bar{h}$, nor the harmonic mean, $1/(\overline{1/h})$. If all mutations have the same constant s and h values, $\hat{h}_{DL}$ provides the true $\bar{h}$, and unbiased estimates of λ and $\bar{s}$ are obtained from equations (1) and (2) ($\hat{\lambda}_{DL}$ and $\hat{s}_{DL}$ in Table 1*C*). In contrast, when s and h are variable, the estimates from Deng & Lynch (1996) are biased to some degree, as has been shown by these authors. However, they seem to have overlooked the fact that the biases occur even if there is no correlation between s and h values ($r_{s,h}=0$). Note that, in order to estimate the mutation rate from (1), the estimate of the average h that has to be used is the harmonic mean of h, i.e. $\hat{h}=1/(\overline{1/h})$. Inserting this value and that for δ into (1), we find that $\hat{\lambda}=\lambda$, i.e. an unbiased estimate of the rate of mutation is obtained. However, using $\hat{h}_{DL}$ as $\hat{h}$ in (1) will introduce bias in the estimate of λ and, in turn, in the estimate of $\bar{s}$ from (2), even if $r_{s,h}=0$. In addition, the estimate of the average h that should be used in equation (3) for the estimation of $\bar{s}$ is the arithmetic mean of h values, i.e. $\bar{h}$. Again, the use of $\hat{h}_{DL}$ will contribute to the bias of the estimates of $\bar{s}$ even if $r_{s,h}=0$.

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Alternative estimates of the average h can be used that may remove the above bias incurred by using $\hat{h}_{DL}$ Under the assumption of MSB, the ratio $(W_{\max}-\overline{W_O})/[2(W_{\max}-\overline{W_I})]$, where W_{max} is the fitness of a genotype free of mutations and $\overline{W_O}$ and $\overline{W_I}$ are the mean fitnesses for outbred and inbred populations (Lynch & Walsh, 1998, pp. 283–287), estimates the unweighted harmonic mean of dominance for newly arisen mutations if W_{max} is assumed to be 1. Therefore, this would be an appropriate estimate to be used for the estimation of λ from equation (1). The corresponding expression of this estimator of the harmonic mean of h for outbred populations subject to selfing ($\hat{h}_R$) is shown in Table 1*C*. Unfortunately, the reliability of this estimator depends on the lack of non-genetic sources of mortality (i.e. $W_{max}=1$). If these are substantial, the estimates will be expected to be biased upwards (Lynch & Walsh, 1998; García-Dorado *et al*., 1999; Fernández *et al*., 2004, 2005). An alternative estimator free of this source of bias can be obtained from the regression of the heterozygous genotypic values (y) for fitness on the sum of their corresponding homozygous genotypic values (x), $b_{y.x}=\sigma_{x,y}/\sigma_x^2$ (Mukai, 1969; Mukai *et al*., 1972; Mukai & Yamaguchi, 1974; García-Dorado & Caballero, 2000) which, under MSB, provides an estimate of the harmonic mean of h values weighted by their homozygous effects. For outbred populations subject to selfing, Deng (1998*a*) developed the corresponding estimator ($\hat{h}_b$) shown in Table 1*C*. This will give estimates of the harmonic mean of h values if there is no correlation between s and h. However, it will give biased estimates otherwise.

For the estimation of the average homozygous effects of mutations from equation (2), an estimate of the arithmetic mean of h values is needed in the denominator of the equation, but no estimator provides an unbiased estimate for this average. However, the inverse of the regression of the homozygous on the heterozygous genotypic values ($1/b_{x,y}$) is assumed to estimate, under MSB, the arithmetic mean of h values weighted by their homozygous effects (Mukai & Yamaguchi, 1974). For an outbred population subject to selfing, the corresponding estimator ($\hat{h}_{1/b}$) is given in Table 1*C*. With $r_{s,h}=0$, $\hat{h}_{1/b}$ estimates the arithmetic mean of h values, but it will be biased otherwise.

Three estimators of the deleterious mutation rate and the average selection coefficient from equations (1) and (2) can therefore be considered (see Table 1*C*). The first ones are the estimators from Deng & Lynch (1996) ($\hat{\lambda}_{DL}$, $\hat{s}_{DL}$), using the corresponding estimate of the average coefficient of dominance, $\hat{h}_{DL}$. These estimators are expected to produce biased estimates in most situations, even if there is no correlation between s and h values. In principle, unbiased estimates of λ could be obtained from (1) by using the estimator $\hat{h}_R$ (i.e. $\hat{\lambda}_R$). In addition, for $r_{s,h}=0$, an unbiased estimate of $\bar{s}$ would be provided by $\hat{s}_R$. However, because $\hat{h}_R$ can be biased when non-genetic sources of mortality are important, a practical alternative is $\hat{h}_b$. Thus, $\hat{\lambda}_b$and $\hat{s}_b$ will provide estimates theoretically unbiased under no correlation between s and h values, but biased otherwise.

(iii) *Mutational models and parameters investigated*

Selection coefficients (s) for new mutations were sampled from a gamma distribution. Only deleterious mutations (positive s values) were assumed for the effects on fitness, and lethal mutations were not included in the analysis. Several shapes for the gamma distribution were considered, with the shape parameter β taking values between ∞ (constant effects) and 0·5, but most results presented refer to $\beta=1$, corresponding to an exponential distribution of selection coefficients. A range of haploid mutation rates (λ) per generation were studied, from $\lambda=0{\cdot}1$ to 0·5 (see García-Dorado *et al*., 1999, 2004; Charlesworth *et al*., 2004; Baer *et al*., 2005; Joseph & Hall, 2004; Denver *et al*., 2004; Schoen, 2005; Halligan & Keightley, 2006), but results will be mainly shown for $\lambda=0{\cdot}1$, a consensus value obtained from mutation-accumulation experiments on several species.

