MODELING EVOLUTION USING THE PROBABILITY OF FIXATION: HISTORY AND IMPLICATIONS

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ABSTRACT

Many models of evolution calculate the rate of evolution by multiplying the rate at which new mutations originate within a population by a probability of fixation. Here we review the historical origins, contemporary applications, and evolutionary implications of these "origin-fixation" models, which are widely used in evolutionary genetics, molecular evolution, and phylogenetics. Origin-fixation models were first introduced in 1969, in association with an emerging view of "molecular" evolution. Early origin-fixation models were used to calculate an instantaneous rate of evolution across a large number of independently evolving loci; in the 1980s and 1990s, a second wave of origin-fixation models emerged to address a sequence of fixation events at a single locus. Although origin-fixation models have been applied to a broad array of problems in contemporary evolutionary research, their rise in popularity has not been accompanied by an increased appreciation of their restrictive assumptions or their distinctive implications. We argue that origin-fixation models constitute a coherent theory of mutation-limited evolution that contrasts sharply with theories of evolution that rely on the presence of standing genetic variation. A major unsolved question in evolutionary biology is the degree to which these models provide an accurate approximation of evolution in natural populations.

INTRODUCTION

ALTHOUGH many of the basic themes of theoretical population genetics had already emerged by 1932, the body of formal theory applied by evolutionary researchers has continued to expand in new directions. Examples of theoretical developments notable for their wide use include the theory of

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kin selection (Hamilton 1964), evolutionary game theory (Maynard Smith and Price 1973), the coalescent (Kingman 1982), and the multivariate generalization of quantitative genetics (Lande and Arnold 1983).

Here we draw attention to a theoretical innovation that has achieved wide use cryptically, without having been recognized as an innovation: specifying the rate of evolution as the product of two factors, the rate at which new alleles originate within the population, and the probability that they reach fixation. By rendering evolutionary change as a two-step procedure of (1) mutation followed by (2) fixation or loss, such models skip lightly over the complex within-population processes that are the predominant focus of theoretical population genetics, and which typically prevent analytical solutions to the dynamics of long-term evolutionary change.

Models of this form are in common use today, appearing in separate patches of the literature, and sometimes associated with labels such as "weak mutation" (Gillespie 1983a), "selection-mutation-drift" (Bulmer 1991), "mutation-selection" (Yang and Nielsen 2008), "mutation-limited" (Kopp and Hermisson 2009b), "new mutation" (Hill 1982), "mutation-driven" (Li 1997) and, in the context of molecular phylogenetics, "mechanistic" (as opposed to empirical) models (Ren et al. 2005). Because none of these names uniquely specify the class of models we have in mind, we call models that determine the rate of evolution by multiplying the rate of mutational origination by the probability of fixation "origin-fixation" models, in reference to their mathematical form (readers should not be confused by the fact that Gillespie, who contributed importantly to the development of these models, uses the terms "origination process" and "fixation process" to refer to something related but different, e.g., Gillespie 1991, 1994a).

Below, we begin with a brief mathematical description of these models, including widely known special cases such as $K=4Nus$ and the move-rule used in the mutational landscape model. Two general classes may be discerned: aggregate-rate models that consider the total rate of evolution over a large number of independently evolving loci, and sequential fixation models that treat a series of substitutions at a single locus.

We then review the diverse ways in which these models have been developed and applied. Whereas the probability of fixation itself is a classical result, true origin-fixation models did not appear until 1969, in the context of the neutral theory, taking the form of aggregate-rate models of neutral or beneficial changes (King and Jukes 1969; see also Kimura and Maruyama 1969). Over time, such models became fundamental to the analysis of molecular evolution. The first influential sequential fixations model emerged in 1983 as a model of genetic adaptation (Gillespie 1983b), while later models of sequential fixations allowing deleterious and neutral mutations were proposed independently in several different fields. As shown below, such models have been applied to a range of issues, including heterogeneity in evolutionary rates, codon usage bias, and phylogenetic inference.

Finally, we draw attention to origin-fixation models as a distinctive theory of evolutionary change. Today, origin-fixation models are frequently invoked without regard to either their underlying assumptions or their implications, as if they represented a generic framework for modeling the evolutionary process. Rather, origin-fixation models depict a specific regime of mutation-limited evolution in which the power of mutation to determine the rate and direction of evolution is particularly strong. Because this regime does not necessarily occur widely in nature, we argue that origin-fixation models should be treated as hypotheses to be tested rather than merely a method to be applied. Determining the degree to which evolution in nature occurs as depicted by origin-fixation models provides an important direction for future research.

THE ORIGIN-FIXATION FORMALISM

To understand the broad appeal of origin-fixation models, it is helpful to recognize that the long-term behavior of a reproducing population of interacting individuals subject to mutation, selection, drift, and recombination is typically mathematically intractable. Even short-term models of population genetics often depend on radical simplifying assumptions such as neutrality, complete linkage

(or full recombination), the absence of epistasis (fitness interactions), and so on.

In origin-fixation models, the key simplifying assumption is that any time a mutation enters the population, it enters the population at a locus where only one other allele is present in the population. The great benefit of making this assumption is that there is a body of classical theory that describes the dynamics of a locus where at most two alleles are segregating and, in particular, there are simple mathematical formulas for the probability that a new mutation will reach frequency one in the population, i.e., achieve fixation. Using such expressions, one can then write down a model in which mutations enter the population one at a time, and each mutation either goes to fixation or is lost depending on its probability of fixation. Such models typically fall into one of two classes. Below we first describe, and then compare, these two classes of models.

AGGREGATE-RATE MODELS

In the first of class of models, we consider an infinite collection of independently evolving loci where the total mutation rate across this infinite collection is finite. Because the mutation rate at any individual locus is infinitesimally small, each new mutation will occur at a different, previously monomorphic, locus. Since the typical goal of such a model is to calculate a total rate of evolution across this infinite collection of loci, we will call these models "aggregate-rate" models.

Let us construct a simple version of such a model and use it to derive some familiar results. Suppose in particular we have a diploid population of size N and we assume that mutations appear with a total rate u across our infinite collection of loci (this rate can be arbitrarily large, yet the assumption of infinite loci ensures that each mutation occurs at a new locus). Then the total rate that such mutations appear in the population as a whole is simply $2Nu$. Of course, only a fraction of these mutations will eventually go to fixation. Thus, to calculate the rate of evolution, K, we need to multiply this origination rate with the probability of fixation of a new mutation. Let us assume that dominance is absent, and that each of these new mutations has the same selection coefficient s. Writing the probability of fixation of a new mutation with selection coefficient s as $\pi(s,N)$, we conclude that the rate of evolution is given by

$$K = 2Nu \times \pi(s,N). \tag{1}$$

Several classical results then follow immediately by making particular choices for s and $\pi(s,N)$. For instance, if we assume that mutations with a positive selection coefficient s are occurring with rate u, we can use Haldane's (1927) approximation $\pi(s,N) = 2s$ to recover the famous formula,

$$K = 2Nu \times 2s = 4Nus. \tag{2}$$

Similarly, if neutral rather than beneficial mutations are arising at rate u, we can use the neutral probability of fixation, $\pi(s,N) = 1/(2N)$, to derive

$$K = 2Nu \times \frac{1}{2N} = u. \tag{3}$$

Although the above models are extremely simple, more complex aggregate-rate models can be constructed, e.g., by allowing the selection coefficients of new mutations to be drawn from a probability distribution (e.g., Ohta 1977; Kimura 1979) or by using these simple formulas as one component of a more complex model (e.g., Hill 1982; Sawyer and Hartl 1992).

SEQUENTIAL FIXATION MODELS

While the aggregate-rate models assume that each mutation occurs at a new locus, models in our second major class instead assume that mutations at any one locus occur sufficiently infrequently that each new mutation is lost or goes to fixation prior to the appearance of the next mutation. Thus, it is still the case that each new mutation occurs at a locus that is otherwise monomorphic. Such models typically are presented as one-locus, multiallelic models where the questions of interest concern the trajectory of a population through some kind of explicit genotypic space (e.g., sequence space). We will call these models "sequential fixation" models. This class of models, although very important (see below), is perhaps less well known than the aggregate-rate models.

