Genetics: Published Articles Ahead of Print, published on November 17, 2011 as 10.1534/genetics.111.132134

Stickbreaking: a novel fitness landscape model that harbors epistasis and is consistent with commonly observed patterns of adaptive evolution

Anna C. Nagel*, Paul Joyce[§,**], Holly A. Wichman*, Craig R. Miller[*,§,1]

* Department of Biological Sciences, University of Idaho, Moscow, Idaho 83843

§ Department of Mathematics, University of Idaho, Moscow, Idaho 83843

** Department of Statistics, University of Idaho, Moscow, Idaho 83843

[1] Corresponding author: crmiller@uidaho.edu

Running Title: Stickbreaking Model of Epistasis

Keywords: epistasis, experimental evolution, adaptive evolution, additive model, multiplicative model

Corresponding Author:

Craig R. Miller

Department of Mathematics

300 Brink Hall

PO Box 441103

Moscow, ID USA 83844-1103

email: crmiller@uidaho.edu

fax: (208) 885-5843

phone: (208) 691-2469

ABSTRACT

In relating genotypes to fitness, models of adaptation need to be both computationally tractable and to qualitatively match observed data. One reason tractability is not a trivial problem comes from a combinatoric problem whereby no matter in what order a set of mutations occurs, it must yield the same fitness. We refer to this as the *bookkeeping problem*. Because of their commutative property, the simple additive and multiplicative models naturally solve the bookkeeping problem. However, the fitness trajectories and epistatic patterns they predict are inconsistent with the patterns commonly observed in experimental evolution. This motivates us to propose a new and equally simple model that we call *stickbreaking*. Under the stickbreaking model, the intrinsic fitness effects of mutations scale by the distance of the current background to a hypothesized boundary. We use simulations and theoretical analyses to explore the basic properties of the stickbreaking model such as fitness trajectories, the distribution of fitness achieved, and epistasis. Stickbreaking is compared to the additive and multiplicative models. We conclude the stickbreaking model is qualitatively consistent with several commonly observed patterns of adaptive evolution.

INTRODUCTION

ADAPTIVE evolution is challenging to understand because it depends on a rich array of biological properties. Among those receiving recent theoretical and experimental attention are the magnitude and distribution of mutational fitness effects, the length of adaptive walks, the rate of fitness increase and the population dynamics that drive it (e.g., BARRETT *et al.* 2006; BETANCOURT 2009; COWPERTHWAITE *et al.* 2005; EYRE-WALKER and KEIGHTLEY 2007; ORR 2002; SCHOUSTRA *et al.* 2009; ORR 2003; ROKYTA

et al. 2008; Rozen *et al.* 2002; Barrick *et al.* 2009; Burch and Chao 1999; Kryazhimskiy *et al.* 2009; de Visser *et al.* 1999; Gerrish and Lenski 1998; Desai *et al.* 2007; Joyce *et al.* 2008). Equally important are epistasis, pleiotropy, parallelism, mutation order, and the number of beneficial mutations available (e.g., Holder and Bull 2001; Kim and Orr 2005; Rokyta *et al.* 2009; Silander *et al.* 2007; Wichman *et al.* 1999, 2005; Miller *et al.* 2011; Khan *et al.* 2011; Chou *et al.* 2011; Kvitek and Sherlock 2011; Rokyta *et al.* 2011; Weinreich *et al.* 2006). Notice that the latter features of adaptation are more meaningful when the identities of the mutations are known and when we consider adaptation as a process subject to replication. For example, epistasis occurs when specific mutations have different effects on different genetic backgrounds (Bonhoeffer *et al.* 2004; Sanjuán *et al.* 2004). The rise of genomic sequencing technologies is having a dramatic effect on the ability of researchers to know the identity of mutations occurring during adaptation.

Knowing the identities of adaptive mutations expands the types of questions that can be addressed, but also creates new challenges. All models of adaptation must assign fitness values to genotypes that have arisen through mutation. In connecting genotype and fitness, a model must have the following property: if the wildtype background acquires mutations A_1, A_2, and A_3 to yield a genotype with fitness $w_{1,2,3}$, every possible order of these mutations must also result in fitness $w_{1,2,3}$. As the number of fixed mutations grows, the number of possible pathways grows in a factorial manner. We call this consistency requirement the *bookkeeping problem*.

At least two groups of population genetic models address the bookkeeping problem; one maps genotype change (i.e., mutation) directly onto fitness (*GF* models), and the second maps genotype onto phenotype, and then phenotype onto fitness (*GPF* models). Here we focus on the simpler GF models. Among these, the *additive model* assumes mutations have an additive effect on fitness. To be more precise, the fitness after mutations

A_1 and A_2 occur on the wildtype background is $w_{1,2} = w_{wt} + \Delta w_1 + \Delta w_2$ where Δw_1 and Δw_2 are the intrinsic effects expressed as fitness differences of mutations A_1 and A_2. The bookkeeping problem is solved by the commutative property of addition (i.e., $\Delta w_1 + \Delta w_2 = \Delta w_2 + \Delta w_1$). Under the *multiplicative model*, the intrinsic effects are selection coefficients affecting fitness in a multiplicative fashion: $w_{1,2} = w_{wt}(1+s_1)(1+s_2)$ where s_1 and s_2 are the intrinsic effects of mutations A_1 and A_2. Multiplication also has the same commutative property [i.e., $(1 + s_1)(1 + s_2) = (1 + s_2)(1 + s_1)$] and thus solves the bookkeeping problem. Both of these solutions to the bookkeeping problem are simple to simulate and test on real data.

Another solution, implicit in the *uncorrelated landscape model* (Gillespie 1991; Joyce *et al.* 2008; Orr 2002), is to assume that the set of mutations arising in an adaptive walk can arise in only one order because each mutation is beneficial on exactly one background. This occurs because the probability of a mutation being beneficial on more than one highly fit background is small enough to be ignored. Thus under the uncorrelated model, once replicate adaptive walks depart from each other, they are 100% divergent. Since the bookkeeping problem involves convergence, the bookkeeping problem is avoided. However, the uncorrelated model makes the extreme prediction for real data that no mutation will be beneficial on two different backgrounds.

Another set of models that avoids the bookkeeping problem are those that assume the number of beneficial mutations on any background is effectively infinite. Under this assumption, the probability of convergent evolution is zero and the bookkeeping problem does not arise. Examples of models that make this assumption include Kryazhimskiy *et al.* 2009, Gerrish and Lenski 1998, Rozen *et al.* 2002, Desai *et al.* 2007.

The *NK model* (Kauffman 1993) is unusual among GF models in that it can produce landscapes with intermediate levels of epistasis. In the NK model, N is the number of sites and K is the number of other sites each site interacts with. When $K = 0$, it is

the additive model and when $K = N - 1$ it is equivalent to the uncorrelated model. When $0 < K < N - 1$, the interaction terms mean that the mutational effects are no longer background independent. The interactions bring more biological realism and allow richer patterns of epistasis, but at the expense of model simplicity. Simulating data when $0 < K < N - 1$, while ensuring the bookkeeping criteria is met, is computationally challenging because it requires assigning fitnesses to the entire fitness landscape. The interactions also pose a problem for analyzing real data because they introduce a large number of parameters that must be estimated.

Kryazhimskiy *et al.* 2009 has also developed a flexible GF modeling framework where the uncorrelated and additive models arise as special cases. These models allow different types of epistasis and decelerating fitness trajectories to be produced. However, because the fitness of beneficial mutations in such models depends only on the current fitness, they do not solve the bookkeeping problem.

Thus there is an array of GF models. Among those that offer simple solutions to the bookkeeping problem (additive, multiplicative, uncorrelated), they generally fail to predict several commonly observed properties of real adaptation. Specifically, in laboratory adaptations parallel evolution is not uncommon, most fitness gain occurs early in a walk, and epistasis is common (Bull *et al.* 1997; Rokyta *et al.* 2009; Wichman *et al.* 1999, 2005; Barrick *et al.* 2009; Lenski and Travisano 1994; Cooper and Lenski 2000; Woods *et al.* 2006; Barrick *et al.* 2009; Betancourt 2009; Cowperthwaite *et al.* 2005; Chou *et al.* 2011; Khan *et al.* 2011; Burch *et al.* 2003; Elena and Lenski 1997; Sanjuán *et al.* 2004; Rokyta *et al.* 2011).