The dominance coefficient of mutations ranged between 0 and 1, i.e. over- and underdominant mutations were not considered. In order to use a flexible distribution that allows a range of h values, a beta distribution was used, specified by the arithmetic mean of h values ($\bar{h}$) and their variance (σ_h^2). Mean values ranged between $\bar{h}=0{\cdot}1$ and 0·5, but results are shown only for $\bar{h}=0{\cdot}2$ and 0·4. Variances varied between $\sigma_h^2=0$ and 0·2 (see Fig. 1*A, B*). In order to establish a negative correlation between s and h values the procedure described by Fernández *et al*. (2004) was carried out.

(iv) *Expected distribution of mutant frequencies*

The expected distribution of mutant frequencies for each sample mutation with parameters s and h in a finite population at mutation–selection–drift balance was obtained by means of Wright's (1937) basic equation:

$$\phi(q)=C\overline{W_O}^{2N}q^{4Nu-1}p^{4Nv-1}, \tag{3}$$

where q is the mutant frequency ranging from 0 to 1, $p=1-q$, $\overline{W_O}=1-2pqsh-q^2s$ is the mean fitness of the outbred population, N is the population size, u is the per-locus deleterious mutation rate, v is the per-locus rate of reverse mutation, and C is a constant such that the summation of $\phi(q)$ for all the spectrum

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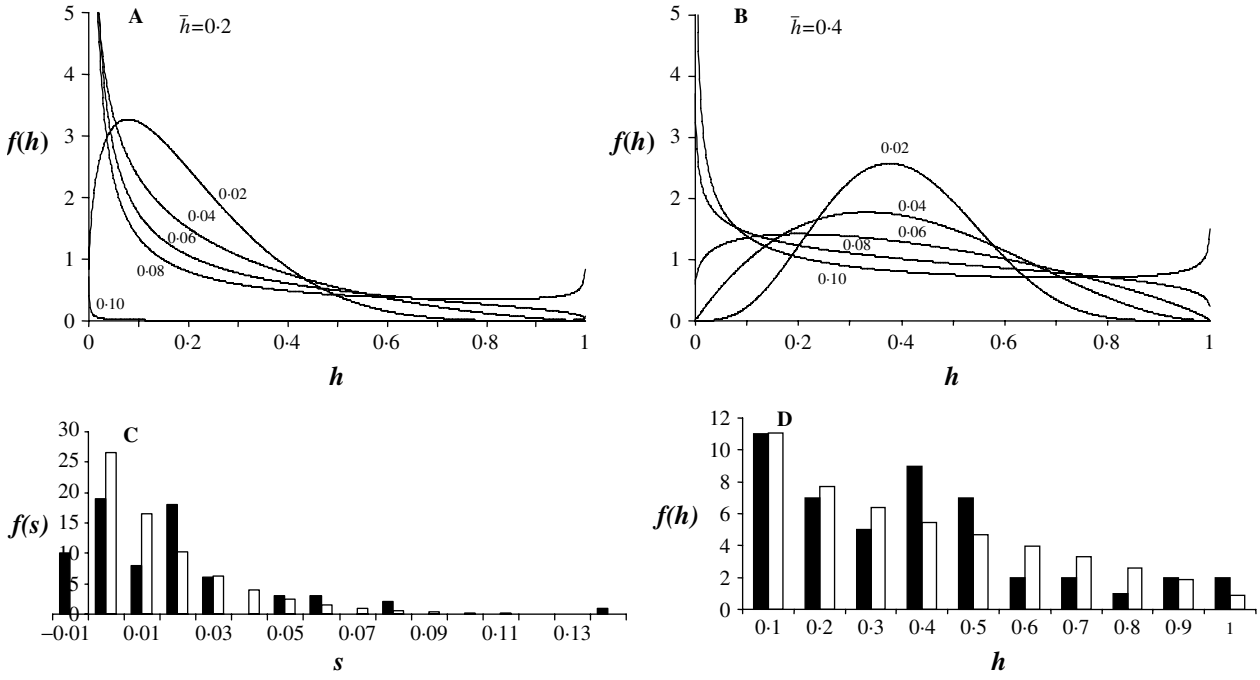

Fig. 1. (*A*), (*B*) Theoretical distribution, $f(h)$, of dominance coefficients following a beta distribution with mean $\bar{h}$ and variance σ_h^2 (figures close to the lines). (*C*) Distribution of s values: observed values (black bars) for single mutations (growth rate from 70 lines of *S. cerevisiae*; data from Szafraniec *et al*., 2003); expected values (open bars) obtained from an exponential distribution with mean effect $\bar{s}=0{\cdot}021$ (the average of the observed data). (*D*) Distribution of h: observed values (black bars) for single mutations (as in plot *C*) using only mutations with $0 \leqslant h \leqslant 1$ (48 lines); expected values (open bars) obtained from a beta distribution of h values with mean $\bar{h}=0{\cdot}33$ and $\sigma_h^2=0{\cdot}065$, which correspond to the mean and variance of the values in the observed distribution.

of gene frequencies is equal to 1. Equation (3) was computed using a discrete frequency model with approximations for the terminal classes ($q=0$ and 1) as indicated in appendix III of Kimura *et al*. (1963).

