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Upper limit of the rate and per generation effects of deleterious genomic mutations

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Summary

Unbiased or upper limit estimates of the rate (U) of genomic mutations to mildly deleterious alleles are crucial in genetic and conservation studies and in human health care. However, only a few estimates of the lower bounds of U are available. We present a fairly robust estimation that yields an upper limit of U and a nearly unbiased estimate of the per generation fitness decline due to new deleterious mutations. We applied the approach to three species of the freshwater microcrustacean *Daphnia* and revealed that the upper limit of U for egg survivorship is 0·73 (SD = 0·30) in 14 *D. pulicaria* populations. For the first four clutches, per generation decline in fecundity due to deleterious mutations ranged from 2·2% to 7·8% in 20 *D. pulex* populations and from 1·1% to 5·1% in 8 *D. obtusa* populations. These results indicate the mutation pressure is high in natural *Daphnia* populations. The approach investigated here provides a potential way to quickly and conveniently characterize U and per generation effects of deleterious genomic mutations on fitness or its important components such as fecundity.

1. Introduction

Estimates of the rate of genomic mutations to mildly deleterious alleles (U) are crucial to testing theories for the evolution of sex (Kondrashov, 1988; Muller, 1964), mate choice (Charlesworth & Charlesworth, 1987; Kirkpatrick & Ryan, 1991; Kondrashov, 1988), outbreeding mechanisms (Kondrashov, 1988) and the accelerated extinction rate of small populations (Lande, 1994; Lynch *et al.*, 1993). They are also important for evaluating the impact of deleterious genomic mutations (DGM) on human health (Crow, 1993, 1995). However, few estimates are available (Crow, 1993; Crow & Simmons, 1983; Drake *et al.*, 1998; Kondrashov, 1988). The few estimates obtained by almost all the current estimation methods are generally lower bounds of U (Bateman, 1959; Charlesworth *et al.*, 1990; Deng, 1998; Deng & Fu, 1998; Deng & Lynch, 1996, 1997; Keightley, 1994; Li *et al.*, 1999; Mukai *et al.*, 1972).

However, it is an unbiased estimation of U or an estimation of the upper bound of U that is more relevant and important in testing many population genetics theories that invoke DGM as an essential assumption and in evaluating the true or maximum mutation load in humans and other organisms. Without an estimation of an upper bound or an unbiased value of U, it is not clear what relevance of U estimates are to testing many genetics theories involving DGM or to evaluating the impact of DGM on human health and continuous survival of finite populations. For example, the deterministic mutation hypothesis for the evolution of recombination depends on a $U > 1{\cdot}0$ (Kondrashov, 1988).

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Formally, any estimate of the lower bound of U cannot be employed to defuse this theory. To test the validity of this kind of theory, either unbiased estimates or estimates of an upper bound of U are necessary. These estimates are essentially unavailable because of the lack of appropriate estimation methods.

The mean and genetic variance of fitness (or its important component traits under directional selection) in large randomly mating natural populations are the result of various evolutionary forces, mainly mutation and selection (Charlesworth & Hughes, 2000). They thus convey important information on how frequently DGM arise per generation and the impact they have on populations fitness traits (Charlesworth *et al*., 1990; Deng & Lynch, 1996). When large populations at approximate mutation–selection (M–S) equilibrium are under consideration, formulae can be derived to provide upper estimates of the rate of DGM. Based on this information, we present here an estimation that yields an upper limit of U and an estimate of the per generation fitness decline due to new deleterious mutations. We applied the approach to three species of the freshwater microcrustacean *Daphnia* and obtained the upper limit of U for egg survivorship.

2. Materials and methods

(i) *Upper bound of U*

As shown elsewhere (Burger & Hofbauer, 1994; Deng & Lynch, 1996; Haldane, 1937; Kimura *et al*., 1963), in a large outcrossing population at approximate M–S equilibrium, the mean fitness of the population, $\overline{W}_o$, is

$$\overline{W}_{\mathrm{o}} = W_{\max} \exp(-U) \tag{1}$$

where $W_{\max}$ is the expected fitness of a genotype free of segregating DGM from the population in the environmental conditions where the measurements are taken. $W_{\max}$ serves as a scaling factor so that fitness measurement can be on any scale instead of just from 0·0 to 1·0, and also so that mean environmental effects of experiments do not influence the estimation (Deng & Lynch, 1996). In addition, it has been shown (Deng & Lynch, 1996) that

$$\sigma_{\mathrm{o}}^2 = \overline{W}_{\mathrm{o}}^2[\exp(U\overline{hs}) - 1] \tag{2}$$

where σ_{o}^2 is the genetic variance of fitness of equilibrium outcrossing populations and $\overline{hs}$ is the arithmetic mean of the product of the variable dominance coefficient h_i and the selection coefficient s_i of DGM across loci.

