

Inferring the distribution of mutational effects on fitness in *Drosophila*

Laurence Loewe* and Brian Charlesworth

Institute of Evolutionary Biology, School of Biological Sciences,
University of Edinburgh, Kings Buildings, West Mains Road,
Edinburgh EH9 3JT, UK

*Author for correspondence (laurence.loewe@evolutionary-research.net).

The properties of the distribution of deleterious mutational effects on fitness (DDME) are of fundamental importance for evolutionary genetics. Since it is extremely difficult to determine the nature of this distribution, several methods using various assumptions about the DDME have been developed, for the purpose of parameter estimation. We apply a newly developed method to DNA sequence polymorphism data from two *Drosophila* species and compare estimates of the parameters of the distribution of the heterozygous fitness effects of amino acid mutations for several different distribution functions. The results exclude normal and gamma distributions, since these predict too few effectively lethal mutations and power-law distributions as a result of predicting too many lethals. Only the lognormal distribution appears to fit both the diversity data and the frequency of lethals. This DDME arises naturally in complex systems when independent factors contribute multiplicatively to an increase in fitness-reducing damage. Several important parameters, such as the fraction of effectively neutral non-synonymous mutations and the harmonic mean of non-neutral selection coefficients, are robust to the form of the DDME. Our results suggest that the majority of non-synonymous mutations in *Drosophila* are under effective purifying selection.

Keywords: *Drosophila*; distribution of mutational effects; lethals; lognormal; gamma; power law

1. INTRODUCTION

Recent advances in evolutionary genetics have led to a number of approaches for estimating the distribution of deleterious mutational effects on fitness (DDME) of non-synonymous mutations, using data on between-species sequence divergence and/or within-species sequence diversity (Bustamante *et al.* 2003; Nielsen & Yang 2003; Piganeau & Eyre-Walker 2003; Sawyer *et al.* 2003; Loewe *et al.* 2006). All assume a specific type of distribution of selection coefficients, which is then used to fit the data. Previous investigations have used a variety of distributions, including the normal, exponential and gamma distributions (Bustamante *et al.* 2003; Nielsen & Yang 2003; Piganeau & Eyre-Walker 2003; Sawyer *et al.* 2003; Loewe *et al.* 2006). The latter is widely

The electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2006.0481> or via <http://www.journals.royalsoc.ac.uk>.

used because of its convenient two-parameter form, which allows a wide range of curve shapes. However, none of these distributions has a special status, since there is currently no basis for a rational choice.

Here, we propose rejection of a candidate DDME if it cannot explain (i) DNA sequence diversity data in two related species with different effective population sizes, and (ii) the frequency of dominant, effectively lethal mutations caused by amino acid mutations. We find that a lognormal DDME satisfies these conditions much better than a gamma distribution or a power law.

2. MATERIAL AND METHODS

We define the DDME as the genome-wide distribution of the heterozygous selection coefficient, s , associated with a new deleterious, non-synonymous mutation. We use diversity data from 17 loci of *Drosophila miranda* and 14 loci of *Drosophila pseudoobscura*, two closely related species of fruitfly with similar habitats, but significantly different effective population sizes (N_e) as estimated from their silent nucleotide site diversities, π_S (Loewe *et al.* 2006). The similarity of the two species means that they probably share the same DDME, so that the larger N_e of *D. pseudoobscura* compared with *D. miranda* causes a larger fraction of sites to experience effective purifying selection. This results in a smaller increase in non-synonymous diversity, π_A , than in π_S . Assuming a given type of DDME for mutations affecting amino acid sites and a fraction of completely neutral, non-synonymous mutations (c_n), we can calculate the expectation of π_A for each species. By equating these to the pair of observed mean values of π_A , we estimate the parameters of the DDME, assuming that it can be described by two parameters. Our method assumes approximate mutation–selection–drift equilibrium and independence among non-synonymous polymorphisms, but is not affected by the details of the frequency distributions of variants. It should, therefore, provide robust estimates of the parameters of the DDME for a fixed value of c_n . Statistical accuracy is assessed by computing 1000 bootstraps. To improve analysis, we used the diversity index, DI (the ratio of the values π_A/π_S for the two species) to eliminate the 12.2% of all bootstraps with $DI \leq 1$, since these cannot be explained by any plausible model. Further details are described in the electronic supplementary material and by Loewe *et al.* (2006).

