Model and test in a fungus of the probability that beneficial mutations survive drift

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Determining the probability of fixation of beneficial mutations is critically important for building predictive models of adaptive evolution. Despite considerable theoretical work, models of fixation probability have stood untested for nearly a century. However, recent advances in experimental and theoretical techniques permit the development of models with testable predictions. We developed a new model for the probability of surviving genetic drift, a major component of fixation probability, for novel beneficial mutations in the fungus Aspergillus nidulans, based on the life-history characteristics of its colony growth on a solid surface. We tested the model by measuring the probability of surviving drift in 11 adapted strains introduced into wild-type populations of different densities. We found that the probability of surviving drift increased with mutant invasion fitness, and decreased with wild-type density, as expected. The model accurately predicted the survival probability for the majority of mutants, yielding one of the first direct tests of the extinction probability of beneficial mutations.

1. Introduction

A fundamental theme of theoretical population genetics that has received little empirical attention is the fixation probability of a beneficial mutation ($p_{fix}$), the likelihood that the mutation will ultimately be present in every individual of the population. First addressed by Haldane, Fisher and Wright nearly 90 years ago, the best-known theoretical result is Haldane’s approximation for $p_{fix}$, $(1 - e^{-2Ns})/(1 - e^{-2Ns}) \approx 2s$, for populations of constant size undergoing weak selection [1–3]. Despite a sustained interest in theoretical models, strict model assumptions and an inability to track individual mutations have prevented empirical tests of their predictions. However, recent advances in theoretical and experimental techniques permit us now to develop models with predictions that can be tested directly [4].

The first component of $p_{fix}$ is determining the probability that novel beneficial mutations survive genetic drift, the random loss of mutants while rare. The processes driving genetic drift are specific to the organism and its growth environment, which must be incorporated into fixation models through a thorough understanding of life-history traits. Although most models (see [4]) use relative mutant fecundity as a predictor for surviving drift, recent work has shown that other measures of fitness may be more applicable (such as survival or generation time) [5,6]. We developed and tested a model for the probability of surviving drift for mutations arising in the fungus Aspergillus nidulans based on the life history of a radially growing colony [7], with mycelial growth rate [8] and initial frequency as model parameters. To test the model, we introduced individual mutant spores with a known fitness advantage into populations of...
wild-type spores of a different colour, and then visually determined the fate of the mutant. This approach let us separate loss due to drift into components with different biological bases. Our model accurately predicted the empirical probability of surviving drift; this is one of the first direct tests of any model of fixation probability.

2. Methods

(a) Experimental protocol

For growth media, we used minimal medium (MM) with pH 5.8, consisting of: NaNO₃ 6.0 g l⁻¹; KH₂PO₄ 1.5 g l⁻¹; MgSO₄ · 7H₂O 0.5 g l⁻¹; NaCl 0.5 g l⁻¹; agar 12 g l⁻¹ and 1 mg l⁻¹ each of FeSO₄, ZnSO₄, MnCl₂ and CuSO₄, and complete medium (CM), which is MM plus: tryptone 10 g l⁻¹; yeast extract 5 g l⁻¹ and vitamin solution (after autoclaving) [9]. After autoclaving, glucose was added to MM at 0.1 g l⁻¹ (0.01%) or to CM at 4 g l⁻¹ (0.4%).

We used 11 mutant strains of A. nidulans with beneficial mutations resulting from a long-term evolution experiment in which spore samples were transferred weekly to MM after approximately 50 nuclear generations. These were adapted from two ancestral strains with either green or yellow spores.

The growth rate of each strain in millimetre per hour was determined by measuring colony diameter after 5 days of growth on CM; these were used to obtain invasion fitness (see §2b for definition).

We estimated the probabilities of escaping the inoculum (pₑ) and forming a sector (pₛ) by inoculating an average of one mutant spore with either 100 or 1000 wild-type spores in a 5 µl droplet on CM. The number of mutant spores was Poisson distributed (λ = 1), which we took into account in tests of the model (see the electronic supplementary material, §3). Forty replicate populations were used per strain-treatment combination, totalling 880 populations. Colonies were photographed (Panasonic DMC-FZ8) after 3 days of growth at 37°C and storage at 4°C for between 1 and 7 days. We categorized colonies into four groups by the presence of mutant mycelium: ‘escaped inoculum (with sector)’, ‘escaped inoculum (without sector)’, ‘blocked in inoculum’ or ‘undetectable’ (figure 1). Proportion of colony area occupied by mutant mycelium was measured with ImageJ (v. 1.44) using ‘Area Fraction’ [10]. Data are available through Dryad (http://dx.doi.org/10.5061/dryad.0rc67).