A range of population sizes ($N=500$ to 10^5) was considered, but results refer mainly to $N=10^4$. In each simulation, $n=1000$ mutational effects (s and h, correlated or not) were sampled from the corresponding distributions and ascribed to 1000 loci. For instance, to simulate a haploid mutation rate of $\lambda=0{\cdot}1$, a value of $u=0{\cdot}0001$ was used in equation (3). A larger number of loci (10 000) was also considered (thus $u=0{\cdot}00001$), but this increased the computing time without altering the results. The rate of reverse mutation used was an order of magnitude lower than u, and no differences in the results were found for relatively lower values.

For each of the 1000 sampled mutations, the mean fitness of outbred and selfed individuals, as well as the mean of T, and all the variances and covariances from Table 1*B*, were obtained by multiplying the corresponding genotypic values by $\phi(q)$ and adding for the whole range of gene frequencies. Overall mean fitnesses were obtained by multiplying over the 1000 mutations (loci), i.e. a multiplicative model was assumed. Estimates of δ, $\bar{h}$, λ and $\bar{s}$ were obtained using logarithmic transformations. The procedure was repeated three times (for three sets of mutations sampled) and averaged over sets. Standard errors of estimates were calculated from the variance among sets.

3. Evaluation of the estimators

Fig. 2 shows the estimates of the average of h values ($\hat{h}$), the mutation rate ($\hat{\lambda}$) and the average of s values ($\hat{s}$) for a range of variances of h when the correlation between s and h is zero ($r_{s,h}=0$). The upper panel gives estimates of the average h obtained with the four estimators (Table 1*C*). Note that they only estimate $\bar{h}$ accurately when all h values are constant ($\sigma_h^2=0$). This has been discussed previously (see Fernández *et al*., 2004, 2005) and is not the purpose of the present paper. Rather, the main interest here is to use these different estimators of the average h to further obtain estimates of λ and $\bar{s}$. In infinite populations under MSB, $\hat{h}_R$ and $\hat{h}_b$ provide estimates of the harmonic mean of h values and, therefore, they decrease when σ_h^2 is increased. In principle, $\hat{h}_{1/b}$ is assumed to provide estimates of $\overline{sh}/\bar{s}$ under MSB and, therefore, of $\bar{h}$ when $r_{s,h}=0$. However, this is only the case for $\bar{h}=0{\cdot}4$ and low σ_h^2. When there is a large proportion of mutations with very low values of h (i.e. low $\bar{s}$ and/or large σ_h^2; see Fig. 1*A, B*) the estimates are biased upwards (and

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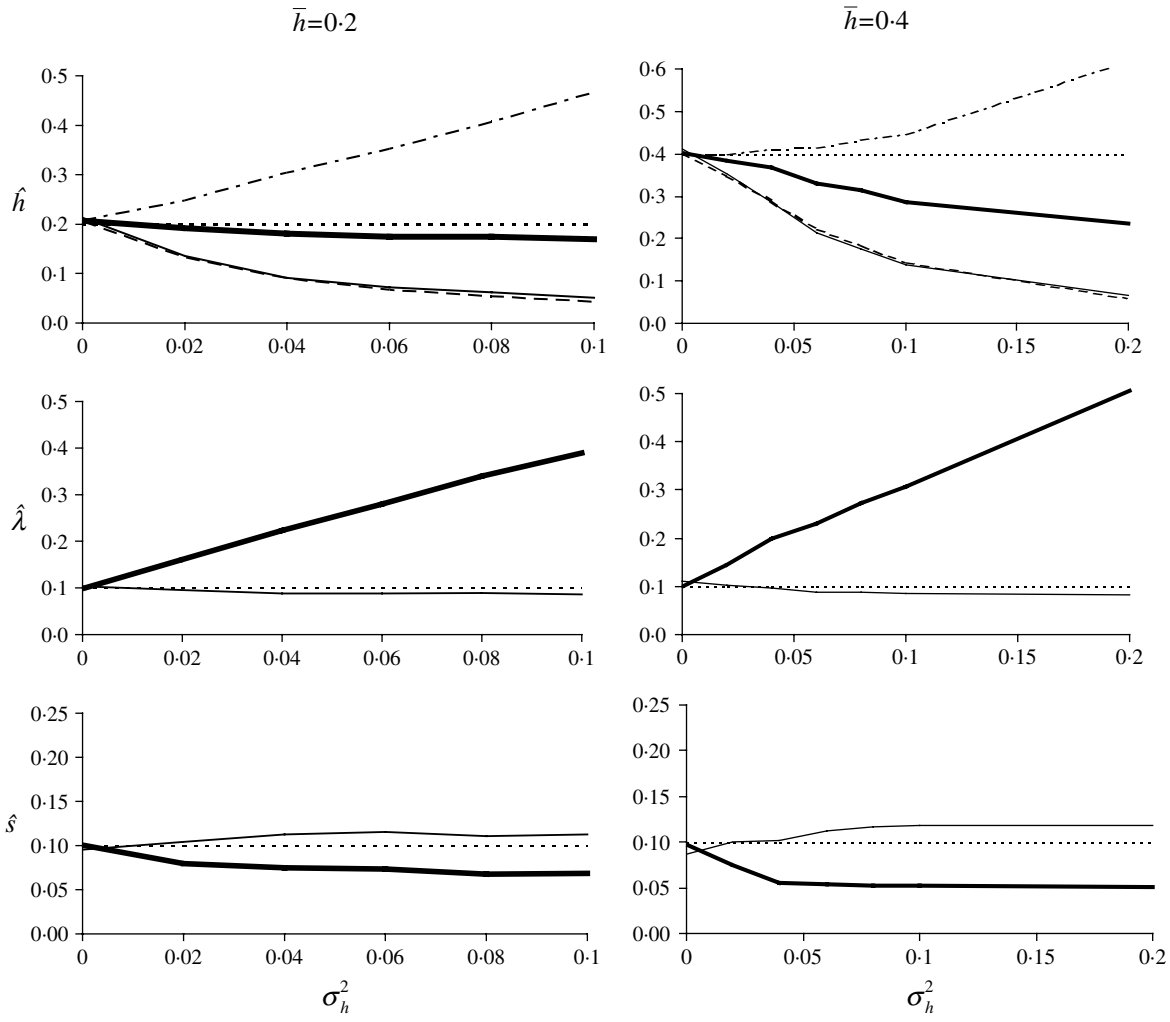