Equations (1) and (2) convey important information on U and per generation mutation effects δ ($\delta = U\overline{hs}$ in outcrossing populations and $\delta = U\bar{s}/2$ in selfing populations, $\bar{s}$ is the mean selection coefficient s_i of DGM across loci). It can be easily seen from (1) and (2) that

$$U = -\ln\left(\frac{\overline{W}_{\mathrm{o}}}{W_{\max}}\right), \tag{3}$$

$$\delta = U\overline{hs} = \ln\left(\frac{\sigma_{\mathrm{o}}^2}{\overline{W}_{\mathrm{o}}^2} + 1\right). \tag{4}$$

For any fitness traits, (4) can be used to quantify the per generation percentage decline δ due to DGM. Note that this estimation is not biased by variable mutation effects across loci for the following reasons. δ is the product of U and $\overline{hs}$. This product can be obtained by using the righthand part of (4), as noted in Deng & Lynch (1996). With given assumptions (such as M–S balance), the sample mean and variance of fitness are unbiased estimates of the mean and variance of fitness regardless of variable mutation effects. Therefore, the estimate is not biased with regard to variable mutation effects. For survivorship, $W_{\max} \leqslant 1$(this is true in any measuring environment); therefore, a quick and convenient estimation of an upper bound of U for viability or survivorship can be obtained:

$$U \leqslant -\ln \overline{W}_{\mathrm{o}}. \tag{5}$$

Similarly, for large selfing populations at approximate M–S equilibrium, using the corresponding equations developed (Charlesworth *et al*., 1990; Deng & Lynch, 1996), it can be shown that, for viability,

$$U \leqslant -2\ln \overline{W}_{\mathrm{s}}. \tag{6}$$

where $\overline{W}_s$ is the mean fitness of a selfing population. For any fitness traits, the per generation percentage decline (δ) due to DGM is

$$\delta = \frac{U\bar{s}}{2} = \ln\left(\frac{\sigma_{\mathrm{s}}^2}{\overline{W}_{\mathrm{s}}^2} + 1\right) \tag{7}$$

where σ_{s}^2 is the genetic variance of fitness of the equilibrium selfing population. Again, this estimation is not biased by variable mutation effects across loci as an unbiased estimate of the product of U and $\bar{s}$ can be obtained. The terms inside the parentheses of (4) and (7) are the genetic coefficient of variation of fitness traits under directional selection.

Therefore, (4), (5), (6) and (7) offer a quick and convenient estimation of an upper bound of U for viability or survivorship in large natural populations, and an estimation of per generation mutation effects on fitness that is not biased by unknown variable mutation effects.

(ii) *Computer simulations*

The statistical properties and robustness of the above approach are investigated extensively under a range of parameter space and biologically plausible situations by computer simulations. These plausible situations include variable mutation effects across loci, epistatic mutation effects, the violation of M–S balance by additional overdominance mutations maintained by balancing selection in the genome, nonequilibrium populations at various stages approaching M–S balance, incomplete selfing/outcrossing and finite populations with linkage disequilibrium between adjacent mutation loci.

Computer simulation procedures for the above-mentioned situations can be found elsewhere (Charlesworth *et al*., 1991, 1992; Deng & Lynch, 1996, 1997; Fraser & Burnell, 1970; Li & Deng, 2000, 2005; Li *et al*., 1999; Kondrashov, 1985) and are briefly described in the next two paragraphs. Parameters used in current simulations are given in the corresponding results tables. Briefly, 200 genotypes are simulated for each run with DGM parameters $U=1{\cdot}0$, $h=0{\cdot}36$ and $s=0{\cdot}03$ except that $h_o=-0{\cdot}20$ and $s=0{\cdot}03$ for overdominance in a mixed dominance and overdominance population. Note that multiplicative interaction is assumed for all simulations except those with epistasis. For epistatic fitness, we use the model (Charlesworth, 1990; Deng & Lynch, 1996) $W(n)=\exp(-an-bn^2/2)$. n is the effective number of heterozygous/homozygous mutations per individual in outcrossing/selfing populations ($n=n_1+n_2/h$ in outcrossing populations and $n=n_1h+n_2$ in selfing population). h is the dominance coefficient of DGM. n_1 and n_2 are, respectively, the number of DGM in the heterozygous and homozygous states in an individual's genome. The parameter a measures the strength of selection against DGM ($a=hs$ in outcrossing populations and $a=s$ in selfing populations). The parameter b provides a measure of the synergistic effects of deleterious alleles. With $b=0$, the model reduces to one of multiplicative interaction effects, and with $b>0$, the effects of deleterious alleles are synergistic, i.e. as more deleterious alleles are added to the genome, the decline in fitness per additional deleterious mutant increases. The ratio r $(=bn/2n)$ provides a measure of the contribution of synergistic effects to mean fitness relative to that of multiplicative effects.