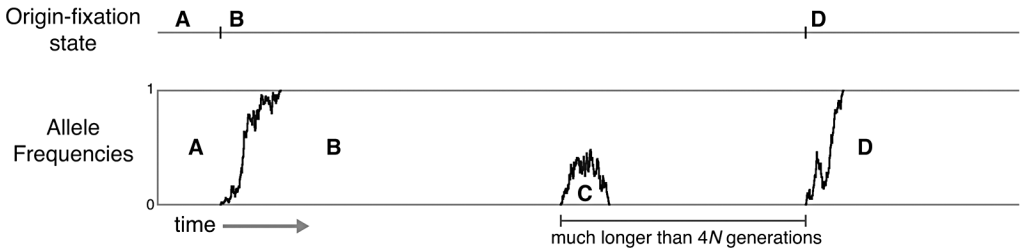

FIGURE 1. A COMPARISON BETWEEN A SEQUENTIAL FIXATIONS MODEL AND THE FULL EVOLUTIONARY DYNAMICS

In a sequential fixations model, the population jumps from one state to another (upper figure) by a process that, in effect, is an instantaneous event of mutation-and-fixation. In an actual population, this process must take some period of time as an allele frequency traverses from a starting value to fixation (lower figure). However, if the gaps between these polymorphic periods are sufficiently long, an origin-fixation model can provide a good approximation to the full evolutionary dynamics.

Because sequential fixation models assume that the time during which any particular mutation segregates in the population is much shorter than the waiting time from one mutation to the next, the population must be monomorphic the vast majority of the time. Thus, in sequential fixation models, one conventionally ignores both the time it takes for a successful mutation to fix and the periods during which the population is polymorphic due to mutations destined for loss. This leads to a model in which a population is viewed as jumping directly from one fixed state to another at the introduction of each new mutation that is destined for fixation (Figure 1).

More formally, sequential fixations models treat evolution as a Markov chain where the states are the possible alleles at a single locus. If u_{ij} is the gametic mutation rate from allele i to allele j, then the total rate at which mutants of allele j originate within a diploid population is given by $2Nu_{ij}$. Furthermore, each such mutant is destined for fixation with probability $\pi(s_{ij},N)$, where s_{ij} is the selection coefficient of alternative allele j relative to prevailing allele i. Thus, fixations from i to j occur at rate:

$$Q(i,j) = 2Nu_{ij} \times \pi(s_{ij},N). \qquad (4)$$

That is, the fixation rate is again equal to the origination rate times the probability of fixation.

The basic behavior of such a Markov chain is easy to understand. A population currently fixed for some genotype i stays at genotype i for an exponentially distributed amount of time, where the mean of this exponential distribution is equal to the inverse of the substitution rate at genotype i, and the substitution rate at i is equal to the sum of the rates to its neighbors, $\sum_{k \neq i} Q(i,k)$. Furthermore, if we want to know the probability that the *next* fixation will be to some genotype j, this probability is given by

$$P(i,j) = \frac{Q(i,j)}{\sum_{k \neq i} Q(i,k)}. \qquad (5)$$

which is simply the ratio between the rate of fixations from i to j and the total substitution rate at i. The great power of these relatively simple dynamics is that it becomes possible to model the evolution of a population through a large space of possible genotypes.

Equation 5 also provides an alternative way to specify an origin-fixation model. Rather than keeping track of absolute time, we can consider a Markov chain where each time step consists of a single substitution. In mathematics, this kind of discrete-time version of a continuous-time process is called the "embedded Markov chain," and P is the transition matrix for this embedded Markov chain. We refer to Equation 5 as the "move-rule" form of origin-fixation dynamics, as it is conveniently used as a rule to choose the next step in an adaptive walk. For instance, the move-rule in Orr's (2002) mutational land-

scape model is a simplified version of Equation 5.

comparing classes of models

An important similarity between the aggregate-rate and sequential fixation models is that both classes of models rely on the same set of well-known expressions for the probability of fixation. Roughly speaking, these are Haldane's (1927) formula for the probability of fixation of an advantageous allele, Wright and Fisher's approximate formulas (Fisher 1930a,b; Wright 1931), Kimura's (1957, 1962) general formula for the diffusion approximation, and Moran's (1959) exact expression for the Moran process.

Despite being based on the same building blocks, aggregate-rate and sequential fixation models rely on different assumptions. In an aggregate-rate model, one assumes that the loci in question evolve independently from each other. This requires that the loci be unlinked (i.e., there is free recombination between them), that the fitness effects of a mutation at one locus do not depend on segregating variants at another locus (i.e., an absence of epistasis), and that selection is weak in the sense that the variance in fitness within the population is small (e.g., Barton 1995; this requirement can be relaxed through the use of an effective population size).

In addition to the assumption that different loci evolve independently, one must also assume that each new mutation happens at a location in the genome where no variation is currently segregating. This second assumption is known in the literature as the "infinite-sites" assumption (Kimura 1969). Thus, aggregate-rate models are a subset of infinite-sites, free-recombination models (a different, but still common, class of models makes the infinite-sites assumption but also assumes complete linkage between sites, e.g., in Watterson 1975).

By contrast, the key assumption underlying sequential fixation models concerns the rate of mutational origination. In sequential fixation models, mutations must occur sufficiently infrequently that each new mutation is lost or becomes fixed in the population before the next new mutation enters the population (whereas in aggregate-rate models, the total rate at which mutations at all loci enter the population, $2Nu$, can be arbitrarily large). For a diploid locus undergoing frequency-independent genic selection, the mutations that segregate for the longest time in a population are neutral mutations that are destined for fixation, which segregate on the order of $4N$ generations (Kimura and Ohta 1969). The expected number of mutations to enter the population during this period is then $4N \times 2Nu = 8N^2u$, so that a condition for the strict applicability of a model of sequential fixations is $8N^2u << 1$ (e.g., see Ishii et al. 1982; Lynch and Abegg 2010). This condition is, however, just a rule of thumb, and the assumptions necessary for an origin-fixation model to faithfully capture the true evolutionary dynamics will in general depend on other factors in addition to the population size and mutation rate. We will return to this point in the Discussion.

It is important to realize that in practice many models of sequential fixations assume free recombination and the absence of epistasis in addition to assuming that mutations enter the population sufficiently infrequently. Such models are typically constructed by considering a collection of independently evolving sequential-fixations Markov chains. For example, Halpern and Bruno (1998) construct a model for the evolution of coding sequences where each codon is modeled as an independent sequential-fixations Markov chain. For such models, the mutation rate u in the condition $8N^2u << 1$ then refers to the mutation rate for each independent Markov chain, rather than the total mutation rate over the collection as a whole.

Origin-fixation models may be distinguished from models that share some, but not all, of their features, as follows. The assumption that mutations at any given locus enter a population extremely rarely is sometimes known as the "weak mutation" assumption. Whereas origin-fixation models are always weak-mutation models, not all weak-mutation models are origin-fixation models. For instance, many weak-mutation models work by integrating over a distribution of gene frequencies in the limit of weak mutation rather than multiplying a rate of mutational origin

by the probability of fixation (e.g., Wright 1931; Li 1987; Zeng et al. 1989; Kondrashov 1995). It is also important to note that there are many classes of models that contain discrete origination events that are not origin-fixation models, such as coalescent models, simulation models that treat the dynamics of segregating alleles in full detail, or recent models of polymorphic asexual populations that, in some ways, extend origin-fixation thinking beyond the weak-mutation regime (e.g., Weissman et al. 2009).

Finally, it may also be worth noting that the origin-fixation formalism might be applied, not only to new mutations in the conventional sense, but also wherever discrete events introduce new heritable types into a population, e.g., events of lateral transfer, migration, infection, and endosymbiogenesis, among others. In principle, the formalism also might be applied to premeiotic clusters of mutations (Woodruff et al. 1996), with suitable adjustments for mutation rate and probability of fixation.

Early History of Origin-Fixation Models

classical period

Mathematical formulas for the probability of fixation were originally proposed by Haldane, Fisher, and Wright (Haldane 1927; Fisher 1930a; Wright 1931; see also Fisher 1922, 1930b; Haldane 1932). Given a formula for the probability of fixation, the construction of an origin-fixation model is trivial—ignoring possible conceptual barriers (e.g., conceptualizing genetic state-space)—yet none of these early works contains an origin-fixation model. For instance, Fisher (1930a:93) derives the probability of fixation by assuming that one new mutation enters the population per generation and then noting that the equilibrium rate at which such mutations reach fixation must be equal to the probability of fixation. But Fisher never takes the next step of writing the rate of evolution, K, as the product of $2Nu$ and the probability of fixation.