Fig. 2. Estimates of the average coefficient of dominance ($\hat{h}$), the haploid genomic mutation rate ($\hat{\lambda}$) and the average homozygous deleterious effect of mutations ($\hat{s}$), assuming a beta distribution of dominance coefficients with mean $\bar{h}$ and no correlation between s and h values ($r_{s,h}=0$), for different values of the variance of dominance coefficients, σ_h^2. Upper panels: dot-striped line ($\hat{h}_{1/b}$), thick line ($\hat{h}_{DL}$), thin continuous line ($\hat{h}_b$), broken line ($\hat{h}_R$), dotted line (true value of $\bar{h}$) (see Table 1 for definitions). Middle and lower panels: thick lines ($\hat{\lambda}_{DL}$ and $\hat{s}_{DL}$), thin continuous lines ($\hat{\lambda}_b$ and $\hat{s}_b$), dotted lines (true value of λ and $\bar{s}$). Other parameters: population size, $N=10^4$; exponential distribution of s values.

also those from $\hat{h}_R$ and $\hat{h}_b$). The reason is that, in this situation, the MSB prediction for the number of mutations present in the population (the pervasiveness; see García-Dorado *et al.*, 2003; Fernández *et al.*, 2004) is usually much larger than the actual one in a finite population, even if the population size is as large as $N=10^4$, as is the case for the results of Fig. 2. Finally, the estimator $\hat{h}_{DL}$ is assumed to provide an estimate of the geometric mean of the estimates provided by $\hat{h}_R$ and $\hat{h}_b$ (see Table 1*C*), and accordingly, its predictions are intermediate between them.

The middle and lower panels of Fig. 2 show the estimates of λ and $\bar{s}$, respectively. The estimators $\hat{\lambda}_R$ and $\hat{s}_R$ are not shown because they require that the only source of decline in fitness in the population is genetic (i.e. $W_{max}=1$), a very dubious assumption. For $W_{max}=1$, estimates of $\hat{\lambda}_R$ were basically unbiased, as expected, and those for $\hat{s}_R$ only gave overestimations for low values of $\bar{h}$ and large σ_h^2 (not shown), because the estimator $\hat{h}_{1/b}$ is upwardly biased in that situation, as was shown above. For $W_{max}<1$, $\hat{\lambda}_R$ gave upwardly biased estimates of λ, and $\hat{s}_R$ downwardly biased estimates of $\bar{s}$ (not shown), as expected.

The estimator $\hat{\lambda}_{DL}$ provides increasingly larger overestimations of λ, and $\hat{s}_{DL}$ underestimations of $\bar{s}$, for increasing values of σ_h^2 (Fig. 2). Interestingly, the underestimations of $\bar{s}$ by $\hat{s}_{DL}$ are not as large as the overestimations of λ by $\hat{\lambda}_{DL}$. The reason for this is that there are two counteracting biases. Because $\hat{\lambda}_{DL}$overestimates λ and $\hat{h}_{DL}$underestimates $\bar{h}$, their effects in the denominator of equation (2) for the estimation of $\bar{s}$ partially cancel out.

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The estimator $\hat{\lambda}_b$ gives almost unbiased estimates of λ, and $\hat{s}_b$ gives only slight overestimations or underestimations of $\bar{s}$. Therefore, with no correlation between s and h values, $\hat{\lambda}_b$ and $\hat{s}_b$ provide reliable estimates. Similar results are obtained for other values of rates of mutations and average mutational effects. For example, for $\lambda=0{\cdot}5$, $\bar{s}=0{\cdot}05$, $\bar{h}=0{\cdot}4$, $\sigma_h^2=0{\cdot}04$ and $r_{s,h}=0$ (other parameters as in Fig. 2), the estimators are $\hat{\lambda}_R=0{\cdot}47\pm0{\cdot}00$, $\hat{\lambda}_b=0{\cdot}52\pm0{\cdot}01$, $\hat{\lambda}_{DL}=0{\cdot}94\pm0{\cdot}04$, $\hat{s}_R=0{\cdot}05\pm0{\cdot}00$, $\hat{s}_b=0{\cdot}05\pm0{\cdot}00$ and $\hat{s}_{DL}=0{\cdot}03\pm0{\cdot}00$. The standard errors of these estimates give an idea of the precision of the values presented in the figures.

The impact of an increasing correlation between s and h values is shown in Fig. 3. An increasingly negative correlation generally reduces the estimates provided by all estimators of the average h except $\hat{h}_R$. The increase of a negative $r_{s,h}$ has the effect of decreasing the estimates of λ and increasing those for $\bar{s}$. Deng & Lynch's (1996) estimates of λ are upwardly biased for all values of $r_{s,h}$ when $\bar{h}$ is low and/or σ_h^2 is high, whereas over- or underestimations of λ and $\bar{s}$ occur depending on the magnitude of the correlation in other instances. The estimates from $\hat{\lambda}_b$ produce slight underestimations for most situations, and those from $\hat{s}_b$ give overestimations that can become substantial for high values of $r_{s,h}$ and low values of σ_h^2.

