Genetics: Published Articles Ahead of Print, published on May 27, 2008 as 10.1534/genetics.108.087122

The distribution of beneficial and fixed mutation fitness effects close to an optimum

Guillaume MARTIN & Thomas LENORMAND

CEFE-CNRS UMR 5175

Address:

Centre d'Ecologie Fonctionnelle et Evolutive, UMR CNRS 5175, 1919 Rte de Mende, 34295 Montpellier, France

Keywords: beneficial mutation effects, adaptation, Fisher model, mutational landscape, quadratic forms.

Short title: beneficial mutation effects distribution

Abstract:

The distribution of the selection coefficients of beneficial mutations is pivotal to study the adaptive process, both at the organismal level (theories of adaptation) and at the gene level (molecular evolution). A now famous result of extreme value theory states that this distribution is an exponential, at least when considering a well adapted wild-type. However, this prediction could be inaccurate under selection for an optimum (because fitness effect distributions have a finite right tail in this case). In this paper, we derive the distribution of beneficial mutation effects under a general model of stabilizing selection, with arbitrary selective and mutational covariance between a finite set of traits. We assume a well adapted wild – type, thus taking advantage of the robustness of tail behaviours, as in extreme value theory. We show that, under these general conditions, both beneficial mutation effects and fixed effects (mutations escaping drift loss) are Beta distributed. In both cases, the parameters have explicit biological meaning, and are empirically measurable; their variation through time can also be predicted. We retrieve the classic exponential distribution as a sub-case of the Beta when there is a moderate to large number of weakly correlated traits under selection. In this case too, we provide an explicit biological interpretation of the parameters of the distribution. We show by simulations that these conclusions are fairly robust to a lower adaptation of the wild-type, and discuss the relevance of our findings in the context of adaptation theories and experimental evolution.

INTRODUCTION

Understanding the distribution of fitness effects of beneficial mutation (hereafter $f_b(s_b)$) is necessary to predict the rate and genetic basis of adaptation (ORR 1998). It is also important to calibrate models of molecular evolution where positive selection is involved (EYRE-WALKER 2006) or to study processes involving the segregation of several beneficial mutations, like clonal interference (GERRISH and LENSKI 1998). So far, this distribution has been studied along two directions (for a historical review see ORR 2005a). The first is based on Fisher's (1930) geometric model of adaptation, while the second uses Gillespie's (1984) mutational landscape model. These two models differ in their basic assumptions, and each has its own limitations (discussed in ORR 2005b). Fisher's model (FM) considers stabilizing selection around an optimum in an *n*-dimensional phenotypic space and focuses on the fitness effect of random phenotypic changes. The mutational landscape model (MLM) directly focuses on the effect of single nucleotide substitutions on fitness. The strength of the FM is to predict the full distribution of fitness effects of mutations (hereafter $f(s)$) including both deleterious and beneficial mutations and their respective proportion, which depends on the level of adaptation of the wild-type (distance to the optimum). The MLM is less general in that it only considers beneficial mutation, but its strength is to avoid explicit assumptions on the phenotype-to-fitness map inherent to the FM. This is made possible when beneficial mutations can be considered drawn from the extreme right tail of $f(s)$. In this case indeed, extreme value theory can be used to predict the (unique) limiting distribution of extreme draws, i.e. $f_b(s_b)$. Importantly, this is robust to a wide range of $f(s)$ (GILLESPIE 1984; ORR 2002). A now famous and remarkably simple prediction of this theory is that $f_b(s_b)$ should be exponential (ORR 2003). This finding has, since then, been widely used (GERRISH and LENSKI 1998; PARK and KRUG 2007; WILKE 2004). Note that this is not the same as the distribution of effects fixed over a bout of adaptation, which is also predicted to be exponential (ORR 1998). In this paper, we determine $f_b(s_b)$ under a general model of stabilizing selection, based on an extension of the FM (MARTIN and LENORMAND 2006b), but we study our model in the same biological conditions as assumed in the MLM, thus allowing the use of extreme value theory in this context. We show that under this general model, the exponential approximation for $f_b(s_b)$ can be substantially inaccurate unless there is a large number of weakly correlated traits under selection. We provide an alternative, in terms of a Beta distribution, that includes the exponential as a limiting case. Before presenting these results, we first discuss the limit of the MLM and classic FM approaches to predict $f_b(s_b)$.

Limitations of the MLM approach: The MLM approach has provided simple, robust and testable conclusions, but as any model, it has limits. First, the MLM is by construction only valid when the wild-type is well adapted to its environment, so that mutations with fitness above the wild-type's are indeed drawn from the rightmost tail of $f(s)$ (GILLESPIE 1984); this may not be the case in a new environment. Second, the MLM makes explicit assumptions on the genetic basis of adaptation (single nucleotide substitutions with equal probability of occurrence). Although it is often seen as more realistic, these genetic assumptions may also limit the scope of the theory. Indeed, even in a simple situation involving only point mutations, corrections were required to compare empirical data to MLM theory because of differences in transition *vs*. transversion rates (ROKYTA *et al.* 2005). Third, even when considering well adapted wild-types, the MLM is not robust to *any* $f(s)$. Only the so-called Gumbel-types of distributions, characterised by an exponential-like tail (BEISEL *et al*. 2007; ORR 2002), are consistent with the historical models by Gillespie and Orr. There are in fact three possible "domains of attraction" determining extreme values behaviour: the Gumbel type discussed above, the Fréchet type, for heavily-tailed distributions, and the Weibull type, for distributions that have a rightmost endpoint. There is no obvious reason to prefer one type over the others (discussed in BEISEL *et al.* 2007). Fourth, the MLM does not provide any prediction on how the distribution of mutant fitnesses should change over several generations, as the population adapts to its environment. In the classic formulation, the effect of adaptation is only to shift the wild-type to higher and higher fitness ranks in an otherwise constant distribution of mutant fitnesses. By contrast, the FM is free of these limits but at the cost of explicit assumptions on the genotype-to-phenotype-to-fitness map, which could be unrealistic.