(b) Modelling

Extinction probabilities are extremely sensitive to life-history and experimental details [4]. To match theory to experiment, we consider a wild-type fungus growing radially in a circular colony with radial growth rate w. The colony begins as an inoculum in the centre of the plate, containing Nᵢ wild-type spores. The inoculum also contains one mutant spore with growth rate m > w.

Since Nᵢ is large, wild-type 'sub-colonies' growing radially from individual spores are likely to physically trap the mutant sub-population within the inoculum. In the electronic supplementary material, we compute the probability that the wild-type hyphae will ultimately block the mutant sub-colony from reaching the inoculum boundary. Assuming that the mutant hyphae will take the 'shortest path' to the boundary, we derive a lower bound for the probability that the mutant escapes the inoculum:

\[ pₑ \approx \pi \left( \frac{\frac{f_i}{N_i}}{\frac{f_i}{N_i}} \right). \]

where fᵢ denotes the invasion fitness, a measure arising from the geometry of colony growth (see the electronic supplementary figure S1 and §S1):

\[ f_i = \sqrt{\frac{(m/w)}{(m/w)}} - 1. \]
of mutants escaping the inoculum droplet, the relative fitness is well-approximated by \( C_20 \). In the electronic supplementary material, we relax this assumption, yielding a less elegant but more accurate numerical estimate of \( p_w \).

We also developed a simulation approach in which a circular plate is modelled as a grid of small pixels. Initially, wild-type spores are distributed randomly across the inoculum area, and a single-mutant spore is added at a random position within the inoculum. Each spore grows radially as a sub-colony until it is surrounded by occupied pixels, at which point it is occluded and ceases growing.

In §3, we demonstrate that, when \( N_l = 1000 \), most mutant spores that escape the inoculum successfully form sectors. However, it is unclear whether a sector guarantees fixation in a serial transfer regime. Letting \( A \) denote the fractional area of the beneficial sector, we also model the serial transfer process in the electronic supplementary material and predict that whenever the product \( N_lA \) is sufficiently large (i.e. \( N_lA/(N_lA - 1) \approx 1 \)), \( p_w \) after serial transfer is well-approximated by \( p_w \).

3. Results

For the 11 mutants, the probability of sector formation \( (p_s) \) increased with invasion fitness \( (f_i) \) and decreased with wild-type frequency \( (N_l) \) (logistic regression: d.f. = 875, for both parameters: \( |z| > 3.6, p < 0.001 \); figure 2a). The probability of escaping the inoculum \( (p_e) \) also increased with \( f_i \) and decreased with \( N_l \) (logistic regression: d.f. = 875, for both parameters: \( |z| > 3.9, p < 0.001 \); figure 2c), as did average sector size (ANCOVA: model \( F_{2,118} = 10.2 \), adjusted \( R^2 = 0.13, p < 0.001 \); figure 2b).

Logically, \( p_e \) was smaller than \( p_f \) for both \( N_l = 100 \) (one-tailed paired t-test: \( t_{10} = 4.96, p < 0.0005 \)) and \( N_l = 1000 \) (one-tailed paired t-test: \( t_{10} = 2.28, p < 0.05 \)). However, for \( N_l = 1000 \), seven of 11 mutant strains had identical \( p_e \) and \( p_f \) and thus the difference was driven by four mutant strains which had large proportions of occluded sectors (see §4). The conditional probability of forming a sector, given that an escape had occurred \( (p_{w|e}) \), increased with \( N_l \), but the effect of \( f_i \) was conditional on \( N_l \): for \( N_l = 100 \), the relationship between \( f_i \) and \( p_{w|e} \) was positive; for \( N_l = 1000 \), it was negative (logistic regression: d.f. = 204, main effects \( N_l \) and \( f_i \), \( p < 0.05, p < 0.05 \); interaction effect \( z = -1.85, p = 0.06 \)).

Figure 2 also demonstrates that, for \( N_l = 100 \) spores/inoculum, \( p_e \) was well-approximated only by the simulation results (dotted line), as expected given the model assumptions that \( N_l \) is large and the mutant colonizes a straight path to the inoculum boundary. For \( N_l = 1000 \), the analytical and numerical models (dashed and solid lines, respectively) provided good estimates of \( p_e \) with four exceptions. Grey lines show the classical prediction for fixation probability in a haploid population of constant size \( N_l \) [11], corrected for the possibility of multiple mutant spores per inoculum (see the electronic supplementary material).

The product of initial wild-type frequency and average sector area, \( N_lA \), ranged from 9.22 to 13.3 for \( N_l = 100 \), and from 66.8 to 238 for \( N_l = 1000 \). Thus, \( N_lA/(N_lA - 1) \approx 1 \)

Note that for large-effect mutations \( (m \gg w) \), \( f_i \) is very close to the relative fitness \( f_i = m/w \), while for small mutational effects \( f_i \ll f_e \).