3. RESULTS

To test whether a DDME that is compatible with observed diversity data also satisfies our second criterion requires an estimate of the rate at which non-synonymous mutations with effectively lethal (i.e. lethal or sterile) heterozygous effects arise. The difficulty is that most lethal mutations are recessive and it is hard to study those that are not. While point mutations, indels and transposable elements (TEs) can all induce dominant, effectively lethal mutations, we are only concerned with non-synonymous mutations. It is virtually impossible to estimate the rate of spontaneous dominant lethal mutations, and most of these are probably due to chromosome breaks (Ashburner 1989). However, the results of ethylmethane sulphonate (EMS) mutagenesis, which mainly but not exclusively induces point mutations, suggest that dominant female sterile mutations arise in *D. melanogaster* at about 1/500th of the rate for recessive lethal mutations (Ashburner 1989). Molecular analyses of two of the genes concerned show that the majority of the mutational lesions involved are non-synonymous mutations (Timinszky *et al.* 2002; Venkei & Szabad 2005).

The approximate overall frequency of such mutations can be assessed as follows. Recent data suggest that spontaneous recessive lethal mutations arise at a rate of about 0.045 per zygote per generation

Table 1. Candidate distributions of mutational effects.

| DDME | probability density function | intuitive meaning |
|-----------|---|--|
| normal | $\phi(s) = (1/(\sigma\sqrt{2\pi}))e^{-((s-\mu)^2/(2\sigma^2))}$ | Arises from the central limit theorem if a mutation has effects on several different traits that affect fitness additively. Determined by location parameter μ (mean) and shape parameter σ (s.d.). |
| gamma | $\phi(s) = (s^{\alpha-1}e^{-s/\beta})/(\beta^\alpha\Gamma(\alpha))$ | Arises naturally as time-to-first-fail for a system with $\alpha-1$ standby backups that have exponentially distributed life times with parameter β (NIST/SEMATECH 2005), where α is the shape and β is the location. |
| pareto | $\phi(s) = ks_{\min}^k/s^{k+1}$ | Arises if a stochastic process that is expected to grow exponentially is killed (or observed) randomly (Reed & Hughes 2002). Determined by location s_{\min} (= smallest value) and shape k . |
| lognormal | $\phi(s) = (1/(s\sigma\sqrt{2\pi}))e^{-((\ln(s)-\mu)^2/(2\sigma^2))}$ | Arises naturally from the central limit theorem if many small pleiotropic fitness-reducing effects have a multiplicative, cumulative effect on damage (NIST/SEMATECH 2005). We use location parameter μ_g (= geometric mean = median = e^μ) and shape parameter σ_g (= e^σ). |