Empirical testing: Overall, from an empirical point of view, it has proved difficult to validate predictions on beneficial mutation effects so far, because they are often rare. The exponential distribution appears to give a reasonable but still imperfect fit to empirical distributions of beneficial effects (KASSEN and BATAILLON 2005; ROKYTA *et al.* 2005). From a more statistical point of view, no alternative theoretical $f_b(s_b)$ had been proposed until recently, when a statistical framework was proposed to test alternative predictions all stemming from extreme value theory (BEISEL *et al.* 2007). Overall, empirical studies so far could neither clearly accept nor reject the predictions of the MLM, so that theoretical arguments may help settle the issue. In particular, it would be important that FM and MLM approaches yield consistent results. We now turn to this question.

$f_b(s_b)$ under the FM, and its limitations : In a recent article, H.A. ORR (2006), sought to bridge the gap between the FM and the MLM, and showed that they share strong similarities regarding $f_b(s_b)$. Indeed, under the classic Fisher's model, the distribution of fitness effects of single mutations is close to a Gaussian, which pertains to the domain of attraction of the Gumbel – type extreme value distribution assumed in the MLM. This property ensures that the derivations of the MLM are at least approximately valid under the biological assumptions of the FM, which reinforces the view that $f_b(s_b)$ should indeed be exponential. However, this conclusion should be taken with caution. First, under the FM, $f(s)$ is necessarily bounded on the right: there is no better mutation than the one bringing the phenotype at the optimum. As we have seen, distributions that are bounded on the right pertain to the Weibull domain of attraction, not to the Gumbel, and this will be true of any model of selection for an optimum. Orr (2006) mentioned this problem and showed that the exponential approximation could nevertheless be accurate under the FM, provided that there is a large number of equivalent and independent traits affected by pleiotropic mutation and selection. However, (i) selective and mutational independence of the traits is often considered biologically unrealistic (ORR 2005b), and (ii) the number of traits affected by mutation may be limited, at least when considering single genes, as is usual in molecular evolution. Overall, the assumption of a large number of independent traits leads to an approximately Gaussian $f(s)$ (simply by the central limit theorem) whereas reviews of empirical $f(s)$ show that they are better approximated by a skewed gamma distribution when only deleterious mutations are observed (EYRE-WALKER and KEIGHTLEY 2007; MARTIN and LENORMAND 2006b). Because it is this Gaussian $f(s)$ that leads to an approximately exponential $f_b(s_b)$ in the FM (ORR 2006), the exponential result may not be robust if traits are fewer and correlated. Indeed, recent models have showed that non-equivalence between traits in the FM could substantially affect the predictions of the FM (MARTIN and LENORMAND 2006b; WAXMAN and WELCH 2005).

Outline of the paper: In this paper, we apply extreme value theory to a general model of selection for an arbitrary optimum, where mutations have pleiotropic effects on an arbitrary number of potentially inequivalent and correlated traits. In this case, $f_b(s_b)$ belongs to the Weibull, rather than the Gumbel domain of attraction. Using a recent approximation for the tail behaviour of quadratic forms in Gaussian vectors (JASCHKE *et al.* 2004), we show that beneficial effects are approximately Beta distributed provided the wild-type is relatively well adapted (as assumed in the MLM). This result is based on tail approximations (similar to the

extreme value theory approach) and is robust to any continuous phenotype-to-fitness function close to an optimum (contrary to the classic FM). Our conclusions are checked using exact simulations, which show that the tail approximation yields surprisingly accurate results, even away from the tail. We discuss our results and compare them with results from the MLM.

MODEL

***f*(*s*) under arbitrary stabilizing selection:** We consider an extension of the FM that has been detailed previously (MARTIN and LENORMAND 2006b). The fitness $W(\mathbf{z})$ of a phenotype $\mathbf{z}$ (of arbitrary dimension n, the number of phenotypic traits under selection) is a multivariate Gaussian function of $\mathbf{z}$, $W(\mathbf{z}) \equiv \exp(-½\ \mathbf{z}^T \cdot \mathbf{S} \cdot \mathbf{z})$ where ^{T} denotes transposition, and $\mathbf{S}$ is an arbitrary positive semi-definite matrix of selective interactions between phenotypic traits. This assumption is justified when close to the optimum, as many continuous fitness functions around a single optimum can be approximated by a Gaussian function close to that optimum (LANDE 1979). This does not preclude the existence of other optima, but it does assume that they are too remote from the mutant "cloud" around the wild - type to influence $f(s)$. We consider an initial genotype (or wild – type), with phenotype $\mathbf{z}_o$ (and fitness $W(\mathbf{z}_o) = W_o$). The distribution of mutant phenotypes ($\mathbf{dz}$) around $\mathbf{z}_o$ is assumed to be multivariate Gaussian with mean $\mathbf{0}$ and arbitrary (positive semi-definite) covariance matrix $\mathbf{M}$. Again, this assumption is not as restrictive as it seems, what is required in fact is that there exist a set of trait definitions for which their mutational effect distribution is Gaussian (MARTIN and LENORMAND 2006b), which only requires that the distribution of mutant effects on the original traits be continuous, unimodal, and approximately centred on the wild – type.

This model is a quite general description of stabilizing selection, when not too far from the optimum, and with universal pleiotropy of mutations (mutations affect all traits simultaneously). Contrary to the classic (isotropic) Fisher model, it allows for differences and correlations between traits for both mutation and selection. Following assumptions of the MLM, we assume that the wild – type is well adapted (close to the optimum), so that beneficial mutation effects are small. Consequently, the selection coefficient of a beneficial mutant relative to the wild-type ($s = W/W_o - 1$) is approximately equal to the log – relative fitness: $s \approx \log(1+s) = \log(W/W_o)$. Therefore, s is approximately a quadratic function of

mutational phenotypic effects **dz** (MARTIN and LENORMAND 2006b), i.e. a quadratic form in Gaussian vectors (MATHAI and PROVOST 1992).

