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THE FITNESS EFFECT OF MUTATIONS ACROSS ENVIRONMENTS: A SURVEY IN LIGHT OF FITNESS LANDSCAPE MODELS

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Abstract.—The fitness effects of mutations on a given genotype are rarely constant across environments to which this genotype is more or less adapted, that is, between more or less stressful conditions. This can have important implications, especially on the evolution of ecological specialization. Stress is thought to increase the variance of mutations' fitness effects, their average, or the number of expressed mutations. Although empirical evidence is available for these three mechanisms, their relative magnitude is poorly understood. In this paper, we propose a simple approach to discriminate between these mechanisms, using a survey of empirical measures of mutation effects in contrasted environments. This survey, across various species and environments, shows that stress mainly increases the variance of mutations' effects on fitness, with a much more limited impact on their average effect or on the number of expressed mutations. This pattern is consistent with a simple model in which fitness is a Gaussian function of phenotypes around an environmentally determined optimum. These results suggest that a simple, mathematically tractable landscape model may not be quantitatively as unrealistic as previously suggested. They also suggest that mutation parameter estimates may be strongly biased when measured in stressful environments.

Key words.—Environmental stress, fitness landscape, genotype-by-environment interaction, mutation, survey.

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Spontaneous mutation is the ultimate source of variation and influences the evolutionary fate of a wide range of phenomena (Charlesworth and Charlesworth 1998; Lynch et al. 1999). More specifically, the evolutionary role of mutation depends on the genomic mutation rate, U, and the distribution of mutations' fitness effects, $f(s)$. In principle, U and $f(s)$ may vary among genotypes within species, among species, and among environments, which may obscure both the interpretation of empirical measurements and theoretical predictions. However, we still have little insights into how U and $f(s)$ may vary in general. In a recent paper (Martin and Lenormand 2006), we showed that $f(s)$ may vary in a predictable way between more or less complex organisms. In this paper, we focus on the variation of mutation effects in different environments.

Since the work of Kondrashov and Houle (1994), several studies have documented differences in mutation effects across various environments for quantitative traits that are more or less related to fitness (reviewed in Lynch et al. 1999; Fry and Heinsohn 2002; Lenormand 2002; Chang and Shaw 2003; Hermisson and Wagner 2004; Korona 2004). The implications of this environment dependence have been debated in an empirical and theoretical context. First, it might explain the discrepancies among estimates of mutational parameters within species, in particular in *Drosophila* (Kondrashov and Houle 1994; Garcia-Dorado et al. 1999), and more generally it has been studied to determine whether laboratory measures could be extrapolated in nature. Second, the environment-dependent variation of mutation effects has been debated in the context of ecological specialization (Fry 1996; Kawecki et al. 1997). In both cases, environmental variation is often measured or qualified in terms of more or less stressful conditions, which is also the approach chosen in this article. The definition of a stressful environment is not straightforward and varies according to authors and fields. Stressful environments can, for example, be defined as environments imposing some constraints on metabolism (e.g., desiccation, high temperature) that can only be coped with at some energetic cost. However, species adapted to extreme conditions may have a lower fitness in less extreme conditions (Parsons 1991). Following a widely used definition in studies of mutational effects (Korona 1999; Szafraniec et al. 2001; Kishony and Leibler 2003), we will consider that an environment is stressful for a given genotype if it reduces its fitness relative to that achieved in a benign (reference) environment.

Mutational parameters are in most cases measured using

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mutation accumulation (MA) experiments in which several sublines are maintained over several generations under minimal selection. Per generation differences in the mean (ΔM) and variance (ΔV) of fitness among sublines during the accumulation can be estimated to infer mutational parameters. In general,

$$\Delta M = U\bar{s} \quad \text{and} \tag{1a}$$

$$\Delta V = U\bar{s}^2[1 + \mathrm{CV}(s^2)], \tag{1b}$$

where U is the genomic rate of nonneutral mutation, $\bar{s}$ is the average fitness effect of single mutations, and CV(s) its coefficient of variation (Mukai et al. 1972). This result relies on only two assumptions: (1) the number of mutation events per genotype is Poisson distributed (with parameter U); and (2) the effects of mutations on fitness are additive (epistasis is neglected). Equations (1a,b) are the basis of the Bateman-Mukai method of estimation of U and $\bar{s}$ when CV(s) is neglected (constant mutation effects), but we will use them in their general formulation above (i.e., without neglecting CV[s]). The same sublines may be assayed in different environments, yielding several estimates of ΔM and ΔV. From equations (1a,b), these estimates may vary because U, $\bar{s}$, and/or CV(s) differ between environments but there is little agreement on the prevalence of either scenario (Fry and Heinsohn 2002). In the following, we give some details on the biological implications of each of the three scenarios.

Intuitively, variation in the genomic mutation rate U between environments should reflect the fact that some mutations have a detectable fitness effect in some environments but are neutral in others. This can happen if the mutational target varies between environments (by mutational target, we mean the fraction of expressed genes or more generally the fraction of genes affecting fitness). Note that the list of genes in this mutational target may vary between environments while leaving the overall size of the mutational target (and hence also U) almost constant. However, because we see no reasons for the mutational target size to be identical in all environments, environment-dependent expression should in principle be detected by variation of U between environments. We will label this hypothesis ''conditional expression'' (CE).

Variation in $\bar{s}$ would reflect that selection intensity differs in more stressful environments (e.g., as suggested in Kishony and Leibler 2003). For instance, a given mutation impairing maltose metabolization may have milder fitness consequences in a benign environment where several sugars are available (it may even be neutral in the absence of maltose) than in a stressful one where maltose is the only source of carbon available. We will label this hypothesis ''conditional average'' (CA).

Finally, variation in CV(s) between environments would reflect that mutational effects are more or less variable (among mutations) in stressful environments. Consider, for instance, a population of bacteria that has adapted in a given environment for a long period of time (e.g., with glucose as the carbon source), so that the average phenotype is close to an optimum on glucose. Most mutations are then likely to be deleterious in glucose, whereas a mixture of deleterious and beneficial mutations may be expected in a new environment (e.g., in maltose). Mutation effects are therefore likely to be less variable in glucose than in maltose. We will label this hypothesis ''conditional variance'' (CV). This hypothesis has been proposed by Fry and Heinsohn (2002) and by Remold and Lenski (2001) to describe a situation similar to the example cited above.

In principle these different scenarios may be distinguished by estimating U, $\bar{s}$, and CV(s) in different environments. However, it is difficult to disentangle them from MA data, because environmental variation may affect both the number of nonneutral mutations and their fitness effect (Lynch et al. 1999). For instance, Bateman-Mukai estimates of U and $\bar{s}$, derived from equations (1a,b),

$$U_{BM} \equiv \Delta M^2/\Delta V = U/[1 + \mathrm{CV}(s)^2] \quad \text{and} \tag{2a}$$

$$\bar{s}_{BM} \equiv \Delta V/\Delta M = \bar{s}[1 + \mathrm{CV}(s)^2] \tag{2b}$$

are biased by the variance of mutational effects (i.e., by CV[s]), which may itself vary across different environments. Therefore, it is impossible to directly assess whether U or $\bar{s}$ actually change across environments (Fry and Heinsohn 2002). Maximum-likelihood (Keightley 1994) or minimum distance (Garcia-Dorado and Marin 1998) methods can partially address this problem. However, with these methods, it is still difficult to state how much U and CV(s) vary relative to one another (Keightley 2004), and Bateman-Mukai estimates remain the most widely available in the literature, so that the empirical issue is still unresolved.

In this paper, we develop a simple approach to discriminate among CE, CA, and CV hypotheses. We then test these hypotheses using a survey of data obtained with MA experiments where mutant fitness was assayed in different environments. We focus in particular on environments that are more or less stressful, that is, in which fitness is reduced compared to a benign (reference) environment (see above).

After presenting the results of our survey and conclusions about CE, CA, and CV hypotheses, we explain when to expect these different scenarios and how the observed patterns can be interpreted. In particular, we interpret these patterns in terms of fitness landscape models that allow one to predict how mutation fitness effects may vary in different environments.

Methods

Simple Predictions Based on Bateman-Mukai Estimates

The three extreme hypotheses that either U, $\bar{s}$, or CV(s) differ between environments generate distinct and straightforward predictions that can be tested with measures of ΔM and ΔV in different environments. Let us consider two environments (1 and 2) in which ΔM (ΔM_1 and ΔM_2) and ΔV (ΔV_1 and ΔV_2) are measured for a given genotype. Define the measurable ratios $\rho_V \equiv \Delta V_1/\Delta V_2$ and $\rho_s \equiv \bar{s}_{BM1}/\bar{s}_{BM2}$ (using the definition of s_{BM} in eq. 2), and the nonmeasurable ratios ρ_U, ρ_s, and ρ_{CV} of U, $\bar{s}$, and $1 + \mathrm{CV}(s)^2$, respectively, in environment 1 versus 2. Then from equation (1), $\log(\rho_V) = \log(\rho_U) + 2\log(\rho_S) + \log(\rho_{CV})$, and from equation (2), $\log(\rho_S) = \log(\rho_s) + \log(\rho_{CV})$. Therefore, we can predict distinct relationships between $\log(\rho_V)$ and $\log(\rho_S)$, for each of the extreme scenarios considered, according to which of ρ_U, ρ_s, and

TABLE 1. Summary of the patterns expected under the three extreme hypothesis; CE, CA and CV (see text). ΔM and ΔV refer to the mutational change in mean and variance of relative fitness, respectively. The ρ values are ratios of estimates in stressful versus benign environments: ρ_V for ΔV and ρ_S for Bateman-Mukai estimates of the average fitness effect of mutations. The predicted relationships are also illustrated on Figure 1.