An increasing degree of kurtosis (decrease in the scale parameter β for the gamma distribution of s values) decreased all estimates of λ and increased the estimates of $\bar{s}$, but the impact was relatively small except for very large kurtosis ($\beta=0{\cdot}5$) (data not shown). Finally, the estimates were almost invariable for population sizes of the order of 10^4 and over, and a relatively low impact (overestimations of λ) was observed for population sizes of the order of 1000 or lower, in agreement with results from Li & Deng (2005).

4. Empirical estimates of σ_h^2 and $r_{s,h}$

Data on the variance of h and the correlation between s and h are difficult to obtain, as they require a precise study of individual mutations. Only a few mutation-accumulation studies approximately meet this requirement but, to my knowledge, no explicit estimates have been provided. I have reanalysed some of the results from Fernández & López-Fanjul (1996), Szafraniec *et al*. (2003), Peters *et al*. (2003) and Shaw & Chang (2006), in order to obtain rough estimates of σ_h^2 and $r_{s,h}$. The distributions of s and of h values from the experimental dataset with more data (Szafraniec *et al*., 2003) are shown in Fig. 1*C* and 1*D* (black bars), respectively. These are compared with the expected distributions obtained from an exponential distribution (gamma with shape parameter $\beta=1$) of s values (Fig. 1*C*, open bars), and a beta distribution of h values (Fig. 1*D*, open bars) using the parameters estimated from the data used. The apparent agreement between observations and expectations is quite remarkable, suggesting that the theoretical distributions used in the previous analytical study were appropriate.

The estimated values for σ_h^2 and $r_{s,h}$ are presented in Table 2. Only the data from Szafraniec *et al*. (2003) provide estimates of homozygous and heterozygous effects for single mutations. The other experiments involve lines possibly carrying more than one fixed mutation, but the estimated number of mutations per line is close to one (see Table 2), so the analysis can be justified. Table 2 shows first the estimates considering all lines available for analysis in the experiments. Estimates of σ_h^2 were very variable, from 0·8 up to 9·1, whereas estimates of $r_{s,h}$ varied from $-0{\cdot}223$ to $+0{\cdot}037$, suggesting generally low values for the correlation. The right-hand part of the table shows estimates after excluding those lines with extreme values of h ($|h|>1$), which were generally those with the lowest homozygous effects. The estimates of σ_h^2 for this subset of lines were of course much lower (between 0·12 and 0·28) but those for $r_{s,h}$ changed relatively little (from $-0{\cdot}382$ to $-0{\cdot}070$), still suggesting generally low values of the correlation (none of them were significantly different from zero).

It should be noted, however, that the low values observed for $r_{s,h}$ could be partially due to the errors in the estimates of s and h. In order to assess this effect, samples of s and h values were taken from the corresponding theoretical distributions with a given correlation as before, but an error variance was attached to these parameters before calculating their final correlation. Thus a normal deviate was added to each sampled s value and to each sampled h value. The approximate standard errors of the estimates of homozygous and heterozygous effects from the experiments of Table 2 were 0·005 (Szafraniec *et al*., 2003), 0·08 (Peters *et al*., 2003), 0·02 (Shaw & Chang, 2006) and 0·1 (Fernández & López-Fanjul, 1996). Thus, a conservative value of 0·1 was taken as the standard deviation of the normal distribution. The results of these simulations are shown in Fig. 4. As can be seen, the error in the estimates of s and h cause a decline in the estimated $r_{s,h}$ with respect to the parametric value (diagonal). However, the bias is not too large for low values of $r_{s,h}$, implying that estimates of, say, $\hat{r}_{s,h}=-0{\cdot}2$ would correspond to true values of about $r_{s,h}=-0{\cdot}3$.

5. Discussion

The knowledge of the sign and magnitude of the biases expected from the application of indirect estimators of deleterious mutation parameters is important in order to interpret the results obtained.