Beyond their mathematical convenience or robustness, these assumptions are supported by data: the model seems to correctly account for the variation of empirical distributions of mutational fitness effects across species (MARTIN and LENORMAND 2006b), across environments (MARTIN and LENORMAND 2006a), and among mutations (fitness epistasis MARTIN *et al.* 2007). Under these assumptions, the probability density function (pdf) of s, $f(s)$, is entirely determined by the n eigenvalues of the matrix product **S.M** and the position $\mathbf{z}_o$ of the initial phenotype relative to the optimum (**Appendix 1** and eq. (A2) of MARTIN and LENORMAND 2006b). There is no analytic expression for $f(s)$ in the general case, but it can be approximated by a displaced gamma distribution (JASCHKE *et al.* 2004; MARTIN and LENORMAND 2006b), as illustrated in **Appendix 1**.

Importantly, the distribution of s is bounded on its rightmost endpoint by $s_o = \log(W_{max}/W_o)$ which is the selection coefficient of the individual with optimal phenotype (with fitness $W(\mathbf{0}) = W_{max}$) relative to the wild-type (with fitness $W(\mathbf{z}_o) = W_o$). As we saw above, this kind of right-bounded distribution is inherent to any model of selection for an optimum.

Distribution of beneficial fitness effects close to the optimum: A tail approximation for the distribution of quadratic forms in Gaussian vectors (such as s) has been derived recently (JASCHKE *et al.* 2004). When the wild – type is well adapted (as $s_o \rightarrow 0$), a simple approximation can be deduced from this tail approximation, for the distribution $f_b(s_b)$ of beneficial mutations ($0 < s_b < s_o$), yielding (**Appendix 2**)

$$\frac{s_b}{s_o} \sim Beta\left(1, \frac{m}{2}\right), \quad \text{when } s_o << 1 \tag{1}$$

where s_o is the fitness distance to the optimum defined above, and m = rank(**S.M**). The distribution of s_b scaled to its range $[0, s_o]$, is a Beta distribution $Beta[1, m/2]$. The two parameters of the distribution have an explicit biological interpretation, and are *a priori* biologically independent of each other: s_o describes the level of adaptation of the wild-type, and m describes the dimensionality of the phenotype-fitness landscape. Here "dimensionality"

is understood as the number of *distinct* traits that are effectively affected by both mutation (matrix **M**) and selection (matrix **S**), i.e. with correlation less than one. When there is no or little correlation between traits, both **S** and **M** are positive definite (with only strictly positive eigenvalues) so that *m* equals *n*, the total number of traits that are pleiotropically affected by mutation. However, with many traits, as correlations between traits increase, either **M** or **S** or both quickly become semi-definite, i.e. their rank *m* is lower than *n*. This means that there are *n* - *m* traits that can in fact be expressed as linear combinations of the *m* distinct traits of the system. Because $m = \text{rank}(\mathbf{S}.\mathbf{M}) \leq \min(\text{rank}(\mathbf{M}), \text{rank}(\mathbf{S}))$, correlations among a large number of traits will result in a relatively small *m*, unless these correlations are weak.

Domain of attraction: The Beta distribution given in Eq. (1) is an example of the so-called Generalized Pareto Distribution (GPD), that encompasses the three possible domains of attraction of extreme value theory (PICKANDS 1975). To use the classic formulation (used e.g. in BEISEL *et al.* 2007), this Beta distribution in eq. (1) is a GPD with location $\mu = 0$, scale $\tau = 2/m$ and shape $\kappa = -2/m$. As long as *m* is not infinitely large, κ is negative so that $f(s)$ falls into the Weibull domain of attraction. However, with an infinitely large *m*, κ would be zero, and $f(s)$ would fall into the Gumbel domain of attraction. This explains why the classic FM, which assumes a very large number of independent traits, is consistent with the MLM (ORR 2006) and close to a Gumbel – type distribution. Consistent with this, the cumulative distribution function of the Beta distribution in eq.(1) converges to that of an exponential distribution when *m* is large (see **Appendix 2**):

$$\frac{s_b}{s_o} \sim Exponential\left(m/2\right), \quad \text{when } s_o << 1 \text{ and } m >> 1 \qquad (2)$$

Importantly, this result also provides an explicit formulation for the rate of the exponential in terms of biological parameters: with large *m*, beneficial effects (s_b) are exponentially distributed with rate $m/2s_o$.

Overall, under our general model of selection for an optimum, and with a well adapted wild-type, we obtain an approximately exponential distribution of beneficial mutations only when there are sufficiently many *independent* and *weakly correlated* traits under selection. When a limited number of traits are affected by the mutational target under consideration (e.g. a single gene), or when there are many but strongly correlated traits, there is no reason to

expect an exponential $f_b(s_b)$. In these cases, one should use the full model (Beta approximation) given in Eq.(1). Because the exponential approximation is a limiting case of the Beta approximation, the two behaviours may be easily compared statistically. We now turn to the study of the fitness effect distribution of those beneficial mutations that reach fixation.

The distribution of fitness effects among beneficial mutations escaping drift loss: Not all beneficial mutations will fix in a population: even in an infinitely large population, most are lost soon after their appearance, due to the stochasticity of offspring number. From $f_b(s_b)$, it is possible to derive the distribution of selection coefficients among those beneficial mutations that escape drift loss when they are still rare (i.e. those that reach fixation in a sexual population). From Eq. (1), assuming a well adapted wild – type and a population not too small, we can use a weak selection approximation ($\pi(s) \propto 2s$) for the fixation probability of beneficial mutations (HALDANE 1927; WHITLOCK 2000) and find another Beta approximation for the distribution of fixed mutation effects s_f (**Appendix 3**):

$$\frac{s_f}{s_o} \sim \text{Beta}\left(2, \frac{m}{2}\right), \qquad \text{when } s_o << 1. \tag{3}$$

The average fitness effect of *fixed* beneficial mutations is then (for small s_o)

$$E\left(s_f\right) \approx \frac{4s_o}{4+m}, \tag{4}$$

which, as expected, is larger than the average fitness effect of all beneficial mutations (from Eq.(1)),

$$E\left(s_b\right) \approx \frac{2s_o}{2+m}. \tag{5}$$

As expected, these two values increase with the wild – type maladaptation s_o, and decrease with increased dimensionality m, which is a part of the "cost of complexity" defined by H.A. ORR (2000). The other part of this cost, not dealt with here, is the reduction of the fraction of beneficial mutations as m increases. When m is large, $E(s_f)$ and $E(s_b)$ are approximately $4s_o/m$ (fixed effects) and $2s_o/m$ (beneficial effects) respectively, which converges to the results derived from the exponential approximation (see **Appendix 3**).