Hypothesis	Prediction across environments	
CE; conditional expression (U varies)	$\Delta M \propto \Delta V$	$\log(\rho_S) = 0$
CA; conditional average ($\bar{s}$ varies)	$\Delta M^2 \propto \Delta V$	$\log(\rho_S) = \frac{1}{2} \log(\rho_V)$
CV; conditional variance (CV[s] varies)	ΔM constant ΔV varies	$\log(\rho_S) = \log(\rho_V)$

ρ_{CV} is assumed to depart from one (expected in the absence of environment-dependent variation). If only U varies between environments (CE hypothesis), $\log(\rho_s)$ and $\log(\rho_{CV})$ should remain negligible relative to $\log(\rho_U)$, so that $\log(\rho_S) = 0$. Similarly, if only $\bar{s}$ varies between the two environments, that is, only $\log(\rho_s)$ is nonzero, (CA hypothesis), then $\log(\rho_S) = \log(\rho_s) = \frac{1}{2} \log(\rho_V)$. Finally, if only CV($S$) varies between environments, that is, only $\log(\rho_{CV})$ is nonzero (CV hypothesis), then $\log(\rho_S) = \log(\rho_{CV}) = \log(\rho_V)$. With several pairs of ρ_S and ρ_V estimates (ratios from several pairs of environments and/or several studies), we can discriminate among the three hypotheses depending on the slope of the empirical relationship between $\log(\rho_S)$ and $\log(\rho_V)$, that is, no relationship (CE) or a linear relationship with slope ½ (CA) or 1 (CV). These predictions are summarized in Table 1. Of course, this empirical relationship (if any) may differ from the three predicted trends (e.g., if U, $\bar{s}$, and CV[s] all vary between environments or differently so in different species or experiments). Therefore, all three extreme hypotheses could easily be rejected, either by any nonlinear relationship between $\log(\rho_S)$ and $\log(\rho_V)$ or by a linear relationship with a slope that differs from ½ or 1.

Stressful Versus Benign Environments

In experiments measuring mutation fitness effects in two environments, one can often be considered more stressful than the other. The most stressful environment is the one in which the nonmutated initial genotype has the lowest absolute fitness. As above, U, $\bar{s}$, or CV(s) may differ between stressful and benign environments. However, these parameters may vary in a consistent direction with stress. For instance, it may be argued that U, $\bar{s}$ or CV(s) should increase in more stressful environments. To detect such a trend, we can take the same approach as above but systematically standardizing our ratios by values in the most benign environment, if such information is available. Denoting with or without a star the value in the benign or stressful environment, respectively, we can therefore compute $\rho_V \equiv \Delta V/\Delta V^*$, $\rho_M \equiv \Delta M/\Delta M^*$, and the corresponding $\rho_S \equiv \bar{s}_{BM}/\bar{s}^*_{BM}$. In this way, we can determine whether U, $\bar{s}$, or CV(s) are systematically changed in stressful versus benign environments and in which direction. For the sake of clarity, all ratios will be computed in this way (i.e., relative to the most benign environment) in the paper.

Survey of Mutational Genotype-by-Environment Interactions for Fitness

MA experiments are the most widely used method to generate a set of mutants from a single isogenic line. The fitness of these mutants can then be estimated, providing estimates of ΔM (the per generation average change in relative fitness due to mutation) and of ΔV (the per generation increment in relative fitness variance due to mutation). In addition, these moments can be measured for a given set of lines, in different environmental conditions, providing a measure of the change of ΔM and ΔV in different environments. We surveyed nine MA experiments (some using mutagenesis) reporting such variation, most of which are also discussed in Fry and Heinsohn (2002). Our survey is summarized in Table 2. When it was not directly provided, we computed the mutational variance in relative fitness ΔV as the squared mutational coefficient of variation of the fitness trait measured, that is, the increase in variance among mutant lines relative to control lines divided by the mean value of the control. Similarly, when not directly provided, we computed the average mutational change in relative fitness ΔM as the difference between the mean value of the fitness measure among mutants and the control value, divided by the control value. We considered that the least stressful environment was the one with the highest absolute fitness of the nonmutated (control) genotype (reported in Table 2). In some cases, fitness was measured in competition with a reference strain, in which case it was not possible to identify the least stressful environment for the control (as stress also affects the competitor). In these cases, the benign environment was defined according to the authors, usually as the ''standard'' laboratory environment to which the control line has adapted for generations or, in some studies, the low-density environment. In most cases, the original papers provided unambiguously the required information. However, in some cases, we had to make some choices or to read some of the data on figures presented in the papers.

In the study of Fry et al. (1996) on *Drosophila melanogaster*, fitness of the nonmutated genotype is not provided. We considered the strain used for the competitive assays as a surrogate for this control genotype. The reproductive output/vial for this strain was read from figures 2A and 2B in the paper. This strain is not related to the MA lines so that it is not a proper control, but it was assumed free of mutation, based on its higher fitness (fig. 2B), and on it having not undergone MA. It may, however, differ from the exact control (ancestor of MA lines) in its level of adaptation to some of environments, which could explain the strong difference observed between MA and ''control'' mean under low temperature (Fry et al. 1996; Fry and Heinsohn 2002). In any case, this use of an improper control should only bias ΔM estimates (not ΔV) because this strain was isogenic or nearly so, and removing the estimates from this study does not affect any of our conclusions. In the study of Korona (1999) on

TABLE 2. Environmental variation on mutational mean and variance in fitness. Estimates of mutational mean ΔM and variance ΔV in fitness (relative to the control), given in various environments: st, standard laboratory environment; D, density; T, temperature; F, food quantity or quality (includes diluted media), substance added to the medium when indicated. Fitness traits: *r*, population growth rate; *w*/vial, reproductive output per vial; *dens*., final population density; *viability*, viability in competition with a reference strain. $\log(\rho_M) = \log(\Delta M/\Delta M^*)$, $\log(\rho_V) = \log(\Delta V/\Delta V^*)$, and $\log(\rho_U) = 2\log(\rho_M) - \log(\rho_V)$ are the log-ratios of ΔM ΔV, and U_{BM} (Bateman-Mukai estimate of U, $U_{BM} = \Delta M^2/\Delta V$), respectively, relative to their values in the benign environment (indicated in bold). When it was provided in the studies, the control fitness measure is given together with the ratio of control fitness in the stressful versus benign environment, as a standardized measure of stress. Data sources are listed below with the corresponding number of MA generations in brackets and with an asterisk when ΔV and ΔM are per generation estimates: $1^{(1)}$ and $1^{(2)}$; experiments 1 and 2 in Fry and Heinsohn (2002) [27–35]*; 2, Fry et al. (1996) [202]*; 3, Yang et al. (2001) [mutagenesis ~100]; 4, Fernandez and Lopez-Fanjul (1997) [104–160]*; 5, Vassilieva et al. (2000) [214]*; 6, Szafraniec et al. (2001) [40]; 7, Korona (1999) [500]; 8, Xu (2004) [600]; 9, Kishony and Leibler (2003) [mutagenesis, assumed to be equivalent to 100 generations]. *n* (2*n*): haploid (diploid) strains of *Saccharomyces cerevisiae*. All estimates are for homozygous or haploid effects except $^{(H)}$ heterozygous effects.

