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The distribution of fitness effects of new beneficial mutations in *Pseudomonas fluorescens*

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Theoretical studies of adaptation emphasize the importance of understanding the distribution of fitness effects (DFE) of new mutations. We report the isolation of 100 adaptive mutants—without the biasing influence of natural selection—from an ancestral genotype whose fitness in the niche occupied by the derived type is extremely low. The fitness of each derived genotype was determined relative to a single reference type and the fitness effects found to conform to a normal distribution. When fitness was measured in a different environment, the rank order changed, but not the shape of the distribution. We argue that, even with detailed knowledge of the genetic architecture underpinning the adaptive types (as is the case here), the DFEs remain unpredictable, and we discuss the possibility that general explanations for the shape of the DFE might not be possible in the absence of organism-specific biological details.

Keywords: mutation; adaptation; evolution

1. INTRODUCTION

The nature of the distribution of fitness effects (DFE) is central to understanding the process of adaptation [1,2]. Although difficulties posed by the collection of a sample of mutations unbiased by selection have thus far rendered the DFE beyond experimental measure [3], the distribution is likely to belong to one of the many probability distributions that have approximately exponentially distributed right-hand tails. Accordingly, extreme value theory (EVT) can be used to provide insight into the distribution of beneficial fitness effects (DBFE) available to a well-adapted genotype [4–6]. Several experimental studies support the prediction that, in some cases, the DBFE conforms to EVT predictions, but they also highlight the shortcomings of the theory [7–13]. For instance, a key requirement for EVT to be applied is that adaptation is typically in response to minimal

environmental change, such that it begins with a genotype that has high fitness. This requirement excludes application of EVT to the consideration of adaptation to novel environments (as occurs during the evolution of antibiotic resistance or during adaptive radiations) from the current theoretical framework.

In this study, we use a novel genetic construct to identify beneficial ‘wrinkly spreader’ (WS) mutants of *Pseudomonas fluorescens*, prior to their exposure to the biasing effects of selection. The WS phenotype confers the ability to colonize the broth surface in oxygen-limited static microcosms. In order to obtain a view of the overall DFE (not just its right-hand tail), we measure the fitness of WS genotypes in this environment, in which the progenitor genotype has low fitness.

2. MATERIAL AND METHODS

(a) Collection of non-selected mutants

We used a reporter construct that fused a kanamycin resistance (*kan*) gene to the *wss* promoter [14]. Upon mutation to the beneficial WS phenotype, transcription of *wss* increases so that mutants having the *P_{wss}-kan* fusion become kanamycin-resistant, and can be identified without selection for the focal beneficial trait (ability to colonize the air–liquid interface). The (non-WS) *P. fluorescens* ancestor containing the WS reporter construct (SM^{MSC}) was inoculated into glass vials containing 6 ml of King’s Medium B (KB) and incubated at 28°C with shaking for 16 h (WS genotypes are maladapted in shaking broth culture); 50 µl of culture was then plated on KB agar containing 15 µg ml⁻¹ kanamycin to select kanamycin-resistant clones.

(b) Fitness assays

Selection coefficients of identified beneficial mutants were calculated as described in the electronic supplementary material. Assays were carried out for 1 day in structured (static) and unstructured (shaken) environments.

(c) Statistical analysis

Estimation of best-fit mutation effect distributions was done using maximum likelihood methods implemented in R v. 2.11.0. Measurement error was accounted for separately by convolution from genotype effects. Details of this approach and comparisons of all distributions fitted are presented in the electronic supplementary material.

3. RESULTS

WS genotypes—adaptive by virtue of their ability to form a self-supporting mat at the air–liquid interface [15]—evolve when *P. fluorescens* SBW25 is propagated in static broth microcosms. A WS reporter construct [14] was used to obtain 100 WS genotypes from the progenitor SM^{MSC} strain without selection for the ability to colonize the air–liquid interface. To ensure that no non-WS, kanamycin-resistant mutants were included, all 100 mutants were individually checked for their ability to colonize the air–liquid interface of broth microcosms.

Next we tested the possibility that some WS genotypes were not detectable owing to their inability to activate kanamycin resistance. A set of 100 independent microcosms inoculated with SM^{MSC} were incubated in non-shaken liquid microcosms for 36 h; all 100 WS obtained in this manner were kanamycin-resistant, despite being selected only for their WS morphology.

