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REVIEW

The fixation probability of beneficial mutations

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The fixation probability, the probability that the frequency of a particular allele in a population will ultimately reach unity, is one of the cornerstones of population genetics. In this review, we give a brief historical overview of mathematical approaches used to estimate the fixation probability of beneficial alleles. We then focus on more recent work that has relaxed some of the key assumptions in these early papers, providing estimates that have wider applicability to both natural and laboratory settings. In the final section, we address the possibility of future work that might bridge the gap between theoretical results to date and results that might realistically be applied to the experimental evolution of microbial populations. Our aim is to highlight the concrete, testable predictions that have arisen from the theoretical literature, with the intention of further motivating the invaluable interplay between theory and experiment.

Keywords: population genetics; fixation probability; extinction probability

1. INTRODUCTION

Mathematical population genetics is a field with an extremely rich historical literature. The first questions about gene frequency distributions were posed in analytical form by Fisher; independent studies were conducted by Wright and Haldane. Fisher, Haldane and Wright together shaped the foundations of the field and are referred to as the 'great trinity' (Crow 1994) of population genetics. The works of these authors (Fisher 1922, 1930; Haldane 1927; Wright 1931) are now considered to be the classic papers in the field.

One of the central ideas addressed by these authors is the fixation probability: the probability that the frequency of a particular allele in a population will ultimately reach 100 per cent. Mathematically, there are several approaches to computing fixation probabilities, and interest in this problem has been sustained for almost a century: the first papers were written in the early 1920s, and there have been important advances in every decade since. Empirically, the fixation probability is necessary in order to estimate the rate at which a population might adapt to a changing environment, the rate of loss of genetic diversity or the rate of emergence of drug resistance.

The last several years have seen two key advances in this field. First, a number of important, and fascinating, theoretical advances have been made, each bringing us one step closer to theoretical predictions that might pertain in a 'real' laboratory population. Second, in parallel with this effort, experimental techniques in microbial evolution have advanced to the point where the fate of a novel mutant strain within a controlled population can be followed over many generations. Thus, these experiments are on the verge of being able to test our theoretical predictions of the fixation probability—predictions that have in many cases stood untested for 80 or 90 years. This is extremely exciting.

Although neutral and deleterious mutations may also reach fixation in finite populations, in the following review we will restrict our attention to beneficial mutations. The selective advantage, s, of a beneficial mutation is typically defined for haploids as follows: if each wild-type individual has on average W offspring per generation, each mutant individual has on average $W(1+s)$ offspring. Throughout this review we will assume that this definition of s holds, unless stated otherwise. For simplicity, for diploid individuals we will use s to denote the advantage of the heterozygote, although the notation hs is also typically used.

In a deterministic model, an initially rare beneficial mutation will increase in frequency in each generation, and fixation is certain. In reality, however, the frequency of any particular lineage fluctuates over time. These fluctuations, 'genetic drift', are very likely to cause the extinction of a beneficial lineage when its frequency is low, and require a stochastic treatment. Once the frequency of the mutant is sufficiently large, further increases are well approximated by a deterministic model. Estimating the fixation probability for a beneficial mutation is thus usually equivalent to estimating the probability that the mutation survives genetic drift when initially rare.

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The underlying distribution of s, i.e. the distribution of selective effects for all possible beneficial mutations, is a topic of current interest, both theoretically and experimentally. Although beyond the scope of this review, we refer the interested reader to several recent papers (Rozen *et al.* 2002; Orr 2003; Rokyta *et al.* 2005; Kassen & Bataillon 2006). A closely related, or even overlapping, issue is adaptation: the rate of fitness increase or overall rate at which beneficial mutations arise and become fixed. While fixation probabilities are essential building blocks in the models of adaptation, such models also require further assumptions, such as an underlying distribution of selective effects or a model for combining the effects of multiple mutations. Estimating the rate of adaptation has a rich literature in its own right, and again we refer the interested reader to a few key references (Orr 1994, 2000; Wilke 2004; Desai & Fisher 2007; Goncalves *et al.* 2007). We touch on this issue again in §5.3.

2. HISTORICAL OVERVIEW

Broadly speaking, there are three approaches to computing fixation probabilities. When the state space of a population (exactly how many individuals have exactly which genotype) can be enumerated, a Markov chain approach can determine the fixation probability exactly. This approach is nicely outlined for the non-specialist reader by Gale (1990), and is typically feasible only when the population size is quite small (but see Parsons & Quince 2007*a*,*b*, discussed in §3.3). When the population size is large, methods based on discrete branching processes are often used. These methods build on the 'Haldane–Fisher' model (Fisher 1922, 1930; Haldane 1927, 1932), which is itself based on a Galton–Watson branching process. We note that any branching process approach provides an approximation to the true fixation probability, as it assumes that the wild-type population is sufficiently large that the fate of each mutant allele is independent of all others. This approach has been widely, and successfully, applied to a number of interesting recent questions regarding the fixation probability (Athreya 1992; Haccou & Iwasa 1996; Lange & Fan 1997; Otto & Whitlock 1997; Wahl & Gerrish 2001; Johnson & Gerrish 2002; De Oliveira & Campos 2004; Wahl & DeHaan 2004; Champagnat & Lambert 2007). Finally, when the population is large and the change in gene frequency is small in each generation (i.e. selection is weak), methods that incorporate a diffusion approximation may be used. These approaches follow from the pioneering 'Wright–Fisher–Kimura' model (Fisher 1922, 1930; Wright 1931, 1945; Kimura 1957, 1962), and are also in wide use today (Yamazaki 1977; Wahl & Gerrish 2001; Gavrilets & Gibson 2002; Whitlock 2003). Significant effort has also been made towards unifying or reconciling the discrete and continuous approaches (Kimura & Ohta 1970; Otto & Whitlock 1997; Wahl & Gerrish 2001; Lambert 2006). We will discuss many of these recent papers in turn in the sections to follow.