In a large population at M–S balance, the number of mutations per individual is generated through a Poisson distribution with a mean of $U/(hs)$ for outcrossing or $U/(2s)$ for selfing. For variable mutation effects, hs and s are replaced by $\overline{hs}$ and $\bar{s}$, respectively, where s is sampled from pdf $p(s)=1/\bar{s}\times\exp(-s/\bar{s})$ and h is set to $e^{-13s}/2$. The fitness of an individual is then determined by $W(n)=W_{\max}\Pi(1-h_is_i)$ (out-crossing) or $W(n)=W_{\max}\ \Pi\ (1-\ h_is_i)\ \Pi\ (1-s_j)$ (selfing), where h_i and s_i are the dominance coefficient and selection coefficient at the ith locus with mutation. This process is repeated until the pre-set number of individuals is obtained (Deng & Lynch, 1996). For overdominance in outcrossing populations, let B be the fitter allele. The numbers of BB, Bb and bb, denoted as n_3, n_4 and n_5, are drawn from a trinomial distribution with frequencies of p^2, $2pq$ and q^2, respectively, where $p=(h_o-1)/(2h_o-1)$ and $q=1-p$. The fitness is modified to $W(n)=W_{\max}[\Pi(1-h_is_i)](1-h_os_o)^{n_3}(1-s_o)^{n_4}]$. For further details and selfing populations, see Li *et al*. (1999).

To simulate populations approaching M–S balance, we start with homozygous individuals free of DGM. These individual then undergo a mutation–selection process for many generations. In each generation cycle, mutation (generated based on a Poisson distribution again), mating (outcrossing or selfing) and selection are simulated sequentially. Samples are obtained at pre-set points in the process and are used to estimate the upper bound of U and δ. For partial outcrossing/selfing populations, the simulation process is similar except that, for the mating step, instead of all individuals being outcrossed or selfed, a parameter S is used to control the proportion of the population undergoing outcrossing/selfing (Kondrashov, 1985; Charlesworth *et al*., 1991; Li & Deng, 2000). When linkage exists among loci, the simulation again starts from homozygous individuals free of DGM and goes through many cycles of mutation, mating and selection. The gametes of each individual are recorded at each generation. New gametes of a specific individual during mating are generated by producing crossovers along the genome based on the recombination rate θ. Zygotes are then generated by sampling from the pooled gametes and whether they survive is determined by selection (Charlesworth *et al*., 1992; Fraser & Burnell, 1970; Li & Deng, 2005).

(iii) *Data from* Daphnia

Accumulated data from different laboratories on the life-history studies of three *Daphnia* species – *D. pulicaria*, *D. pulex* and *D. obtusa* – are used in our study. For all the *D. pulicaria* and *D. pulex* populations, all the study clones were sampled from permanent lakes (*D. pulicaria*) or ephemeral ponds (*D. pulex*) in western Oregon. Samples were collected during January and February 1996. From each population, mature females were isolated into single 250 ml beakers containing 200 ml of aged filtered pond water supplemented with the green alga *Scenedesmus* as a food resource and maintained in the laboratory by clonal reproduction at 12 °C with a photoperiod of 12L : 12D. Data were obtained by standard life-table

Table 1. *Estimation of* U *for viability or survivorship under various conditions in large populations*