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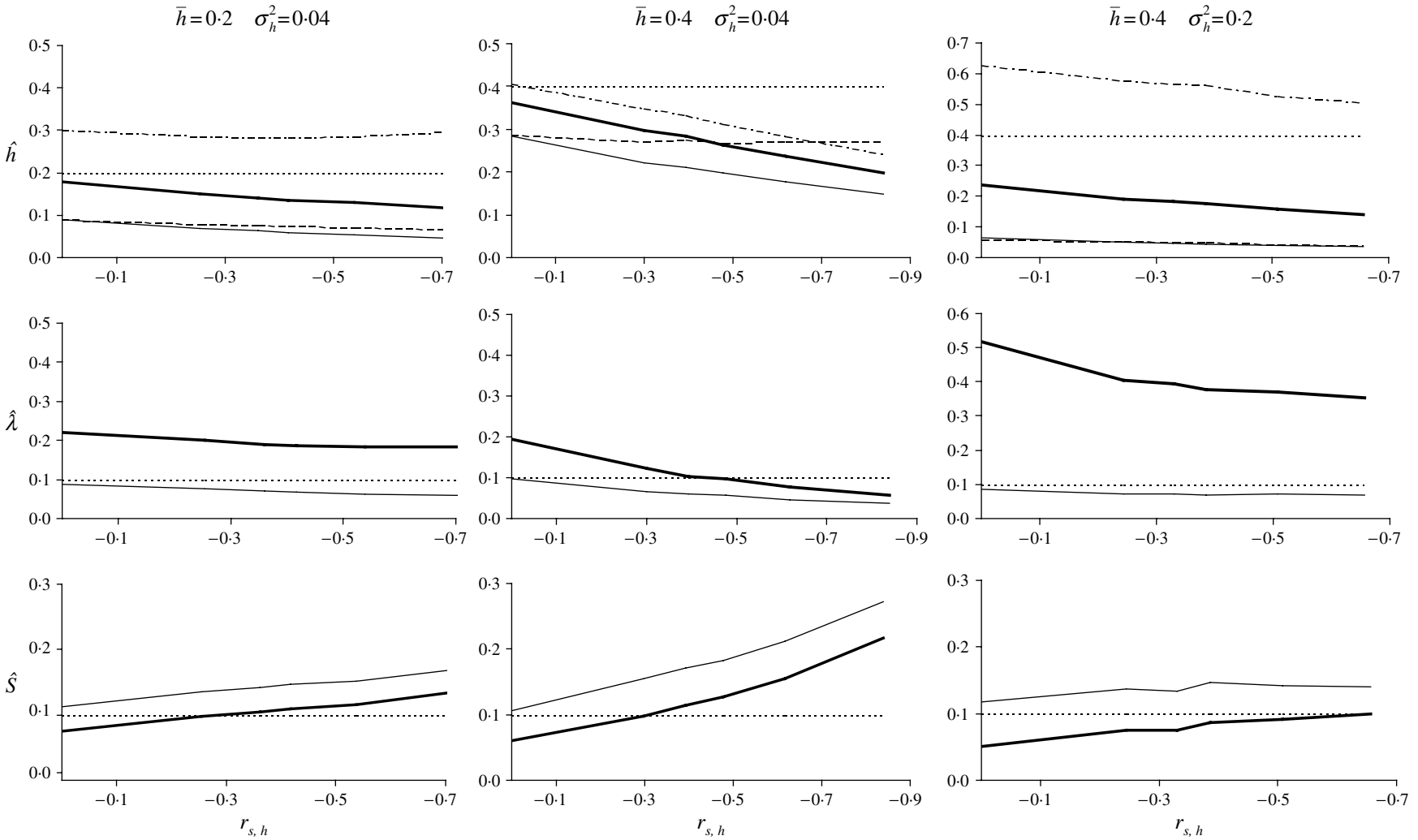

Fig. 3. Estimates of the average coefficient of dominance ($\hat{h}$), the haploid genomic mutation rate ($\hat{\lambda}$) and the average homozygous deleterious effect of mutations ($\hat{s}$), assuming a beta distribution of dominance coefficients with mean $\bar{h}$ and variance σ_h^2, for different values of the correlation between s and h ($r_{s,h}$). Definitions and parameters as in Fig. 2.

Table 2. *Estimates of the variance of dominance coefficients, σ_h^2, and the correlation between s and h values, $r_{s,h}$, obtained from several mutation-accumulation experiments*

Species	Trait	Type	Mutations per line	All lines			Excluding lines with $\lvert h\rvert > 1$			
				n	$\hat{\sigma}_h^2$	$\hat{r}_{s,h}$	n	$\hat{h}$	$\hat{\sigma}_h^2$	$\hat{r}_{s,h}$
D. melanogaster	Egg-to-adult viability	Spontaneous	$\sim 1{\cdot}6^a$	12	3·6	−0·028	9	0·23	0·28	−0·070
D. melanogaster	Fecundity	Spontaneous	$\sim 1{\cdot}6^a$	12	9·1	−0·223	5	0·09	0·23	−0·232
S. cerevisiae	Growth rate	EMS	1	70	1·0	−0·143	58	0·23	0·12	−0·115
C. elegans	Relative fitness	EMS	$\sim 1{\cdot}5^b$	19	2·0	+0·037	14	−0·15	0·15	−0·269
A. thaliana	Reproductive Biomass	Spontaneous	$\sim 1{\cdot}4^c$	8	0·8	−0·075	6	0·27	0·12	−0·382

Mutations per line, expected number of mutations fixed in each line.
n, number of lines included in the analysis.
[a] From $\hat{\lambda} \approx 0{\cdot}016$ (García-Dorado *et al.*, 1999) for $t \approx 100$ generations.
[b] From Peters *et al.* (2003).
[c] From $\hat{\lambda} \approx 0{\cdot}08$ (Shaw *et al.*, 2002) for $t = 17$ generations.
Estimates of the average h ($\hat{h}$), $\hat{\sigma}_h^2$ and $\hat{r}_{s,h}$ obtained from tables 4 and 6 of Fernández & López-Fanjul (1996) (*Drosophila melanogaster*), figure 3 of Szafraniec *et al.* (2003) (raw data provided by R. Korona) (*Saccharomyces cerevisiae*), figure 3 of Peters *et al.* (2003) (*Caenorhabditis elegans*), and tables 2 and 4 of Shaw & Chang (2006) (*Arabidopsis thaliana*).

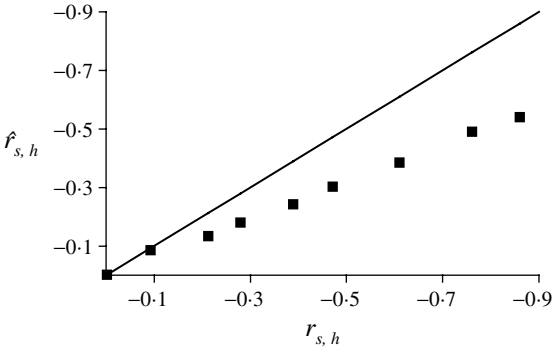

Fig. 4. Correlation between *s* and *h* values estimated after attaching an error variance to the sampled parameters, versus true correlation values. Samples of *s* and *h* values were taken from the corresponding distributions (exponential distribution for *s*, beta distribution for *h*) with a given correlation, $r_{s,h}$. An error variance was then attached to these parameters before calculating their observed correlation, by adding to each one a normal deviate with mean zero and standard deviation 0·1. The diagonal represents the value of the correlations without error variance.