Simulations: To check the accuracy of the above results, we simulated distributions of beneficial mutations in the Fisher model with correlated traits as in (MARTIN and LENORMAND 2006b). We drew the mutational and selective covariance matrices (**M** and **S**) from $n \times n$ Wishart distributions $W_p(n,\mathbf{I})$ (where **I** is the $n \times n$ identity matrix) so that the rank of **S.M** is $m = \min(p,n)$. **M** and **S** were then scaled to obtain an average deleterious effect of mutations $\bar{s} = \mathrm{tr}(\mathbf{S.M})/2 = 0.05$, where tr(.) denotes matrix trace. Then the phenotype of the wild-type $\mathbf{z}_o$ was drawn as a Gaussian vector and scaled so that $\log(W_{max}/W_o) = \log(1/W_o) = s_o$, for a given fitness distance to the optimum. Finally, for each single mutant, we drew a mutation effect vector **dz** from a multivariate Gaussian distribution $N(\mathbf{0},\mathbf{M})$, and computed s as $s = \log(W(\mathbf{z}_o + \mathbf{dz})/W(\mathbf{z}_o))$. The resulting distributions of s are illustrated in **Supplementary Figure 1**.

We chose p in order to get a distribution of fitness effects (among all mutations) with a large skewness, as is typically observed in empirical studies (e.g. SANJUÀN *et al.* 2004). More precisely, a given distribution of deleterious s (when $s_o = 0$) corresponds to a given effective number of traits n_e (depending on the magnitude of correlations in **M** and **S**) which determines the shape of $f(s)$ (MARTIN and LENORMAND 2006b). With **M** and **S** drawn as Wishart deviates $\mathbf{S},\mathbf{M} \sim W_p(n,\mathbf{I})$, $n_e \approx n/(1+2n/p)$ (for details, see Appendix 2 of MARTIN and LENORMAND 2006b), so we chose p as the integer part of $2\, n\, n_e/(n-n_e)$ to obtain a given n_e and n. All figures are given for the same two examples corresponding to alternative levels of pleiotropy. In the low pleiotropy case, $n = 4$ and $n_e = 2.5$, so that $m = n = 4$ (**S** and **M** are positive definite). In the high pleiotropy case (still keeping a small n_e), $n = 40$ and $n_e = 4$, so that $m = p = 9$ (**S** and **M** are positive semi-definite). Therefore, these two cases correspond to a lower (resp. higher) number of traits jointly affected by mutation and selection and to a lower (resp. higher) dimensionality m. They are denoted low (resp. high) pleiotropy on the figures.

From a set of 400,000 simulated single mutants we kept only those with fitness higher than the wild – type as beneficial mutants. To compute the fitness effect distribution among mutants that escape drift loss, we computed the exact fixation probability P_{fix} of each of the n_b beneficial mutants (with selection coefficient s) by numerically solving $P_{fix} = e^{-(1+s)P_{fix}}$, according to (HALDANE 1927). Then we sampled n_b times the beneficial mutants according to their individual fixation probability P_{fix}.

RESULTS

Accuracy of the Beta approximation for beneficial and fixed effects s_b: The Beta approximation given in Eq. (1) gives a very good fit to the simulations when s_o is small relative to the average of all mutations ($s_o << \bar{s}$), so that beneficial mutations are rare and at the rightmost tail of $f(s)$. **Figure 1** (top panels) illustrates $f_b(s_b)$ in this situation and **Supplementary Figure 1**, shows the corresponding $f(s)$. However, the prediction is still fairly accurate for larger values of s_o (and larger proportions of beneficial mutations), as illustrated in **Supplementary Figure 2**, for $s_o = \bar{s}$. As expected (Eq.(2)), when compared to the Beta, even the best – fitting exponential distribution provides a less accurate description of $f_b(s_b)$ when m is small (**Figure 1(a)**), but a similarly satisfying one, even with a moderately large m ($m = 9$, **Figure 1(b),** the two approximations are almost indistinguishable). Nevertheless, even in the latter case, a closer investigation (**Figure 2**) shows that the exponential approximation inaccurately captures the distribution on its rightmost part (for the largest beneficial effects), while the Beta approximation (eq. (1)) is accurate on the whole range of beneficial effects. This has little influence on the accuracy of the exponential model for beneficial effects (with large m), but is more problematic when deriving the distribution of *fixed* effects. Indeed, as for beneficial effects, the Beta approximation for the distribution of fixed effects (eq.(3), bottom panels of **Figure 1**) gives a good fit to individual simulations (compare plain lines and dots in **Figure 1(c) and (d)**). As a comparison, the distribution of s_f under the (best-fitting) exponential approximation for s_b (eq. A.3.4, **Appendix 3**) gives a less good fit to simulations (dashed lines, **Fig. 1(c) and (d)**), worse when the dimensionality is low (**Fig. 1(c)**, low pleiotropy case). The exponential approximation gives less accurate results for fixed effects

(**Fig. 1**, bottom panels) than for beneficial effects (**Fig. 1**, top panels) because the exponential inaccurately describes the distribution of large beneficial s (**Fig. 2**) which are over-represented among fixed mutations.

Robustness of the results away from the optimum: As in the MLM, the results presented here are all weak selection approximations in that they assume that the wild – type is close to the optimum (small s_o), so that beneficial mutations are all of small effect. We checked the robustness of these results when s_o gets larger: **Supplementary Figure 2** shows that while the Beta approximation for beneficial effects (s_b, Eq. (1)) is less (but still reasonably) accurate when $s_o = \bar{s}$, the Beta approximation for fixed effects (s_f in Eq. (3)) remains fairly accurate in this case. More surprisingly, the average value of fixed effects ($E(s_f)$, **Figure 3**) remains close to the tail approximation result (Eq.(4)), even for fairly large values of s_o (up to ten times the average effect of all mutations: $s_o = 0.5 = 10\,\bar{s}$). As expected again, the prediction from the exponential approximation is less accurate, at least with a small m for beneficial effects, and in both cases for fixed effects. It becomes less and less accurate as s_o increases (constant difference on log- scale, **Figure 3**). The same pattern holds for $E(s_b)$ (not shown). Overall, while the shape of the distributions, away from the tail, is less accurately described by eqs. (1) and (3) (see **Supplementary Figure 2**), their means are still fairly robustly predicted for large s_o.