This leads us to propose a novel GF model for combining mutational effects that we call stickbreaking. The *stickbreaking model* is premised on the familiar idea that mutations have intrinsic effects. But rather than assuming fitness differences are background independent (like the additive model), or that differences scale by background fitness

(like the multiplicative model), differences in the stickbreaking model scale by how near the current background is to a hypothesized upper fitness boundary. For example, if mutation A_1 has stickbreaking coefficient u_1 and the fitness distance from the wildtype to the boundary is d, then the mutation will increase fitness by the amount du_1 (Figure 1). We use theory and simulations to show that stickbreaking both solves the book-keeping problem and produces some qualitative features commonly observed in adaptive evolution.

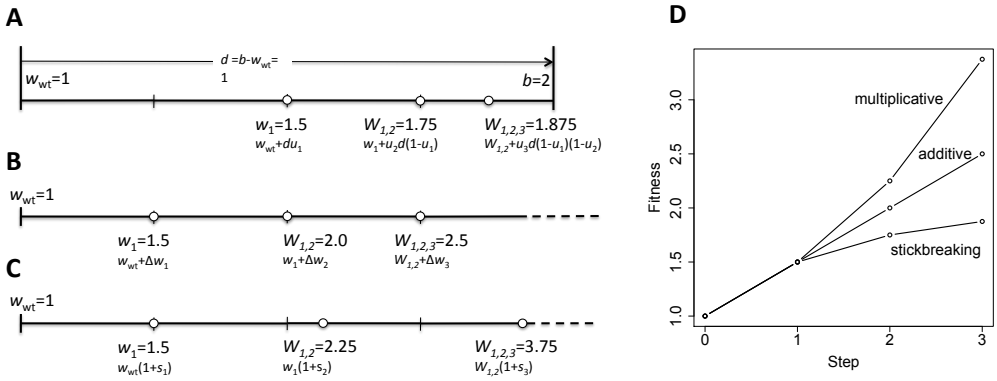

FIGURE 1 Simple comparison of (A) stickbreaking, (B) additive, and (C) multiplicative models when all three begin with the same initial fitness ($w_{wt} = 1$) and fix three mutations of the same intrinsic effect (0.5) (i.e., $u_1 = u_2 = u_3 = \Delta w_1 = \Delta w_2 = \Delta w_3 = s_1 = s_2 = s_3 = 0.5$). In (A-C), the bold horizontal lines are fitness and the circles are genotypes with the first, second, and third mutations to fix. In the stickbreaking model (A), each mutation closes the distance from the current fitness to the boundary by the mutation's intrinsic effect. Panel (D) displays the same information as (A-C) by plotting step vs. fitness, and better illustrates how stickbreaking produces diminishing effects and a decelerating fitness trajectory.

Stickbreaking: We begin by introducing the stickbreaking model and compare it to the additive and multiplicative GF models. Suppose the maximum fitness achievable in the current environment is w_{max} while the current fitness is w_{wt}. Let $d = w_{max} - w_{wt}$ be the maximum possible fitness gain through adaptation. Let u_i be the stickbreaking coefficient of A_i such that its fitness on the wildtype background, w_i, is given by $w_i = w_{wt} + du_i$ where $u_i \leq 1$. In the stickbreaking model, stickbreaking coefficients are assumed to be background independent. If a second mutation, A_j, with stickbreaking coefficient u_j occurs on the A_i background, its fitness is given by, $w_{i,j} = w_{wt} + d(u_1 + u_2(1-u_1))$. To see why, notice that after the first mutation fixes, the remaining distance to the boundary is $d(1-u_i)$ and the second mutation therefore increases the fitness by $u_j d(1-u_i)$. Adding this increase to the fitness of the first mutation, $w_{wt} + du_i + u_j d(1-u_i)$, and simplifying gives $w_{wt} + d(u_1 + u_2(1-u_1))$. But since $u_1 + u_2(1-u_1) = 1 - (1-u_1)(1-u_2)$, we can rewrite the fitness of the double mutant as $w_{i,j} = w_{wt} + d(1-(1-u_i)(1-u_j))$. In general, if m mutations with identities $A_1, A_2, ..., A_m$ and stickbreaking coefficients $u_1, u_2, ..., u_m$ accumulate on the wildtype background, the fitness is given by,

$$w_{1,2,\cdots,m} = w_{wt} + d\left(1 - \prod_{i=1}^{m}(1-u_i)\right). \tag{1}$$

The intrinsic effect of each mutation A_i thus closes the distance between the current background and the fitness limit by a proportion u_i. This process is analogous to a stickbreaking exercise. With a stick of length d laid along a number line, the first mutation dictates where, in a fractional sense, it is broken. Setting the left portion of the stick aside, the next mutation determines where the remaining right portion is broken. The process continues with subsequent mutations breaking the remaining right portion into ever smaller pieces. Unless a stickbreaking coefficient of 1 is available, fitness

will never actually reach the fitness maximum.

The stickbreaking model solves the bookkeeping problem because, as equation (1) shows, the final fitness depends on the *product* of intrinsic effects and is therefore order independent. Notice mutations with intrinsic effects between 0 and 1 are beneficial. It is less obvious that intrinsic effects may be zero or negative, representing neutral and deleterious mutations respectively. We also note that the stickbreaking metaphor appears in other modeling contexts, for example, to describe niche partitioning and species abundance in ecology (PATIL and TAILLIE 1977; MACARTHUR 1957) and in population genetics to derive the distribution of age-ordered alleles under the infinite alleles model (DONNELLY and JOYCE 1989). To our knowledge, stickbreaking has not previously been applied to the subject of adaptive evolution.

Stickbreaking compared to additive and multiplicative models: Because of the mathematical similarities between the stickbreaking, additive, and multiplicative models, it is possible to assess when they yield similar results and when they do not. Fitness effects are expressed as fitness differences (Δw) in the additive model, selection coefficients (s) in the multiplicative model, and stickbreaking coefficients (u) in the stickbreaking model. In each case, the model's respective fitness effects are assumed to be background independent. More precisely, if b is the the genetic background and i is the arising mutation, then $\Delta w_{i|b} = w_{i,b} - w_b$, $s_{i|b} = (w_{i,b} - w_b)/w_b$, and $u_{i|b} = (w_{i,b} - w_b)/(w_{max} - w_b)$.

Under the additive model, the fitness after $A_1, A_2, ..., A_m$ mutations with fitness differences $\Delta w_1, \Delta w_2, \cdots, \Delta w_m$ have accumulated on the wildtype background is,

$$w_{1,2,\cdots,m} = w_{wt} + \sum_{i=1}^{m} \Delta w_i. \tag{2}$$

Under the multiplicative model, the fitness after m mutations with selection coefficients $s_1, s_2, \cdots, s_m$ have accumulated is given by,

$$w_{1,2,\cdots,m} = w_{wt} \prod_{i=1}^{m} (1 + s_i). \tag{3}$$

The stickbreaking, additive, and multiplicative models converge to the same model when effect sizes are small and walks are not too long. This occurs when the product of effect size and walk length is small. Note if the product in equation (3) is expanded and all higher order terms are assumed to be zero, then fitness under the multiplicative model is approximated by a sum,

$$w_{1,2,\cdots,m} = w_{wt} \prod_{i=1}^{m} (1 + s_i) \approx w_{wt} \left(1 + \sum_{i=1}^{m} s_i \right). \tag{4}$$

Similarly, if equation (1) is expanded and higher order terms ignored, then fitness under stickbreaking is also approximated by a sum,

$$w_{1,2,\cdots,m} = w_{wt} + d \left(1 - \prod_{i=1}^{m} (1 - u_i) \right) \approx w_{wt} + d \sum_{i=1}^{m} u_i. \tag{5}$$

Combining equations (4), (5), and (2) gives,

$$w_{wt} \left(1 + \sum_{i=1}^{m} s_i \right) \approx w_{wt} + d \sum_{i=1}^{m} u_i \approx w_{wt} + \sum_{i=1}^{m} \Delta w_i. \tag{6}$$

If fitness effects are small and walks not long, it implies that $w_{wt} s_i \approx d u_i \approx \Delta w_i$.

Definitions of fitness: Before continuing, it is important to clarify our approach to defining fitness. We have and continue to denote fitness in a generic sense as w. Fitness is more precisely defined in two ways that we call *Darwinian* and *Malthusian* fitness. *Darwinian fitness* is λ in a discrete population growth model, $N_t = N_0 \lambda^t$, where N_0 and

N_t are the population sizes at time 0 and time t. *Malthusian fitness* is r in the continuous growth model, $N_t = N_0 e^{rt}$. One can be easily transformed to the other by $\lambda = e^r$, or $\ln(\lambda) = r$. They can also be defined in relative terms where the change in frequency of a mutant to a reference type gives the ratio of growth rates (HARTL and CLARK 1997); their meaning and log relationship is the same.