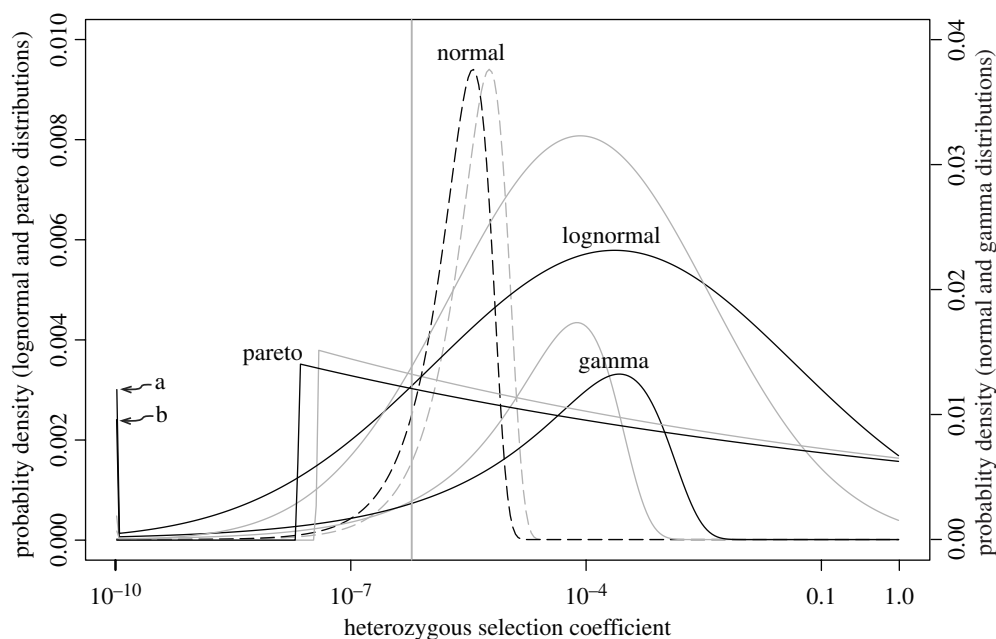


Figure 1. Probability densities fitted to the diversity data for various distributions of mutational effects (DDMEs). Solid lines indicate cases where the data could be fitted; dashed lines could not be fitted. Black lines assume $c_n=0$, grey lines $c_n=2.5\%$. The scales for the lognormal and pareto distributions are on the left-hand y-axis and those for the normal and gamma distributions are on the right-hand y-axis. The vertical grey line denotes $N_{e,s}=0.5$ for *D. miranda*. The spikes at 10^{-10} integrate over all probability mass down to 0, where a is for the lognormal ($c_n=0$) and b is for the gamma ($c_n=0$). The normal DDMEs shown give the closest fit to the data that we could find for the given c_n values. They consist of 48% advantageous mutations (truncated). Fine-tuning DDME location and c_n allows fits of many DDMEs (including constant s) to diversity data, but not to the frequency of lethals.

in *D. melanogaster*, but many of these are probably due to TE insertions (Charlesworth *et al.* 2004). The rate of TE insertions is about 0.2 per zygote per generation (Maside *et al.* 2000). Assuming that 25% of the genome is coding sequence (Misra *et al.* 2002) and 25% of all genes are vital (Oh *et al.* 2003) gives an estimate of 0.0125 recessive lethal TE-insertions per zygote per generation. If 25% of non-TE mutations are indels (Charlesworth *et al.* 2004), this suggests that the recessive lethal mutation rate due to base substitutions is around $0.75 \times (0.045 - 0.0125) = 0.024$. The rate of mutation to dominant female sterile non-synonymous mutations in *Drosophila* is thus about 5×10^{-5} . If dominant lethal and dominant male sterility mutations arise at a similar rate, the net rate of mutation to effectively lethal dominant mutations is about 2×10^{-4} .

There is 27.8 Mb of exon sequence in *D. melanogaster* (Misra *et al.* 2002); about 70% of these sites can generate non-synonymous mutations without shifting the reading frame. With a mutation rate of 1.5×10^{-9} (Loewe *et al.* 2006), this results in a total mutation rate for non-synonymous mutations of $U = 0.058$ per zygote per generation. The fraction λ of non-synonymous mutations that are dominant effective lethals is thus about 3.4×10^{-3} . The precise value of this parameter is not important; however, the fact that effectively lethal non-synonymous mutations occur at a detectable, but low rate means that any candidate DDME that predicts either no or many such mutations can be rejected.

Table 1 gives an overview of the various types of DDME that were tested against the data. Figure 1 plots the best-fitting estimates for visual inspection.