DISCUSSION

In this paper, we derive the distribution of beneficial mutation fitness effects under a general model of selection for a phenotypic optimum with an arbitrary set of traits undergoing selection and pleiotropic mutation. The main restriction of the model is that the wild-type in which mutations appear is assumed to be well adapted to the environment considered, an assumption shared with the mutational landscape approach.

Comparing the Beta and exponential distributions for beneficial effects: under these fairly general conditions, and although the exact system depends on many parameters, the

distribution of beneficial effects, $f_b(s_b)$, is accurately approximated by a simple Beta distribution (Eq. (1)). This distribution is an example of the Generalized Pareto Distribution of the Weibull type, not of the Gumbel type as is classically assumed in most of the literature on adaptation theory. However, in the limit of a large number of weakly correlated traits (increased dimensionality m), our Beta approximation converges to the exponential, consistent with previous results (ORR 2006). Overall, the distribution of beneficial effects (s_b, Eq. (1)) will substantially differ from an exponential when only a limited number of traits is considered (**Figure 1 (a), Supplementary Figure 2**). Indeed, the convergence to an exponential is quick as dimensionality increases (**Figure 1 (b)**, m = 9). However this convergence to the exponential is slower for the distribution of fixed beneficial effects s_f (**Figure 1**, bottom panels). This occurs because, even for large m, the exponential distribution tends to particularly overestimate the proportion of largely beneficial effects (the really extreme right tail of $f(s)$) compared to the Beta (**Figure 2**), and these effects are strongly over-represented among fixed mutations.

Therefore, when assuming selection for an optimum, one should use the Beta approximations proposed here (eqs (1-4)), whenever possible, as they provide better accuracy in the general case, while retaining the simplicity that made the exponential approximation theoretically attractive. However, when considering beneficial effect distributions (and with caution for fixed effects), and provided the dimensionality m is even moderately large (an issue we discuss more fully below), the exponential can provide an even simpler, and similarly accurate approximation.

An important aspect of our result is that, beyond classic results from extreme value theory, our model provides a biological interpretation of the two parameters that emerge in the tail approximation: s_o is the selection coefficient of the optimal genotype relative to the wild-type; m measures the level of pleiotropy (i.e. the number of not fully dependent dimensions of the phenotypic space under selection). Note that when $n > m$, there are $n - m$ traits that are completely determined by linear combinations of the first m traits: m is therefore akin to a "degree of freedom", the number of traits that suffice to fully describe the fitness landscape. Although included in the model for the sake of generality, the extra $n - m$ traits are somehow meaningless in terms of pleiotropy. These two parameters (m and s_o) are, *a priori*, biologically independent. For instance, because s_o measures adaptation of the wild – type, we may predict how this parameter changes through time as individuals adapt, while m could be expected to remain constant, at least over short evolutionary timescales. Beyond

characterizing the distribution of beneficial effects, this model therefore provides a means to predict how this distribution changes through time, as in the FM, while preserving the robustness provided by the use of tail behaviours (extreme value theory) as in the MLM. This is true including when the exponential approximation is valid (large m, **Figure 1(b)**): our model and simulations then show that beneficial effects s_b are exponentially distributed, with rate $m/2s_o$.

Robustness of the results: There are few other assumptions in the model, apart from the existence of an optimum, to which the wild – type is well adapted. Indeed, the model is approximately valid near a local maximum of any continuous fitness function, and for any selective or mutational covariance between traits (by construction). Another relevant issue is modularity: our model assumes total pleiotropy of all mutations on all the traits considered. If distinct mutational targets (e.g. genes) affect at least partly distinct sets of traits, then there is modularity in the effect of mutation (WELCH and WAXMAN 2003). We suspect that the total $f(s)$ would then be a sum of each modules' $f(s)$, weighted by the probability of mutation in each module. The effect of such modularity would have to be studied in more detail, but even then, there would still be a maximum value of s, so that $f(s)$ would likely pertain to the Weibull domain of attraction, leading to a distribution of beneficial effects of the type of Eq. (1). However, the biological interpretation of the two parameters is probably less straightforward in this case.

A surprising property of our model is the robustness of the predictions when away from the tail. The approximate distribution of both beneficial and fixed effects is still reasonably accurate when they are of the same order as the mean effect of all mutations ($s_o = \overline{s}$, **Supplementary Figure 2**), for which the proportion of beneficial mutations is higher than 10%. Even more surprisingly, the mean of these distributions (both beneficial and fixed effects), are accurately predicted, even further away from the tail (up to $s_o = 10\overline{s}$, **Figure 4**). Overall, the average log-fitness gain per adaptive fixation ($E(s_f)$, Eq. (4)) is approximately $4s_o/(4+m)$, for fairly arbitrary levels of adaptation of the wild – type. However, the prediction would probably fail, away from the tail, if the phenotype-to-fitness function $W(\mathbf{z})$ was not close enough to a Gaussian, which is possible when away from the optimum. Whether or not fitness functions are Gaussian remains an open question, although a review of mutation effects in stressful environments (wild – type illadapted) did suggest that it may be a

reasonable approximation even away from the optimum (MARTIN and LENORMAND 2006a). Overall, while the simple results derived here may apply also in new and stressful environments (away from the optimum), they are a priori more likely to be valid in benign ones (close to it).