	Dominance				Mixed dominance and overdominance			
W_{max}	Constant	Variable	Epistasis		Constant dominance		Variable dominance	
Outcrossing population								
1·0	1·00 (0·01)	1·00 (0·01)	$0{\cdot}92_{r=0{\cdot}10}$ (0·01)	$0{\cdot}82_{r=0{\cdot}30}$ (0·01)	$0{\cdot}98_{\alpha=0{\cdot}79,\ \beta=0{\cdot}95}$ (0·01)	$0{\cdot}96_{\alpha=0{\cdot}59,\ \beta=0{\cdot}89}$ (0·01)	$0{\cdot}97_{\alpha=0{\cdot}80,\ \beta=0{\cdot}90}$ (0·01)	$0{\cdot}93_{\alpha=0{\cdot}60,\ \beta=0{\cdot}90}$ (0·01)
0·8	1·22 (0·01)	1·22 (0·01)	$1{\cdot}14_{r=0{\cdot}10}$ (0·01)	$1{\cdot}04_{r=0{\cdot}30}$ (0·01)	$1{\cdot}21_{\alpha=0{\cdot}79,\ \beta=0{\cdot}95}$ (0. 01)	$1{\cdot}18_{\alpha=0{\cdot}59,\ \beta=0{\cdot}89}$ (0. 01)	$1{\cdot}19_{\alpha=0{\cdot}80,\ \beta=0{\cdot}90}$ (0. 01)	$1{\cdot}15_{\alpha=0{\cdot}60,\ \beta=0{\cdot}90}$ (0. 01)
0·6	1·51 (0·01)	1·51 (0·01)	$1{\cdot}43_{r=0{\cdot}10}$ (0·01)	$1{\cdot}33_{r=0{\cdot}30}$ (0·01)	$1{\cdot}49_{\alpha=0{\cdot}79,\ \beta=0{\cdot}95}$ (0. 01)	$1{\cdot}46_{\alpha=0{\cdot}59,\ \beta=0{\cdot}89}$ (0. 01)	$1{\cdot}48_{\alpha=0{\cdot}80,\ \beta=0{\cdot}90}$ (0. 01)	$1{\cdot}44_{\alpha=0{\cdot}60,\ \beta=0{\cdot}90}$ (0. 01)
Selfing population								
1·0	1·00 (0·02)	1·00 (0·02)	$1{\cdot}01_{r=0{\cdot}10}$ (0·02)	$1{\cdot}01_{r=0{\cdot}30}$ (0·02)	$1{\cdot}06_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0·02)	$1{\cdot}12_{\alpha=0{\cdot}63,\ \beta=0{\cdot}89}$ (0. 02)	$1{\cdot}06_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0. 02)	$1{\cdot}12_{\alpha=0{\cdot}62,\ \beta=0{\cdot}89}$ (0. 02)
0·8	1·22 (0·02)	1·22 (0·02)	$1{\cdot}46_{r=0{\cdot}10}$ (0·02)	$1{\cdot}46_{r=0{\cdot}30}$ (0·02)	$1{\cdot}28_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0. 02)	$1{\cdot}34_{\alpha=0{\cdot}63,\ \beta=0{\cdot}89}$ (0. 02)	$1{\cdot}28_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0. 02)	$1{\cdot}34_{\alpha=0{\cdot}62,\ \beta=0{\cdot}89}$ (0. 02)
0·6	1·51 (0·02)	1·51 (0·02)	$2{\cdot}03_{r=0{\cdot}10}$ (0·04)	$2{\cdot}03_{r=0{\cdot}30}$ (0·02)	$1{\cdot}57_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0. 02)	$1{\cdot}63_{\alpha=0{\cdot}63,\ \beta=0{\cdot}89}$ (0. 02)	$1{\cdot}57_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0. 02)	$1{\cdot}63_{\alpha=0{\cdot}62,\ \beta=0{\cdot}89}$ (0. 02)

The reported values are the means and associated 1 standard deviation (numbers within parentheses) over 1000 repeated simulations (this is the case for Tables 2–5).
'Dominance' indicates that only DGM with a dominance coefficient between 0 and 1 is simulated, and 'Mixed dominance and overdominance' indicates that additional overdominant mutations are also simulated. Mutation effects are indicated as constant, variable and epistatic. α and β are defined in detail in Li *et al.* (1999) and represent, respectively, the proportion of total heterosis and that of the standing genetic variation in natural populations, both on the log fitness scale, which are attributable to dominance mutations. The parameter values are $U=1{\cdot}00$, $h=0.36$, $s=0.03$ for all simulations except in the case of mixed dominance and overdominance mutations, where $h=-0{\cdot}20$ and $s=0.03$ for overdominance mutations. For variable mutation effects, a leptokurtic exponential distribution across loci is used (Deng & Lynch, 1996) $p(s_i)=\exp(-s_i/\bar{s})/\bar{s}$ and $h_i=\exp(-13s_i)/2)$. In each assay, 200 random genotypes are sampled for measurement. W_{max} is the parameter value used in the simulation, and the estimates of the upper limits of U are obtained by assuming $W_{max}=1{\cdot}0$ for viability or survivorship. In Tables 2–6, unless otherwise specified, the simulation conditions are the same as specified here.

experiments (Lynch *et al.*, 1989), in which each clone has two sublines represented. Clonal sublines were maintained by asexual reproduction under the assay conditions for two generations prior to measurement. In the third generation, individuals were measured daily under a microscope for various life-history traits including the number of live off spring released in different clutches and egg survivorship of the first clutch (the ratio of the number of released live off spring to the number of eggs conceived, measured for the populations of *D. pulicaria* only). Each individual was maintained in 100 ml of aged, filtered water from the Amazon Pond (AM), a local temporary pond in Eugene, OR, supplemented with a pure laboratory culture of the green alga *Scenedesmus* to a density of ~150 000 cells/ml. The food/water mixture was replaced every other day. The experiment was conducted at 15 °C with a 12L : 12D photoperiod. One-way analysis of variance for unbalanced data was used to partition the phenotypic variance into within- and among-clone components of variance, the latter being the genetic variance among the clones (Deng & Lynch, 1996, 1997). The experimental procedures for the *D. obtusa* data are similar and the details as well as the geographic locations of populations can be found in Spitze (1993).