Species	Environment	Trait	Control fitness	% fitness in benign	ΔM	$\log(\rho_M)$	ΔV	$\log(\rho_V)$	$\log(\rho_U)$	Source
Drosophila melanogaster	**low D**	*viability*			**−0.0029**		**5.9 × 10^{-5}**			$1^{(1)}$
D. melanogaster	st	*viability*			−0.001	−0.462	3.5 × 10^{-4}	0.78	−1.70	$1^{(1)}$
D. melanogaster	low T	*viability*			−0.004	0.140	1 × 10^{-3}	1.25	−0.97	$1^{(1)}$
D. melanogaster	ethanol	*viability*			−0.0037	0.106	3.8 × 10^{-4}	0.81	−0.59	$1^{(1)}$
D. melanogaster	**low D**	***viability***			**−0.0015**		**5.1 × 10^{-5}**			$1^{(2)}$
D. melanogaster	st	*viability*			−0.0032	0.329	6.9 × 10^{-4}	1.13	−0.47	$1^{(2)}$
D. melanogaster	low T	*viability*			−0.0041	0.437	8.9 × 10^{-4}	1.24	−0.37	$1^{(2)}$
D. melanogaster	ethanol	*viability*			−0.0039	0.415	1.8 × 10^{-3}	1.55	−0.72	$1^{(2)}$
D. melanogaster	**st**	***w/vial***	**165**		**−0.0017**		**5.4 × 10^{-5}**			2
D. melanogaster	low T	*w/vial*	157.5	95%	−0.004	0.385	2.9 × 10^{-5}	−0.27	1.04	2
D. melanogaster	tomato	*w/vial*	138.095	84%	−0.0014	−0.082	5.8 × 10^{-5}	0.04	−0.20	2
D. melanogaster	ethanol	*w/vial*	120	73%	−0.0017	0.000	5.4 × 10^{-5}	0.00	0.00	2
D. melanogaster $^{(H)}$	**low D**	***viability***			**−0.0136**		**3.1 × 10^{-3}**			3
D. melanogaster $^{(H)}$	low F+high D	*viability*			−0.0129	−0.023	1.8 × 10^{-2}	0.76	−0.80	3
D. melanogaster $^{(H)}$	low F	*viability*			−0.009	−0.179	7.5 × 10^{-4}	−0.62	0.26	3
D. melanogaster	**st**	***fecundity***			–	–	**1 × 10^{-4}**		–	4
D. melanogaster	high T	*fecundity*			–	–	2.28 × 10^{-4}	0.358	–	4
D. melanogaster	NaCl	*fecundity*			–	–	1.82 × 10^{-4}	0.261	–	4
D. melanogaster	low F	*fecundity*			–	–	1.72 × 10^{-4}	0.235	–	4
D. melanogaster	**st**	***early viab.***			–	–	**9.8 × 10^{-5}**		–	4
D. melanogaster	high T	*early viab.*			–	–	2.79 × 10^{-4}	0.454	–	4
D. melanogaster	NaCl	*early viab.*			–	–	4.62 × 10^{-5}	−0.326	–	4
D. melanogaster	low F	*early viab.*			–	–	6.56 × 10^{-5}	−0.174	–	4
D. melanogaster	**st**	***late viab.***			–	–	**1.6 × 10^{-5}**		–	4
D. melanogaster	high T	*late viab.*			–	–	1.94 × 10^{-5}	0.083	–	4
D. melanogaster	low F	*late viab.*			–	–	1 × 10^{-4}	0.796	–	4
Caerorhabditis elegans	**st**	***r***	**1.309**		**−0.0008**		**1.6 × 10^{-5}**			5
C. elegans	low T	*r*	0.39	30%	−0.0015	0.273	5.3 × 10^{-4}	1.52	−0.97	5
Saccharomyces cervisiae 2*n* $^{(H)}$	**st**	*r*	**0.418**		**−0.0024**		**1.9 × 10^{-6}**			6
S. cervisiae 2*n* $^{(H)}$	high T	*r*	0.247	59%	−0.19	1.901	9.4 × 10^{-6}	0.63	3.17	6
S. cervisiae 2*n* $^{(H)}$	**st**	***dens.***	**1.537**		**0**		**2.3 × 10^{-7}**			6
S. cervisiae 2*n* $^{(H)}$	high T	*dens.*	1.179	77%	−0.083		3.9 × 10^{-3}	4.22		6
S. cervisiae 2*n*	**st**	***r***	**0.705**		**−0.067**		**1 × 10^{-2}**			7
S. cervisiae 2*n*	low F	*r*	0.503	71%	−0.0457	−0.164	3.6 × 10^{-2}	0.55	−0.88	7
S. cervisiae n	**st**	***r***	**0.675**		**−0.262**		**7.3 × 10^{-3}**			7
S. cervisiae n	low T	*r*	0.147	22%	−0.236	−0.045	3.4 × 10^{-2}	0.67	−0.76	7
S. cervisiae n	high T	*r*	0.502	74%	−0.5	0.280	2.8 × 10^{-1}	1.58	−1.02	7
S. cervisiae n	low F	*r*	0.534	79%	−0.194	−0.130	1 × 10^{-2}	0.15	−0.41	7
S. cervisiae n	glycerol	*r*	0.272	40%	−0.272	0.016	7.6 × 10^{-3}	0.01	0.02	7
Cryptococcus neoformans	**st**	***r***	**0.5333**		**−0.3085**		**3.7 × 10^{-2}**			8
C. neoformans	low F+low T	*r*	0.14861	28%	−0.32	0.016	7.3 × 10^{-2}	0.29	−0.26	8
C. neoformans	low T	*r*	0.27083	51%	−0.167	−0.267	1 × 10^{-2}	−0.54	0.00	8
C. neoformans	low F	*r*	0.20625	39%	−0.44	0.155	1.2 × 10^{-1}	0.50	−0.19	8

TABLE 2. Continued.

Species	Environment	Trait	Control fitness	% fitness in benign	ΔM	$\log(\rho_M)$	ΔV	$\log(\rho_V)$	$\log(\rho_U)$	Source
Escherichia coli	st	*r*		73%	−0.275	0.008	–	–	–	9
E. coli	acidic	*r*		61%	−0.300	0.046	–	–	–	9
E. coli	**high F**	***r***			**−0.270**		–	–	–	9
E. coli	NaCl	*r*		48%	−0.250	−0.033	–	–	–	9
E. coli	redox agent	*r*		54%	−0.210	−0.109	–	–	–	9
E. coli	antibiotic	*r*		43%	−0.100	−0.431	–	–	–	9
E. coli	antibiotic	*r*		48%	−0.150	−0.255	–	–	–	9
E. coli	low T	*r*		12%	−0.150	−0.255	–	–	–	9

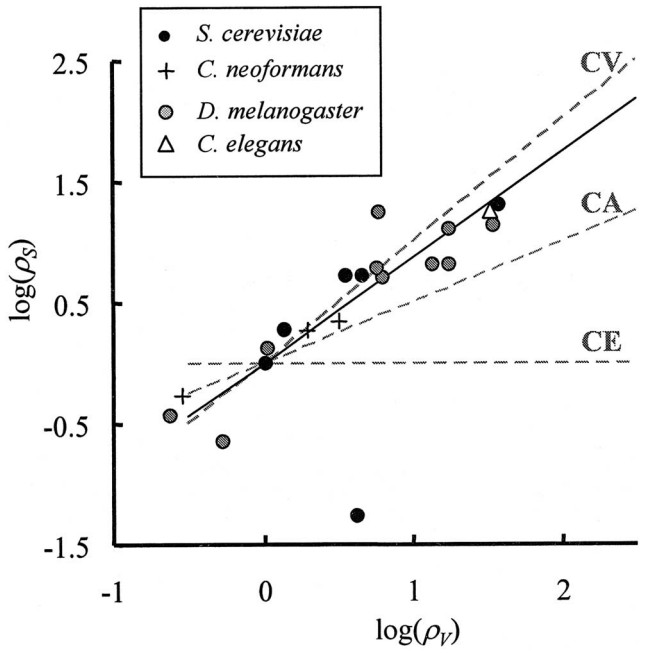

FIG. 1. Effect of stressful conditions on mutational variance in fitness and Bateman-Mukai estimates of $\bar{s}$. The log-ratio of ΔV estimates ($\log[\rho_V]$, *x*-axis) and of s_{BM} ($\log[\rho_S]$, *y*-axis), the Bateman-Mukai estimates of $\bar{s}$, in the stressful versus benign environments are from Table 2 (with s_{BM} computed from equation 2) for various species (indicated on the graph). The solid line gives the observed linear relationship between estimates (excluding the outlier, top of the graph). Dashed lines give the predicted linear relationships for each of the three hypotheses (CE, CA, CV, see text) indicated on the graph.

Saccharomyces cerevisiae, the among-line variance of MA haploid lines (M lines) was directly read on figure 1 in the paper, and the variance among control (F) lines was set to zero (from fig. 1). For diploid strains, the among-line variances of M/M and F/F strains were read on figure 3 in the paper. In the study of Xu (2004) on *Cryptococcus neoformans*, two environments (37°C and 25°C) were used during the mutation accumulation itself. We pooled results from all MA lines (a total of 16 lines). The absolute fitness of the controls, which was not available in the paper, was provided by the author. In Fernandez and Lopez-Fanjul (1997), no control was available and we did not find an alternative control measure as in Fry et al. (1996), so we only report effects of mutations on ΔV computed by neglecting the variance among control lines. This study is therefore not used in the relationship between $\log(\rho_S)$ and $\log(\rho_V)$ (see Fig. 1), but only to assess the effect of stressful conditions on the sign of $\log(\rho_V)$ (see Fig. 2). Conversely, in Kishony and Leibler (2003), ΔV is not given, so that we only report ΔM. Finally, in two other studies of mutation effects across environments (Chang and Shaw 2003; Kavanaugh and Shaw 2005), there is no clear evidence of any fitness variance induced by mutation, in any environment, so we discarded these studies.

As explained above, to estimate the effect of the environment on ΔM and ΔV we used log-ratio estimates of ΔM ($\log[\rho_M]$) and ΔV ($\log[\rho_V]$) in a given stressful environment relative to the estimate in the benign environment (denoted

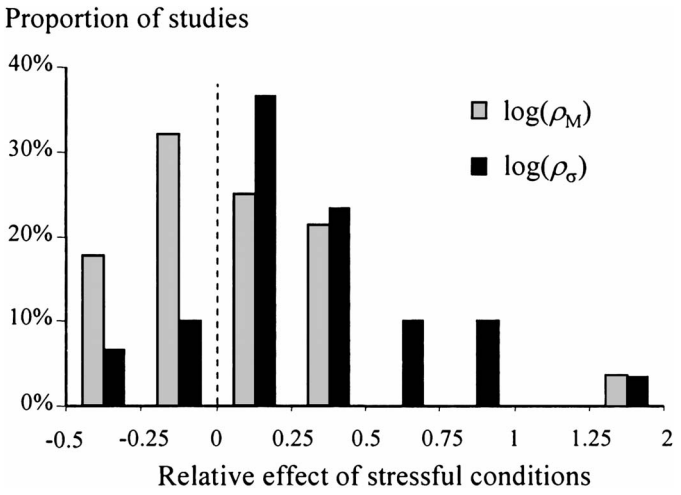

FIG. 2. Distribution of the relative change in mutational mean and standard deviation of fitness in stressful versus benign environments. Mutation effects on mean (ΔM) and variance (ΔV) in relative fitness are given relative to their values in the benign environment (ΔM^* and ΔV^*). Values on the *x*-axis refer to the log relative change in mean $\log(\rho_M) = \log(\Delta M/\Delta M^*)$ (28 estimates) or standard deviation $\log(\rho_\sigma) = \frac{1}{2}\log(\Delta V/\Delta V^*)$ (30 estimates). Positive values correspond to an increase in the mean or variance of mutation effects in stressful conditions. All but one (96%) of the $\log(\rho_M)$ estimates fall within the range $(-0.5, 0.5)$.

ΔM^* and ΔV^*, respectively). These measures are therefore standardized within each study, which allows us to compare different experiments that may differ in the species used, the experimental design, the fitness measure, the number of MA generations (which may even be poorly known, e.g., in mutagenesis or microbe studies), or with regard to specific features of the organism studied (e.g., ploidy or genome size that affect the mutational target size). Note also that the results in each experiment are based on a single set of lines having accumulated mutations in a common controlled environment so that there is no influence of environment-dependent molecular mutation rates (except potentially in Xu 2004).