In this paper, the definition of fitness is important when we consider (i) how fitnesses arise during an adaptive walk and (ii) what type of fitness is measured when a walk is 'observed'. In modeling walks (i), we maintain generality by considering mutations acting in an additive, multiplicative, or stickbreaking manner on either Darwinian or Malthusian fitness. This yields six combinations. Note, because multiplicative effects on λ and additive effects on r are equivalent, there are actually five different models. For clarity, however, we describe them as a set of six models. After an adaptive walk occurs, we imagine measuring fitness (ii). Throughout this paper we measure Malthusian, but not Darwinian, fitness to simplify our results and because Malthusian fitness is the predominant definition used in the experimental evolution literature.

Fitness trajectories: The predicted fitness under the additive, multiplicative, and stickbreaking models after m steps can be approximated if we assume the pool of beneficial mutations (M) is large enough that sampling is effectively done with replacement (i.e., $M \gg m$). Then, under strong selection, weak mutation conditions (SSWM), the expected effect of a mutation that arises, escapes drift, and sweeps to fixation is given by $\nu = \sum_{i=1}^{M} x_i^2 / \sum_{j=1}^{M} x_j$ (GILLESPIE 1991) where x_i represents the intrinsic effect under either of the three models (i.e. Δw_i, s_i, or u_i). We therefore replace Δw_i, s_i, and u_i in equations 1, 2, and 3 with ν. Note that when mutations affect λ, but we measure r, a log transformation is necessary. These approximations as well as model abbreviations

are given in Table 1.

TABLE 1

Expected fitness after m steps given the model generating fitness effects

Model	Model Abbreviation	Expected fitness (r)
Additive on λ	**add on λ**	$\ln(\lambda_{wt} + m\nu)$
Multiplicative on λ	**mult on λ**	$\ln(\lambda_{wt}) + m\ln(1+\nu)$
Stickbreaking on λ	**stick on λ**	$\ln[\lambda_{wt} + d(1-(1-\nu)^m)]$
Additive on r	**add on r**	$r_{wt} + m\nu$
Multiplicative on r	**mult on r**	$r_{wt}(1+\nu)^m$
Stickbreaking on r	**stick on r**	$r_{wt} + d(1-(1-\nu)^m)$

Fitness is measured on the Malthusian, r, scale. Expected fitness is obtained by replacing mutational effects in equations (1)-(3) with the mean intrinsic effect of a fixing mutation, ν. The second column gives model abbreviations used throughout the paper.

Distributions of fitness during replicate walks: We would like to know the distribution of fitness achieved at step m when the total number of beneficial mutations available is M under each of the three models. Notice this differs from the familiar distribution of fitness effects and the distribution of fitnesses across the landscape; rather, it is the distribution of fitness achieved among replicate walks after m steps when all walks begin at the same genotype. The details of this derivation are provided in the APPENDIX. Denote the intrinsic effect of mutation i as x_i, where $x_i = \Delta w_i$, $x_i = s_i$, $x_i = u_i$ under the

three models. Assume the x_i values are drawn from a distribution and replicate walks occur using this fixed set of mutations (i.e. on a fixed landscape). Let Y_j be the intrinsic effect of the mutation that fixes at step j. Note that s_i and u_i differ from Δw_i by a scaling factor which cancels when calculating the scale free quantity Y_j. If M is large and m is an order of magnitude smaller, such that as both $M \to \infty$ and $m \to \infty$, $m \ln(M)/M \to 0$, then $Y_1, Y_2, \cdots, Y_m$ will be approximately independent and identically distributed with $P(Y_j = x_i) = x_i M/\bar{x}$ for $j = 1, 2, \cdots, m$. Based on the central limit theorem, this implies that the distribution of $\sum_{j=1}^{m} Y_j$ will be approximately normal, $\prod_{j=1}^{m}(1+Y_j)$ will be approximately log normal, and $1 - \prod_{j=1}^{m}(1-Y_j)$ will be approximately negative log normal. Thus, when M is large and m is small, but not extremely small (i.e., when the pool of beneficial mutations is large and the number to have fixed is moderately small), fitness of replicate walks under the additive, multiplicative, and stickbreaking models follow the normal, log normal, and negative log normal distributions with density functions and parameter values provided in Appendix. These limiting distributions can be obtained as a function of time, not mutational step, using a scale transformation.

Epistasis: Epistasis occurs when a mutation has different fitness effects in different genetic backgrounds. One way to measure epistasis is therefore to assess the fitness effect of a mutation across different backgrounds. A second way to examine epistasis is as a deviation of observation from prediction: (i) measure the fitness effects of two or more mutations on the same genetic background, (ii) predict their combined fitness effect under an assumed model based on their individual effects, (iii) measure their combined fitness effect, and (iv) define epistasis as the disparity between predicted (ii) and observed (iii). The first approach is more intuitive, the latter is more commonly used in the literature as a measure of epistasis. We pursue both here.

Epistasis as different effects of the same mutation across backgrounds: For any mutation, we specifically wish to know how its fitness effects change across the steps of a walk beginning with the wildtype and continuing until the mutation actually fixed. Following convention, we define fitness effects as differences in r. As above, we consider data arising under each of six models. We assume the pool of beneficial mutations is large and SSWM conditions operate such that the expected fitness effect of a mutation at each step is given by ν. An adaptive walk of length $m-1$ occurs. If we imagine a mutation of average (fixed) effect, ν, is then inserted (i.e. genetically engineered) as the m^{th} mutation on the $m-1$ background, the expected value of Δr that results is contained in Table 2.

Table 2

Expected fitness effects for a mutation fixing after $m-1$ steps

Model	**Expected fitness effect (Δr)**
Additive on λ	$\ln[(\lambda_{wt}+m\nu)/(\lambda_{wt}+m\nu-\nu)]$
Multiplicative on λ	$\ln(1+\nu)$
Stickbreaking on λ	$\ln[(\lambda_{wt}+d-d(1-\nu)^m)/(\lambda_{wt}+d-d(1-\nu)^{m-1})]$
Additive on r	ν
Multiplicative on r	$r_{wt}\nu(1+\nu)^{m-1}$
Stickbreaking on r	$\nu d(1-\nu)^{m-1}$

Left column gives the model under which data arise. Fitness effects (right column) are defined as the expected fitness differences in r as a consequence of the m^{th} mutation.

Epistasis as departure of observed from predicted effects of combined mutations: An alternative way to measure epistasis is as a departure of observation from prediction: $\epsilon = r_{obs} - r_{pred}$. Predicted values are based on additivity on r while observed data arise according to one of the six models. We are interested in how the disparity between observed and predicted fitness depends on the model under which fitness effects arise and the number of mutations considered, m. Again, we assume SSWM conditions and a large pool of beneficial mutations such that the expected effect of a randomly fixing mutation is ν. Table 3 gives the expected values for ϵ for each of the six models.

Table 3

Expected deviations from additivity on r (ϵ)

Model	**Expected ϵ**
Additive on λ	$\ln(\lambda_{wt} + m\nu) - n\ln(1 + \nu/\lambda_{wt})$
Multiplicative on λ	**0**
Stickbreaking on λ	$\ln[\lambda_{wt} + d - d(1-\nu)^m] - r_{wt} - m\ln(1 + d\nu/\lambda_{wt})$
Additive on r	**0**
Multiplicative on r	$r_{wt}(1+\nu)^m - r_{wt}(1 + m\nu)$
Stickbreaking on r	$d - d(1-\nu)^m - md\nu$

ϵ is defined as $r_{obs} - r_{pred}$. Left column gives the model under which data arise while the right column gives the expected value of ϵ as a function of the number of fixed mutations (m) and the mean intrinsic effect of mutations that fix (ν).

SIMULATIONS

Overview: Simulations written in R (R Development Core Team 2009) were used to study the patterns of fitness trajectory, distribution of fitness effects, and epistasis and to compare these to the theoretical results derived above. All simulations were done in the following basic framework. First, we assumed SSWM dynamics (Gillespie 1991) such that the population is described by a procession of fixed beneficial mutations. Second, a fitness landscape was defined by a relatively small number of beneficial mutations ($M = 50$) with fitness effects, x, randomly drawn from a distribution. Neither the pool of mutations nor their inherent effects change as adaptive walks proceed. Third, the time until the next mutation fixed was simulated by drawing random exponential waiting times for all $M - m$ available mutations with rate $N\mu_b\pi(s_i)$ where N was set at 10^5, the per site per generation beneficial mutation rate, μ_b, was set to 2×10^{-7}, and the fixation probability for mutation A_i, $\pi(s_i)$, is given by $(1 - e^{-2s_i})/(1 - e^{-2s_iN})$ (Kimura 1962) where s_i is the selection coefficient of A_i as traditionally defined (i.e. fractional changes in λ or differences in r; Chevin 2010). The mutation that fixed was that with the shortest waiting time.