Table 2. Numerical details of the distributions of deleterious mutational effects. All estimates in this table are based on diversity data for *D. miranda* (mir, $N_e=837\ 000$) and *D. pseudoobscura* (pso, $N_e=4\ 770\ 000$; Loewe *et al.* 2006). Numerical results assume a mutation rate of $u=1.5\times 10^{-9}$, a mutational bias of $\kappa=2$ (the ratio of mutation rates to and from deleterious amino acids) and $N_{e,s}=0.5$ as the border to effective neutrality (Loewe *et al.* 2006). Values in parentheses give the lower 5th percentiles and the upper 5th percentiles from 1000 bootstraps. The different columns have the following meanings. DDME, $c_n\%$: type of distribution and the fraction of completely neutral non-synonymous mutations assumed. Shape, location: the parameters that determine the distributions (see table 1). Species: the focal species whose N_e was used to compute the next six columns. $N_{e,s}$ (am), (hm): the arithmetic and harmonic mean, respectively, of all effectively deleterious, but non-lethal selection coefficients, multiplied by effective population size. These values (and those in the next three columns) use all s values that are larger than the border to effective neutrality and smaller than lethal ($s=1$). $N_{e,s}$ (5%), (95%): the lower and upper 5% percentiles of the truncated DDME. CV: coefficient of variation of the truncated DDME. c_{ne} (%): the fraction of effectively neutral non-synonymous mutations. λ (%): fraction of the DDME with $s\geq 1$ (effectively dominant lethals).

| DDME c_n (%) | shape | location | species | $N_{e,s}$ (am) | $N_{e,s}$ (hm) | $N_{e,s}$ (5%) | $N_{e,s}$ (95%) | CV | c_{ne} (%) | λ (%) |
|-------------------|--------------------------|--|---------|---------------------------|---------------------|------------------------|-----------------------------|----------------------|----------------------|---------------------------------------|
| log N 0% | 202 (9.34/2490) | 0.000231 (8.29×10^{-6} /0.00448) | mir | 29900 (108/56600) | 8.47 (3.65/10.7) | 1.21 (0.781/1.54) | 190000 (362/398000) | 3.34 (2.45/12.3) | 13.2 (8.62/17.4) | 5.73 (9×10^{-6} /23.8) |
| | | | pso | 159000 (546/309000) | 12.6 (7.80/16.2) | 1.99 (1.31/2.73) | 999000 (1810/2190000) | 3.47 (2.53/12.9) | 7.44 (1.73/11.0) | |
| log N 2.5% | 44.9 (4.14/1480) | 8.41×10^{-5} (5.90×10^{-6} /0.00421) | mir | 9880 (16.6/53700) | 7.44 (3.22/11.5) | 1.19 (0.801/1.71) | 35100 (62.4/370000) | 5.26 (2.26/11.5) | 12.1 (6.03/16.7) | 0.681 (2×10^{-15} /22.4) |
| | | | pso | 52900 (85.9/306000) | 13.4 (8.69/21.1) | 2.31 (1.49/4.54) | 184000 (328/2130000) | 5.44 (2.33/11.9) | 6.43 (2.64/11.3) | |
| gamma 0% | 0.299 (0.0782/0.741) | 0.00089 (1.60×10^{-5} /209000) | mir | 255 (11.6/95700) | 8.35 (3.49/17.9) | 1.31 (0.843/3.01) | 1100 (36.9/558000) | 1.67 (1.08/2.15) | 12.7 (7.91/17.0) | $<10^{-100}$ ($<10^{-100}$ /61) |
| | | | pso | 1370 (55.3/521000) | 14.4 (8.69/25.0) | 2.5 (1.55/5.47) | 6220 (188/3110000) | 1.74 (1.14/2.22) | 7.57 (2.35/13.4) | |
| gamma 2.5% | 0.448 (0.0996/1.32) | 0.000171 (5.45×10^{-6} /956) | mir | 70.6 (6.96/76500) | 6.86 (3.19/17.2) | 1.21 (0.877/2.96) | 274 (18.4/463000) | 1.39 (0.832/2.08) | 11.4 (5.73/16.6) | $<10^{-100}$ ($<10^{-100}$ /45.2) |
| | | | pso | 382 (35.4/392000) | 14.7 (9.48/28.4) | 2.73 (1.77/7.31) | 1550 (97.2/2450000) | 1.45 (0.865/2.13) | 6.58 (2.87/13.4) | |
| pareto 0% | 0.0458 (0.0154/0.101) | 2.12×10^{-8} (5.61×10^{-11} /1.09 $\times 10^{-7}$) | mir | 43400 (27500/58700) | 5.54 (4.21/6.47) | 0.823 (0.72/0.938) | 323000 (183000/422000) | 2.9 (2.59/3.58) | 14.3 (11.0/19.4) | 44.5 (19.5/69.9) |
| | | | pso | 210000 (124000/274000) | 5.82 (4.65/7.20) | 0.861 (0.759/1.01) | 1490000 (766000/2020000) | 3.18 (2.80/3.97) | 7.21 (0.454/13.4) | |
| pareto 2.5% | 0.0494 (0.0148/0.114) | 3.71×10^{-8} (7.43×10^{-11} /1.60 $\times 10^{-7}$) | mir | 42300 (25100/57900) | 5.43 (3.93/6.48) | 0.819 (0.708/0.937) | 304000 (157000/421000) | 2.94 (2.59/3.79) | 15.2 (12.4/19.6) | 43 (16.3/70.8) |
| | | | pso | 204000 (114000/275000) | 5.7 (4.80/7.52) | 0.857 (0.769/1.23) | 1480000 (668000/2030000) | 3.22 (2.80/4.18) | 7.53 (2.58/14.1) | |