RESULTS

In Figure 1, we illustrate how $\log(\rho_S)$ varies with $\log(\rho_V)$ in the surveyed experiments. We find a clear linear relationship between $\log(\rho_S)$ and $\log(\rho_V)$. Because both $\log(\rho_S)$ and $\log(\rho_V)$ are measured with error, we use the reduced major axis to measure the slope of the relationship between the two variables (regression type II; Sokal and Rohlf 1995). Because there is an obvious outlier, we report the estimated slope with or without it. In addition, we report the slope assuming or not a zero intercept. Table 3 summarizes the estimates and their 95% bootstrap confidence limits. The fitted linear relationships between $\log(\rho_S)$ and $\log(\rho_V)$ give a good fit to the data, explaining 55% or 88% of the total variance (with or without the outlier, respectively), and the estimated slope ranges between 0.86 and 1.02 (depending on the model). This slope is not significantly different from one (expected under the CV hypothesis) except for the model with zero intercept excluding the outlier for which the slope 95% confidence interval is (0.80, 0.99). In all cases, the estimated slope is significantly different from ½ (CA hypothesis) and from zero (CE hypothesis) ($P < 0.0001$). These results indicate that the observed pattern is very close to that expected under the CV hypothesis—the corresponding predicted relationship, $\log(\rho_S) = \log(\rho_V)$ explains 82% of the total variance, when excluding the outlier—with a slope perhaps slightly less than one, however. This result strongly supports the idea that changing environments mainly changes the variance of mutation fitness effects (CV hypotheses) rather than their average effect (CA hypothesis) or their net expression level over the genome (CE hypothesis). Finally, note that this first conclusion does not depend on correctly assessing stressful versus benign environments: the observed relationship $\log(\rho_S) \approx \log(\rho_V)$ is expected even when standardizing with a nonbenign environment.

To scale the range of variation of the mean and variance of mutation effects, we considered the standard deviation, $\sigma = \Delta V^{1/2}$, and its relative change under stressful conditions: $\log(\rho_\sigma) = \frac{1}{2}\log(\rho_V)$. Figure 2 shows the distribution of $\log(\rho_M)$ and $\log(\rho_\sigma)$ in our survey. In the large majority of experiments (25 of 30 estimates), stressful conditions result in an increase of the mutational variance (or σ) in fitness (i.e., CV hypothesis with a directional effect of stress): most $\log(\rho_\sigma)$ values are positive (two-tailed Wilcoxon signed-rank test, $P < 0.0001$). On the contrary, $\log(\rho_M)$ does not show the same pattern, increasing in only half of the cases (14 of 28 estimates) and not showing any significant positive or negative sign (two-tailed Wilcoxon signed-rank test, $P = 0.73$). Therefore, contrary to their effect on ΔV, stressful conditions do not result in a consistent trend toward increased or decreased ΔM. Finally, note that the variation of $\log(\rho_M)$ is also smaller than that of $\log(\rho_\sigma)$ (see Fig. 2), although means and standard deviations are of the same scale. Ninety-six percent of $\log(\rho_M)$ estimates fall in the range $(-0.5, 0.5)$ (i.e., all but the outlier mentioned above), whereas more than 23% (7 of 30) of $\log(\rho_\sigma)$ estimates fall outside this range.

INTERPRETATION IN TERMS OF FITNESS LANDSCAPES

Our survey reveals that stressful conditions tend to inflate the variance in mutational fitness effects, CV(*s*), while leav-

TABLE 3. Summary of statistics for the regression in Figure 1.

Model	Intercept	95% min intercept	95% max intercept	Slope (reduced major axis)	95% min slope	95% max slope	R^2
All data	−0.16	−0.75	0.11	1.03	0.78	1.37	0.55
All data	0	–	–	0.91	0.82	1.06	0.56
Without outlier	0.02	−0.13	0.13	0.86	0.75	1.00	0.88
Without outlier	0	–	–	0.87	0.80	0.99	0.88

ing almost unaffected either U or $\bar{s}$. This pattern is consistent across species and experiments. Overall, this result suggests that the CV hypothesis is the prominent explanation for environmental variation of mutation fitness effects. The next step is to interpret this result. When do we expect such a pattern? Is it compatible with a mutation fitness effect model? In this section, we briefly present a fitness landscape model that provides a framework to interpret these empirical patterns.

A Fitness Landscape Model of Mutation Fitness Effects

A straightforward way to evaluate the effect of the environment on the distribution of mutation fitness effects $f(s)$, is to consider fitness landscape models, similar to Fisher's (1930) geometric model, whereby the fitness of a given phenotype falls off with the phenotypic distance to an optimum determined by the environment. Assuming a distribution of mutational effects on phenotypic traits, this approach provides a natural way to predict $f(s)$ at a given distance from the phenotypic optimum (Orr 2000; Welch and Waxman 2003). We can model a phenotype as a set of n phenotypic traits z_i represented by a column vector $\mathbf{z} = \{z_i\}_{i\in[1,n]}$, with fitness given by an arbitrary (twice differentiable) fitness function $W(\mathbf{z})$. Each MA line accumulates mutations causing a phenotypic displacement $\mathbf{dz} = \{dz_i\}_{i\in[1,n]}$ from an initial phenotype $\mathbf{z}_o$. In MA experiments, $\mathbf{z}_o$ can be thought of as the phenotype of the strain from which MA lines are derived. The fitness of this initial phenotype $\mathbf{z}_o$ is $W(\mathbf{z}_o)$ and a mutant line has phenotype $\mathbf{z}_o + \mathbf{dz}$ and fitness $W(\mathbf{z}_o + \mathbf{dz})$. W is the absolute fitness but our review focuses on the effect of mutation accumulation on relative fitness w, that is, on the distribution of the fitness deviation of MA lines relative to the initial genotype. This deviation for a line with phenotype $\mathbf{z}_o + \mathbf{dz}$ is $dw = [W(\mathbf{z}_o + \mathbf{dz}) - W(\mathbf{z}_o)]/W(\mathbf{z}_o)$, which is the selection coefficient of the mutant line relative to its wild-type ancestor. Note that we do not denote it ''s,'' which refers to the effect of single mutations, whereas dw may result from several mutations accumulated in a given line. If the deviations remain small, the effect of $\mathbf{dz}$ on relative fitness approximately equals its effect on absolute log-fitness ($\ln[W(\mathbf{z})]$ denoted $\ln W(\mathbf{z})$), $dw \approx \ln(1 + dw) = \ln W(\mathbf{z}_o + \mathbf{dz}) - \ln W(\mathbf{z}_o)$. Under the same assumption of small deviations, $\mathbf{dz}$ remains small around the initial phenotype $\mathbf{z}_o$, so that $\ln W(\mathbf{z}_o + \mathbf{dz}) - \ln W(\mathbf{z}_o)$ can be approximated by a second-order multivariate Taylor Series around $\mathbf{z}_o$, yielding

$$\begin{aligned} dw &\approx \ln(1 + dw) \\ &= \sum_{i=1}^{n} \frac{\partial \ln W(\mathbf{z}_o)}{\partial z_i} dz_i \\ &\quad + \frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} \frac{\partial^2 \ln W(\mathbf{z}_o)}{\partial z_i \partial z_j} dz_i dz_j + o(dz^2). \end{aligned} \tag{3}$$

From this equation, we can compute the mean and variance of dw over mutant line effects $\mathbf{dz}$, which are the quantities ΔM and ΔV, respectively, reported in our survey. From here, we assume that mutation effects, on phenotypic traits ($\mathbf{z}$), are unbiased, $\mathrm{E}(dz_i) = 0$. This appears to be the most parsimonious assumption, as there is no clear trend expected or observed for the effect of mutations on, for example, morphological traits (for further discussion, see Martin and Lenormand 2006). Note that we make no assumption on the effect of mutation on fitness, the latter being derived from the model, not assumed. Then, keeping only terms up to the second order in dz, equation (3) yields

$$\Delta M \approx \frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n} \frac{\partial^2 \ln W(\mathbf{z}_o)}{\partial z_i \partial z_j} \mathrm{E}(dz_i dz_j) + o[\mathrm{E}(dz^2)] \tag{4a}$$

$$\Delta V \approx \sum_{i=1}^{n}\sum_{j=1}^{n} \frac{\partial \ln W(\mathbf{z}_o)}{\partial z_i} \frac{\partial \ln W(\mathbf{z}_o)}{\partial z_j} \mathrm{E}(dz_i dz_j) + o[\mathrm{E}(dz^2)]. \tag{4b}$$

This approximation shows that both the average and variance of the relative fitness of MA lines can be decomposed into two parts reflecting the genotype-phenotype relationship ($\mathrm{E}[dz_i dz_j]$), and the phenotype-(log)fitness relationship (derivatives of $\ln W[\mathbf{z}]$ at phenotype $\mathbf{z}_o$).

The terms $\mathrm{E}(dz_i dz_j)$ are the variances and covariances of the effects of mutation accumulation on the underlying phenotypic traits, because $\mathrm{E}(dz_i) = \mathrm{E}(dz_j) = 0$. They quantify globally how and how much mutation accumulation affects the phenotype distribution among MA lines. They absorb the mutation rate and the way in which mutations have a phenotypic effect, in a given environment (patterns of expression and pleiotropy). Equation (4) shows that the mean and variance of mutation effects are both proportional to $\mathrm{E}(dz_i dz_j)$: both ΔM and ΔV scale with the amount of phenotypic change produced by mutation accumulation.

$\ln W(\mathbf{z})$ describes how phenotypic changes (dz_i) translate into relative fitness changes (dw) in a given environment. First, from equation (4), the average relative fitness effect of mutations depends on the local curvature of the log-fitness function, $\partial^2 \ln W(\mathbf{z})/\partial z_i \partial z_j$. Indeed, symmetrical variation in dz can only translate into a bias in dw if the phenotype-fitness relationship $\ln W(\mathbf{z})$ is nonlinear. Second, the mutational variance in relative fitness (ΔV) is proportional to the product of first derivatives of $\ln W(\mathbf{z})$ taken at $\mathbf{z}_o$. This result is intuitively simple: variance in the underlying phenotypic traits (z_i) transforms into variance in fitness according to the local slope of the fitness function (to $\partial \ln W[\mathbf{z}]/\partial z_i$) irrespective of its sign (hence the square).