To our knowledge, the first paper to describe the general relationship between K, $2Nu$, and the probability of fixation is Wright's well-known paper, "The distribution of gene frequencies under irreversible mutation" (Wright 1938). In this paper, Wright notes that, in the special case of his model where dominance is absent, "$K = \frac{4Nvs}{1 - e^{-4Ns}}$ approaching $v(1 + 2Ns)$ as $4Ns$ decreases" (Wright 1938:258). Here, K is the instantaneous equilibrium rate of fixations at a single site and v is analogous to u, the mutation rate, in our notation. Clearly, this formula reduces to $K = u$ (Equation 3 above) as Ns approaches 0 and $K = 4Nus$ (Equation 2 above) for large Ns (the formula $K = u$ for the neutral case is also stated explicitly as Wright's Equation 15). However, these results were not derived by assuming the origin-fixation formalism, but by considering the full probability distribution for the frequency of the new allele during the period that it segregates in the population.

Surprisingly, Wright's 1938 paper does not appear to be the source of later origin-fixation models. Looking at all citations to this paper from 1938 to 1985, we do not find a single article that cites Wright for an origin-fixation formalism or for the formula $K = 4Nus$ (51 records, Web of Science search, accessed 4 March 2014). This is particularly odd because Kimura, who did much to popularize both formulas, habitually cited Wright (1938) for the general form of the distribution of allele frequencies under reversible mutation that occurs earlier in the paper.

Indeed, we are not aware of any origin-fixation model proposed prior to the molecular era (see next section). Before this time, the typical uses for the probability of fixation were to inquire as to the number of times a particular advantageous mutant must enter the population before it is relatively assurred of reaching fixation (Haldane 1927:839, 1932, 1990:114; Fisher 1930a:77; Wright 1949, in the section on nonrecurrent mutation) or to remark on the extremely small probability of fixation for disadvantageous mutations (Fisher 1930a:93-94; Wright 1938).

In addition to being absent from the mathematical theories of Wright, Fisher, and Haldane, origin-fixation models are broadly incompatible with their stated views of

how evolution occurs in nature. In general, Wright, Fisher, and Haldane believed that evolutionary change is due largely to natural selection acting on segregating (i.e., standing) variation (Wright 1960, 1980:829), and were impressed both with the speed of this process, and with the ability of the joint action of natural selection and recombination to make genotypes common that otherwise would have been vanishingly rare (e.g., Fisher 1930a:96; Haldane 1932:94-95). Fisher and Wright also placed a strong emphasis on fitness interactions (epistasis) among segregating alleles, which precluded the possibility that an allele would have a constant selection coefficient during its sojourn in a population (as per the end of the section on nonrecurrent mutation in Wright 1949; or Fisher 1930a:95). Thus, for Fisher and Wright, formulas assuming an independent fixation probability for each new mutation had little relevance to evolution in actual populations. Finally, whereas Fisher and Haldane rejected drift as being too slow to substantially affect evolution in natural populations (Haldane 1932:117), origin-fixation models rely on the waiting time between mutations being even longer than the time-scale of drift.

As the mid-20th-century evolutionary synthesis progressed, it came to include a verbal theory of evolutionary genetics even less compatible with origin-fixation thinking. Although today evolutionary geneticists consider it an open question whether adaptation occurs primarily through "new mutations, fixation of standing variation or modest changes at many loci" (Olson-Manning et al. 2012:869), the architects of the mid-century consensus argued that the abundance of variation in the "gene pool" and the ability of recombination to generate further multilocus variation ensured that selection would never need to wait for a new mutation. For these authors, evolution was not a process wherein individual mutations fixed one at a time; rather, evolution became synonymous with a shift from one multilocus equilibrium of segregating variation to another (e.g., see Dobzhansky 1955:282; Mayr 1963: 613; Stebbins 1966:29-31, 1982:160; Dobzhansky et al. 1977:6, 72).

THE MOLECULAR REVOLUTION

At the same time that mainstream evolutionary biology was emphasizing the role of segregating variation in the evolutionary process, a very different vision of evolution was emerging, starting in the late 1950s, based on comparing protein sequences from different species. A key aspect of this view was a powerful new mode of inference, by which a macromolecular subsequence (part of a gene, protein, or RNA) is inferred to be not "critical" or not "essential" in determining some property of the macromolecule if the subsequence differs among species while the property does not (e.g., Anfinsen 1959:149-155). Evolutionary change was understood as a sequence of distinct amino acid substitutions, each determined by a mutation (e.g., Zuckerkandl and Pauling 1962, 1965; Margoliash 1963). Natural selection was said to "accept" or "reject" mutations, and was invoked mainly in the role of a filter preventing harmful changes (e.g., Eck and Dayhoff 1966:161, 200).

It was in this more favorable climate that an explicit origin-fixation formalism rose to prominence in association with the neutral theory of molecular evolution, which was proposed in parallel by Kimura (1968) and King and Jukes (1969). Indeed, origin-fixation models played an integral role in both the proposal and development of the neutral theory (see Dietrich 1994 for a history of this era).

In his original proposal of the neutral theory, Kimura (1968) argued that the substitutional load (i.e., excess mortality) necessary to account for the observed genome-wide rate of substitution would be unacceptably large unless most substitutions are neutral, noting that for neutral mutations the rate of evolution is equal to the rate of mutation ($K=u$). Whereas some sources (e.g., Charlesworth and Charlesworth 2010) cite Kimura's 1968 paper as the source of an origin-fixation derivation of $K=u$, the origin-fixation formalism is not presented explicitly, and only (arguably) arises if one tries to reconstruct the missing steps in Kimura's argument. The mere presence of the relation $K=u$ does not indicate origin-fixation thinking because $K=u$

holds for neutral evolution under broad conditions, even if one does not assume a mutation-limited regime. Among Kimura's works, the equation $K = 4Nus$ first appears in a subsequent technical paper (Kimura and Maruyama 1969) where it is derived using the origin-fixation formalism.

In contrast to the ambiguity in Kimura's presentation, King and Jukes (1969) derive both $K = u$ and $K = 4Nus$ using a fully explicit, and clearly explained, origin-fixation argument as part of their parallel proposal of the neutral theory. Furthermore, while Kimura's proposal of the neutral theory emerged primarily from a theoretical argument, the proposal by King and Jukes (1969) invoked a variety of empirical arguments based on patterns of molecular evolution, e.g., the observation that the least important parts of molecules seemed to be changing the most rapidly. One such argument was that, for any given protein, the rate of evolution appeared to be remarkably constant (Zuckerkandl and Pauling 1962, 1965), a result that seemed more consistent with the neutral formula $K = u$ than with $K = 4Nus$. Indeed, by 1971, the contrast between a constant neutral rate and a variable selective one had become a leading argument for the neutralists, with this argument often expressed in an explicit origin-fixation framework (Kimura and Ohta 1971a,c; see also Kimura and Ohta 1971b; Kimura 1971).

The introduction of Ohta's theory of slightly deleterious fixations (see Ohta 1992; Ohta and Gillespie 1996 for a review) led to an additional class of origin-fixation models that focused on deleterious, rather than advantageous, fixations. These models were proposed with the positive goal of explaining the observed data better than the simpler model $K = u$ (e.g., Ohta 1973). Note that this was in contrast to the case of $K = 4Nus$, which was used by neutralists as a straw man to argue against, at a time when selectionists themselves had not embraced the concept of evolution as a stochastic proposal-acceptance process (e.g., Maynard Smith 1975:106).

Another notable advance during this era was the introduction of the so-called "shift" models, which extended the calculation of the substitution rate to the situation where the selection coefficients of new mutations are drawn from a distribution rather than taking a constant value (Ohta 1977; Kimura 1979; see also Ohta and Kimura 1971; Kimura and Ohta 1972). The name comes from the fact that, after each non-neutral substitution, the old distribution of fitnesses must shift in order for the new distribution of selection coefficients to remain the same.

Finally, it is important to note that the origin-fixation models used during this period are best understood as aggregate-rate models, even though the assumptions of infinite sites and free recombination underlying such models are not always stated explicitly. For instance, Kimura and Ohta (1971a) state both assumptions, while Ohta and Kimura (1971) state neither, even though the two articles appear back-to-back in the inaugural issue of the *Journal of Molecular Evolution*; of the original papers that derive $K = 4Nus$, Kimura and Maruyama (1969) state only the infinite sites assumption, while King and Jukes (1969) state neither assumption.