3. Results

(i) *Simulation results*

Results in Table 1 indicate that the estimation is relatively robust and generally yields estimates of the upper bounds for U that are close to the true U. The estimation is not biased by unknown variable mutation effects. With the substantial presence of additional overdominance mutations in the genome, estimation of the upper limit of U is little influenced. Consistent with previous results (Charlesworth *et al.*, 1990; Deng, 1998; Deng & Lynch, 1997), putative epistasis does not much influence the estimation of U. In outcrossing populations, when the true W_{max} is 1·0, the estimates of the upper limit of U are slightly smaller than the true U value with epistatic mutation effects or mixed dominance and overdominance mutations in the genome. Assuming the unknown W_{max} to be at its maximum (1·0) for viability or survivorship, estimates of the upper limit of U increase with decreasing true W_{max} values. However, the increase is roughly linear when the true W_{max} is greater than 0·5. Thus the upper limit estimate for U (by assuming the unknown W_{max} to be 1·0) is generally no more than twice the true value of U.

Table 2. *Estimation of* δ *under various conditions in large populations*

Dominance				Mixed dominance and overdominance			
Constant	Variable	Epistasis		Constant dominance		Variable dominance	
Complete selfing population							
0·011 (0·001)	0·010 (0·001)	$0{\cdot}013_{r=0{\cdot}10}$ (0·001)	$0{\cdot}017_{r=0{\cdot}30}$ (0·002)	$0{\cdot}011_{\alpha=0{\cdot}79,\ \beta=0{\cdot}95}$ (0·001)	$0{\cdot}012_{\alpha=0{\cdot}59,\ \beta=0{\cdot}89}$ (0·001)	$0{\cdot}009_{\alpha=0{\cdot}80,\ \beta=0{\cdot}90}$ (0·001)	$0{\cdot}010_{\alpha=0{\cdot}60,\ \beta=0{\cdot}90}$ (0·001)
Complete selfing population							
0·015 (0·002)	0·030 (0·003)	$0{\cdot}018_{r=0{\cdot}10}$ (0·002)	$0{\cdot}023_{r=0{\cdot}30}$ (0·003)	$0{\cdot}016_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0·002)	$0{\cdot}017_{\alpha=0{\cdot}63,\ \beta=0{\cdot}89}$ (0·002)	$0{\cdot}032_{\alpha=0{\cdot}77,\ \beta=0{\cdot}94}$ (0·003)	$0{\cdot}034_{\alpha=0{\cdot}62,\ \beta=0{\cdot}89}$ (0·003)

$W_{\max}$ is set to 1·0 as its parameter value does not influence the estimation of the per generation decrease in fitness due to DGM (equations 4 and 7).

Nearly unbiased estimates of δ are obtained for most situations. Strong putative epistasis causes the δ estimation to be slightly upwardly biased (Table 2). This bias is generally small, except that in partial selfing/outcrossing populations with variable effects, estimates of relatively large upward bias could be obtained (Table 3). When mating in a population is partial selfing/outcrossing and $W_{\max}$ is set to 0·8, the estimates of U are biased; however, the estimated U is less than $2U$ (Table 3). The degree of bias decreases when the selfing rate (S) approaches 0·5 in predominantly outcrossing populations ($S<0{\cdot}5$) and approaches 1·0 in predominantly selfing populations ($S>0{\cdot}5$).

In populations approaching M–S balance, the estimates are very close to (slightly smaller than) those obtained under M–S balance; thus the bias is usually smaller than the true parameter values (Table 4). Even starting from populations free of deleterious mutations, it takes only about 100 generations in selfing populations and about 500 generations for outcrossing populations to obtain the same estimates as those obtained under M–S balance. In addition, linkage disequilibrium due to mutation and selection in finite populations has little effect on the estimates in outcrossing populations (Table 5).

(ii) *Results from* Daphnia *data*

Averaged over 14 populations of *D. pulicaria*, the upper limit of U for egg survivorship of the first clutch is 0·73 (SD = 0·30) (Table 6). For the first four clutches, per generation decrease in fecundity due to DGM ranges from 2·2% (SD = 6·2%) to 7·8% (9·4%) in 20 *D. pulex* populations and from 1·1% (2·9%) to 5·1% (4·1%) in 8 *D. obtusa* populations. Averaged over all the study populations of *D. pulex* and *D. obtusa*, DGM decrease a clutch size by 3·6% per generation. These results indicate that DGM are an important force in shaping fitness of natural *Daphnia* populations.