The method proposed by Deng & Lynch (1996) has been evaluated for a wide range of scenarios, but always under the same model of coefficients of dominance: an exponential function such that, for a given mutation with homozygous effect *s*, the degree of dominance is $h = \frac{1}{2}\exp(-13\,s)$. This strict relationship between *s* and *h* implies that the variation allowed for *h* values is quite low and the negative correlation between *s* and *h* values is very close to −1. For example, using an exponential distribution of *s*, with mutational parameters $\bar{s} = 0{\cdot}1$ and $\bar{h} = 0{\cdot}4$, the variance of *h* values with the function used by Deng and co-workers is $\sigma_h^2 = 0{\cdot}007$, and the correlation between *s* and *h* values is $r_{s,h} = -0{\cdot}98$. The above function is related to that proposed by Caballero & Keightley (1994) for which the values of *h* are taken from a uniform distribution between zero and $\exp(-k\,s)$, where *k* is a constant to obtain the desired $\bar{h}$. This latter function allows for a larger σ_h^2 and a lower $r_{s,h}$ than that of Deng and co-workers, and has been used in some simulation and analytical studies (e.g. Fernández *et al.*, 2004, 2005). However, in order to evaluate a flexible distribution of *h* values, the beta distribution used in this paper was found to be more appropriate, allowing for any level of σ_h^2 and $r_{s,h}$ (note also the good agreement between expectations and observations in Fig. 1*D*).

The analyses carried out by Deng and co-workers on the bias of the estimates from the Deng & Lynch (1996) method generally found underestimation of λ (estimates around 0·4–0·8 of the true value) and overestimation of $\bar{s}$ (estimates up to about 3 times the true value), depending on the particular models and mutational parameters considered (Deng & Lynch,

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1996, 1997; Deng, 1998*b*; Deng & Fu, 1998; Li & Deng, 2000, 2005). Therefore, these results would suggest that the method almost always underestimates λ and overestimates $\bar{s}$. Here, the use of a wide range of bivariate distributions of s and h values has shown that this is not necessarily the case. For large values of a negative $r_{s,h}$, low values of σ_h^2, and large $\bar{h}$, the estimates obtained by the Deng & Lynch (1996) method agree with those observed by the previous studies, i.e. an underestimation of λ and an overestimation of $\bar{s}$ (see Fig. 3). However, the method can also produce highly upwardly biased estimates of λ when $r_{s,h}=0$ (Fig. 2) and for $r_{s,h}<0$ when $\bar{h}$ is low and/or σ_h^2 is large (Fig. 3). It may produce also underestimations of $\bar{s}$ for low values of $r_{s,h}$ (Figs. 2, 3). A modification of the method consisting of using a different estimator of the average h in the equations for predicting λ and $\bar{s}$ provides almost unbiased estimates of λ for the reference case of $r_{s,h}=0$ but underestimations for $r_{s,h}<0$ (Fig. 3). It also gives substantial overestimations of $\bar{s}$ for large values of $r_{s,h}$ and low σ_h^2 (Fig. 3).

Because the estimators proposed by Deng & Lynch (1996) were derived for constant mutational effects and dominance, these authors suggested some corrections to account for variable mutational effects. The corrections imply knowledge of the coefficient of variation of dominance coefficients, $\sigma_h^2/\overline{h}^2$, and the coefficient of covariation between s and h, $\sigma_{s,h}/\overline{sh}$. Accordingly, the estimates of $\hat{h}_b$ can be corrected if one could know the covariance between s^2 and h, $\sigma_{s^2,h}$, and the mean of s^2 values, $\overline{s^2}$ (Caballero *et al*., 1997; Fernández *et al*., 2005). The possibility of knowing any of these parameters with enough precision is, however, very unlikely, making the above corrections of very little use in practice. Deng *et al*. (2002) also developed an extension of the Deng & Lynch (1996) method that allows for a variable distribution of mutational effects. In fact, this method could provide estimates of the covariance between s and h values, although the sign of this covariance cannot be estimated reliably in the case of outcrossing populations. Unfortunately, the method requires previous knowledge of an unbiased estimate of a key parameter, such as λ, $\bar{s}$, $\bar{h}$, $\overline{sh}$, or the harmonic mean of h. Deng *et al*. (2002) also provided an empirical regression procedure to estimate λ, so that this could be used for the subsequent estimation method. However, this regression procedure requires an unbiased estimate of the kurtosis of the distribution of homozygous effects (β), a parameter that is extremely hard to estimate.

This paper has focused on the scenario of a naturally outbred population that can be subjected to selfing. The application of other forms of inbreeding, such as full-sib mating (Deng, 1998*b*), is not expected to change the conclusions. The case where a naturally inbred population is subjected to outbreeding is another scenario considered by Deng & Lynch (1996), extending the work of Charlesworth *et al*. (1990). In this case, the mean fitness of a naturally selfed population is $\overline{W_S}\approx 1-\lambda$, and the expected mean fitness of the crosses between individuals from this population is $\overline{W_O}\approx 1-2\lambda\bar{h}$, with corresponding variances $\sigma_{WS}^2\approx\lambda\bar{s}$ and $\sigma_{WO}^2\approx 2\lambda(\overline{h^2 s})$, and outbreeding depression $\delta\approx\lambda[2\bar{h}-1]$. The estimate of the average h proposed by Deng & Lynch (1996) is

$$\hat{h}_{DL}=\sqrt{\sigma_{WO}^2/(2\sigma_{WS}^2)}=\sqrt{(\overline{h^2 s})/\bar{s}}.$$

Note that, in this case, the estimator will produce unbiased estimates of $\bar{h}$ when $r_{s,h}=0$, which is the average required to estimate λ. Thus, unbiased estimates of λ could be obtained, in principle, from $\hat{\lambda}_{DL}=\delta/(2\hat{h}_{DL}-1)$, and those of $\bar{s}$ from $\hat{s}_{DL}=\sigma_{WS}^2/\hat{\lambda}_{DL}$ (Deng & Lynch, 1996). In addition, Deng and co-workers' analyses considering a high negative $r_{s,h}$ suggest that the method works better in this scenario than in the outbreeding scenario.