Subsequent Development and Current Applications

After featuring prominently in the advent of molecular evolution, origin-fixation models of the aggregate-rate form became a staple of textbook treatments of molecular evolution (Nei 1987; Li 1997; Gillespie 1998; Graur and Li 2000; Charlesworth and Charlesworth 2010). Such models remain in wide use today, with the major innovations being the use of such models to track the change in phenotype or fitness with time (e.g., Hill 1982; Lande 1994), or the incorporation of aggregate-rate components within more complex population-genetic models that also make predictions about polymorphism (e.g., Sawyer and Hartl 1992; Piganeau and Eyre-Walker 2003).

More recent progress in the extension of the origin-fixation framework has centered on the introduction and development of models of sequential fixation. Like the aggregate-rate models, the intellectual roots of models of sequential fixation go back to the molecular revolution. Whereas in the case of aggregate-rate models, the main in-

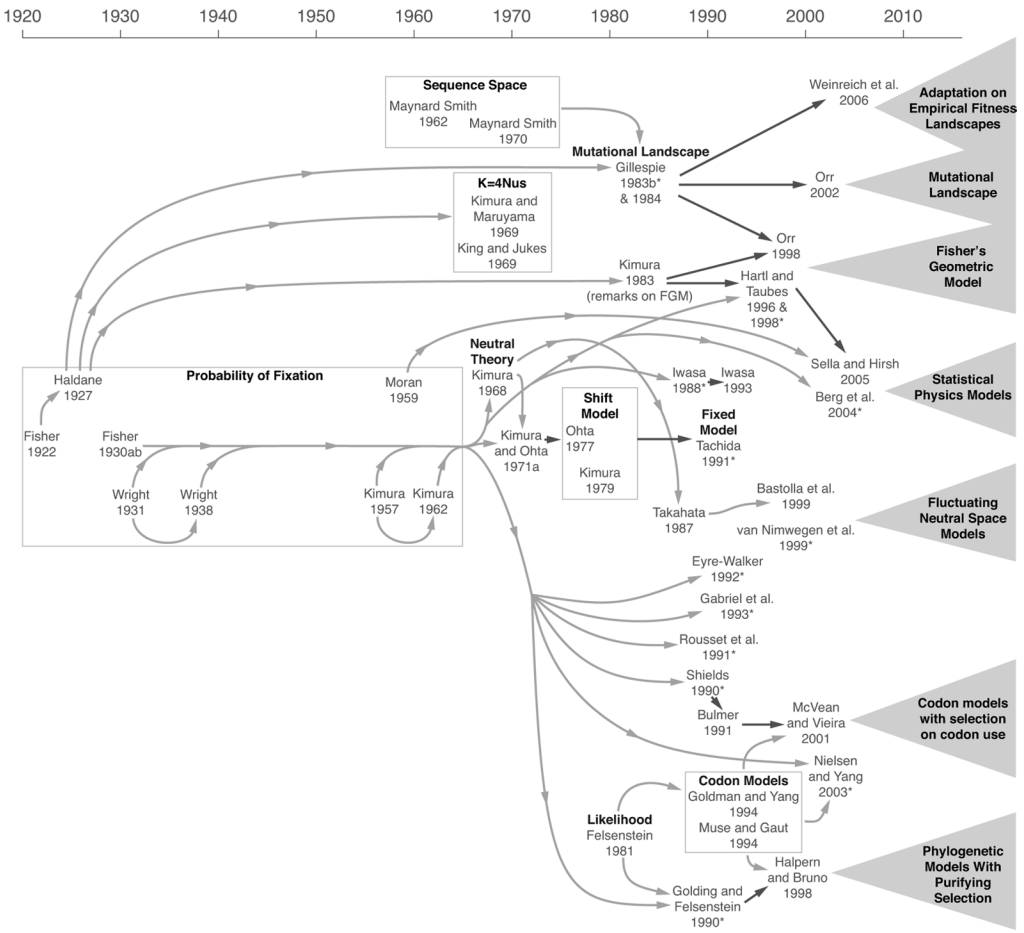

Figure 2. Historical Timeline of Origin-Fixation Models

Light, curved arrows indicate that one publication draws methods from another; dark, straight arrows indicate that one origin-fixation model builds on another. The shaded triangles at the right indicate segments of the contemporary literature that could be represented by multiple publications not listed. Whereas we depict the initial proposal of aggregate-rate models in the 1969 work of Kimura, Maruyama, King, and Jukes, we do not attempt to follow their subsequent influence, as such models are used widely and recognized in textbooks of molecular evolution (see text). Independent proposals of models with sequential fixations are marked with an asterisk (*).

spiration was observations about the tempo (i.e., rate) of molecular evolution, models of sequential fixation instead draw on an observation about its mode: molecular evolution largely occurs by a succession of single amino acid (or later nucleotide) substitutions (Zuckerkandl and Pauling 1962, 1965), so that a population can be viewed as having traced an evolutionary path through "sequence space," a concept first proposed by Maynard Smith (1962, 1970).

Unlike the aggregate-rate models, which can be associated historically and conceptually with the advent of the neutral theory, the history of models of sequential fixation is more complex (see Figure 2). Whereas origin-fixation models using Haldane's $2s$ as the fixation probability clearly originate with Gillespie's pioneering work in the 1980s (Gillespie 1983b, 1984a), models including deleterious or neutral fixations were described independently at least

seven times in the late 1980s and early 1990s (Iwasa 1988; Golding and Felsenstein 1990; Shields 1990; Rousset et al. 1991; Tachida 1991; Eyre-Walker 1992; Gabriel et al. 1993) and arguably several more times subsequently (Hartl and Taubes 1998; van Nimwegen et al. 1999; Nielsen and Yang 2003; Berg et al. 2004; see also the introduction to Ishii et al. 1982; and an early, flawed attempt in Vogel and Zuckerkandl 1971). Thus, models of sequential fixation have been proposed independently or semi-independently so many times between the mid-1980s and today that no single suggestion for a canonical source seems justified. In what follows, we attempt to summarize the complex history of models of sequential fixation by discussing five different clusters of models whose literature and methods have developed relatively independently.

MODELS OF PURE ADAPTATION

The first influential models of sequential fixation, proposed by Gillespie (1983b, 1984a) had two distinctive features. First, they were restricted to beneficial changes, using Haldane's probability of fixation, $2s$. Because the use of this formula assumes that Ns is large, and the use of the origin-fixation formalism implies that Nu is small, they became known as strong-selection weak-mutation (SSWM) models. Second, the fitnesses of genotypes were assumed to be random draws from the tail of a single distribution (implying that the fitness landscape is uncorrelated). This justified the use of extreme value theory, which allowed predictions that were largely independent of the exact form of this distribution (Gillespie 1983b; reviewed in Orr 2005a).

Whereas Gillespie (1983b) was certainly aware of the origin-fixation models of Kimura and others, his SSWM formalism was constructed to cover a broader set of cases, including cases in which the population is typically polymorphic, e.g., models of symmetric overdominance (Gillespie 1983a, 1991). In other words, Gillespie's models (like Wright's 1938 model) converged on origin-fixation dynamics without being derived from earlier origin-fixation models, and without taking the origin-fixation formalism as a starting point.

Gillespie (1984a) introduced the "mutational landscape model," a SSWM model in which each allele has a fixed number of neighbors and each substitution results in a new set of neighbors (studies in this framework typically use a move-rule formalism based on Equation 5). In the mutational landscape model, evolution continues until the population becomes fixed at a genotype whose neighbors are all less fit. In recent years, this model has gained attention due to extensions that address the fitness effects and rank of fixed mutations (Orr 2002, 2005b; Rokyta et al. 2006; Unckless and Orr 2009) or apply to broader classes of fitness landscapes and starting conditions (Orr 2006; Joyce et al. 2008; Jain and Seetharaman 2011).

Another recent body of work retains the SSWM assumptions but dispenses with the assumption that fitnesses are drawn from an unchanged distribution. Often these studies focus on the set of possible adaptive paths (and their relative probabilities) from a given starting point (Weinreich 2005; Weinreich et al. 2005, 2006; Stoltzfus 2006; Unckless and Orr 2009; Draghi and Plotkin 2013). The same approach may be applied to analyze evolutionary dynamics on empirical fitness landscapes, when the fitness is known for all possible subsets of a fixed set of mutations (Weinreich et al. 2006; DePristo et al. 2007; de Visser et al. 2009; Lozovsky et al. 2009; Brown et al. 2010).