Table 2 reports the distributional parameters, as well as scaled measures of selection intensities, for the DDMEs that can be fitted to the diversity data (which is not the case for the normal distribution with $c_n=0$ or 2.5%). The gamma distribution fits the diversity data well (Loewe *et al.* 2006). The number of dominant effective lethals predicted by the best gamma DDME is, however, very small, because the diversity data can only be fitted by a gamma distribution with a relatively small width (table 2), so that the right-hand end of the distribution falls off quickly (figure 1). Inspection of the results for the gamma distribution shows that 6.5% of the 802 bootstraps that could be fitted to the data for $c_n=0$ lead to potentially realistic genomic lethal rates ($U\lambda$ between 10^{-5} and 0.004), but most results gave unacceptably low values. Thus, the gamma DDME does not easily predict plausible numbers of these mutations.

Recent searches for general principles have frequently uncovered or attempted to fit power laws (Reed & Hughes 2002; Mitzenmacher 2004), and so a power law such as the pareto distribution (table 1) might be a good candidate DDME. While we found that a pareto DDME can fit the diversity data reasonably well, it failed to predict plausible fractions of lethals (table 2). In contrast, a lognormal DDME can fit both observed diversities and fractions of lethals. This result seems to be relatively insensitive to different assumptions about c_n , and the bootstraps for $U\lambda$ largely overlap the range of values that are consistent with the data. For a lognormal DDME and $c_n=0$, a much larger fraction of bootstraps (60% out of 610) predicts a plausible genomic lethal rate than with a gamma DDME. The fact that this fraction is not higher is probably due to the limited size of our dataset and the correspondingly noisy statistics.

4. DISCUSSION

Is there a theoretical reason for the relatively good performance of the lognormal distribution? The central limit theorem, which states that a variable affected by independent additive effects of several other variables is normally distributed, implies that a variable controlled multiplicatively by several independent factors follows the lognormal distribution (Koch 1969; Mitzenmacher 2004; NIST/SEMATECH 2005). Darwinian fitness must be affected by many different factors operating at different levels of biological organization. We suggest that the extent of a reduction in fitness caused by a deleterious mutation, as measured by s , is a function of the amount of damage that it causes at several independent functional levels. If the total amount of damage were a multiplicative function of the amounts of damage at each level, a lognormal distribution of s would result (NIST/SEMATECH 2005).