In conducting simulations, we had to decide whether to conduct replicate walks on one landscape or single walks on replicate landscapes. In other words, should we average over replicate walks or replicate landscapes? We argue that conducting replicate walks on the same landscape is more analogous to experimental evolution where these models may ultimately be tested empirically. Consequently, we simulated a single landscape and ran 1000 replicate walks on this landscape, collecting and summarizing relevant information. We then repeated this entire process over several landscapes and confirmed that the observed qualitative patterns that are our focus here do not depend on the particular landscape (results not shown). To generate a landscape, 50 beneficial mutations were

drawn from the positive region of a negative log normal (APPENDIX). If $X \sim$ Normal (μ, σ), then $1 - e^X$ is a sample from the negative log normal. Parameters for the negative log normal ($\mu = 0.75, \sigma = 0.6$) were choosen so that 10% of the probability is positive (Figure 2). This distribution was used because it produces values ≤ 1 as required by the stickbreaking model while also being consistent with the additive and multiplicative models. Once generated, we used this single set of 50 values to simulate replicate walks under the six models: additive, multiplicative, and stickbreaking affecting Darwinian or Malthusian fitness. For all models the initial fitness was set at 1, and for both stickbreaking models, the fitness boundary was set at 2 such that $d = 1$. Walks were simulated until all 50 beneficial mutations fixed.

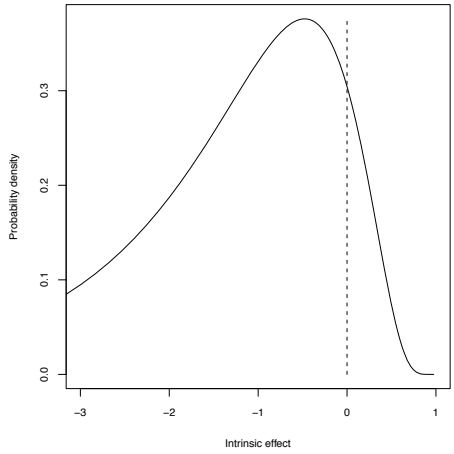

FIGURE 2 Negative log normal distribution from which effects are drawn. All mutations are drawn from 10% of the distribution > 0 (indicated by dashed line).

Analysis of simulations: Three analyses of simulated data were conducted. First, we compared mean fitness trajectory for each of the six models. Because final fitness differs dramatically between models, trajectories were rescaled for every simulated walk to range from zero to one. Second, to assess the distribution of fitness, we sampled

fitness for each of the 1000 walks at steps 5, 10, 20, and 30 and generated histograms from the results. Third, epistasis was measured in the same two ways we quantify it in the theory section above: (i) as fitness effects and (ii) as departure from additivity on r. In approach (i), we took a mutation that arose later in a walk, simulated engineering it into each of the preceding backgrounds, and measured its resulting fitness effect. We arbitrarily used the mutation fixing 10^{th} and we defined *fitness effect* as the difference in r. In the latter approach (ii), we compared observed fitness with predicted fitness on the r scale. For each simulated walk, we considered the first m mutations that fixed for $m = 2, 3, \cdots 10$. We then imagine measuring the effect of each of these m mutations on the wildtype background (i.e., as first-step mutations) yielding $\Delta r_{1|wt}, \Delta r_{2|wt}, \cdots, \Delta r_{m|wt}$. Under the additive model, the predicted fitness when all m mutations are combined is just, $r_{1,2,\cdots,m(\text{pred})} = r_{wt} + \Delta r_{1|wt} + \Delta r_{2|wt} + \cdots \Delta r_{m|wt}$. Epistasis, as a function of the number of mutations, is then $\epsilon_m = r_{1,2,\cdots,m(\text{obs})} - r_{1,2,\cdots,m(\text{pred})}$.

RESULTS AND DISCUSSION

Our objective in this work is to propose and explore a new model of combining mutational effects which we call stickbreaking. Stickbreaking is premised on the idea that, in the current environment and on short evolutionary timescales, there is a fitness boundary imposed by the laws of biochemistry and by restrictions on how radically the architecture of the genome can be altered by mutation. This limits how dramatically phenotype can be changed over a short evolutionary time span. Within the scope of available phenotypes, the optimal one corresponds to the fitness boundary. For example, if a set of mutations affect the rate a virus attaches to its host, the accumulation of many such mutations will not indefinitely push the attachment rate higher; rather a

boundary on attachment and therefore fitness will be imposed by the kinetics of collisions of objects in random motion. Such boundaries help provide a basic rationale behind the stickbreaking model.

Stickbreaking may also arise when organisms are moderately redundant such that they may solve a given problem multiple ways. Once substantial progress is made toward one solution (through mutation), pursuing alternative solutions to the same problem may be beneficial, but not nearly as much as the first. In the attachment example above, we might imagine multiple residues where binding can occur to the host; a virus that attaches poorly requires a mutation at only one of these residues to dramatically increase attachment. Subsequent mutations offering alternative ways to bind will provide diminshing beneficial effects. Conversely, when an organism is very near the optimal fitness because it has found several, semi-redundant solutions to a problem, a deleterious mutation that disrupts one solution will have a relatively small negative effect on fitness. It is also noteworthy that patterns qualitatively similar to stickbreaking can emerge from metabolic control theory (Kacser and Burns 1981). When a mutation changes the activity of an enzyme in a pathway, its effect on the pathway's flux is smaller than on the enzyme itself and it diminishes the nearer the pathway is to the maximum flux.

In stickbreaking, these biological assumptions of a boundary and diminishing effects are translated mathematically by allowing mutations to further and further subdivide the distance to the boundary in a multiplicative manner (equation 1, Figure 1). Because it involves a product, stickbreaking has the commutative property and, like the additive and multiplicative models, thereby solves the bookkeeping problem. However, this process of subdivision leads to different walk properties than those models.

Fitness trajectory: Different models lead to dramatically different trajectories of

fitness as a function of mutational step over an adaptive walk (Figure 3A). When mutations affect r, the trajectories for the additive, multiplicative, and stickbreaking models are approximately linear, exponential, and rapidly decelerating respectively. When mutations instead affect λ, the trajectories are shifted: additive becomes modestly decelerating, multiplicative becomes approximately linear, and deceleration under stickbreaking becomes very slightly exaggerated. Notice that the theoretical expectations from Table 1 (grey lines) in panel A are qualitatively correct; the disparities between them and the simulations (black lines) reflects the limited pool of beneficial mutations and the biased nature in which selection fixes mutations.

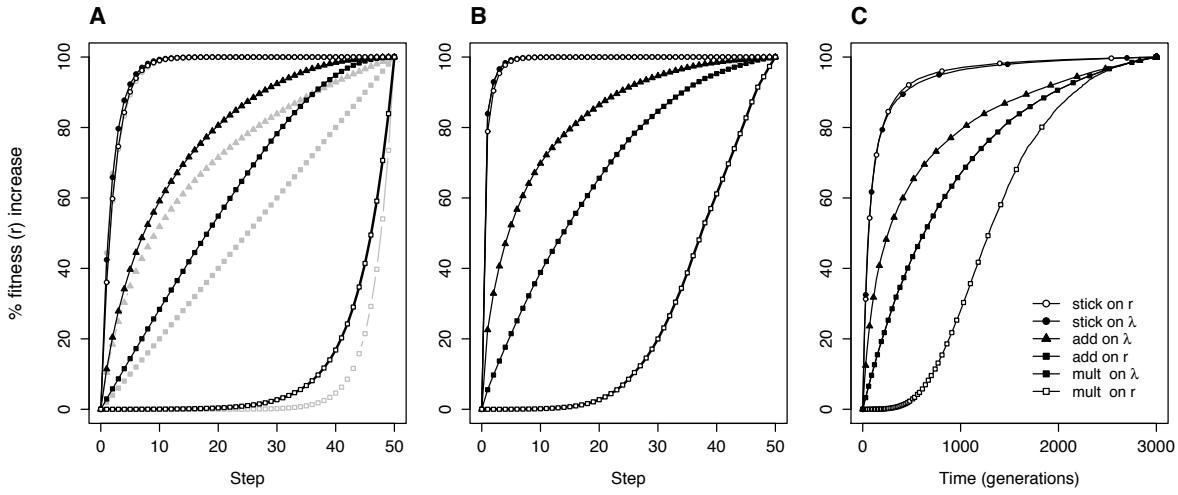

FIGURE 3 Mean fitness trajectory scaled as % of final fitness achieved under the six models indicated in inset legend in panel C (see Table 1 for model abbreviations). Symbols on trajectories identify model and denote mutations. Note 'add on r' and 'mult on λ' are the same model with same trajectory. (A) Mean fitness trajectories by mutational step based on simulations (black lines) and theoretical predictions from Table 1 that assumes a constant mean effect for all mutations (grey lines). SSWM conditions assumed. (B) Simulated trajectories under extreme clonal interference where mutations fix in order of descending effect size. (C) Same fitness trajectories as in panel A except plotted by time, not step, for the first 3000 generations.