A similar set of approaches have been used to analyze an influential model of stepwise evolution known as the "NK" landscape (Kauffman and Levin 1987; Kauffman 1993). Although analyses of this model in the physics literature were originally conducted without a biologically inspired move-rule (Macken and Perelson 1989; Weinberger 1991; Flyvbjerg and Lautrup 1992; Kauffman 1993; Perelson and Macken 1995), these analyses were later adapted to incorporate origin-fixation dynamics within a SSWM framework (e.g., Welch and Waxman 2005; Orr 2006; Stoltzfus 2006; Kryazhimskiy et al. 2009; Jain and Seetharaman 2011). Finally, while the above models all consider a fixed fitness landscape, SSWM models have occasionally also been used to investigate evolution

in a changing environment (e.g., Kopp and Hermisson 2009b).

It is important to note that a SSWM origin-fixation formalism sometimes also appears in the "adaptive dynamics" literature as a step in deriving the canonical equation of adaptive dynamics (Dieckmann and Law 1996; Champagnat et al. 2001). However, the assumption of vanishingly small mutation sizes in adaptive dynamics gives these models a deterministic character that is qualitatively different from other origin-fixation models, so we do not review this literature here.

Various models not restricted to advantageous fixations

As was mentioned earlier, models of sequential fixations that include deleterious and neutral fixations arose independently many times, starting in the late 1980s and early 1990s. These scattered introductions lead to a number of distinct literatures; we review a few of the smaller literatures here, while tackling the larger literatures in the next several sections.

One set of models of sequential fixation was focused on understanding the statistical properties of sequence evolution. The first of these models emerged out of the analysis of the so-called "fixed" model (Ohta and Tachida 1990), where the fitness of each new mutant is drawn at random from a fixed distribution (this model is also known as the "house of cards model"; Kingman 1977, 1978) in contrast to the "shift" models discussed earlier (Ohta 1977; Kimura 1979). The introduction of this new class of models paralleled a change in Ohta's emphasis from deleterious fixations (Ohta 1973) to compensatory or nearly neutral evolution involving a mix of slightly deleterious and slightly advantageous fixations (Ohta 1992). Subsequently, origin-fixation models with sequential fixations were introduced to analyze the fixed model in the weak-mutation limit (Tachida 1991, 1996; Gillespie 1994b). Notable later developments include the use of an origin-fixation model to explore the relationship between the NK model and the fixed model (Welch and Waxman 2005), and the use of an origin-fixation model to investigate a broader class of models that subsumes both the shift and fixed models (Kryazhimskiy et al. 2009).

A related set of origin-fixation models was focused on explaining the index of dispersion of the molecular clock (e.g., Gillespie 1984b, 1986), which is a measure of how irregularly substitutions occur in time. The main early contribution to this question using a model of sequential fixations was made by Iwasa (1993; drawing on Iwasa 1988), who suggested that the substitution rate tends to increase after deleterious fixations. A different, neutralist, explanation was given by Takahata's (1987) fluctuating neutral space model, which proposed to explain the overdispersion of the molecular clock by invoking a change in the space of neutral possibilities (and, thus, the neutral mutation rate) after each neutral substitution. A clear mechanistic justification for this second type of model was provided when Bastolla and colleagues (Bastolla et al. 1999, 2002, 2003a,b) argued, on biophysical grounds, that different protein sequences will have different numbers of mutational neighbors that share the same fold and, hence, will have different neutral substitution rates. However, neither Takahata nor Bastolla and colleagues invoked an origin-fixation framework. Meanwhile, van Nimwegen et al. (1999) proposed a model of sequential fixations (for a population evolving on a network of selectively neutral alleles embedded within a larger space of highly unfit alleles) that could be interpreted as being isomorphic with the earlier phenomenological models by Takahata and by Bastolla et al., although this connection was not noted. Finally, Wilke (2004) put the pieces together and pointed out that the earlier efforts by Bastolla and Takahata could be construed in origin-fixation terms using the framework of van Nimwegen et al. (1999). Subsequently, studies in the same vein (Bloom et al. 2007; Raval 2007), including by Bastolla and colleagues (Bastolla et al. 2007; Bastolla 2014), have invoked origin-fixation dynamics explicitly.

Another set of origin-fixation models arising from the literature of the early 1990s were models of sequential fixation meant to

address the evolution of codon bias, particularly the simple two-allele case with preferred and nonpreferred codon types (Shields 1990; Bulmer 1991). Although the simplicity of this framework led to its re-use (e.g., in Eyre-Walker and Bulmer 1995; Takano-Shimizu 1999; Charlesworth and Eyre-Walker 2007; Kondrashov et al. 2010), the two-allele case is so simple that the origin-fixation formalism is unnecessary: a full diffusion-based analysis of population dynamics is possible and provides greater insight (McVean and Charlesworth 1999). Similarly, Rousset et al. (1991) proposed a simple origin-fixation model for the coevolution of paired bases in noncoding RNA molecules, but subsequent work on this problem from a population-genetic perspective has tended to be based on explicit two-locus models that include recombination (following Kimura 1985; Higgs 1998).

Several other models of sequential fixation were also proposed in the early 1990s. Eyre-Walker (1992) proposed an early neutral origin-fixation model in the process of arguing that increasing constraint can sometimes increase the rate of evolution. Gabriel et al. (1993) discussed a model of Muller's Ratchet in which deleterious fixations accumulate by sequential fixations. Force et al. (1999) and Lynch and Force (2000) later went on to use a neutral model of sequential fixations in their analysis of the duplication-degeneration-complementation (DDC) model for the preservation of duplicate genes in the limit of low mutation rates.

We also note in passing that, just as one can derive adaptive dynamics models by assuming that the probability of fixation is proportional to the selective advantage and all mutations have infinitesimally small effects, one also can modify such models to include deleterious fixations, which yields a diffusion process rather than the deterministic dynamics that appear when only advantageous fixations are considered. Oddly, this type of model has been proposed only rarely (although see Proulx and Day 2001; Day and Otto 2007:655, 667).

APPLICATIONS TO FISHER'S GEOMETRIC MODEL

In a famous argument for Darwinian infinitesimalism, Fisher (1930a) imagined a multidimensional phenotypic space in which fitness is a decreasing function of the distance from an arbitrary optimum, concluding that the smallest mutations were the most likely basis of evolutionary change on the grounds that, starting from a suboptimal phenotype, a smaller change in a random direction is more likely to be beneficial, particularly when the phenotypic space is high-dimensional. This framework was revived in the modern literature by Kimura (1983), who noted that if one assumes that selection coefficients for new mutations are proportional to the size of a mutation's effect on phenotype, then in fact mutations with intermediate effect sizes have the highest joint probability of being beneficial and escaping random loss.

Many subsequent authors have addressed Fisher's geometric model in Kimura's origin-fixation framework, where only a single substitution is considered (Hartl and Taubes 1996; Welch and Waxman 2005; Martin and Lenormand 2008; Chevin et al. 2010). As a further development, models of sequential fixation that consider the longer-term dynamics of evolution under Fisher's geometric model were introduced by Hartl and Taubes (1998) and Orr (1998). Subsequent models of sequential fixations have been posed both in terms of models of adaptation toward the optimum using Haldane's $2s$ (as in Orr 1998, 2000; Welch and Waxman 2003), and as models of nearly neutral evolution that include deleterious fixations (Hartl and Taubes 1998; Poon and Otto 2000; Sella and Hirsh 2005; Gros and Tenaillon 2009; Gros et al. 2009; Sella 2009; Lourenço et al. 2011; Razeto-Barry et al. 2011). Many of the latter class of studies have discussed the difference between the fitness at the optimum and the expected fitness of a population at equilibrium (the drift load) as a function of population size and the number of dimensions in the phenotypic space; Tenaillon et al. (2007) were able to use these results to estimate empirically the dimensionality of the pheno-

typic space for two viruses. Barton (2001) and Gu (2007a,b) also treat Fisher's geometric model, but in something closer to an aggregate-rate framework.

Models from phylogenetics

The models described in this section and the next rely on the fact that models of sequential fixation can be easily configured to satisfy a technical characteristic known in mathematics as reversibility, which refers to a property of a Markov chain at equilibrium when time is reversed. Allowing each type of transition to be reversed at some rate is a necessary (though not sufficient) condition for reversibility. Therefore, a model cannot be reversible if it includes only categorically deleterious changes, or only categorically beneficial ones.

In molecular phylogenetics, it is convenient to have a model of sequence evolution that takes the form of a reversible Markov chain because the likelihood of any particular phylogeny under such a model can be found efficiently using Felsenstein's (1981) pruning algorithm. This made origin-fixation models an obvious choice to be incorporated into models of phylogenetic reconstruction.