For example, if the log-fitness is concave around $\mathbf{z}_o$, that is, $\partial^2 \ln W(\mathbf{z})/\partial z_i \partial z_j < 0$, then mutations are deleterious on average ($\Delta M < 0$, see eq. 4), and the variance ΔV increases with the distance to the optimum $|\mathbf{z}_o|$. Indeed, the absolute slope of $\ln W$ ($|\partial \ln W[\mathbf{z}]/\partial z_i|$) increases as the initial phenotype $\mathbf{z}_o$ gets away from the optimum. This argument is illustrated on Figure 3 for the case of a quadratic log-fitness function. Note that concavity or convexity is defined locally in the range of phenotypes produced by mutations and in the environment where fitness is measured. The landscape may be rugged at a finer scale (i.e., with changing concavity), for instance, at the level of DNA sequences. However, we focus here on the fitness function measured at the scale of newly arising phenotypic variation. Note also that, from equation (4), there should be no variance in fitness at the optimum (where all $\partial \ln W/\partial z_i$ are zero). However, this conclusion only arises from our approximation in $o(dz^2)$. When all the first order derivatives are zero, the higher order terms ($\mathrm{O}[dz^3]$,

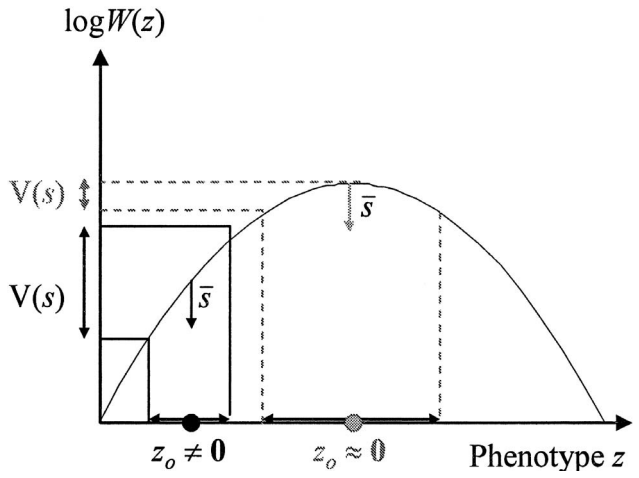

FIG. 3. Effect of stress on the mean and variance of s with a quadratic fitness function. A fitness landscape for a single phenotypic trait z is represented with a Gaussian fitness function (quadratic log-fitness function) $\ln W(z) \propto -z^2$. Mutations cause a phenotypic variation around the initial phenotype (x-axis). In gray, the initial phenotype is close to the optimum ($z_o = 0$ benign environment); in black, the initial phenotype is maladapted to the environment ($z_o \neq 0$, stressful environment). The resulting mean $\bar{s}$ and variance $V(s)$ of mutation fitness effects are represented in each case on the y-axis.

$O[dz^4]$, etc.) become leading order terms in ΔV, so that there still is some mutational variance in fitness at the optimum.

Overall and to summarize, ΔM is proportional to the curvature of $\ln W(\mathbf{z})$ at $\mathbf{z}_o$, whereas ΔV is proportional to the square of the slope of $\ln W(\mathbf{z})$ at $\mathbf{z}_o$, and both ΔM and ΔV are proportional to the mutational variance accumulated on the underlying phenotypic traits (hence to U). We now interpret the effect of the environment in this landscape approximation.

Fitness Effects in Different Environments in a Fitness Landscape Model

Let us first consider that the main effect of the environment is to affect the genotype-phenotype relationship, the $E(dz_i dz_j)$. Such an effect may be expected when some phenotypic traits are plastic, so that the same mutation has different phenotypic effects depending on the environment; then the (co)variances of mutation effects on phenotypic traits, $E(dz_i dz_j)$, may be environment dependent. In this case, from equation (4) the variations of ΔM and ΔV across environments should be proportional, that is, both proportional to the environmentally determined variation of $E(dz_i dz_j)$. Quite logically, this corresponds to the expected pattern under the CE hypothesis (see Table 1), which assumes that only U is affected by the environment. Such variation of U may therefore reflect environmental variation of the proportion of expressed mutations or of their effect variance on the underlying traits (z_i).

However, different environments may also correspond to different optima for the phenotypic traits z_i. If we assume that the environment only alters the optimum and not the fitness function $\ln W(\mathbf{z})$ around this optimum, then different environments equivalently correspond to different positions of the initial phenotype $\mathbf{z}_o$. In particular, and following our definition, stressful and benign environments correspond to situations where the initial phenotype is at a small or large distance from the optimum, respectively. As the mean ΔM and variance ΔV of mutant relative fitnesses depend on the first and second derivatives of $\ln W$ at $\mathbf{z}_o$, the way in which ΔM and ΔV vary with the environment gives us information on the way the derivatives of $\ln W$ vary with $\mathbf{z}_o$. This in turn gives us information on the type of log-fitness function $\ln W(\mathbf{z})$. Therefore, different log-fitness functions correspond to distinct predictions regarding the relationship between ΔM and ΔV across environments, or equivalently between $\log(\rho_S)$ and $\log(\rho_V)$. Just as environmental effects on the genotype-phenotype relationship correspond to variation of U (CE hypothesis), variation of the distance to the optimum corresponds to variation of $\bar{s}$ and $\mathrm{var}(s)$ (CE and CV hypotheses, respectively).

We illustrate the above argument in Figure 4, showing the influence of the distance to the optimum on the mean ($\bar{s}$, Fig. 4a) and variance ($\mathrm{var}[s]$, Fig. 4b) of single mutation fitness effects, for various log-fitness functions. Specifically, we consider that $\ln W$ is a power function (of order k) of the distance to the optimum on each trait, that is, a linear combination of $|z_i|^k$, which provides an easy way to consider different shapes for $\ln W$ by varying a single parameter (k) and reduces to the Gaussian case with $k = 2$. The distance to the optimum is defined as the log-fitness of a genotype lying at the optimum (W_{max}) relative to that of the initial genotype $W(\mathbf{z}_o)$: $s_o = \log[W_{max}/W(\mathbf{z}_o)]$ (Martin and Lenormand 2006). We also allow the direction of $\mathbf{z}_o$ (for a given s_o) to vary randomly across environments, as well as the coefficients of each of the $|z_i|^k$, with the constraint that their sum remains constant across traits z_i. Consequently, the variance among points (for a given s_o), mainly reflects that all directions in the phenotypic landscape are not equivalent (each point represents a particular direction $\mathbf{z}_o$, with different coefficients of each of the $|z_i|^k$). Figure 4a shows that varying the shape of the log-fitness function (i.e., varying the parameter k) strongly affects the change in $\bar{s}$ with the distance to the optimum (with s_o). In all these examples, as $\ln W$ is concave, mutations are deleterious on average ($\bar{s} < 0$) and the variance of their effect increases with s_o, as predicted by equation (4). In the particular case $k = 2$, which corresponds to a Gaussian $W(\mathbf{z})$, $\bar{s}$ does not change with s_o because $\ln W(\mathbf{z})$ is quadratic and therefore has a constant second derivative.

Interpreting the Empirical Patterns

Our survey revealed that mutations are always deleterious on average in all environments and that stressful conditions tend to inflate the variance in mutational fitness effects, that is, $\mathrm{CV}(s)$ or equivalently $\mathrm{var}(s)$, while leaving almost unaffected either U or $\bar{s}$. This corresponds to the CV hypothesis. Under our landscape model, this pattern would be expected if $E(dz_i dz_j)$ does not change in different environments and with a globally quadratic $\ln W(\mathbf{z})$, that is a Gaussian function $W(\mathbf{z})$ with a constant width across environments and with an environment-dependent optimum. With this specific model: (1) U is constant in different environments because $E(dz_i dz_j)$