Regardless of the question of the true nature of the DDME, the results presented in table 2 are encouraging in that some of the more important parameters derived from its properties are relatively invariant with respect to the type of distribution and are also fairly well bounded by the bootstrap procedure. In particular, the harmonic mean of $N_e s$ for effectively deleterious mutations is fairly similar for the different distributions

and its bootstrap intervals are bounded well above 1. As noted previously, this parameter is close to the mean selection coefficient associated with polymorphic mutations that are not effectively neutral i.e. have $N_e s > 0.5$ (Loewe *et al.* 2006). It plays an important role in processes such as background selection and Muller's ratchet (Charlesworth & Charlesworth 2000). Similarly, the proportion of effectively neutral mutations is consistently estimated to be less than 20% and usually less than 10% (table 2). These conclusions are consistent with results from other methods (Bustamante *et al.* 2003; Nielsen & Yang 2003; Piganeau & Eyre-Walker 2003; Sawyer *et al.* 2003; Loewe *et al.* 2006).

It is likely that datasets of larger diversity and more accurate estimates of the fraction of dominant effective lethals will lead to more precise estimates in the future. Obviously, it is possible that there could be a mixture of distributions, with widely different means, contributing to the overall DDME and mimicking the results we have obtained by fitting the lognormal. For practical purposes, it is preferable to use a single distribution that matches the data successfully.

The results strengthen the conclusion that most amino acid mutations segregating in natural populations of *Drosophila* have sufficiently large deleterious effects on fitness that they behave quasi-deterministically ($N_e s > 1$), although large N_e values imply that selection against deleterious mutations in *Drosophila* is mostly weak (Loewe *et al.* 2006; mean $s \approx 10^{-4}$). Despite the very small selection coefficients associated with most mutations, our best estimates for the lognormal DDME predict appreciable numbers of mutations with detectable fitness effects; we expect 11 or 7% of all non-synonymous mutations to have heterozygous effects between $s=0.01-0.1$, assuming $c_n=0\%$ or 2.5%, respectively. Recent experiments on isolating EMS mutations in specific *Drosophila* genes by TILLING have shown that a substantial fraction of non-synonymous mutations can have drastic homozygous fitness effects (Winkler *et al.* 2005). Together with earlier findings that EMS-induced mutations with drastic homozygous fitness effects typically have small, but detectable fitness losses when heterozygous (Simmons *et al.* 1978), this is consistent with the predictions of the lognormal DDME, as is the fact that many human dominant Mendelian disorders are caused by single amino acid changes (Yampolsky *et al.* 2005). The hypothesis that there is a substantial minority of amino acid mutations with experimentally detectable heterozygous fitness effects can be tested in model organisms such as yeast or *Drosophila*, by measuring the fitness effects of induced non-synonymous mutations of known identity.

We thank Jay Taylor for helpful discussions and the Royal Society and the Leverhulme foundation for support. We have no competing financial interests.

- Ashburner, M. 1989 *Drosophila*: a laboratory handbook. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
 Bustamante, C. D., Nielsen, R. & Hartl, D. L. 2003 Maximum likelihood and Bayesian methods for estimating