The first phylogenetic model to include an explicit origin-fixation formalism was by Golding and Felsenstein (1990). This inspired Halpern and Bruno (1998; cf. Bruno 1996) to construct an influential model that assigns—to each amino acid in a particular position—a scaled selection coefficient (Ns), with the transition rates between codons determined by multiplying the mutation rate by the probability of fixation (see also Rodrigue et al. 2010; Tamuri et al. 2012, 2014; Rodrigue 2013). Similar methods have also been applied to investigate selection for codon usage by assigning a fitness to each codon regardless of its place in the protein (McVean and Vieira 2001; Nielsen et al. 2007; Yang and Nielsen 2008) and adapted to study the evolution of transcription factor-binding sites (Moses et al. 2003; Doniger and Fay 2007; Kim et al. 2009).

We note that an explicit origin-fixation argument is not always presented in papers inspired by the Halpern and Bruno (1998) model. This is because their Equation 10 allows the construction of a rate matrix incorporating natural selection given just a mutational rate matrix and an observed vector of equilibrium frequencies under selection. Some models derived from the Halpern and Bruno (1998) model (e.g., Moses et al. 2004; Wong and Nielsen 2007) simply use this equation rather than recapitulating the entire model. A special case of this relation also was rediscovered, in an origin-fixation framework, by Knudsen and Miyamoto (2005), who related it to generalized weighted frequencies ("+gwF") models (Goldman and Whelan 2002).

Another approach influenced by Halpern and Bruno (1998) attempts to incorporate knowledge about the structure of evolving macromolecules to make phylogenetic inferences as well as inferences about the strength of selection. In this approach, each possible nucleic acid sequence is assigned a scaled selection coefficient based on its compatibility with a specified, and presumably selectively favored, protein structure or RNA secondary structure (Robinson et al. 2003; Yu and Thorne 2006; Choi et al. 2007, 2008; Thorne et al. 2007; Rodrigue et al. 2009; Pollock et al. 2012; reviewed by Thorne et al. 2012), so that the probability of fixation of each mutant depends on its predicted effects on the structure of the relevant macromolecules.

Origin-fixation models have also been used to interpret the parameter known as ω (omega), which can be viewed as the ratio of the fixation probabilities of nonsynonymous versus synonymous substitutions (Nielsen and Yang 2003; Subramanian 2013; see also Kryazhimskiy and Plotkin 2008), and have been used to understand the impact of long-term stabilizing selection and biased gene conversion on phylogenetic inference (Lawrie et al. 2011). Speaking more broadly, the rise of origin-fixation models in phylogenetics is best seen as part of the increasing popularity of "mechanistic" models that attempt to directly model certain causal aspects of the evolutionary process, rather than treating evolutionary rates as purely phenomenological (see the recent review by Rodrigue and Philippe 2010).

MODELS BASED ON STATISTICAL MECHANICS

In addition to being useful in a phylogenetic context, reversible origin-fixation Markov chains are easy to analyze using a suite of techniques from statistical physics, an observation that has been noted independently on at least three occasions (Iwasa 1988; Berg and Lässig 2003; Berg et al. 2004; Sella and Hirsh 2005; see also Vogel and Zuckerkandl 1971). In particular, in the special case in which a mutational model that would be reversible in the absence of natural selection is used and each allele is assigned a fixed fitness, the choice of any standard form of the fixation probability (as long as it allows both advantageous and deleterious fixations) results in a reversible origin-fixation Markov chain. One may then view fitness as being analogous to the negative of the energy of a state, and population size as being analogous to inverse temperature, so that the stationary distribution for the evolutionary Markov chain corresponds to a Boltzmann distribution, and a quantity, analogous to free energy, can be defined whose expectation is nondecreasing over time.

Whereas these properties are really properties of reversible Markov chains in general and not origin-fixation models per se (Iwasa 1988; Barton and Coe 2009), the simple and tractable description of the origin-fixation dynamics and equilibrium distribution presented by Berg et al. (2004) and Sella and Hirsh (2005) have proved useful in a variety of contexts. These include studying the evolution of transcription factor-binding sites (Mustonen and Lässig 2005; Mustonen et al. 2008; Nourmohammad and Lässig 2011), codon usage bias (Wilke and Drummond 2006; Gilchrist 2007; Gilchrist et al. 2009; Shah and Gilchrist 2011), protein evolution (Mendez et al. 2010; Ramsey et al. 2011; Pollock et al. 2012), deleterious mutation accumulation (Tenaillon et al. 2007), the evolution of epistasis (Gros and Tenaillon 2009; Gros et al. 2009), gene network evolution (Le Nagard et al. 2011), Fisher's Geometric Model (Sella 2009), and evolutionary dynamics on high-dimensional fitness landscapes (McCandlish 2011, 2013).

DISCUSSION

In origin-fixation models, evolutionary change consists of events in which a variant is introduced into a population by mutation, and then is accepted or rejected based on its individual effects. Such models seem obvious today, yet they remained undiscovered for over 40 years after initial work on the probability of fixation. Through a complex and uneven process that began with the proposal of the neutral theory, origin-fixation models have risen to prominence and now represent a widely used branch of theoretical evolutionary genetics.

Above, we reviewed the history, development, and technical uses of these models without addressing their validity or realism. In fact, the rise in popularity of origin-fixation models (with a few exceptions) has not been accompanied by increased attention to their problematic assumptions and distinctive implications. Instead of being treated as a conjecture subject to evaluation, they are invoked instrumentally, as though they were a generic tool that may be inserted into a method of inference without introducing any dangers.

However, the true dynamics of evolutionary change are shaped to an unknown degree by phenomena that origin-fixation models simply ignore. Various phenomena that depend intrinsically on polymorphism may become relevant when the weak-mutation assumption fails. Examples include the evolution of quantitative traits based on abundant standing variation, overdominance (which can result in prolonged polymorphism), stochastic tunneling (where mutations that arise on a deleterious background sweep to fixation, e.g., Iwasa et al. 2004; Weinreich and Chao 2005), selection for mutational robustness (van Nimwegen et al. 1999), and interference between multiple beneficial mutations at either a single locus (Gerrish and Lenski 1998; Desai and Fisher 2007; Rouzine et al. 2008) or several partially linked loci (Hill and Robertson 1966).

Even when the weak-mutation assumption holds, there are other reasons to question the validity of origin-fixation models. Gillespie (2004) argues that selection coefficients do

not remain sufficiently constant over time to justify a model of fixation based on a single selection coefficient. Origin-fixation models neglect the time that it takes for a new mutation to reach fixation (see Figure 1), and thus may miscalculate the rate of evolution at short time scales (Stephan and Kirby 1993). The same approximation means that incomplete lineage sorting (i.e., the persistence of a polymorphism through a speciation event), relevant in the context of phylogenetic analysis, is overlooked.

By ignoring the complications of within-population processes, origin-fixation models provide a formal view of the evolutionary traversal of a genotypic or phenotypic space via multiple changes. Yet, the same assumption that makes the origin-fixation formalism useful also guarantees that it is not a generic model of the evolutionary process, but a specialized model—specialized for a particular regime of "mutation-limited" evolution that side-steps various phenomena considered important in theoretical population genetics. In strict terms, this regime is narrow and very difficult to justify, yet the same might be said for the mid-20th-century orthodoxy of population genetics (noted above, and described further below). Because of the prevalence of origin-fixation models in the contemporary literature, it is important to determine the extent to which this origin-fixation regime actually occurs in nature.

Thus, in the following two sections, we treat origin-fixation models, not merely as a useful technical innovation, but as a theory of evolution whose importance is subject to investigation. To invoke an origin-fixation model is to invoke a particular mode of evolutionary change with distinctive implications; these implications may be used to evaluate the extent to which the true evolutionary dynamics fall within the origin-fixation regime.

DISTINCTIVE IMPLICATIONS OF ORIGIN-FIXATION MODELS

By merit of both their assumptions and their mathematical form, accepting origin-fixation models as an accurate representation of evolution in nature would have definite implications for our understanding of the evolutionary process. Here, we focus on three implications regarding the rate of evolution, the direction of evolution, and the role of selection.

The first implication is that, under origin-fixation models, the rate of evolution is directly proportional to the mutation rate. In a simple aggregate-rate model, doubling the rate of mutation doubles the rate of evolution; doubling the mutation rate in a simple model of sequential fixations generally results in behavior equivalent to the original model run at twice the speed. However, by embedding an origin-fixation model within a larger model that includes environmental change, one may discover conditions under which most fixations occur after an environmental change, so that the long-term rate of evolution is decoupled from the mutation rate and is instead controlled by the rate of environmental change (e.g., Gillespie 1984b, 1991, 2004; Razeto-Barry et al. 2012). Nonetheless, in such models the short-term rate of adaptation *during* the adaptive transient remains proportional to the mutation rate.