A survey of the experimental evolution literature indicates that, in most cases, the

observed fitness trajectory decelerates as adaptation proceeds. This result has been observed in *Escherichia coli* (Lenski and Travisano 1994; Barrick *et al.* 2009; de Visser *et al.* 1999), the DNA bacteriophages ϕX174 and G4 (Holder and Bull 2001; Bull *et al.* 1997; Wichman *et al.* 1999), RNA bacteriophage (Burch and Chao 1999; Betancourt 2009), and the animal RNA virus, VSV (Elena *et al.* 1998). The exceptions we are aware of are approximately linear trajectories in *Saccharomyces cerevisiae* (Desai *et al.* 2007) and in one study on VSV (Novella *et al.* 1995). Of the models considered here, both stickbreaking models show rapidly decelerating trajectories and the additive model on λ shows a moderately decelerating trajectory.

This suggests that one of these three models is likely nearer the truth than the model most commonly assumed in the literature, additivity on r (multiplicative on λ) with its approximately linear trajectory. There are at least two reasons to be somewhat cautious regarding this conclusion. First, our results are based on SSWM dynamics while many experimental and real world systems involve interference dynamics with more than one mutation contending simultaneously. Under interference dynamics, selection is more efficient at fixing bigger effect mutations early in a walk compared to SSWM conditions (Rozen *et al.* 2002; Barrett *et al.* 2006). We can obtain a bound on this effect by assuming the pool of contending mutations is the entire pool of beneficial mutations and selection therefore fixes them in descending order from the largest to the smallest. Panel B in Figure 3 shows this trajectory. As expected, interference shifts all the trajectories toward a decelerating pattern although the effect is modest.

Second, trajectories are affected by whether fitness is considered a function of mutational step (as we have done thus far) or time. Plotting fitness against time instead of step bends most of the trajectories toward a more concave, decelerating shape (Figure 3C). Under all models, there is a tendency to fix mutations from larger to smaller intrinsic effect. When all else is equal, this leads to selection coefficients (as tradition-

ally defined, see SIMULATIONS) tending from large to small and, therefore, for waiting times between fixation events tending from short to long. In the 'add on r' model, this is the only effect, and the trajectory decelerates moderately. In the 'add on λ' model there is also the effect that as fitness grows in an additive way, the proportional effect of each added mutation (the selection coefficient) becomes smaller. The stickbreaking models are most dramatically affected by the time scale because as they approach their boundary, selection coefficients become very small and waiting times very long. At the other extreme lies the 'mult on r' model where selection coefficients actually get larger as the walk proceeds causing the walk to accelerate in time for most of its duration. We leave a statistical treatment of trajectory data for later work and here emphasize three things: (i) most of the models show decelerating trajectories, (ii) the slow down is exaggerated both by clonal interference and by using time rather than step as the explanatory variable, and (iii) with or without these influences, the stickbreaking models show much more dramatic decelerating effects than the other models.

Distribution of fitness over replicate walks: When mutations affect Malthusian fitness, r, and fitness is measured as r, the theoretical distributions from replicate walks (APPENDIX) are log normal, normal, and negative log normal for the additive, multiplicative, and stickbreaking models (solid lines in Figure 4 A-C). When mutations affect λ instead, these qualitative patterns are only slightly changed with heavier left tails (Figure 4 D-E). These predictions are based on asymptotic assumptions that (i) the total number of beneficial mutations, M, is large, (ii) the step where fitness is measured is far smaller than the number of beneficial mutations, $m \ll M$, and (iii) m is large enough for the law of large numbers to apply. In reality, M will often be modest (e.g. $10 < M < 100$) and m may be relatively small (e.g. ≤ 30). The simulations shed light

on what effect violating these assumptions has.

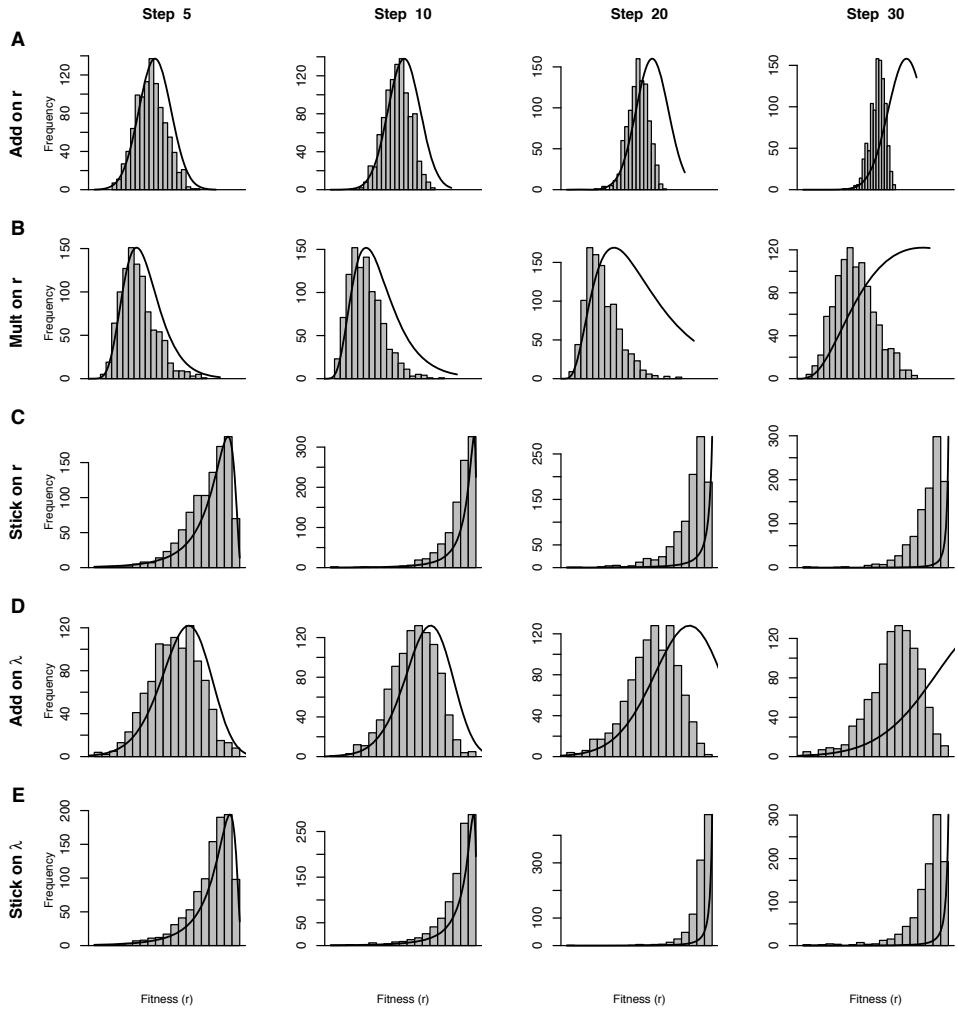

FIGURE 4 Distributions of fitness achieved at indicated step (top) under the six models (left) where the equivalent 'add on r' and 'mult on λ' are consolidated. Grey bars show frequency of fitness over 1000 walks on the same landscape while black lines are predicted distributions based on asymptotic results in APPENDIX. X-axis (Malthusian fitness) is same for all panels. When mutations affect r, the additive, mutliplicative, and stickbreaking models (A-C) yield normal, log normal, and negative log normal distributions of fitness.

Early in a walk ($m \leq 10$) there is good agreement between the observed and predicted distributions (Figure 4) in terms of both mean and variance. As a walk approaches its

midpoint, observed means are noticably smaller than the predicted means because the theory assumes constant effect sizes while, in simulated walks, fitness increase slows as large effect mutation are removed from the available pool. Still, the shapes of the distributions remain the same even when m is large. The different models make qualitatively different predictions about the distribution of fitness during replicate adaptive walks: both stickbreaking models predict heavy left tails, the multiplicative on r model a heavy right tail, and both additive models an approximately normal distribution. Whether mutations effect r and λ is relatively minor. Notice also that the distributions are in terms of number of mutations fixed (steps), not time elapsed. As shown in the fitness trajectories subsection above, different models fix mutations in different lengths of time (Figure 3C) and will therefore achieve the distributions shown in (Figure 4) at different rates (see APPENDIX for details).