Finally, we tested for a correlation between WS fitness and the level of *wss* transcription. If the most (or least) fit WS genotypes have a higher rate of *wss* expression, this might affect the extent of kanamycin resistance, raising the possibility that some WS genotypes may grow too slowly to be detected. Of the 100 WS genotypes, 31 were randomly selected and plated at a low dilution on kanamycin-containing agar

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2010.0547> or via <http://rsbl.royalsocietypublishing.org>.

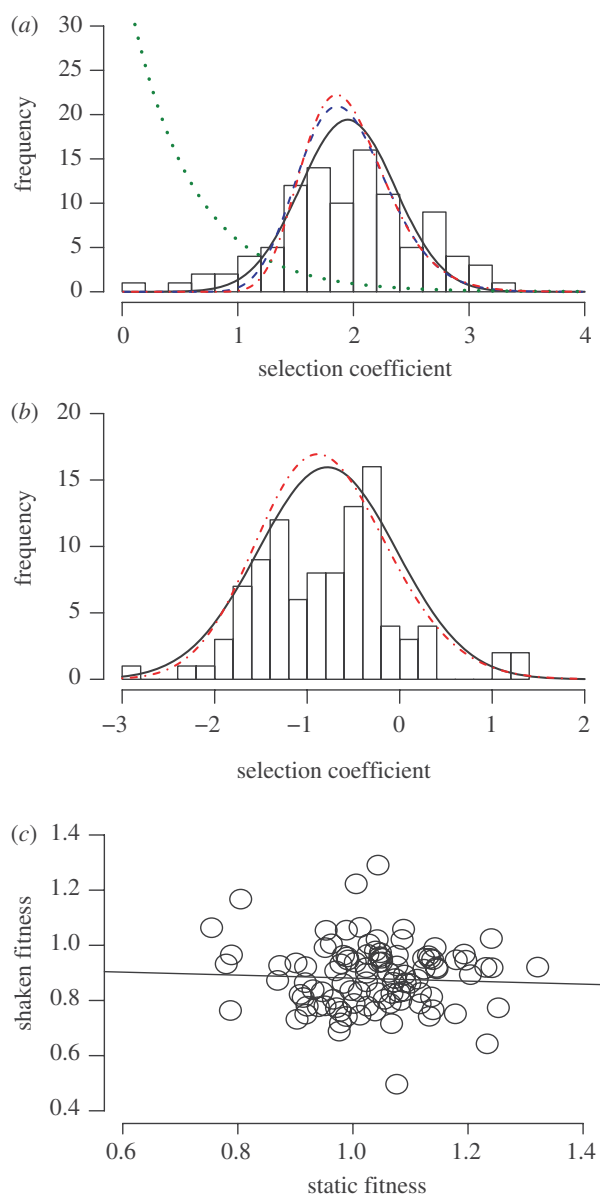


Figure 1. The distribution of fitness effects for non-selected WS genotypes. Curves are fitted using parameters that best fit the data for fitness measurements obtained in the (a) static and (b) shaken broth environments. Lines show best-fit distributions as determined by maximum likelihood as follows: black solid, normal; red dot dashed, lognormal; blue dashed, gamma; and green dots, exponential. The normal distribution provides a marginally better fit to the data. (c) The measurements taken for genotypes in the static and shaken environments show no correlation.

plates. The diameter of four colonies from each WS genotype was measured and the average plotted against the fitness as determined in a static microcosm relative to a marked WS (see below): no correlation was observed (Pearson's correlation coefficient, $r^2 = 1.6 \times 10^{-5}$, $p = 0.981$).