How large is m? An important issue is whether m is large or not, as it determines the accuracy of the exponential approximation. When m is at least moderately large ($m \geq 10$), then the classic exponential (Eq. (2)) could be a sufficient approximation, when describing beneficial effects (although it would be less accurate for fixed effects, as we already mentioned). Although a large m seems intuitively likely because many traits are under selection, it needs not be so: as we have seen above, (i) with even weak mutational and selective correlations, a large set of traits is necessarily mutually dependent, which reduces m, and (ii) traits may be organized in modules. That empirical $f(s)$ are not Gaussian suggests that these effects are important. In fine, whether m is large enough that the exponential is a sufficient description of $f_b(s_b)$ is mainly an open empirical question; we now turn to this issue.

Empirical estimation of *m*: One may estimate m empirically, with a similar approach as proposed to estimate n_e (MARTIN and LENORMAND 2006b): from empirical distributions of single mutant fitnesses (empirical $f(s)$). Indeed, at any distance from the optimum, m may be estimated by fitting a Generalized Pareto Distribution to the extreme right tail of mutation fitness effects. The shape of the GPD, $\kappa = -2/m$, will then provide an estimate for m. Such a fit can be performed using the method of BEISEL et al. (2007) or routines proposed in the "POT" R package (http://r-forge.r-project.org/projects/pot/), for example. Alternatively, m may be measured from evolution experiments data (TENAILLON *et al.* 2007). However, some simulations would be needed to check whether this latter method does estimate the required quantity (i.e. m not n) when traits are correlated.

Predicting the proportion of beneficial mutations? The tail approximation used in this paper (JASCHKE *et al.* 2004) can also be used to derive the proportion p_b of beneficial mutations when s_o is small (Eq. A.2.3 of **Appendix 2**). Unfortunately, this prediction is much less robust than that on $f_b(s_b)$, giving strongly inaccurate results unless p_b is of the order of 10^{-}

^{3} or less (simulations not shown). Consistent with this, JASCHKE et al. (2004) showed that their tail approximation gave a poor fit to quadratic forms distributions, unless considering values very close to the rightmost endpoint. Because our prediction for $f_b(s_b)$ depends on the same approximation, modulo the scaling constant d (see **Appendix 2**), we believe the poor robustness of the approximation for $f(s)$ and p_b comes from the expression for d being only valid very close to the rightmost endpoint of the distribution. Anyhow, this lack of fit means that our results do not provide a satisfactory expression for p_b, and that the displaced gamma approximation should be preferred for this purpose (MARTIN and LENORMAND 2006b), although it yields a slightly more complicated expression.

Extreme value theory and clonal interference: Clonal interference is the mechanism by which beneficial mutations occurring in different individuals compete for ultimate fixation in asexuals. P. GERRISH and R. LENSKI (2001; 1998) showed that, in the limit of fairly low mutation rates, this process implies that the mutations that fix are the ones with the largest selection coefficient among all those that appear during a selective sweep. Such sieving process therefore consists in drawing the maximum value among a set of draws from a distribution, which is exactly what is described by extreme value theory or tail approximations (SMITH 2003). Therefore, the MLM and the model discussed in this paper may prove useful for describing the distribution of fixed mutations in asexuals, in a much more general context than for sexuals, i.e. at *any* distance from the optimum. As extreme s values (largely beneficial) are strongly over-represented among mutations that escape clonal interference, it will probably be safer to use the general model (Beta approximations) than the exponential limit in this context, as the latter is less accurate when it comes to describing the very right tail of $f(s)$ (**Figure 2**).

Conclusion: Overall, our study clarifies the conditions under which the different ways to model the distribution of fitness effects of beneficial mutations give similar or different results and why. In particular, we stress that beneficial mutations may not be exponentially distributed. Under selection for an optimum, the fitness effects of both beneficial and fixed mutations are Beta distributed, which is only close to exponential when there is a large number of weakly correlated traits subject to selection and pleiotropic mutation.

Acknowledgments: We thank Allen Orr and Paul Joyce for their helpful comments on a previous version of this manuscript. G.M. was funded by a CNRS Post Doctoral grant to Sylvain Gandon, and T.L. was supported by a starting grant from the European Research Council.

REFERENCES:

BEISEL, C. J., D. R. ROKYTA, H. A. WICHMAN and P. JOYCE, 2007 Testing the extreme value domain of attraction for distributions of beneficial fitness effects. Genetics **176:** 2441-2449.

EYRE-WALKER, A., 2006 The genomic rate of adaptive evolution. Trends in Ecology & Evolution **21:** 569-575.

EYRE-WALKER, A., and P. D. KEIGHTLEY, 2007 The distribution of fitness effects of new mutations. Nature Reviews Genetics **8:** 610-618.

FISHER, R. A., 1930 *The genetical theory of natural selection*. Oxford University Press, Oxford.

GERRISH, P., 2001 The rhythm of microbial adaptation. Nature **413:** 299-302.

GERRISH, P. J., and R. E. LENSKI, 1998 The fate of competing beneficial mutations in an asexual population. Genetica **103:** 127-144.

GILLESPIE, J. H., 1984 Molecular Evolution over the Mutational Landscape. Evolution **38:** 1116-1129.

HALDANE, 1927 A mathematical theory of natural and artificial selection V. Selection and mutation. Proceedings of the Cambridge Philosophical Society **26:** 220-230.

JASCHKE, S., C. KLUPPELBERG and A. LINDNER, 2004 Asymptotic behavior of tails and quantiles of quadratic forms of Gaussian vectors. Journal of Multivariate Analysis **88:** 252-273.

KASSEN, R., and T. BATAILLON, 2005 The distribution of fitness effects among beneficial mutations prior to selection in experimental populations of bacteria. In review.

LANDE, R., 1979 Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution **33:** 402-416.

MARTIN, G., S. F. ELENA and L. LENORMAND, 2007 The distribution of epistasis fits predictions from a fitness landscape model in bacteria and viruses. Nature Genetics **39:** 555-560.

MARTIN, G., and T. LENORMAND, 2006a The fitness effect of mutations in stressful environments: a survey in the light of fitness landscape models. Evolution.

MARTIN, G., and T. LENORMAND, 2006b A general multivariate extension of Fisher's geometrical model and the distribution of mutation fitness effects across species. Evolution **60:** 893-907.