Table 3. *Estimation of* U *and* δ *in partial outcrossing/selfing populations*

	U		δ	
S	Constant	Variable	Constant	Variable
0·1	1·16 (0·01)	1·17 (0·01)	0·014 (0·002)	0·017 (0·002)
0·3	1·06 (0·01)	1·06 (0·01)	0·018 (0·002)	0·041 (0·005)
0·7	1·50 (0·02)	1·50 (0·03)	0·019 (0·002)	0·046 (0·004)
0·9	1·31 (0·02)	1·30 (0·03)	0·017 (0·002)	0·036 (0·003)

Two subpopulations are simulated, in one of which the mating is outcrossing and in the other of which it is selfing, with S the selfing rate in the study population. The outcrossing and selfing subpopulations will contribute the proportions of $1-S$ and S to the study population, respectively (Charlesworth *et al*., 1991; Kondrashov, 1985; Li & Deng, 2000). For $S<0{\cdot}5$, the population is predominantly outcrossing and equations (4) and (5) are applied; for $S>0{\cdot}5$, mating is predominantly selfing and equations (6) and (7) are applied. $W_{\max}=0{\cdot}8$ in this table.

4. Discussion

The approach investigated here may open a door to quickly and conveniently characterizing U and per generation effects of DGM on fitness or its important components such as fecundity. For the estimation of the upper limit of U, which is generally less than twice the true value of U, the approach applies to essentially any populations and species. However, care should be taken to employ the estimates of the upper limit of U from any higher eukaryotes to test some population genetic theories such as those on the evolution of recombination. Due to various mechanisms (i.e. variable efficiency of DNA repair systems or genome sizes, which are shaped by evolutionary forces and related to life histories adopted: Drake *et al*., 1998; Kibota & Lynch, 1996), U may differ substantially in different organisms (Drake *et al*., 1998), especially between higher and lower organisms. Therefore, while characterization of the patterns of U across diverse

Table 4. *Estimation of* U *and* δ *in non-equilibrium populations approaching mutaion–selection*(*M–S*) *balance*

	Selfing populations					Outcrossing populations			
	U		*δ*			*U*		*δ*	
G	Constant	Variable	Constant	Variable	*G*	Constant	Variable	Constant	Variable
50	1·01 (0·02)	1·00 (0·02)	0·012 (0·001)	0·023 (0·002)	50	0·64 (0·00)	0·54 (0·00)	0·005 (0·000)	0·003 (0·000)
75	1·12 (0·02)	1·11 (0·02)	0·013 (0·001)	0·027 (0·003)	200	1·11 (0·01)	1·01 (0·01)	0·010 (0·001)	0·008 (0·001)
150	1·22 (0·02)	1·22 (0·02)	0·015 (0·001)	0·030 (0·003)	500	1·22 (0·01)	1·20 (0·01)	0·011 (0·001)	0·010 (0·001)
300	1·23 (0·02)	1·23 (0·02)	0·015 (0·001)	0·030 (0·003)	1000	1·22 (0·01)	1·22 (0·01)	0·011 (0·001)	0·010 (0·001)
M–S balance	1·23 (0·02)	1·23 (0·02)	0·015 (0·001)	0·030 (0·003)	M–S balance	1·22 (0·01)	1·22 (0·01)	0·011 (0·001)	0·010 (0·001)

Starting with a population free of deleterious mutations, mutation and selection are applied from generation to generation in simulations (Charlesworth *et al*., 1991; Kondrashov, 1985; Li & Deng, 2000) and assays are made after certain generations specified in the table and after simulated populations reach M–S balance (labelled as 'M–S' in the table). *G* denotes the generations passed under the forces of mutation and selection for a starting population free of deleterious mutations. $W_{max} = 0{\cdot}8$ in this table.

Table 5. *Estimation of* U *and* δ *in finite populations with linkage disequilibrium*

	Selfing populations				Outcrossing populations			
	U		*δ*		*U*		*δ*	
θ	Constant	Variable	Constant	Variable	Constant	Variable	Constant	Variable
0·01	1·69 (0·13)	2·03 (0·12)	0·016 (0·003)	0·032 (0·004)	1·23 (0·02)	1·20 (0·02)	0·011 (0·000)	0·008 (0·001)
0·10	1·74 (0·13)	2·03 (0·22)	0·015 (0·003)	0·030 (0·002)	1·22 (0·01)	1·20 (0·01)	0·011 (0·000)	0·008 (0·000)

θ is the recombination rate between adjacent loci. Finite populations are simulated as in Charlesworth *et al*. (1992), Fraser & Burnell (1970) and Li & Deng (2005). Starting from no deleterious mutations, a population of 1600 individuals each with 5200 mutable loci is simulated under mutation and selection from generation to generation. For each set of data, 10 different populations are simulated. For each population, 100 assays are done under quasi-balance (Charlesworth *et al*., 1992; Fraser & Burnell, 1970; Li & Deng, 2005). $W_{max} = 0{\cdot}8$.

organisms is very important, care should be taken to employ these estimates in different contexts in a meaningful way. For example, while estimates of *U* from higher eukaryotes are valuable in inferring the impact of DGM on human health or on rare or endangered species, they may be of limited value for testing population genetics theories on the evolution of recombination. This is because recombination may have evolved in very primitive and lower organisms.