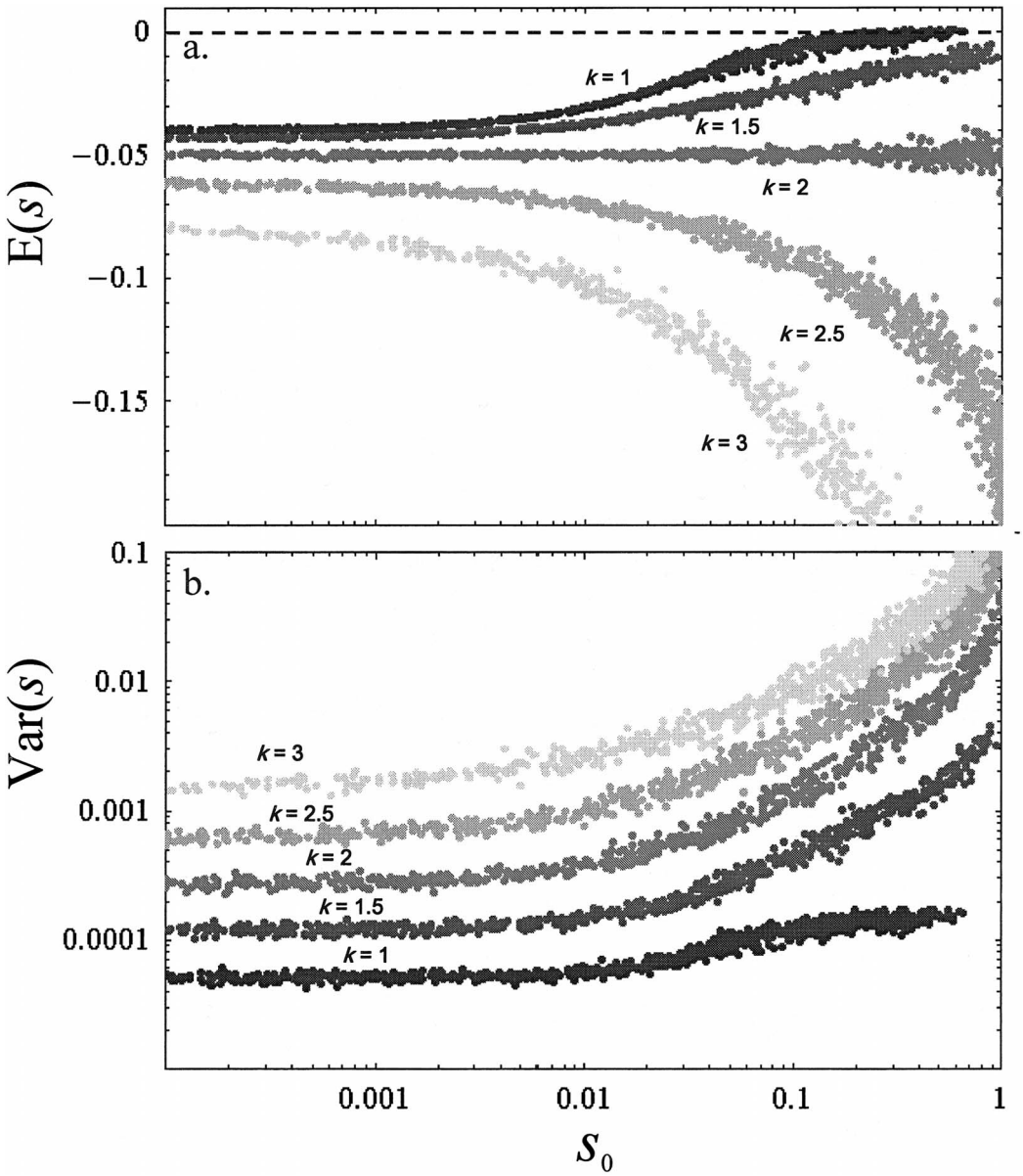

FIG. 4. Variation of $\bar{s}$ and var(s) with the fitness distance to the optimum (s_o) with diffrent fitness functions. The fitness functions are of the form $W(\mathbf{z}) = \exp(-\frac{1}{2}\sum_{i=1}^{n} |z_i|^k \lambda_i)$, where $k = 1$, 1.5, 2, 2.5 or 3, as indicated on the graph. The parameter k indicates whether the fitness function has a wider ($k > 2$) or narrower ($k < 2$) plateau around the optimum compared to a Gaussian fitness function ($k = 2$). Several steps are needed to obtain one dot on the figure. First, two random variance covariance matrices **S** and **M** of size $n = 50$ are drawn and scaled such that if $k = 2$, $E(\ln[1 + s]) \approx \bar{s}$ would be -0.05. The eigenvalues of the product **S**·**M** give the λ_i in the expression of $W(\mathbf{z})$. Then the n trait values of the initial phenotype ($\mathbf{z}_o$) are drawn randomly, giving its fitness distance to the optimum $s_o = \log[W_{max}/W(\mathbf{z}_o)]$ where $W_{max} = W(\mathbf{0}) = 1$. Then 1000 mutants are drawn around this initial phenotype (i.e., 1000 deviation vectors $\mathbf{dz} = \{dz_i\}_{i\in[1,n]}$, where the dz_i are drawn into independent Gaussians $N[0, 1]$). The relative fitness of each mutation is computed as $s(\mathbf{dz}) = W(\mathbf{z}_o + \mathbf{dz})/W(\mathbf{z}_o) - 1$, and used to compute the mean $\bar{s}$ and variance var(s) of s among mutants. Increasing the number of traits (n) does not change qualitatively the outcome but magnifies the differences among different fitness functions (not shown).

is constant, (2) var(s) increases in more stressful environments because ln $W(\mathbf{z})$ is concave; and (3) $\bar{s}$ is negative and constant because ln $W(\mathbf{z})$ is concave with constant second derivatives (see Fig. 4, $k = 2$).

A continuum of log-fitness functions ln $W(\mathbf{z})$ would predict a continuum of relationships between $\log(\rho_S)$ and $\log(\rho_V)$. Figure 5 illustrates this by showing the different relationships between $\log(\rho_S)$ and $\log(\rho_V)$ simulated for various types of log-fitness functions (i.e., different k values). Figure 5 shows that the relationships between $\log(\rho_S)$ and $\log(\rho_V)$ differs strongly whether $k = 1$, 1.5, or 2 but that it is more difficult to discriminate among higher k values ($k = 2$, 2.5, or 3). The relationship obtained from our survey $\log(\rho_S) = 0.87 \log(\rho_V)$, is close to the CV hypothesis $\log(\rho_S) = \log(\rho_V)$ (corresponding to $k = 2$). It is very different from the relationship expected for k values lower than 2. However, it may be con-

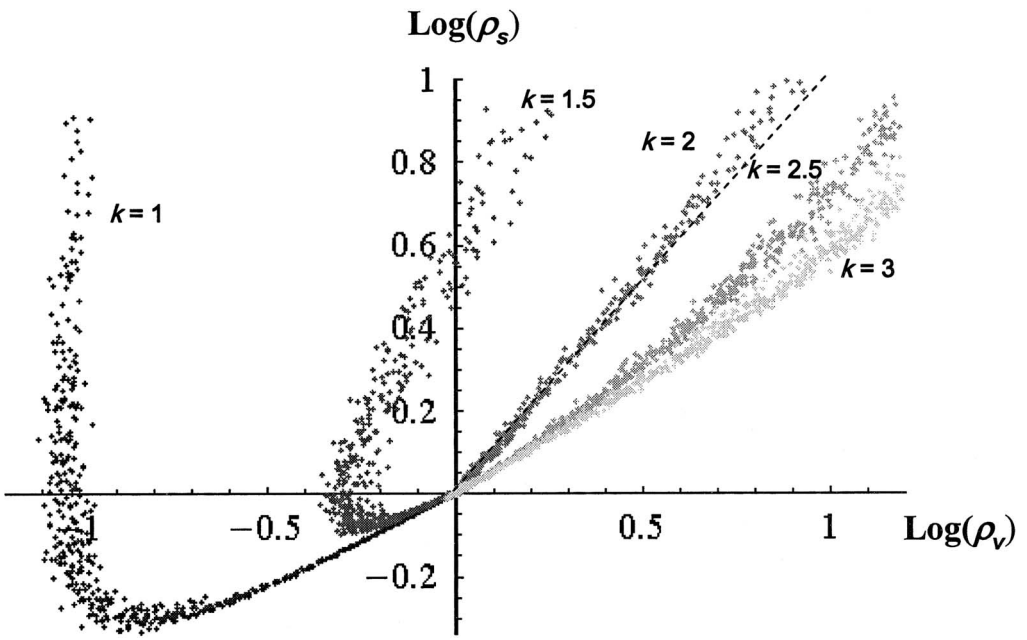

FIG. 5. Variation of $\log(\rho_S)$ against $\log(\rho_V)$ with different fitness functions. We use the same simulations as in Figure 4 (with the same gray-level code for each k value). Log(ρ) ratios are computed relative to the case $s_o = 0$. The dashed line corresponds to $\log(\rho_S) = \log(\rho_V)$ (CV hypothesis).

sistent with k values slightly larger than 2 (e.g., maybe $k = 2.5$) because it is more difficult to distinguish among larger values of k, at least in very stressful conditions (i.e., away from the origin on Fig. 5). However, it is important to keep in mind that the number of mutations sampled in a typical MA experiment is not very large, which introduces an additional source of noise in the patterns that can be observed. Figure 6 illustrates the expected relationship between $\log(\rho_S)$ and $\log(\rho_V)$ for a Gaussian fitness function ($k = 2$), when only 40 mutations are sampled around the initial phenotype (instead of 1000 in Figs. 4, 5). It shows that there is considerable uncertainty in the precise relationship between $\log(\rho_S)$ and $\log(\rho_V)$ and that this uncertainty is much larger in very stressful conditions (i.e., away from the origin on the figure). Overall, given these various sources of uncertainty and given the uncertainty of the empirical estimates themselves, we believe that it is not justifiable to make a very precise statement about $W(\mathbf{z})$. However, the observation that the main effect of stress is to increase CV(s) with relatively little effect on U and $\bar{s}$ suggest that a landscape model with a constant $E(dz_i dz_j)$ and a Gaussian fitness function would be consistent with the data available.

DISCUSSION

The two main findings of this study are that stressful conditions tend to inflate var(s) while leaving almost unaffected either U or $\bar{s}$ and that this pattern is consistent with a simple Gaussian fitness landscape (quadratic log-fitness). In such landscape model: (1) the fitness function is Gaussian (or nearly so) around an optimum that is determined by each environment; (2) the parameters of this Gaussian fitness function around each optimum are little affected by the environment; and (3) the mutational variances and covariances on phenotypic traits do not vary much across environments. Conditions (1) and (2) ensure that only var(s) and not $\bar{s}$ changes across environments, and condition (3) ensures that U does not change with the environment. We now discuss the plausibility of this interpretation and to what extent our findings are consistent with less restrictive assumptions.