MATHAI, A. M., and S. B. PROVOST, 1992 *Quadratic forms in random variables*. Marcel Dekker, New York.

ORR, H. A., 1998 The population genetics of adaptation: The distribution of factors fixed during adaptive evolution. Evolution **52:** 935-949.

ORR, H. A., 2000 Adaptation and the cost of complexity. Evolution **54:** 13-20.

ORR, H. A., 2002 The population genetics of adaptation: The adaptation of DNA sequences. Evolution **56:** 1317-1330.

ORR, H. A., 2003 The distribution of fitness effects among beneficial mutations. Genetics **163:** 1519-1526.

ORR, H. A., 2005a The genetic theory of adaptation: A brief history. Nature Reviews Genetics **6:** 119-127.

ORR, H. A., 2005b Theories of adaptation: what they do and don't say. Genetica **123:** 3-13.

ORR, H. A., 2006 The distribution of fitness effects among beneficial mutations in Fisher's geometric model of adaptation. Journal of Theoretical Biology **238:** 279-285.

PARK, S., and J. KRUG, 2007 Clonal interference in large populations. Proceedings of the National Academy of Sciences, USA **104:** 18135 - 18140.

PICKANDS, I., J.,, 1975 Statistical inference using extreme order statistics.
. Annals of Statistics
3: 119 - 131.

ROKYTA, D. R., P. JOYCE, S. B. CAUDLE and H. A. WICHMAN, 2005 An empirical test of the mutational landscape model of adaptation using a single-stranded DNA virus. Nature Genetics **37:** 441-444.

SANJUÀN, R., A. MOYA and S. F. ELENA, 2004 The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. Proceedings of the National Academy of Sciences of the United States of America **101:** 8396-8401.

SMITH, R. L., 2003 statistics of extremes, with applications in environment, insurance and finance, pp. in *Science*.

TENAILLON, O., O. K. SILANDER, J. UZAN and L. CHAO, 2007 Quantifying Organismal Complexity using a Population Genetic Approach. Plos One **2:** e217. doi:210.1371/journal.pone.000021.

WAXMAN, D., and J. J. WELCH, 2005 Fisher's microscope and Haldane's ellipse. American Naturalist **166:** 447-457.

WELCH, J. J., and D. WAXMAN, 2003 Modularity and the cost of complexity. Evolution **57:** 1723-1734.

WHITLOCK, M. C., 2000 Fixation of new alleles and the extinction of small populations: Drift load, beneficial alleles, and sexual selection. Evolution **54:** 1855-1861.

WILKE, C. O., 2004 The speed of adaptation in large asexual populations. Genetics **167:** 2045-2053.

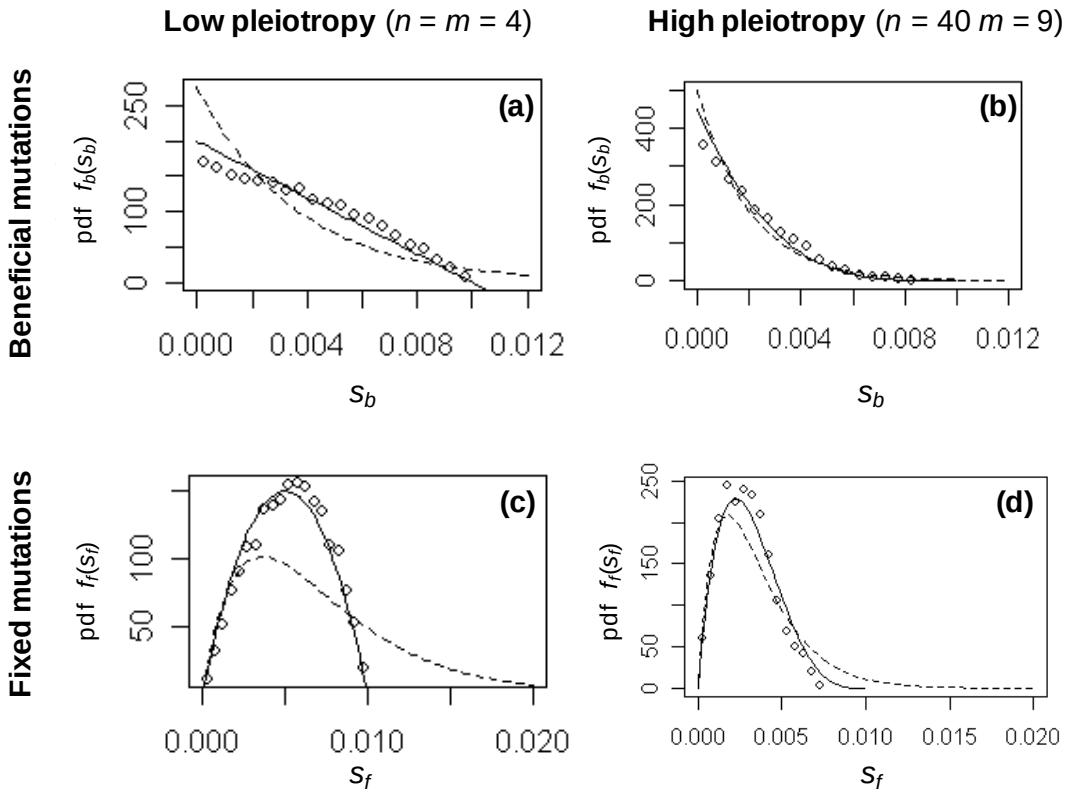

Figure 1. Distribution of beneficial (s_b) and fixed (s_f) mutation fitness effects. The fitness effects distributions of beneficial (s_b, top panels) and fixed (s_f, bottom panels) mutations in exact simulations (from 400,000 random mutations, circles) are compared to alternative approximations: the Beta (plain lines, Eqs.(1) and (3) for s_b and s_f, resp.) and the exponential (dashed lines, best-fitting exponential for s_b and eq. (A.3.4) for s_f). Left panels ((a), (c)) correspond to a low pleiotropy case: n = 4 weakly correlated traits (m = 4). Right panels ((b), (d)) correspond to a higher pleiotropy case: n = 40 traits that are more strongly correlated (m = 9). The wild-type was well adapted (fitness distance to the optimum: s_o = 0.01, with an average (deleterious) effect of all mutations: $-\overline{s}$ = - 0.05). The Beta approximation leads to a more accurate prediction than the exponential in both cases: the increase in accuracy is almost undetectable for high pleiotropy (right panels), but substantial for low pleiotropy (left panels).