$W_{max} = 1{\cdot}0$ means that the genotype free of segregating mutations in the population has viability or survivorship 100%. However, it is unlikely that any outcrossing species or population has a genotype free of fixed (homozygous) DGM for viability or survivorship. Some DGM (especially slightly deleterious mutations) are likely to have been fixed in the genome during the long history of evolution and population dynamics (such as bottlenecks, where DGM can relatively easily get fixed by random genetic drift; Crow & Kimura, 1970). In addition, it is difficult for a genotype, even if free of heterozygous mutations, to achieve a viability or survivorship of 100% due to unfavourable components of the environment (especially in natural habitats) where the measurements are to be taken. Therefore, taking these realistic conditions into account, the estimation by equation (5) will almost always yield an estimate of the upper bound of *U*.

The species used in our study, *Daphnia*, is a freshwater microcrustacean. For our study populations, *D. pulex* and *D. obtusa* live in intermittent environments and *D. pulicaria* in a seasonally changing permanent environment. These populations of *Daphnia* species reproduce by cyclical parthenogenesis (Lynch *et al*., 1989) to cope with seasonality of their habitats. Generally, populations are established via the revival of diapausing eggs produced by sexual reproduction towards the end of the previous growing season. They undergo several generations of asexual reproduction during the season of population expansion before engaging in sexual reproduction when the habitats start to deteriorate (Deng, 1996, 1997; Hutchinson, 1967). Asexual fecundity during asexual reproduction is crucial for propagating genotypes clonally and is

Table 6. *Estimates of the upper bound of* U *for survivorship in* D. pulicaria *and* Uhs *for fecundity in* D. pulex *and* D. obtusa

D. pulicaria population	$U \leqslant$	*D. pulex* population	*Uhs* for different clutch sizes				*D. obtusa* population	*Uhs* for different clutch sizes			
			First	Second	Third	Fourth		First	Second	Third	Fourth
DL (39)	0·851	CP (42)	0·026	0·050	0·070	−0·025	BUF (20)	−0·025	0·031	0·114	0·017
EL (35)	0·607	OA (36)	−0·035	0·047	−0·057	0·115	CT (26)	−0·016	0·045	0·048	0·033
GL (32)	0·553	SW (44)	0·014	0·056	−0·136	0·059	HAP (23)	0·029	0·041	0·016	0·036
HL (45)	0·740	PW (48)	0·010	−0·036	0·076	0·179	MAY (25)	0·021	0·124	0·068	0·074
KL (27)	0·810	CC (40)	0·065	−0·016	0·001	0·119	NH (22)	0·068	0·029	−0·042	0·067
LCL (28)	0·931	MV (28)	−0·018	−0·011	0·090	0·070	NP (20)	−0·011	0·004	−0·099	0·014
LL (49)	0·292	MP (42)	0·014	0·069	0·016	0·325	OJ (19)	0·011	0·030	0·021	0·044
MCL (41)	0·607	OP (36)	0·038	0·023	0·051	0·023	TRE (18)	0·010	0·105	0·052	0·091
ML (17)	0·924	PG (42)	0·011	0·005	0·025	0·157					
PL (11)	0·711	AM (25)	0·060	0·032	−0·002	0·009					
SDL (39)	0·548	SG (45)	−0·004	0·058	−0·053	0·043					
SL (48)	0·662	WM (40)	0·058	0·038	0·152	0·034					
SSL (26)	0·383	BT (43)	0·031	0·023	0·007	0·190					
TL (17)	1·542	GI (47)	0·023	0·003	0·021	−0·096					
		FC (38)	0·017	0·118	0·071	0·030					
		KC (18)	0·080	0·054	0·072	0·056					
		SL (35)	0·039	0·046	−0·026	0·067					
		WH (29)	0·015	0·000	0·045	−0·025					
		LO (30)	0·056	0·049	0·006	0·057					
		SH (36)	−0·019	0·072	0·013	0·177					
Mean	0·726		0·024	0·034	0·022	0·078		0·011	0·051	0·022	0·047
(SD)	(0·300)		(0·030)	(0·036)	(0·062)	(0·094)		(0·029)	(0·041)	(0·066)	(0·028)

The numbers in parentheses after the population name abbreviations are the numbers of clones (the same as sample sizes) from the respective study populations.