Epistasis: Epistasis occurs when the fitness effect of a mutation depends on the genetic background. We investigate epistasis in two ways: first as the effect of a single mutation across a procession of backgrounds, and second as departures from additivity when a set of single mutations are combined. For the first approach, we simulate replicate walks of 10 mutational steps under each model on a single landscape. We then imagine taking the mutation that fixed 10^{th} and engineering it into each of the preceding backgrounds in the walk. (Our choice of the 10^{th} mutation is arbitrary, but using other stop points does not change the qualitative patterns observed; data not shown).

The black lines in Figure 5 show the means of simulation results when fitness effects are defined as differences in r while the grey lines give the theoretical relationships (Table 2). The results show how the observed fitness effects change along the walk under the different models for the same mutation (or as the intrinsic effect is held constant).

Effect sizes grow exponentially for the 'mult on r' model, are constant for the 'add on r' ('mult on λ') model, decay moderately 'add on λ', and show rapidly diminishing effects for both stickbreaking models. Of course, these patterns closely reflect the previously discussed fitness trajectories. Here we are considering how the vertical distance (fitness) between steps qualitatively changes along a walk when intrinsic effect is held constant. It also noteworthy that because differences in r are, in fact, selection coefficients, Figure 5 illustrates how selection coefficients change across a walk under each model. As discussed above, this, in turn, explains how waiting times between mutations change across a walk (Figure 3C).

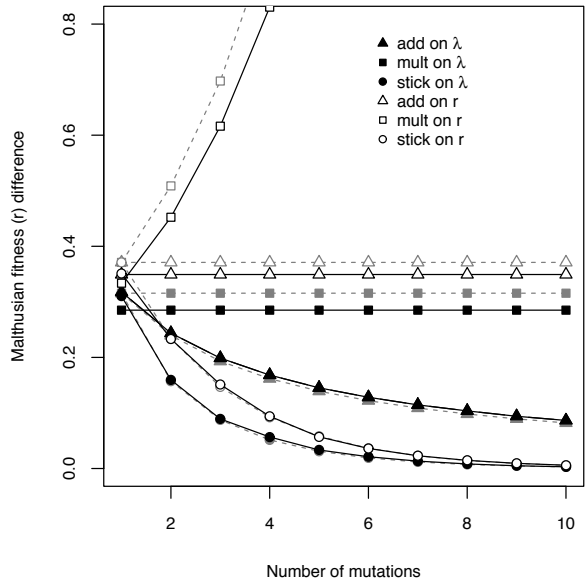

FIGURE 5 Mean fitness effect of the mutation fixing at step 10 inserted into the procession of preceeding backgrounds beginning with the wildtype. Grey dashed lines are theoretical predictions from Table 2. Stickbreaking on either r or λ and, to a lesser extent, additivity on λ produce diminishing returns epistasis.

In the literature, epistasis is more commonly quantified as the departure from addi-

tivity when single mutations are combined. We again simulated replicate walks under each model on a single landscape. For the first m mutations that fixed, we imagined engineering each into the wildtype and measuring their fitness effects (as difference in r). In keeping with the literature, we predicted fitness based on the additivity of r model (i.e., summing fitness effects). Epistasis is then defined as $\epsilon = r_{obs} - r_{pred}$. For beneficial mutations $\epsilon < 0$ and $\epsilon > 0$ are termed antagonistic and synergistic epistasis, respectively.

The patterns of ϵ (Figure 6) are similar to those observed in Figure 5. The stickbreaking models show strong antagonistic epistasis, 'add on λ' shows moderate antagonistic epistasis, 'add on λ' ('mult on r') shows no epistasis (by definition), and 'mult on r' shows strong synergistic epistasis. In fact, it is easy to understand why ϵ (Figure 6) and fitness effect (Figure 5) must follow the same basic pattern. Consider two mutations, A_1 and A_2. If $\epsilon < 0$ (antagonistic epistasis), then $r_{obs} < r_{pred}$. Letting Δr denote fitness effect on r and r_{wt} the wildtype fitness, this implies that $r_{wt} + \Delta r_{1|wt} + \Delta r_{2|1} < r_{wt} + \Delta r_{1|wt} + \Delta r_{2|wt}$ which implies $\Delta r_{2|1} < \Delta r_{2|wt}$, or a diminishing effect. Similar arguments can be made for $\epsilon = 0$ and $\epsilon > 0$.

In the experimental evolution literature, the commonly observed patterns of epistasis are: (1) diminishing effects, where the same mutation has smaller effects on more fit backgrounds and conversely larger effects on less fit ones; (2) antagonistic epistasis is more frequent than synergistic epistasis (Burch *et al.* 2003; Sanjuán and Elena 2006). For example, Bull *et al.* 2000 found that the fitness effect of one mutation (1727T) in the bacteriophage ϕX174 decreased across four backgrounds of increasing fitness. Recently, Chou *et al.* 2011, Khan *et al.* 2011, and Kvitek and Sherlock 2011 all showed a general pattern of diminishing returns epistasis when beneficial mutations were inserted into closely related backgrounds. Similar results are found in double mutant studies. Trindade *et al.* 2009 found that when antibiotic resistance mutations in *E. coli* are combined, 42% of those showing significant epistasis are antagonistic, while only 15%

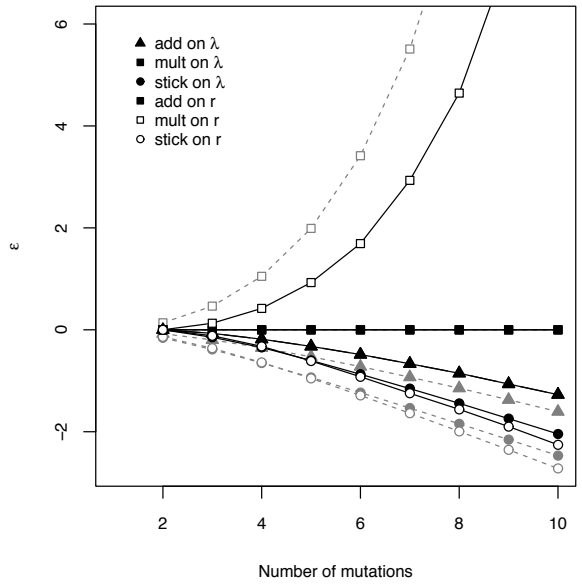

FIGURE 6 Mean epistasis ($\epsilon = r_{obs} - r_{pred}$) as a function of the number of mutations (m) comprising the measured genotype. Predicted values assume additivity on r. Genotypes are always formed from the first m mutations in a walk. Grey dashed lines are theoretical predictions from Table 3.

show synergistic epistasis. ROKYTA *et al.* 2011 inserted nine beneficial single mutations in a G4-like bacteriophage to form 18 double mutants and found antagonistic epistasis for all 18. Finally, a synthesis of 21 studies by SANJUÁN and ELENA 2006 indicated that antagonistic epistasis is more prevalent in viruses and prokaryotes, while synergistic or no epistasis is more common in eukaryotes. Thus studies have tended to show patterns of epistasis broadly consistent with the two stickbreaking models and additivity on λ.

It is important to clarify that the values of ϵ and hence the patterns of antagonistic vs. synergistic epistasis depend on the null model used to calculate predicted fitness. It is easy to see what the patterns would be under other nulls by noting that the 'predicted' and 'observed' labels in Figure 6 are arbitrary. The figure can also be thought of as

showing the fitness divergence between different models as mutations of the same intrinsic effect are introduced. For any null and alternative model, the distance between them corresponds to the values of ϵ.

Conclusion: The stickbreaking model is based on the simple idea that mutational fitness effects should diminish the nearer the background is to the maximum fitness boundary. It solves the bookkeeping problem while also producing patterns of fitness trajectory and epistasis broadly consistent with experimental findings. The next important step is to develop statistical methods for fitting and testing the stickbreaking model on real data. Like the additive and multiplicative models, stickbreaking is too simple to be biologically correct. Rather, our hope is that stickbreaking is mathematically tractable like those models, but also captures a basic biological property and provides an explanatory power that those models seem to miss.

LITERATURE CITED

Barrett, R. D. H., L. K. M'Gonigle and S. P. Otto, 2006 The distribution of beneficial mutant effects under strong selection. Genetics **174**: 2071–2079.

Barrick, J. E., D. S. Yu, S. H. Yoon, H. Jeong, T. K. Oh *et al.*, 2009 Genome evolution and adaptation in a long-term experiment with escherichia coli. Nature **461**: 1243–U74.

Betancourt, A. J., 2009 Genomewide patterns of substitution in adaptively evolving populations of the RNA bacteriophage MS2. Genetics **181**: 1535–1544.