The second major implication is that biases in mutation rates can bias the course of evolution, including adaptive evolution. For example, consider a model of sequential fixations in which our population is currently fixed at allele i and can mutate to a variety of other alleles. The odds that the population will next become fixed at allele j rather than allele k (based on the move-rule in Equation 5) are given by

$$\frac{P(i,j)}{P(i,k)} = \frac{2Nu_{ij}\pi(s_{ij},N)}{2Nu_{ik}\pi(s_{ik},N)} = \frac{u_{ij}}{u_{ik}} \times \frac{\pi(s_{ij},N)}{\pi(s_{ik},N)}. \tag{6}$$

This can be described as the product of two factors: a ratio of mutation rates and a ratio of fixation probabilities. For the case of beneficial fixations, this can be reduced to

$$\frac{P(i,j)}{P(i,k)} = \frac{u_{ij}}{u_{ik}} \times \frac{s_{ij}}{s_{ik}}, \tag{7}$$

by assuming that the probability of fixation is well-approximated by $2s$ (see Yampolsky and Stoltzfus 2001). Thus, doubling the mutation rate to an allele or doubling its probability of fixation both have the same effect on the odds that it is the next allele to be fixed. This

means that a sustained bias in mutation can cause a long-term trend, even when all fixations are due to selection (Stoltzfus 2006). Note that one could construct analogous formulas for aggregate-rate models that would address the ratio between the rates of evolution for two different classes of mutations, e.g., transitions versus transversions or insertions versus deletions.

Equations of this general form provide a useful framework for assessing the relative importance of mutation and selection and, more specifically, for comparing the impact of biases in the origination process to the impact of biases in the fixation process (Yampolsky and Stoltzfus 2001; Stoltzfus and Yampolsky 2009; Farlow et al. 2011; Streisfeld et al. 2011). For instance, Streisfeld et al. (2011) use Equation 7 as the framework for a test of mutational and selective causes of a bias in the observed pattern of adaptive changes in floral evolution. A related result, discussed further below, is the finding of Rokyta et al. (2005) that both biases in mutation and differences in fixation probability affect the chances of parallel evolution in a laboratory phage population.

The final major implication of origin-fixation models concerns the role of natural selection in evolution. Clearly, in these models, selection acts as a stochastic sieve or filter that accepts or rejects new mutations one at a time depending on their fitness effects (and the population size).

These implications of origin-fixation models are not shared by all theories of evolution. In particular, the mid-20th-century orthodoxy of population genetics rejected each of the above implications on the grounds that evolution is intrinsically polygenic and epistatic (reliant on gene interactions): "Natural selection directs evolution not by accepting or rejecting mutations as they occur, but by sorting new adaptive combinations out of a gene pool of variability which has been built up through the combined action of mutation, gene recombination, and selection over many generations" (Stebbins 1966:31; see also Dobzhansky et al. 1977:6; Mayr 1963: 613; Simpson 1964:1536).

First, in this view, the buffering effect of the "gene pool" means that change is initiated by the environment (not by a new mutation), and the rate of evolution is unlinked from the mutation rate (e.g., Dobzhansky et al. 1977:77; Stebbins 1966:29; Mayr 1994:38), a view that led some to oppose the idea of a molecular clock (see Dietrich 1998). Second, in regard to the causes of orientation or direction in evolution, the mid-20th-century consensus holds that "selection is the only direction-giving factor in evolution" (Mayr 1980:3), i.e., the course of evolution is determined externally, by the environment, whereas the contrary notion of any intrinsic sources of direction is dismissed (e.g., Simpson 1967: 159). Various authors invoked theoretical population genetics to support this position (reviewed in Yampolsky and Stoltzfus 2001). Based on the equation for the equilibrium gene frequency in an infinite population at mutation-selection balance, Fisher argued that mutation cannot control the direction of evolution, on the grounds that mutation rates are small compared to selection coefficients (Fisher 1930a,b; see also Wright 1931; Haldane 1933). This argument, invoking the relative weakness of the "force" of mutation, was repeated later by others (Huxley 1942: 56; Mayr 1960:355; Simpson 1967:159; Gould 2002:509).

Importantly, the logic of mutation and selection as opposing pressures is repeated in textbooks and used by researchers, leading to the common assumption that biases in variation are ineffectual except when the opposing pressure of selection is effectively absent, i.e., the special case of neutral evolution (e.g., as in the rationale given in Maynard Smith et al. 1985:282; for other examples, see Stoltzfus 2006). Clearly, the origin-fixation view, via Equation 6, has a completely different implication: a bias toward a particular type of evolutionary change may reflect a bias in mutational origin, whether fixations occur by selection or drift.

Finally, in regard to the role of selection, the mid-20th-century consensus rejects the idea that selection acts as a sieve or filter on separate mutations, a notion that is condemned as "downright misleading" on the grounds that, due to epistasis in a polymorphic context, an allele has no fixed meaning, independent of the rest of the gene pool

(Dobzhansky 1974). Instead, natural selection is understood as an artist or creative agent (see Chapter 4 of Gould 1977), with the gene pool supplying the abundant raw materials for selection to craft an adaptive response to any change in conditions.

The contrast between these two positions, only rarely treated as a multifaceted clash of worldviews (e.g., Nei 2013), is suggested more subtly in the literature on the genetics of adaptation which frequently references a distinction between new mutations and standing variation (Orr and Betancourt 2001; Hermisson and Pennings 2005; Barrett and Schluter 2008; Pritchard and Di Rienzo 2010; Jerome et al. 2011; Nuismer et al. 2012; Olson-Manning et al. 2012). Some authors who emphasize the role of standing variation have suggested that "[m]ost of the current theory on the genetics of adaptation assumes that adaptation occurs exclusively from new mutations rather than from standing variation" (Barrett and Schluter 2008:39; see also Hermisson and Pennings 2005). Indeed, such an assumption is evident in Orr's (2002) characterization of three possible move-rules for evolutionary walks. After describing three such rules—choose the fittest neighbor (the perfect or greedy rule), choose any fitter neighbor at random (the random rule), or choose a fitter neighbor with a probability proportional to the fixation probability for a new mutation—Orr refers to the first two as "idealized" rules, and to the third as "adaptation by natural selection," as though this were the only possible implication of selection in evolutionary theory. Instead, the mid-20th-century orthodoxy definitively rejects any single-locus move-rule, and suggests instead that evolution in nature works by something like a multilocus greedy rule (crafting an optimal combination of allele frequencies from the diversity of the gene pool).

It is also useful to compare evolution in the origin-fixation regime to neutral evolution. Of course, there is a precise overlap for the case of origin-fixation models in which fixation takes place by drift, but many simple neutral models that allow polymorphism share the implications described above. In such models, the rate of evolution is proportional to the (neutral) mutation rate, the direction of evolution is determined by mutational biases in mutation, and natural selection acts as a sieve that removes deleterious mutations.

More generally, however, neutral evolution is a different kind of concept than the origin-fixation regime. Whether neutral evolution will happen is mainly an issue of the sizes of fitness differences in relation to population size, while the relevance of the origin-fixation regime is an issue of the relationship of mutation rates to population size and, for aggregate-rate models, independence between loci. Just as there are origin-fixation models that are not neutral models (e.g., the mutational landscape model), one may construct models of neutral evolution that depend specifically on phenomena of polymorphism excluded from origin-fixation models. For instance, Kimura's model of compensatory neutral evolution invokes a new mutation that occurs in the background of a segregating deleterious allele, reversing its deleterious effect (Kimura 1985). This model was introduced specifically because the rate of paired changes (e.g., in structural RNAs) was too high to reflect the rate of an origin-fixation process based on double mutations.