Figure 2. Both (a) the proportion of colonies with a mutant sector, \( p_s \), and (b) the average proportion of colony area made up by mutant mycelium, \( A \), increase with mutant invasion fitness, \( f_i = \sqrt{(m/w)^2 - 1} \) and decrease with \( N_l \) (black symbols, \( N_l = 100 \); grey symbols, \( N_l = 1000 \)). (c) For \( N_l = 100 \), the proportion of mutants escaping the inoculum droplet, \( p_e \) (circles), closely matches simulation results and exceeds \( p_s \) (triangles, from (a)). (d) For \( N_l = 1000 \), observed \( p_e \) (circles) closely matches modelled \( p_e \) and observed \( p_s \) (triangles, from (b)), barring four exceptional data points (see §4). Error bars are ±s.e.; invasion fitness has a s.e. ≤ 0.029 for all points (not shown). Dashed line, analytical model; solid line, numerical model; dotted line, simulation results; grey line, classical prediction [11].
when $N_i = 1000$, suggesting that $p_e$ is a good estimator of the ultimate fixation probability after serial transfer for these populations.

4. Discussion

Long-standing models for the probability of escaping genetic drift have remained untested owing to a mismatch between appropriate empirical data and model assumptions. We developed and tested a new model for the probability of surviving genetic drift for beneficial mutations in the fungus *A. nidulans* based on a thorough understanding of its life-history characteristics during colony expansion on a surface. Our model incorporated initial frequency and invasion fitness and accurately predicted the probability of surviving genetic drift for 11 beneficial mutants.

While genetic drift is typically characterized by the random sampling of alleles [4], in our system drift arises primarily from the random position of spores within the inoculum. If we consider inocula as ‘generations’ in a serial transfer regime, the random sampling of alleles from one generation to the next occurs largely through the random placement of spores in the inoculum, since spores that are close to the boundary have a greater chance of contributing to the next generation. Thus, our model captures the physical mechanism underpinning genetic drift. We also identified a second component of drift in our system, which blocked mutants after they had escaped the inoculum. Experimentally, we were able to observe the effect of $N_i$ and $f_i$ on these components independently, which led to the discovery of a surprising dynamic between $N_i$ and drift after escaping the inoculum. Although the overall probability of escaping drift decreased with $N_i$ as expected, the conditional probability of sector formation ($p_{\text{esc}}$) increased with $N_i$, meaning the chance of being blocked outside the inoculum was marginally greater for $N_i = 100$ than for $N_i = 1000$. This results from an increased number of ‘circuitous escapes’ permitted by the lower wild-type density, where the mutant does not take the shortest path to escape the inoculum. These mutants tend to form narrowed, irregularly shaped growing fronts (as in figure 1b) that presumably result from single hyphae and are thus more easily occluded by the wild-type.

The probability of a circuitous escape is also expected to increase with $f_i$, but this effect likely depends on $N_i$, i.e. there is a minimum $f_i$ that permits a circuitous escape for each $N_i$. This is a possible explanation for the increased $p_e$ we observed in four of the $N_i = 1000$ populations, and for the negative relationship between $f_i$ and $p_{\text{esc}}$ overall. These results suggest that the relationships between drift and fitness or initial frequency are not always straightforward—the spatial patterns of growth must also be considered.

Determining the probability of escaping genetic drift is the first component of a model for the ultimate fixation of beneficial mutations, $p_{\text{fix}}$. How much additional loss should be expected due to population bottlenecks if populations would be serially transferred until the mutant fixes or goes extinct? Our theoretical results suggest that $p_e$ is a good estimator of ultimate fixation probability for populations with large $N_iA$ (where $A$ is sector area). Furthermore, intuition from serial transfer experiments with this system suggests that visible sectors are unlikely to be lost due to bottlenecks, so that $p_e$ is likely to predict $p_{\text{fix}}$ when $N_i \approx p_e$. Populations from the $N_i = 1000$ satisfy both these requirements, suggesting that $p_e$ is a good estimate of $p_{\text{fix}}$ for these populations. Further work will measure fixation probability directly and test its predicted dependence on $p_e$ and $p_i$. Quantifying the relationship between the probability of fixation of genotypes and competitive fitness when common will aid our understanding of the variation in life-history traits among different organisms.

The classical prediction for $p_{\text{fix}}$ [11] yielded a surprisingly good fit to our data when $N_i = 100$; however, this prediction changes negligibly for $N_i = 1000$ and fits the data very poorly in this case. In contrast, our predictions vary sensitively with wild-type density, as do the experimental data. Although our model is specific to our experimental system, this work nevertheless demonstrates the predictive power of models of fixation probabilities that are tailored to the experimental system, and the power of current experimental techniques to examine the most fundamental assumptions of population genetics.

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References