Bonhoeffer, S., C. Chappey, N. T. Parkin, J. M. Whitcomb and C. J. Petropoulos, 2004 Evidence for positive epistasis in HIV-1. Science **306**: 1547–1550.

Bull, J. J., M. R. Badgett and H. A. Wichman, 2000 Big-benefit mutations in a bacteriophage inhibited with heat. Molecular Biology and Evolution **17**: 942–950.

Bull, J. J., M. R. Badgett, H. A. Wichman, J. P. Huelsenbeck, D. M. Hillis *et al.*, 1997 Exceptional convergent evolution in a virus. Genetics **147**: 1497–1507.

Burch, C. L. and L. Chao, 1999 Evolution by small steps and rugged landscapes in the RNA virus ϕ6. Genetics **151**: 921–927.

Burch, C. L., P. E. Turner and K. A. Hanley, 2003 Patterns of epistasis in RNA viruses: a review of the evidence from vaccine design. Journal of Evolutionary Biology **16**: 1223–1235.

Chevin, L., 2010 On measuring selection in experimental evolution. Biology Letters **7**: 210–213.

Chou, H., H. Chiu, N. Delaney, D. Segrè and C. Marx, 2011 Diminishing returns epistasis among beneficial mutations decelerates adaptation. Science **332**: 1190–1192.

Cooper, V. S. and R. E. Lenski, 2000 The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature **407**: 736–739.

Cowperthwaite, M. C., J. J. Bull and L. Ancel Myers, 2005 Distributions of beneficial fitness effects in RNA. Genetics **170**: 1449–1457.

de Visser, J. A. G. M., C. W. Zeyl, P. J. Gerrish, J. L. Blanchard and R. E. Lenski, 1999 Diminishing returns from mutation supply rate in asexual populations. Science **283**: 404–6.

Desai, M. M., D. S. Fisher and A. W. Murray, 2007 The speed of evolution and maintenance of variation in asexual populations. Curr. Biol. **17**: 385–394.

Donnelly, P. and P. Joyce, 1989 Continuity and weak convergence of ranked and size-biased permutations on an infinite simplex. Stochastic Processes and their Applications **31**: 89–103.

Elena, S. F., M. Davila, I. S. Novella, J. J. Holland, E. Domingo *et al.*, 1998 Evolutionary dynamics of fitness recovery from the debilitating effects of muller's ratchet. Evolution **52**: 309–314.

Elena, S. F. and R. E. Lenski, 1997 Test of synergistic interactions among deleterious mutations in bacteria. Nature **390**: 395–398.

Eyre-Walker, A. and P. D. Keightley, 2007 The distribution of fitness effects of new mutations. Nature Reviews Genetics **8**: 610–618.

Gerrish, P. J. and R. E. Lenski, 1998 The fate of competing beneficial mutations in an asexual population. Genetica **102/103**: 127–144.

Gillespie, J. H., 1991 *The causes of molecular evolution*. Oxford University Press, New York.

Hartl, D. L. and A. G. Clark, 1997 *Principles of population genetics, third edition*. Sinauer Associates, Inc., Sunderland.

Holder, K. and J. Bull, 2001 Profiles of adaptation in two similar viruses. Genetics **159**: 1393–1404.

Joyce, P., D. R. Rokyta, C. J. Beisel and H. A. Orr, 2008 A general extreme value theory model for the adaptation of DNA sequences under strong selection and weak mutation. Genetics **180**: 1627–1643.

Kacser, H. and J. Burns, 1981 The molecular basis of dominance. Genetics **97**: 639–666.

Kauffman, S. A., 1993 *The origins of order*. Oxford University Press, New York.

Khan, A., D. Dinh, D. Schneider, R. Lenski and T. Cooper, 2011 Negative epistasis between beneficial mutations in an evolving bacterial population. Science **332**: 1193–1196.

Kim, Y. and H. A. Orr, 2005 Adaptation in sexuals vs. asexuals: clonal interference and the Fisher-Muller model. Genetics **171**: 1377–1386.

Kimura, M., 1962 On the probability of fixation of mutant genes in a population. Genetics **47**: 713–719.

Kryazhimskiy, S., G. Tkačik and J. Plotkin, 2009 The dynamics of adaptation on correlated fitness landscapes. Proceedings of the National Academy of Sciences of the USA **106**: 18638–18643.

Kvitek, D. and G. Sherlock, 2011 Reciprocal sign epistasis between frequently experimentally evolved adaptive mutations causes a rugged fitness landscape. PLoS Genetics **7**: e1002056.

Lenski, R. E. and M. Travisano, 1994 Dynamics of adaptation and diversification: A 10,000-generation experiment with bacterial populations. Proceedings of the National Academy of Sciences of the USA **91**: 6808–6814.

MacArthur, R., 1957 On the relative abundance of bird species. Proceedings of the National Academy of Sciences of the USA **43**: 293–295.

Miller, C., P. Joyce and H. Wichman, 2011 Mutational effects and population dynamics during viral adaptation challenge current models. Genetics **187**: 185–202.

Novella, I. S., E. A. Duarte, S. F. Elena, A. Moya, E. Domingo *et al.*, 1995 Exponential increases of RNA virus fitness during large population transmissions. Proceedings of the National Academy of Sciences of the USA **92**: 5841–5844.

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.

Patil, C. and C. Taillie, 1977 Diversity as a concept and its implications for random communities. Bulletin of the International Statistical Institute **47**: 497–515.

R Development Core Team, 2009 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna (Austria).

Rokyta, D., P. Joyce, S. Caudle, C. Miller, C. Beisel *et al.*, 2011 Epistasis between beneficial mutations and the phenotype-to-fitness map for a ssdna virus. PLoS Genetics **7**: e1002075.

Rokyta, D. R., Z. Abdo and H. A. Wichman, 2009 The genetics of adaptation for eight microvirid bacteriophages. Journal of Molecular Evolution **69**: 229–239.

Rokyta, D. R., C. J. Beisel, P. Joyce, M. T. Ferris, C. L. Burch *et al.*, 2008 Beneficial fitness effects are not exponential for two viruses. Journal of Molecular Evolution **67**: 368–376.

Rozen, D. E., J. A. G. M. de Visser and P. J. Gerrish, 2002 Fitness effects of fixed beneficial mutations in microbial populations. Curr. Biol. **12**: 1040–1045.

Sanjuán, R. and S. F. Elena, 2006 Epistasis correlates to genomic complexity. Proceedings of the National Academy of Sciences of the USA **103**: 14402–14405.

Sanjuán, 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 USA **101**: 8396–8401.

Schoustra, S. E., T. Bataillon, D. R. Gifford and R. Kassen, 2009 The properties of adaptive walks in evolving populations of fungus. PLoS Biology **7**.

Silander, O. K., O. Tenaillon and L. Chao, 2007 Understanding the evolutionary fate of finite populations: The dymanics of mutational effects. PLoS Biology **5**: e94.

Trindade, S., A. Sousa, K. Xavier, F. Dionisio, M. Ferreira *et al.*, 2009 Positive epistasis drives the acquisition of multidrug resistance. PLoS Genetics **5**: e1000578.

Weinreich, D. M., N. F. Delaney, M. A. DePristo and D. L. Hartl, 2006 Darwinian evolution can follow only very few mutational paths to fitter proteins. Science **312**: 111–114.

Wichman, H. A., M. R. Badgett, L. A. Scott, C. M. Boulianne and J. J. Bull, 1999 Different trajectories of parallel evolution during viral adaptation. Science **285**: 422–424.

Wichman, H. A., J. Millstein and J. J. Bull, 2005 Adaptive molecular evolution for 13,000 phage generations: a possible arms race. Genetics **170**: 19–31.

Woods, R., D. Schneider, C. Winkworth, M. Riley and R. Lenski, 2006 Tests of parallel molecular evolution in a long-term experiment with escherichia coli. Proceedings of the National Academy of Sciences of the USA **103**: 9107–9112.

APPENDIX

Distribution of total fitness effects after m steps of adaptation: We show here that there are three limiting distributions for the fitness achieved after m steps in a walk: the normal distribution under additivity, the log normal under the multiplicative model, and negative log normal under stickbreaking.