Plausibility of the Fitness Landscape Model Proposed

It may seem surprising that a simplified fitness landscape model explains well empirical patterns across different species and environments. In the following section, we discuss the realism of some of the assumptions underlying this model, in particular, the existence of a single optimum and the constancy of parameters across environments. There are several lines of evidence indicating that simple fitness landscape models (or equivalently stabilizing selection models on many traits) may be more realistic than sometimes claimed. First, the idea that an environmental change determines a new phenotypic optimum is supported by the long-term dynamics of experimental adaptation to new environments. In both microbes (reviewed in Elena and Lenski 2003) and *Drosophila* (Gilligan and Frankham 2003), fitness typically plateaus in the long run, suggesting an approach to a new optimum. Second, the distribution of mutation fitness effects is gamma-like in many species, which is consistent with the predictions of an approximately Gaussian fitness landscape model (Martin and Lenormand 2006). Third, an increase in the proportion of beneficial mutations under stressful conditions as predicted by fitness landscape models has been documented in *Escherichia coli* (Remold and Lenski 2001, 2004). Fourth, there is ample evidence for stabilizing selection on various traits (Kingsolver et al. 2001; Hereford et al. 2004) or landscape-like relationships between enzymatic activities and fitness

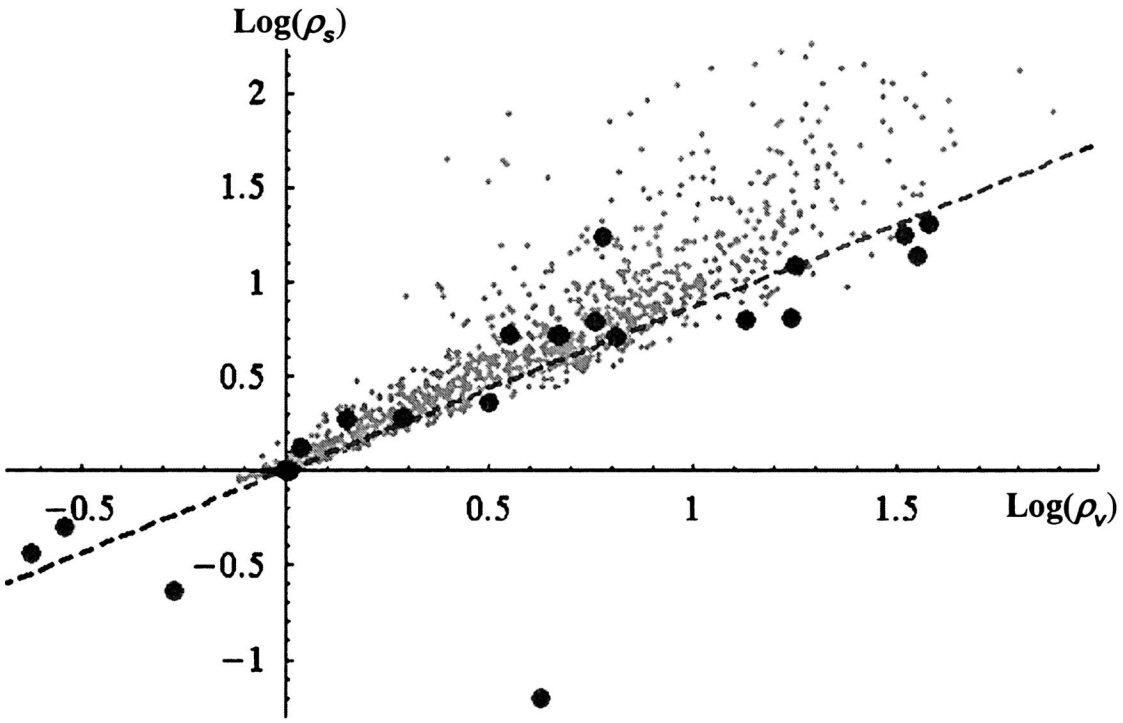

FIG. 6. Variation of $\log(\rho_S)$ against $\log(\rho_V)$ for a Gaussian fitness function ($k = 2$). This figure illustrates the same type of simulations as in Figures 4 and 5, except that for each point only 40 mutants are drawn around the initial phenotype (instead of 1000). Log(ρ) ratios are computed relative to the case $s_o = 0$. This figure illustrates that drawing a small number of mutations around the initial phenotypes, which is typical of most mutation accumulation experiments, results in a large uncertainty in the expected relationship between $\log(\rho_S)$ and $\log(\rho_V)$. The large dark dots represent the observed values surveyed in Table 2. The dashed line corresponds to the observed relationship $\log(\rho_S) = 0.87 \log(\rho_V)$ (see Table 3, without outlier, intercept = 0).

(Dykhuizen and Hartl 1983; Dean et al. 1986, 1988; Dykhuizen et al. 1987; Dykhuizen and Dean 1990).

Overall, the idea that adaptation may be modeled by some simple Gaussian or quadratic fitness function around an environment-dependant optimum seems not so unrealistic, although it would have been difficult to predict that a Gaussian fitness function assumption would quantitatively match the data so closely. However, to explain the empirical pattern, it is also necessary to assume that mutational and selective covariances on phenotypic traits remain constant across environments (conditions (2) and (3) above). This seems quite unrealistic, as we know that different genes are expressed in different environments and that a given trait may be strongly or weakly selected depending on the environment (i.e., the width of the Gaussian, not only its maximum, may change with the environment). First, it is possible that only a small portion of the genome has environment-dependent expression. Microarray studies in microorganisms suggest that only about 1% of the genome is activated or repressed in many specific stress responses (Wright 2004). However, this observation does not rule out that mutations phenotypic effects (if not expression) may strongly depend on the environment. Nevertheless, conditions (2) and (3) may be less restrictive than they seem. The only requirement is in fact that the net effect of the environment on all traits remains approximately constant, not that selective and mutational parameters on each of them be invariant. This is much less restrictive, particularly when considering a large number of traits. With the Gaussian fitness landscape model, $W(\mathbf{z})$ can be fully specified with a covariance matrix of selection intensity **S** (which is the multivariate measure of width of the fitness function). As a consequence, the distribution of mutation fitness effects $f(s)$ in a given environment depends on this matrix **S**, on the mutational covariance matrix **M** describing the (co)variance of all traits by mutation (the $\mathrm{E}[dz_i dz_j]$ in eq. 4), and on the optimal values for each trait. More specifically, $f(s)$ depends on the distribution of the eigenvalues of the matrix **S·M**, on the number of traits and the distance to the optimum (Martin and Lenormand 2006). Conditions (2) and (3) are therefore overly restrictive. The empirical pattern that we observe would be consistent with different **S** and **M** matrices in different environments as long as the distribution of **S·M** eigenvalues and the number of traits stay approximately constant across environments. This condition would be met asymptotically (i.e., with many traits) if elements of **S** correspond to random draws in a distribution, which is arbitrary but identical in all environments, with the same requirement for **M** (Martin and Lenormand 2006). This argument, which stems from random matrix theory (Bai 1999), indicates that random variation of **S** (width of the fitness function) and **M** (mutational variance) across environments (in addition to a change in the optimum) may be undistinguishable from exactly constant **S** and **M** as far as mutations' fitness effects are concerned. This fact was illustrated in Figures 4 and 5, where we allowed for such random variation of mutational and selective parameters, and found robust relationships be-

tween $\bar{s}$, var(s), and the fitness distance to the optimum (s_o). In any case, although a Gaussian fitness landscape model does not fully describe the relationship between phenotype, fitness, and the environment, it may nonetheless be a sufficiently robust simplification as it captures empirical patterns surveyed in this paper. As such, it may be a useful and reasonably accurate model for mutation fitness effects.

However, a Gaussian fitness landscape model does not account for all the data. For instance, in our survey (see Table 2; Figs. 1, 5), a minority of experiments found that ΔV was lower in more stressful conditions (instead of higher in a Gaussian fitness landscape). In many cases, the difference is not large and may be simply accounted for by measurement error on the relative fitness of the mutant lines or the low number of mutations sampled among the mutant lines. It is also possible that the stressful and benign environments were ill-attributed in some cases (i.e., when no stress measure was provided; see Table 2). However, it is also possible that these measures genuinely reflect that ΔV is sometimes lower in more stressful conditions. It is possible to theoretically expect these results with a fitness function with a narrower plateau around the optimum than the Gaussian ($k < 2$, see Fig. 5), although the data available do not globally support this possibility. Of course, it is also possible that the fitness function differs among species and environment, which could then easily account for these observations. In fact, the observation that most data could be interpreted with a single landscape model is the most intriguing result emerging from our survey. However, it seems quite likely that there is variation, even if it is modest, in the shape of the landscape among species and environments, and more data are clearly needed to settle this issue.

In line with this discussion, it is worth mentioning the mutation accumulation of Burch and Chao (2004) on RNA bacteriophage Φ6. In this experiment, the authors measure ΔM and ΔV using more- or less-adapted initial genotypes in a single environment, instead of the same initial genotype in different environments as in our survey. This type of experiment can be interpreted in a similar way as experiments involving different environments (the variation in the distance to the optimum [s_o] is given by the fitness of distinct initial genotypes in a single environment, instead of distinct environments determining the fitness of a single line). Contrary to the main result of our survey, they found that s_{BM} decreased with increasing maladaptation of the initial genotype (s_o). Just as in the present review, ρ_S and ρ_V can be computed from their MA data (not shown), and their mutual relationship across initial genotypes (instead of across environments) can be obtained. This relationship is more consistent with a linear log-fitness function ($k = 1$), than with the quadratic function ($k = 2$) suggested by our survey. As far as this interpretation is correct, the discrepancy between the effects of maladaptation generated by environmental change versus mutation accumulation suggests that a comparison between the two types of experiments would deserve further investigation. Overall, the fact that the fitness function compatible with these data on Φ6 may be quite different from what we found in our survey also indicate that the shape of fitness functions may differ across species (viruses may show a particular pattern compared to ''higher'' species), although further experiments are needed in both higher organisms and microbes to assess the generality of this conclusion. The framework we propose in this paper may also be useful for this purpose.