The general implications of origin-fixation models overlap most easily with the Mendelian-mutationist view of evolution developed a century ago by early geneticists such as Punnett, Johannsen, Bateson, and Morgan. Their view allowed smooth Darwinian change, but also emphasized the creative and dispositional role of mutation (via discrete effects, mutational biases, and the uniqueness of individual variants), with selection often cast in the role of a filter that accepts or rejects mutations (Stoltzfus and Cable 2014). The architects of the 20th-century orthodoxy believed that they could dispense with the importance of individual mutations for determining the direction of evolution—what Fisher (1930a) called "evolution worked by mutation" (Chapter 1)—on the grounds that, via recombination among segregating alleles, selection can move a population well beyond its initial phenotypic range, without any new mutations (Stoltzfus and Cable 2014; see also Provine 1971:108-129). This is why mid-20th-century writings are rich in statements (some quoted above) that reject

the origin-fixation view, even before it was formalized in 1969: when the architects of this orthodoxy were arguing for their distinctive "shifting gene frequencies" view throughout the 1960s and 1970s, they were arguing against both the earlier Mendelian-mutationist view (Stoltzfus and Cable 2014) and the emerging "molecular evolution" view. However, the degree to which evolution can be viewed as an origin-fixation process cannot be established by either verbal plausibility arguments or mathematics, but must be determined empirically.

TESTING THE ORIGIN-FIXATION HYPOTHESIS

As explained in the previous section, origin-fixation models have distinctive implications because they reflect a distinctive set of assumptions about how evolution actually works. Thus, an important research program for the future is to assess the extent to which evolution in nature may be characterized as an origin-fixation process.

Unfortunately, the set of systems that origin-fixation models have been applied to in the literature provides little insight into this problem. For instance, in the case of modeling sequence evolution in *Drosophila* species, both theory (Durrett and Schmidt 2007) and empirical observations (e.g., the appearance of independent, single nucleotide insecticide residence mutations in Karasov et al. 2010) indicate that evolution is not under weak mutation even for regions of the genome that are only a few nucleotides long. Yet, origin-fixation models are often applied to make inferences, not only about *Drosophila* but also unicellular microorganisms with even larger population sizes (e.g., yeast and *E. coli*) for which the weak-mutation assumption is even less likely to apply. How, then, can one test whether a particular system is evolving according to an origin-fixation process?

One approach is to begin with a theoretical understanding of conditions that would justify an origin-fixation model and then assess whether those conditions hold for a particular evolving system. This is problematic even when the relevant population parameters can be estimated because origin-fixation models often are employed in situations where they cannot be subsumed within a more general population-genetic model that is sufficiently tractable to derive the relevant condition for their validity.

Furthermore, in the few simple models where we do understand the boundaries of the origin-fixation regime, these boundaries do not depend solely on genome-wide characteristics like population size and mutation rate. For instance, while the strict condition $8N^2u << 1$ mentioned earlier for the validity of sequential fixations is meant to preclude the possibility of complications of polymorphism, the degree to which the origin-fixation regime extends to situations that violate this condition depends on the structure of the fitness landscape (Kim and Orr 2005; Desai and Fisher 2007; Kopp and Hermisson 2007, 2009a; Rouzine et al. 2008; Weissman et al. 2009; Park et al., 2010). At the same time, the condition $8N^2u << 1$ is based on the assumption that there is no heterozygote advantage (which could result in prolonged polymorphism); the validity of this assumption again depends on the structure of the genotype-phenotype-fitness map (Sellis et al. 2011). A similar situation arises for aggregate rate models, where deviations from the aggregate rate predictions due to linkage and epistasis will also depend on the distribution of selection coefficients introduced by mutation (Barton 1994, 1995; Stephan et al. 1999; Neher et al. 2010; Weissman and Barton 2012; see also Neher 2013). Finally, there remains the aforementioned concern, relevant to both classes of models, that selection coefficients remain sufficiently constant over the course of a substitution (see Gillespie 2004).

Fortunately, there are other ways to assess the validity of an origin-fixation model, including (1) using simulations to clarify the limits of the origin-fixation regime, (2) comparing the likelihood of an origin-fixation model to that of another model, when applied to data from natural populations, (3) evaluating the internal consistency of an origin-fixation model when applied to data from natural populations, and (4) rare cases in which experimental data are of sufficient depth to allow a test.

Simulations are likely to be extremely use-

ful in determining the applicability of origin-fixation models because they overcome many of the difficulties with the strategy based on analytically deriving the limits of the origin-fixation regime. Even if a population-genetic model is too complex to derive some simple, analytical condition for the boundaries of the origin-fixation regime, violations of origin-fixation behavior should still be easy to detect by simulation.

In some cases, it may be possible to compare an origin-fixation model with a more complex population-genetic model to determine which model provides a better fit to data on natural divergence. Such an approach has become possible only recently, due to the advent of phylogenetic models that can accommodate both polymorphism and mutation (e.g., Bryant et al. 2012, which is limited to biallelic sites; or De Maio et al. 2013, which also includes selection). As these methods mature, a broader range of comparisons will become possible.

A different approach is provided by tests of internal consistency, which provide a way to evaluate origin-fixation models without requiring a fully specified alternative model. An example of such a test would be to assess the effect of transition-transversion bias in the context of phylogenetic inference methods. Such methods often implement a fitted parameter for transition-transversion bias, which in the case of origin-fixation models (e.g., Yang and Nielsen 2008) is understood as a bias in the origination process. When strict origin-fixation dynamics apply to all evolutionary changes, it will not matter if (for instance) the synonymous changes are mostly neutral and the nonsynonymous ones are mostly beneficial: the dependence of rates on the mutational bias factor (as in Equation 6) is independent of the mode of fixation. Thus, if the transition-transversion bias parameter is inferred separately for two different categories of changes (e.g., synonymous versus nonsynonymous) the inferred values should be the same.

Finally, in some cases, an origin-fixation model may be evaluated experimentally. For instance, Rokyta et al. (2005) repeatedly carried out one-step adaptation with a bacteriophage, comparing the results to the predictions of Orr's (2002) mutational landscape model. Orr's model provided a good fit to the data, but only after biases in mutation rates (absent in the original model) were taken into account. In fact, the best-fitting model did not employ the distinctive features of the mutational landscape model (e.g., extreme value theory), but was simply the origin-fixation move-rule (Equation 5) with empirical parameter values (mutation rates and selection coefficients) and an improved formula for the probability of fixation. A number of studies conclude against origin-fixation dynamics based on deviations from origin-fixation predictions (e.g., de Visser et al. 1999; Miralles et al. 1999), however, for studies where multiple fixation events have occurred, care must be taken to avoid rejecting too simplistic an origin-fixation model, e.g., one that does not include epistasis. This problem can be circumvented by directly observing competition between multiple segregating mutations (Kao and Sherlock 2008; Lang et al. 2013). This second approach is probably preferable and should become increasingly practical as sequencing costs continue to fall.

The application of the approaches described above, we suspect, will show that many systems previously analyzed using origin-fixation models are not evolving in a pure origin-fixation regime. However, inferences made from these models are not necessarily incorrect: there must be conditions under which origin-fixation models provide a rough approximation to the true evolutionary dynamics, and this regime of approximate applicability may be quite large. Thus, another important area for future research is to assess the strengths and weaknesses of origin-fixation models in those the regions of population-genetic parameter space in which their behavior is approximate, with a view to identifying their suitability for specific purposes.

In general, studies to address the robustness of origin-fixation models to violations in the underlying assumptions will have to be tailored to specific inferences. This is because many different behaviors are possible outside the origin-fixation regime and these different

behaviors will have different effects on different inferences (e.g., incomplete lineage sorting may be relevant to inferring phylogenies but irrelevant to studies of adaptation).

Nonetheless, developing a qualitative understanding of how certain simple phenomena change as one leaves the weak-mutation regime may still be worthwhile. For instance, Yampolsky and Stoltzfus (2001) showed that, as the weak-mutation assumption for advantageous mutations breaks down, the influence of mutational biases on the direction of evolution is reduced relative to origin-fixation expectations (Equation 7) because natural selection is very effective at increasing the frequency of the more fit of a pair of advantageous mutants that are simultaneously segregating in a single population. However, it is currently unknown what would occur in the more general context of Equation 6, e.g., for the case of deleterious mutations or a mixture of deleterious and advantageous mutations. This type of basic understanding could be broadly useful in understanding the implications of violating the weak-mutation assumption.

A final issue relevant to hypothesis testing is whether the predictions of origin-fixation models are unique. Many of the distinctive implications of origin-fixation models would still apply if the rate of evolution was merely proportional to (rather than equal to) the product of (1) the rate of mutational origin and (2) some function of the selection coefficient. As discussed above, these conditions hold for some neutral models, which explains certain similarities between the implications of neutral evolution and the implications of evolution in the origin-fixation regime. It is therefore important to determine the broader set of population-genetic conditions outside the origin-fixation regime where some subset of the basic implications of origin-fixation models continue to hold.

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