Denote the 'intrinsic' fitness effect of the beneficial mutation A_i by x_i. For the additive model $x_i = \Delta w_i$, for the multiplicative model $x_i = s_i$, and for the stickbreaking model $x_i = u_i$. Note that u_i and s_i are just different ways to scale Δw_i. That is $u_i = \Delta w_i/(d - w_{wt})$ and $s_i = \Delta w_i/w_{wt}$. Therefore

$$\frac{u_j}{\sum_{i=1}^{M} u_i} = \frac{\Delta w_j/(d - w_{wt})}{\sum_{i=1}^{M} \Delta w_i/(d - w_{wt})} = \frac{\Delta w_j}{\sum_{i=1}^{M} \Delta w_i}$$

and similarly

$$\frac{s_j}{\sum_{i=1}^{M} s_i} = \frac{\Delta w_j/w_{wt}}{\sum_{i=1}^{M} \Delta w_i/w_{wt}} = \frac{\Delta w_j}{\sum_{i=1}^{M} \Delta w_i}.$$

Throughout we will assume that the walks evolve according to SSWM conditions. We will also assume that for each value of M, $x_1, x_2, \cdots, x_M$ is fixed. That is, we will use the same set of intrinsic fitness effects for replicate walks.

Consider an adaptive walk of length m. Let Y_i be the intrinsic fitness effect of the mutation arising at step i. The joint distribution of $Y_1, Y_2, \cdots, Y_m$ can be described as

$$P(Y_1 = x_{i_1}, Y_2 = x_{i_2}, \cdots, Y_m = x_{i_m}) = \frac{x_{i_1}}{\sum_{i=1}^{M} x_i} \frac{x_{i_2}}{\sum_{i \neq i_1}^{M} x_i} \frac{x_{i_3}}{\sum_{i \neq i_1, i_2}^{M} x_i} \cdots \frac{x_{i_m}}{\sum_{i \neq i_1, \cdots, i_{m-1}}^{M} x_i}. \tag{7}$$

Note that $Y_1, Y_2, \cdots, Y_m$ are dependent random variables. The dependence comes from the fact that once a mutation is used in a walk it will not be used again, thus reducing

the number of available mutations at each step. However, if M is large enough we show below that $Y_1, Y_2, \cdots, Y_m$ are approximately independent and identically distributed. Let $x_{(1)} = \max\{x_1, x_2, \cdots, x_M\}$. Note that $x_{i_1} + \cdots + x_{i_{m-1}} \leq (m-1)x_{(1)}$. Therefore,

$$\bar{x} \geq \frac{1}{M} \sum_{i \neq i_1, \cdots, i_{m-1}}^{M} x_i \geq \bar{x} - (m-1)x_{(1)}/M. \tag{8}$$

Here is where the relationship between M and m becomes important. We will assume that m is an order of magnitude smaller than M. More precisely, we assume that as $M \to \infty$ then $m \ln(M)/M \to 0$ and $m \to \infty$. It follows from extreme value theory that for large M, $x_{(1)} \approx c \ln M$. (More precisely $x_{(1)}/\ln(M)$ converges to a constant c as $M \to \infty$.) Taking the limit as $M \to \infty$ in inequality (8) reveals that $\lim_{M\to\infty} \bar{x} - \frac{1}{M} \sum_{i \neq i_1, \cdots, i_{m-1}}^{M} x_i = 0$. Thus for large M, $\frac{1}{M} \sum_{i \neq i_1, \cdots, i_{k-1}}^{M} x_i \approx \bar{x}$, for $k = 1, 2, \cdots, m$. If we replace the denominators in equation (7) with $M\bar{x}$, then this leads to the assumption $Y_1, Y_2, \cdots, Y_m$ are approximately independent and identically distributed with $P(Y = x) \approx \frac{x}{M\bar{x}}$. Note that $E(Y) = \frac{\sum_{i=1}^{M} x_i^2/M}{\bar{x}}$ and $\mathrm{Var}(Y) = \frac{\sum_{i=1}^{M} x_i^3/M}{\bar{x}} - \left(\frac{\sum_{i=1}^{M} x_i^2/M}{\bar{x}}\right)^2$. Both converge as $M \to \infty$.

Normal, log normal, negative log normal: Below we review the three central distributions associated with m steps of an adaptive walk. The **normal distribution** is given by

$$f_X(x|\mu, \sigma^2) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2}. \tag{9}$$

If X follows the normal distribution, we say that $V = e^X$ follows the **log normal distribution** with probability density function given by

$$f_V(v|\mu, \sigma^2) \frac{1}{\sqrt{2\pi}\sigma v} e^{-(\ln(v)-\mu)^2/(2\sigma^2)} \tag{10}$$

and if V follows the log normal we say that $W = 1-V$ follows the **negative log normal distribution** with probability density function given by

$$f_W(w) = \frac{1}{\sqrt{2\pi}\sigma(1-w)} e^{-(\ln(1-w)-\mu)^2/(2\sigma^2)}. \tag{11}$$

Note that the parameters μ and σ appear in all three probability densities, but must be interpreted differently in each. While μ represents the mean and σ^2 represents the variance of a normal, the mean of the log normal distribution is $E(V) = E(e^X) = e^{\mu+\sigma^2/2}$ and the variance of a log normal is $\mathrm{Var}(V) = E(e^{2X} - (Ee^X)^2 = e^{2\mu+\sigma^2}(e^{\sigma^2}-1)$. If W is negative log normal then the mean is $E(W) = 1 - E(V) = 1 - e^{\mu+\sigma^2/2}$ and the variance of a negative log normal is the same as that of a log normal.

Now if Y_i represents the fitness differences then the fitness after m steps is given by $w_{1,2,\cdots,m} = w_{wt} + \sum_{i=1}^m Y_i$. Under the additive model, the central limit theorem applies and the distribution of $w_{1,2,\cdots,m}$ will be approximately normal with mean $\mu = w_{wt} + mE(Y) = w_{wt} + m\frac{\sum_{i=1}^M x_i^2/M}{\bar{x}}$ and $\sigma^2 = m\mathrm{Var}(Y) = m\frac{\sum_{i=1}^M x_i^3/M}{\bar{x}} - m\left(\frac{\sum_{i=1}^M x_i^2/M}{\bar{x}}\right)^2$. However, if the multiplicative model applies then $w_{1,2,\cdots,m} = w_{wt}\prod_{i=1}^m(1+Y_i)$. This implies $w_{1,2,\cdots,m}/w_{wt} = e^{\sum_{i=1}^m \ln(1+Y_i)}$. The central limit theorem now applies to $\sum_{i=1}^m \ln(1+Y_i)$. Thus $\mu = mE(\ln(1+Y)) = m\sum_{j=1}^M x_j\ln(1+x_j)/(N\bar{x})$ and

$$\sigma^2 = m\mathrm{Var}(\ln(1+Y)) = m\sum_{j=1}^M \left((\ln(1+x_j))^2 x_j/(N\bar{x}) - \left(\sum_{j=1}^M x_j\ln(1+x_j)/(N\bar{x})\right)^2\right).$$

So $w_{1,2,\cdots,m}/w_{wt}$ is distributed log normal.

Under the stickbreaking model, $(w_{i,1,\cdots,m} - w_{wt})/d = 1 - \prod_{i=1}^m(1-Y_i)$ is approximately negative log normal, where $\mu = mE(1-Y)$ and $\sigma^2 = m\mathrm{Var}(1-Y)$ and the formulas are analogous to those of the log normal.

The assumption that M is large enough so that m is an order of magnitude smaller

yet m is still large enough for the central limit theorem to apply is not always going to be achieved. Simulations can help in determining the degree to which violation of assumptions matter.

Number of steps versus time to adaptation: Under SSWM conditions the time it takes a mutation with selection coefficient s to arise and fix in the population is exponentially distributed with mean $1/(N\mu s)$, where μ is the beneficial mutation rate and N is the population size. Now if there are a total of M beneficial mutations available, the time in generations to fixation of the first beneficial mutation is on average $1/(N\mu M\bar{s})$ where $\bar{s}$ is the average selection coefficient among the M available mutations. All of our theory is based on the asymptotic results formed by taking the limit as M goes to infinity. As M goes to infinity the time to fixation converges to zero. So a time scale change is required. If we assume that one unit of time is equivalent to $N\mu M$ generations then the mean time for the first beneficial mutation to fix using this time scale will be exponentially distributed with mean $1/\bar{s}$. In the limit as M goes to infinity $\bar{s}$ converges to the mean of the distribution of beneficial mutations, which we will denote by γ. We now use an extension of the central limit theorem that states $\sum_{i=1}^{M_t} \frac{Y_i - m\mu}{\sqrt{M_t}\sigma}$ converges to the normal distribution as $t \to \infty$. This shows that the time limit prediction of the additive model is normal. Applying the analogous central limit theorem result to $\sum_{i=1}^{M_t} \ln(1+Y_i)$ shows that the multiplicative model leads to a log-normal distribution. Applying the analogous central limit theorem result to $\sum_{i=1}^{M_t} \ln(1-Y_i)$ shows that the stickbreaking model leads to a negative log-normal distribution.