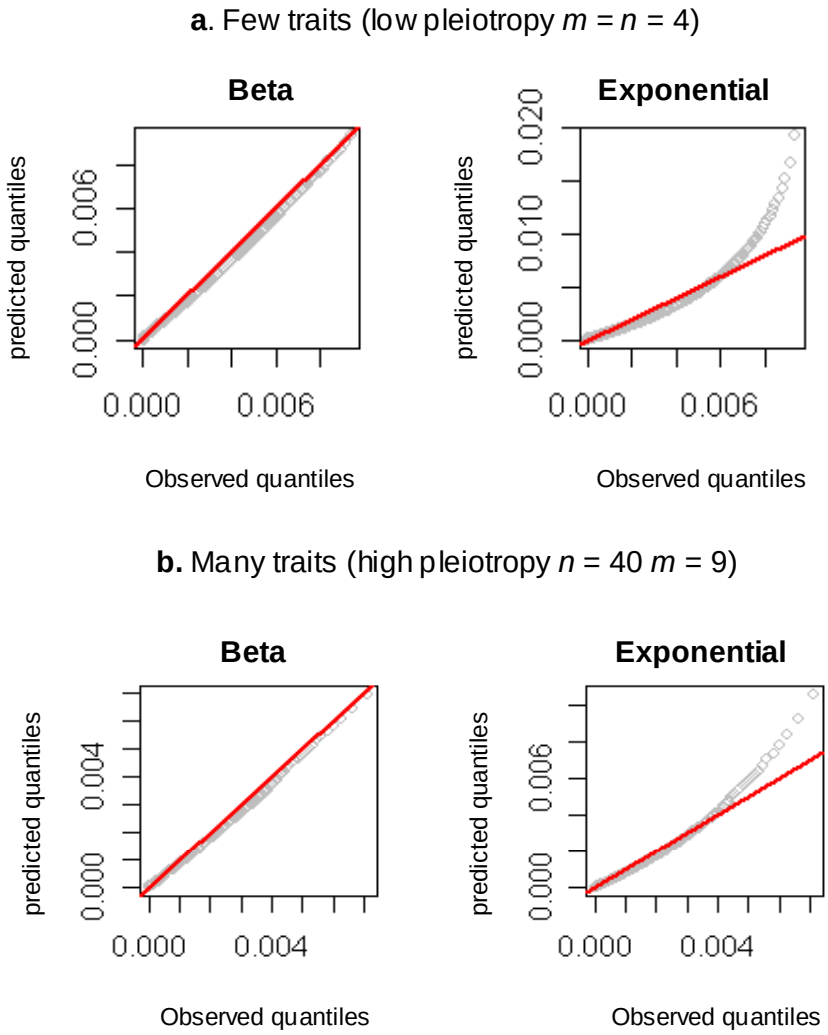

Figure 2. Quantile – quantile plots for the Beta vs. Exponential approximations of $f_b(s_b)$.

This figure is based on the same cases as in the top panels of **Figure 1.** The quantiles of $f_b(s_b)$ from simulations (x-axis) are compared to either the Beta prediction from eq. (1) or the best – fitting exponential (y-axis). When the points lie on a line with slope 1 (given on the graph), the observed and expected distributions are the same. The Beta approximation is accurate on the whole range of fitness effects while the Exponential is inaccurate in the rightmost part of the distribution (largest s values).

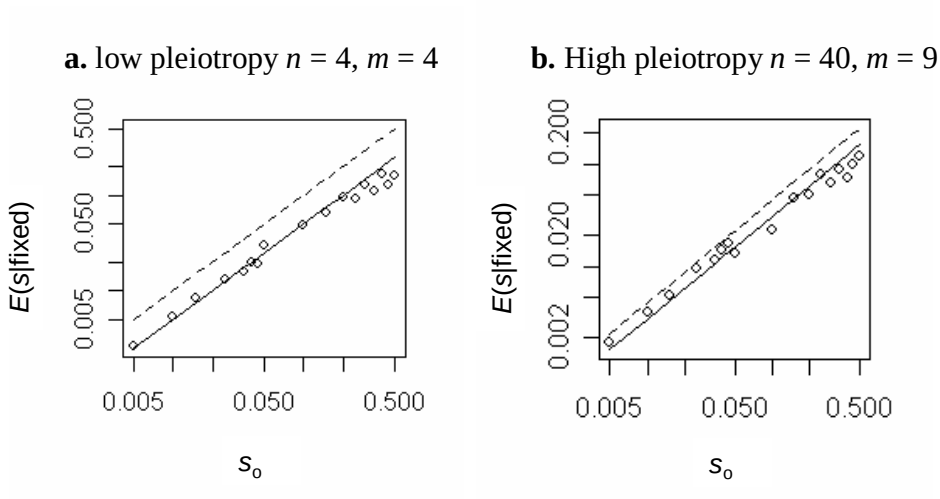

Figure 3. Variation of E(s_f) with the distance to the optimum s_o. The average selection coefficient of fixed mutations, E(s_f), is given for the same parameter values as in **Figures 1 and 2**, except that the distance to the optimum (x-axis) varies from $s_o = 0.005 = 0.1\,\bar{s}$, to $s_o = 0.5 = 10\,\bar{s}$. Dots give $E(s_f)$ from simulations, while lines give the prediction from either the Beta approximation (Eq. (4), plain line), or the best – fitting Exponential (Eq. A.3.5, dashed lines). Panels (a) and (b) correspond to the same two levels of pleiotropy as in **Figures 1 and 2**. Note the log-log scale. Eq. (4) is accurate even for surprisingly large values of s_o. In contrast, results from the exponential approximation are moderately accurate in the high pleiotropy case (right panels), and inaccurate in the low pleiotropy case (left panel).