- the distribution of selective effects among classes of mutations using DNA polymorphism data. *Theor. Popul. Biol.* **63**, 91–103. (doi:10.1016/S0040-5809(02)00050-3)
- Charlesworth, B. & Charlesworth, D. 2000 The degeneration of Y chromosomes. *Phil. Trans. R. Soc. B* **355**, 1563–1572. (doi:10.1098/rstb.2000.0717)
- Charlesworth, B., Borthwick, H., Bartolome, C. & Pignatelli, P. 2004 Estimates of the genomic mutation rate for detrimental alleles in *Drosophila melanogaster*. *Genetics* **167**, 815–826. (doi:10.1534/genetics.103.025262)
- Koch, A. L. 1969 The logarithm in biology II. Distributions simulating the log-normal. *J. Theor. Biol.* **23**, 251–268. (doi:10.1016/0022-5193(69)90040-X)
- Loewe, L., Charlesworth, B., Bartolomé, C. & Noël, V. 2006 Estimating selection on non-synonymous mutations. *Genetics* **172**, 1079–1092. (doi:10.1534/genetics.105.047217)
- Maside, X., Assimacopoulos, S. & Charlesworth, B. 2000 Rates of movement of transposable elements on the second chromosome of *Drosophila melanogaster*. *Genet. Res.* **75**, 275–284. (doi:10.1017/S0016672399004474)
- Misra, S. *et al.* 2002 Annotation of the *Drosophila melanogaster* euchromatic genome: a systematic review. *Genome Biol.* **3**, research0083. (doi:10.1186/gb-2002-3-12-research0083)
- Mitzenmacher, M. 2004 A brief history of generative models for power law and lognormal distributions. *Internet Math.* **1**, 226–251.
- Nielsen, R. & Yang, Z. 2003 Estimating the distribution of selection coefficients from phylogenetic data with applications to mitochondrial and viral DNA. *Mol. Biol. Evol.* **20**, 1231–1239. (doi:10.1093/molbev/msg147)
- NIST/SEMATECH 2005 8.1.6 What are the basic lifetime distribution models used for non-repairable populations? In *e-handbook of statistical methods*, see <http://www.itl.nist.gov/div898/handbook/apr/section1/apr16.htm>.
- Oh, S. W. *et al.* 2003 A P-element insertion screen identified mutations in 455 novel essential genes in *Drosophila*. *Genetics* **163**, 195–201.
- Piganeau, G. & Eyre-Walker, A. 2003 Estimating the distribution of fitness effects from DNA sequence data: implications for the molecular clock. *Proc. Natl Acad. Sci. USA* **100**, 10 335–10 340. (doi:10.1073/pnas.1833064100)
- Reed, W. J. & Hughes, B. D. 2002 From gene families and genera to incomes and internet file sizes: why power laws are so common in nature. *Phys. Rev. E* **66**. (doi:10.1103/PhysRevE.66.067103) art no.-067103.
- Sawyer, S. A., Kulathinal, R. J., Bustamante, C. D. & Hartl, D. L. 2003 Bayesian analysis suggests that most amino acid replacements in *Drosophila* are driven by positive selection. *J. Mol. Evol.* **57**, S154–S164. (doi:10.1007/s00239-003-0022-3)
- Simmons, M. J., Sheldon, E. W. & Crow, J. F. 1978 Heterozygous effects on fitness of EMS-treated chromosomes in *Drosophila melanogaster*. *Genetics* **88**, 575–590.
- Timinszky, G. *et al.* 2002 The importin-beta P446L dominant-negative mutant protein loses RanGTP binding ability and blocks the formation of intact nuclear envelope. *J. Cell Sci.* **115**, 1675–1687.
- Venkei, Z. & Szabad, J. 2005 The *Kavar*^D dominant female-sterile mutations of *Drosophila* reveal a role for the maternally provided α -tubulin⁴ isoform in cleavage spindle maintenance and elongation. *Mol. Genet. Genomics* **273**, 283–289. (doi:10.1007/s00438-005-1109-x)
- Winkler, S. *et al.* 2005 Target-selected mutant screen by TILLING in *Drosophila*. *Genome Res.* **15**, 718–723. (doi:10.1101/gr.3721805)
- Yampolsky, L. Y., Kondrashov, F. A. & Kondrashov, A. S. 2005 Distribution of the strength of selection against amino acid replacements in human proteins. *Hum. Mol. Genet.* **14**, 3191–3201. (doi:10.1093/hmg/ddi350)

SUPPORTING ONLINE MATERIAL

Loewe, L & Charlesworth, B

Inferring the distribution of mutational effects on fitness in *Drosophila*
Biology Letters (accepted April 2006)

METHODS

Data. We used data from population surveys of 17 genes from *Drosophila miranda* and 14 genes from *D. pseudoobscura* (respective sample sizes 11-12 and 7-139). Diversity values for individual genes were weighted according to their estimated inverse sampling variances, as described previously, in order to estimate mean values of silent and non-synonymous nucleotide site diversities across genes (Loewe *et al.* 2006).