thus assumed to be under directional selection to increase, as is the survivorship. Within cyclical parthenogenetic *Daphnia* populations, mating is almost always random (Lynch & Spitze, 1994); this is also generally true for our study populations as examined by molecular polymorphisms at allozyme and/or microsatellite marker loci (Deng & Lynch, 1996; Spitze, 1993). An unbiased estimate of the total genetic variance requires that clones of the genotypes be available, so that the environmental variation will be clearly separated from the total genetic variance (Lynch & Walsh, 1998). Cloning of genotypes is generally not a problem in selfing organisms, though it poses a challenge in most outcrossing populations. However, in outcrossing cyclical parthenogens, genotypes can be preserved and replicated (forming a clone) easily by asexual reproduction. Thus, cyclical parthenogenetic *Daphnia* is an ideal model system for estimating the per generation decline in fitness due to DGM by equation (4). It should be noted that estimation of the upper bound of U for viability or survivorship does not require estimation of genetic variance and thus can be applied to any outcrossing populations.

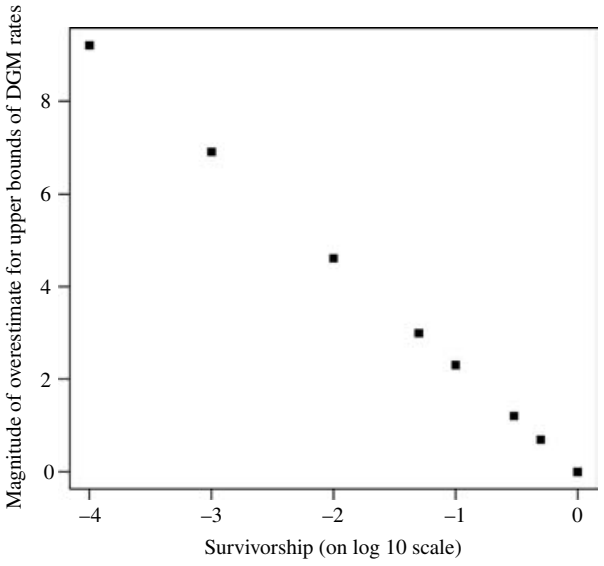

Fig. 1. The effects of survivorship on estimates of upper bounds of DGM rate. Each data point indicates the magnitude of overestimation for the upper bound of DGM rate when the real maximum survivorship is less than 1 but 1 is used in the estimation process. Note that the unit on the x-axis is log 10 of the survivorship.

One concern of applying our approach is that the maximum survivorship can vary dramatically among species; specifically, it can be much less than 1 in many species such as insects, amphibians and flowering plants. This low survivorship can be mainly due to environmental factors such as carrying capacity. This may result in overestimated upper bounds for U. However, based on the intrinsic properties of our

approach and with careful consideration of experimental designs, our approach can still be applied to a range of biologically plausible situations. First, the survivorship enters into the formula (equation 3) as a logarithmic term. Therefore, low survivorship (when overestimated) will not have the dramatic effect it would seem to on its original scale. For example, an estimate of maximum survivorship with a 10-fold increase from the true value only increases the estimate of the upper bound of U by a factor of about 2 (Fig. 1). Even with an estimate of maximum survivorship 10 000 times higher than its true value, the upper bound of U is only inflated by a factor of about 9. Therefore, the estimate of the upper bound of U is quite insensitive to the maximum survivorship assumed. Second, there are many species whose survivorship is relatively high, such as mammals. For these kinds of species, an estimate of the maximum survivorship should not be too far (i.e. more than one order of magnitude) from its true value. Hence, no substantial bias for the upper limit of the DGM rate is expected. With no other methods available for estimating the upper bound of the DGM rate, our approach can at least be used as a starting point for further investigation. Third, since survivorship is very likely to be higher in a laboratory than in the wild, estimates of survivorship from laboratory experiments can be used as $W_{\max}$. The estimate of the DGM rate obtained using equation (3) for outcrossing populations or $U \approx -2\ln(W_s/W_{\max})$ for selfing populations can then be considered as the estimate of the upper bound of the DGM rate in the wild.

Our results are consistent with earlier studies suggesting high deleterious mutation pressures on fitness (Charlesworth *et al*., 1990; Deng & Lynch, 1997; Houle *et al*., 1992; Johnston & Schoen, 2005; Kibota & Lynch, 1996; Lynch, 1985; Lynch *et al*., 1998; Mukai *et al*., 1972; Shabalina *et al*., 1997) and are inconsistent with those suggesting otherwise (Fernandez & Lopez-Fanjul, 1996; Keightley & Caballero, 1997). The results here indicate that DGM, and natural selection curbing their accumulation in natural populations, are important forces shaping population fitness and thus survivability of natural populations. Therefore, prevention of accumulation of DGM should be taken seriously for the protection of human health and conservation of small populations of rare and endangered species.

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