Information on the variation of h for mutations affecting fitness traits and on the correlation between s and h is scarce and rather hard to obtain. Mukai (1969) provided a value of $\hat{\sigma}_h^2=0{\cdot}044\pm 0{\cdot}014$ from estimates of the average coefficient of dominance obtained by the ratio of heterozygous to homozygous chromosomal viabilities ($\hat{h}_R$) from lines of *Drosophila melanogaster* that had accumulated mutations for 32 and 52 generations. The estimate depended on a prediction of the number of mutations carried by the lines (estimated to be 4·72 in generation 32 and 7·34 in generation 52), which was obtained assuming a haploid genomic mutation rate for viability of $\lambda\approx 0{\cdot}35$ (Mukai, 1964). Other analyses of individual spontaneous mutations from mutation-accumulation experiments concur in suggesting a considerable variability in the coefficient of dominance of deleterious mutations, ranging from recessive to dominant, with putative cases of overdominance and underdominance (Fernández & López-Fanjul, 1996; Caballero & Keightley, 1994; Szafraniec *et al*., 2003; Peters *et al*., 2003; Phadnis & Fry, 2005; Shaw & Chang, 2006). The large variation of h values observed in some of these experiments is summarized by the estimates of σ_h^2 of Table 2, although these can be inflated by error variance.

With regard to the correlation between s and h values, very little information is available. From multiple *P*-element insertion data in *D. melanogaster* (Mackay *et al*., 1992) a tendency was found for mutations of large effect on viability to have lower coefficients of dominance (Caballero & Keightley, 1994), but this observation referred to lines with coefficients of selection $s>0{\cdot}3$, as estimates of h for insertion lines of smaller effect had extremely large sampling errors.

An analysis of single *P*-element insertions (therefore lacking the problems attached to the inferences from lines with multiple insertions) suggested a low correlation between s and h (Lyman *et al*., 1996). The average coefficient of dominance for mutations affecting viability was obtained from the linear regression of heterozygous on homozygous effects and turned out to give estimates very close to zero. The fact that the quadratic coefficients of regression were very small and could be attributed to a few lines with large homozygous effect, suggested that the correlation between s and h values was not substantial (Lyman *et al*., 1996). In contrast, an analysis of gene deletions in yeast showed a widespread substantial negative correlation between s and h values (Spearman rank correlations around $-0{\cdot}5$ to -1 correlation) with a large variation among h values (Phadnis & Fry, 2005). The results of the analysis carried out in this paper seem to suggest that the correlation between s and h, though negative, is closer to zero than to -1. This conclusion, however, should be viewed with extreme caution: first, because of the scarcity of data and their attached estimation errors, and second, because the analysed lines (mutations) may not be a random sample of the whole spectrum of mutations, but instead a subset of those with the largest effects.

A main requirement for the application of the Deng & Lynch (1996) method and all its modifications and extensions is that no sources of variation other than the occurrence of mutations and their elimination by directional selection are acting on the population, and that estimates of fitness effects in the laboratory are the same as in nature. A first problem is that deleterious allele frequencies in natural populations depend on overall fitness rather than on the individual fitness trait under study and that, in addition, fitness measures in the laboratory are not necessarily the same as in nature. If it is assumed that the effects of mutations on overall fitness in nature are proportionately higher (say twice as large; see Mukai & Yamaguchi, 1974; Charlesworth & Hughes, 2000) than for a single fitness trait studied in the laboratory, estimates of λ would be underestimated by about a half, but there would be almost no consequences either for the estimates of the average h (Fernández *et al*., 2004, 2005) or for those of $\bar{s}$ (in this latter case because both the estimates of λ and σ^2_{WO} are reduced by a half, not affecting the estimate of $\bar{s}$; see equation 2). These results were checked by repeating the analyses of Figs. 2 and 3 but using a value of s twice as large in the calculations from equation (3).

The most important caveat of the Deng & Lynch (1996) method is the existence of balancing selection (through, for example, overdominance, antagonistic pleiotropy, or environmental heterogeneity), which may imply substantial biases in the estimates, generally inflating the estimates of λ and reducing those of $\bar{s}$ with respect to the estimates obtained under MSB conditions (see Mukai & Yamaguchi, 1974; Deng, 1998*a*; Li *et al*., 1999; Fernández *et al*., 2005). However, some populations may be close to MSB conditions (see e.g. Kusakabe & Mukai, 1984; Rodríguez-Ramilo *et al*., 2004) and the method could then be applied with some confidence in these instances. In addition, the possibility of complementing the estimates with those obtained directly from mutation-accumulation experiments may still provide a useful tool to test the hypothesis of MSB for the segregating populations. Furthermore, direct estimates from mutation-accumulation studies are also subject to biases: first, because many mutations of small effect may pass undetected in these experiments, and second because selection within and between mutation-accumulation lines will remove mutations of large effect (see Caballero *et al*., 2002).

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