Increased Var(s) under Stressful Conditions

Our results suggest that stressful conditions (at least those considered in our survey) mainly increase the variance of deleterious mutation fitness effects and have a modest influence (if any) on their average effect (CA hypothesis) or on their total level of expression (CE hypothesis). The finding that stressful conditions tend to increase mutational variance is of course not totally new. For instance, Hermisson and Wagner (2004) proposed a general mechanism for the increase in mutational variance on a quantitative trait (not necessarily fitness related) after an environmental change. However, they focused on hidden standing genetic variation, whereas our study focuses on newly arisen mutational effect, and on their fitness consequence only. An increase in ΔV under stress has also been mentioned previously by several authors (reviewed in Fry and Heinsohn 2002), although without testing between the possible causes of this increase. Our results are also consistent with Remold and Lenski (2001), who showed that the variance in fitness among lines carrying a single mutation increased in stressful conditions, whereas the average deleterious effect remained unchanged (but ΔV and ΔM estimates are not given in that article). Moreover, Remold and Lenski (2004) also studied the fitness effect of single mutations across five genetic backgrounds and in two environments. Among the 18 mutations studied, some were conditionally neutral (sensu Kawecki et al. 1997), but only on specific genetic backgrounds. However, many mutations (7 of 18) were simply neutral in both environments (unconditionally neutral). Together, although based on a limited amount of studies, these results suggest that stressful conditions do increase the variance of mutation fitness effects, but not necessarily (and maybe rarely) because of an increase in the expression of deleterious mutations. There are many examples of genes being activated in response to stress (e.g., oxidative, temperature, or osmotic stresses), both in the yeast (Toone and Jones 1998) and in mammals (Sonna et al. 2002). Mutations on these genes should indeed be neutral in benign environments, but stress responses are also known to downregulate some other genes (e.g., in the response to oxidative stress; Morel and Barouki 1999). Other stresses may result in the switch to a new resource utilization pathway. In both situations, different genes are being either up- or downregulated and the net outcome thus may not necessarily be an increase in the total number of expressed mutations in stressful conditions.

Conditional Neutrality, Genotype-by-Environment Interactions, and Ecological Specialization

Differences in mutation effects across environments are a necessary ingredient for the evolution of ecological specialization (Futuyma and Moreno 1988). Ecological specialization or local adaptation may occur if the direction of selection changes for an allele between environments (antagonistic pleiotropy). It may also occur if the intensity of selection

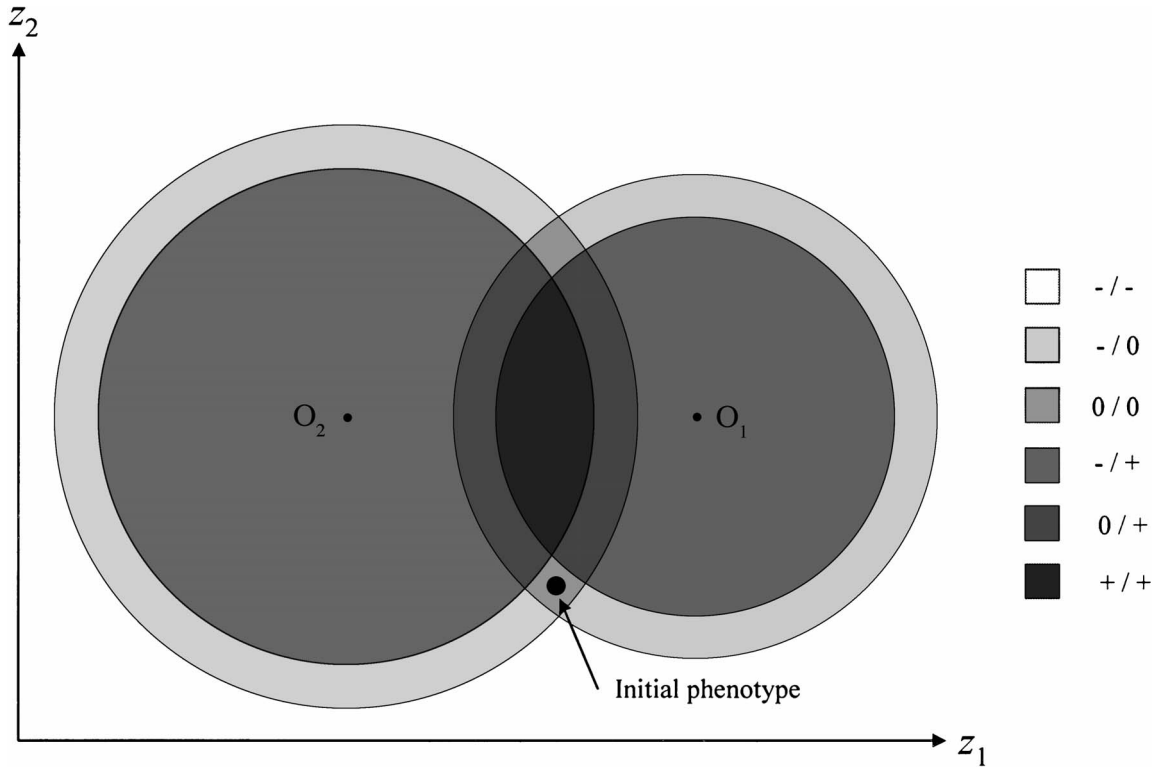

FIG. 7. Mutational genotype × environment interactions for fitness in a fitness landscape model. The figure represents a phenotypic space with two phenotypic traits (z_1 and z_2) with two different optima in two distinct environments (O_1 and O_2). Mutations on a given initial phenotype (black dot) in a given environment may be deleterious (−), neutral (0), or advantageous (+), depending on whether it brings the phenotype away from or closer to the corresponding optimum (O_1 or O_2). The joint effect of a mutation in both environments depends on the position of the mutant in the phenotypic plan. Each colored area represents a zone in which all mutations have the same qualitative joint effect, encoded as the effect in one environment/effect in the other one. See text for details.

against deleterious mutations at several loci covaries negatively between environments (Fry et al. 1996; Whitlock 1996). The first scenario requires the existence of a trade-off and the occurrence of mutations that are beneficial in at least some environments, whereas the second scenario works best when mutations are neutral in one environment but deleterious in another (i.e., under conditional neutrality Kawecki et al. 1997) and accords with the common view that most mutations are deleterious. Many empirical studies document the evolution of ecological specialists in constant environments or of locally adapted genotypes in heterogeneous environments (reviewed in Kassen 2002; Lenormand 2002), and the prevalence of conditional neutrality versus antagonistic pleiotropy has been much debated in this context (cost of specialization: Cooper and Lenski 2000; MacLean et al. 2004). There is evidence for the different types of mutation presented above. In particular, conditionally neutral genetic variation at quantitative trait loci (some of which is revealed by stressful conditions) has been documented in several experiments (reviewed in Hermisson and Wagner 2004). However, some other quantitative trait loci with significant fitness effects may show less environment dependence, and evidence for conditional neutrality on traits with very limited impact on fitness (e.g., bristle number) is not evidence for conditional neutrality for fitness. There is also evidence for antagonistic pleiotropy (Cooper and Lenski 2000; Gazave et al. 2001; MacLean et al. 2004). However, the relative frequency of these different types of mutations is not clearly documented apart from a recent study (Remold and Lenski 2004).

If simple fitness landscape models are a reasonably accurate approximation, as our results suggest, they could provide a rationale to predict the proportion and impact of each of the above type of mutation. Indeed, genotype × environment interactions for mutation fitness effects are inherent to a fitness landscape model in which different environments are characterized by different optimal values for the underlying traits. Figure 7 sketches the different types of mutation fitness effects (relative to an initial phenotype $\mathbf{z}_o$) that may occur in a simple two-traits landscape with two different optima, O_1 and O_2, determined by two contrasted environments. First, a given mutation **dz** may increase the phenotypic distance from both O_1 and O_2 if the phenotype $\mathbf{z}_o + \mathbf{dz}$ lies in the white area of Figure 7. Such a mutation would be deleterious in both environments (−/− area), although this deleterious effect may be more severe in one of them. Second, a given mutation may increase the distance from only one of the two optima. Such a mutation would be conditionally neutral or deleterious (−/0 area). Third, a mutation may not significantly change the distance from either optimum and be neutral (0/0 area). Fourth, a mutation may decrease the distance from only one of the optima and be conditionally neutral or beneficial (0/+ area). Fifth, a mutation may decrease the distance from one optimum but increase the distance from the other. Such a mutation would be antagonistic

pleiotropic (+/− area). Finally, a mutation may decrease the distance from both optima and be unconditionally beneficial (+/+ area). The proportion of these different types of mutation depends on the relative position of the optima and the initial phenotype and may be predictable in a given landscape and for a given fitness effect threshold defining a neutral mutation.

The evolution of specialization mainly relies on −/0 mutations (conditional neutrality) and +/− mutations (antagonistic pleiotropy). Our results suggest that whether the environment is stressful or not does not change the total number of expressed deleterious alleles (i.e., of conditionally neutral mutations). However, this does not rule out the potential for ecological specialization by conditional neutrality (sensu Kawecki et al. 1997), as different deleterious mutations may be expressed in different environments, even if their total number remains roughly constant across environments. In any case, our survey is mainly concerned with unconditionally deleterious −/− mutations and is not directly relevant to these other contexts, but we note that fitness landscape models may be useful to quantitatively predict the relative prevalence of conditional neutrality and antagonistic pleiotropy.

Implications for the Estimation of Mutational Parameters

Finally, and perhaps more importantly, our results suggest that stressful conditions tend to increase the coefficient of variation of mutation fitness effects, CV(s). This effect could lead to strong underestimation of the mutation rate by the Bateman-Mukai method when fitness is assayed in stressful conditions, sometimes by up to two orders of magnitude. Therefore, it seems a priori wisest to rely on U estimates based on fitness assays in an environment to which the control genotype is well adapted. Fortunately, most estimates have been done in this context. Considering that U does not vary much across environments compared to CV(s) may also be useful when analyzing MA data across environments using maximum likelihood (Keightley 1994; Vassilieva et al. 2000) or minimum distance (Garcia-Dorado and Marin 1998). In any case, a significant change in U versus CV(s) across environments can be tested with these methods to further investigate the results of the present study.

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