To determine the DBFEs of the 100 non-selected WS mutants, we assayed the fitness of each mutant relative to a *lacYZ*-labelled reference WS strain in static broth microcosms. A maximum likelihood method (see §2) was used to fit gamma, exponential, lognormal, skew normal and normal distributions to the resulting data. The gamma, lognormal and exponential distributions were rejected as best describing

Table 1. Mutations identified by sequencing WS genotypes isolated from microcosms 1–20 of the 100 WS mutants used in this study (13 mutations were found).

nucleotide change	gene
C608A	<i>wspF</i>
$\Delta 3066$ -3074	<i>mwsR</i>
$\Delta 228$ -261	<i>awsX</i>
$\Delta 3068$ -3076	<i>mwsR</i>
$\Delta 3066$ -3074	<i>mwsR</i>
$\Delta 228$ -261	<i>awsX</i>
$\Delta 99$ -138	<i>awsX</i>
C160G	<i>awsR</i>
$\Delta 140$ -185	<i>awsX</i>
$\Delta 228$ -261	<i>awsX</i>
$\Delta 155$ -173	<i>awsX</i>
$\Delta 99$ -138	<i>awsX</i>
$\Delta 99$ -138	<i>awsX</i>

the DBFE in favour of the normal distribution (figure 1; electronic supplementary material, table S1). In order to test whether the normal distribution was specific to the spatially structured environment we determined the DBFE for the same 100 genotypes in spatially unstructured (shaken) microcosms. We found that the DBFE of these genotypes in this alternative environment were also best described by the normal distribution. A possible explanation for this result is that the fitness of each genotype in the structured environment is negatively or positively correlated with its fitness in the unstructured environment. This possibility was rejected because of the significant lack of correlation in the rank order of genotype fitness measured in the different environments (Spearman's rank correlation, $\rho = -0.030$, $p = 0.415$). In light of the absence of a correlation in fitness across environments, it is interesting that the DFE of mutant genotypes in the shaken environment was also best described by the normal distribution.

Although our sample of WS mutants was independently collected, it is possible that a mutational hotspot could bias our sample. To address this, we sequenced 20 of the 100 WS mutants at four loci known to acquire WS-generating mutations: *wspF*, *awsX*, *awsR* and *mwsR* [16]. We found 13 mutations; five of these were unique, two occurred twice and one occurred three times (table 1). This distribution did not differ from a random expectation of mutation counts leading to a rejection of the hypothesis that the observed distribution was biased by mutations of a few types (Poisson distribution goodness-of-fit, $\chi^2 = 0.713$, $p = 0.398$).

4. DISCUSSION

Theoretical predictions of the DFEs of new beneficial mutations depend on a number of assumptions. One of these is that the wild-type is well adapted to the prevailing conditions [3]. Such an assumption does not hold here; our findings are therefore not a test of existing theory [4–6]. Instead, because WS genotypes colonize a niche largely unavailable to the ancestral type, only beneficial mutations are viable. All deleterious or neutral mutations remain as non-viable as the ancestor. This means that the set of 100 WS described here are not only an unbiased

sample of beneficial mutations, but also provide an empirical glimpse of the shape of the overall DFE.

The need for theory applicable to situations where new beneficial mutations arise from a maladapted ancestral type is clear; but even in the absence of theory our detailed understanding of the genotype-to-phenotype map underpinning the evolution of WS genotypes ought to be sufficient to attempt an explanation for the observed normal distribution.

All known WS genotypes arise owing to overactivation of di-guanylate cyclases (DGCs) [16–20]. Despite the fact that the genome contains 39 DGCs [21], all single-step WS genotypes arise as a consequence of mutations in just three DGC-containing loci (*Wsp*, *Aws* and *Mws*), and most are contained in one of three genes (*wspF*, *awsX* or *mwsR*). Just why three out of many potential pathways are trodden by evolution is described in detail elsewhere [16], but this fact alone draws attention to a bias in the spectrum of WS variants delivered to selection. The very existence of such a bias suggests that the shape of the DBFE will be influenced by the genetic architecture underlying the trait(s) of interest; however, just why the realized DBFE should conform to a normal distribution is not clear. One possibility is that this distribution reflects the genetic architecture underpinning WS evolution.

Our finding that the shape of the DBFE is normal in both spatially structured and unstructured environments is curious. Two other studies have measured the fitness effects of bacterial genotypes collected while minimizing selection bias [9,10]. Both found that the fitness of all genotypes was approximately normally distributed when measured in an environment in which the ancestor had a fitness of zero. Although larger datasets covering a wider range of organisms are required, these observations hint at generalities for the DFE over different organisms and traits. This would open up the possibility for a more general model of adaptive walks than is currently available.

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