Estimation of DDME parameters. We applied the methods for arbitrary selection models of mutation-selection-drift equilibrium with back mutations, as described previously (Loewe *et al.* 2006), using the infinite sites assumption that new mutations do not arise at a segregating site (McVean & Charlesworth 1999). In summary, we applied results from McVean & Charlesworth (1999) to compute non-synonymous diversity π_A for a given selection coefficient s for a heterozygous deleterious mutation, with the following extensions. A fraction c_n of all non-synonymous sites is assumed to be incapable of acquiring deleterious mutations. Sites fixed for slightly deleterious mutations can mutate to equally deleterious alternatives at a rate of $u(1-c_n)(\kappa-1)/\kappa$, where u is the mutation rate and κ is the mutational bias (the ratio of the respective mutation rates forwards and backwards between wild-type and a deleterious amino-acid replacement mutation at a given site). Silent diversity π_S was computed in the same way, assuming $s = 0$. To compare a mean π_A for a given DDME with the data, we estimated N_e from the neutral prediction $\pi_S = 4N_e u$, and integrated over the DDME from $s = 0$ to $s \geq 1$ (lethal) using the values of κ , u and c_n specified in Table 2 (for justification of these choices of parameters, see (Loewe *et al.* 2006)). We assumed autosomal inheritance, intermediate dominance (selection coefficients against mutant heterozygotes and homozygotes of s and $2s$, respectively), absence of linkage disequilibrium, and equality of N_e and census population size. The validity of these assumptions has been discussed previously (Loewe *et al.* 2006). The observed diversities for two species allow estimation of the parameters of the DDME in question. Estimation involves optimizing the

parameters of a given choice of DDMEs until the \log_{10} of the sum of absolute differences between predictions and observations for π_S/π_A in both species becomes so small that the predictions approximate the observations to 6 significant digits (Loewe *et al.* 2006).

Lethal mutational effects. The values of s generated from the DDME are to be regarded as measures of “damage”, such that these are equivalent to selection coefficients for $s \leq 1$; for $s > 1$, the damage results in lethality, so the selection coefficient is equivalent to 1 in such cases.

Completely neutral mutations. We cannot estimate c_n and the DDME shape and location from our data at the same time. Since the least is known about DDMEs, we chose to estimate shape and location, while assuming potentially realistic values of c_n like 0% or 2.5%. We also tested higher values like 5% and 7.5%, but these severely decreased the fraction of bootstraps that could be fitted to the data.

Diversity Index. Bootstraps were carried out independently for each species, with 1000 resampled datasets created by sampling loci at random with replacement (Loewe *et al.* 2006). Fits of the different distributions were then attempted with each bootstrap replicate. To eliminate bootstraps that cannot be fitted to models that include purifying selection, we defined the Diversity Index as $DI_{12} = (\pi_{A1}/\pi_{S1})/(\pi_{A2}/\pi_{S2})$, where 1 and 2 denote the species with the smaller and larger N_e values, *D. miranda* and *D. pseudoobscura* respectively. This allows easy filtering for situations ($DI \leq 1$), where resampling led to results that are incompatible with any sensible model, since all models with purifying selection that assume a common DDME for the two species predict $DI_{12} > 1$. These were removed from the bootstraps, together with all other samples that could not be fitted to the diversity data by our automated procedure. For our variance-weighted data, $DI_{12} = 1.67$ and $N_{e2}/N_{e1} = 5.70$. Among 1000 bootstraps of these data, 12.2% had $DI \leq 1$.

References

- Loewe, L., Charlesworth, B., Bartolomé, C., & Nöel, V. 2006 Estimating selection on non-synonymous mutations. *Genetics* **172**, 1079-1092.
- McVean, G. A. T. & Charlesworth, B. 1999 A population genetic model for the evolution of synonymous codon usage: patterns and predictions. *Genet. Res.* **74**, 145-158.