The most widely known result regarding the fixation probability is Haldane's celebrated approximation, obtained for weak selection using a discrete-time branching process. Haldane (1927) demonstrated that the probability of ultimate fixation, π, of an advantageous allele is given by $\pi \approx 2s$, when the allele is initially present as a single copy in a large population.

Haldane's elegant result necessarily relies on a number of simplifying assumptions. The population size is large and constant, generations are discrete and the number of offspring that each individual contributes to the next generation is Poisson distributed. This last simplification masks an assumption on which the fixation probability critically depends: individuals in such a branching process cannot die before having offspring. In effect, individuals die in such models only by having zero offspring. But since the probability of having zero offspring is completely determined by the mean of the Poisson distribution, there is no room in Haldane's approach to independently specify a survival probability. This will become important as we review some recent work that relaxes this assumption.

This work by Haldane, as well as Wright (1931) and Fisher (1992), was later generalized in a number of different directions, most notably by Kimura (Kimura 1957, 1962, 1964, 1970; Kimura & Ohta 1970). Kimura's approach was to use a diffusion approximation to model small changes, over many generations, in the frequency of a particular allele. To understand Kimura's foundational result, we must briefly introduce N_e, the variance effective population size. If we imagine a diploid population in which, for example, mating is not random or the sex ratio is not 1 : 1, these effects may change the variance in the number of offspring alleles per parental allele. N_e is then the size of an 'ideal' population—a large population of constant size, in which mating is random and we have equal numbers of males and females—that would give the same variance as the real population in question. Kimura's most widely known result is that the probability of ultimate fixation, π, of an allele with an initial frequency p and an additive selective effect s is

$$\pi \approx \frac{1-e^{-4spN_e}}{1-e^{-4sN_e}}. \tag{2.1}$$

(Moran (1960) and Gillespie (1974), among others, obtain the same expression.)

For large diploid populations, equation (2.1) implies that the fixation probability for a new mutation that arises *as a single copy* decreases with larger effective population sizes. However, the decay of this function is extremely rapid; for example, for $s=0.01$, a population size of 100 is already sufficient that the denominator is approximately 1. For all but extremely small populations or nearly neutral mutations, we then find that $\pi \approx 2sN_e/N$ for a mutation occurring as a single copy. Thus, π depends on the ratio of effective population size to census size. It is also clear that when $N_e = N$, we obtain Haldane's approximation $\pi \approx 2s$ for weak selection (Haldane 1927). By contrast, the fixation probability for an allele that is present *at a given frequency* increases with population size. (Note, however, that a single copy of an allele corresponds to a smaller frequency in a larger population, and thus $\pi \approx 2s$ still holds.)

A final note on the approximation $\pi \approx 2sN_e/N$ is that s reflects the selective advantage of the beneficial

allele, while N_e is most often inversely proportional to the variance in offspring number. This foreshadows the important work of Gillespie (1974, 1975) who predicted that the ratio of the mean to the variance in offspring number is necessary in determining both the long-term effects of selection on a beneficial allele and the fixation probability. This idea, particularly as applied to long-term selective effects, has been expanded in a number of elegant recent papers (Proulx 2000; Lande 2007; Orr 2007; Shpak & Proulx 2007).

Much progress has been made since the work of Kimura and the great trinity. As we will review in the following sections, the fixation probability has now been estimated in populations of fluctuating size, for populations whose size cycles among a set of constant values and, more recently, fluctuates according to a density-dependent birth–death process. Populations experiencing exponential or logistic growth or decline have been treated, as have populations that are subject to sustained growth periods followed by a population bottleneck—a sudden reduction in population size. A large body of work treats populations subdivided into demes, most recently including heterogeneous selection among demes and asymmetrical migration. Recent work has also addressed multiple segregating alleles, specifically treating quasi-species interactions and clonal interference, as described in the sections to follow.

3. POPULATIONS OF CHANGING SIZE

3.1. Growing, declining or cyclic population sizes

Fisher (1930) suggested that the probability of fixation of beneficial alleles would increase in growing populations and decrease in declining populations. Analysis by Kojima & Kelleher (1962) confirmed Fisher's proposition. Fisher's claim was further justified through the theoretical studies of logistically changing populations by Kimura & Ohta (1974).

Ewens (1967) used a discrete multitype branching process to study the survival probability of new mutants in a population that assumes a cyclic sequence of population sizes, as well as a population that initially increases in size and thereafter remains constant. For the former case, Ewens found the probability of fixation of a beneficial mutation to be

$$\pi = \frac{2s\tilde{N}}{\bar{N}}, \tag{3.1}$$

where $\tilde{N}$ is the harmonic mean and $\bar{N}$ is the arithmetic mean of the population sizes in the cycle. Ewens found that when the population sizes during the cycle differ considerably, the value of π may be considerably less than $2s$, implying that a constant population size is favourable for the survival of new mutants.

Ewens' relaxation of the assumption of constant population size was an important step towards generalizing fixation probability models; however, he still maintained the other classic assumptions and only explored two cases of changing population sizes. The approximation in equation (3.1) led Kimura (1970) to a conjecture that equation (2.1) may be used for populations that assume a cyclic sequence of values, with N_e replaced by $\tilde{N}$. Otto & Whitlock (1997) later built on the work of Ewens and Kimura by addressing the question of the fixation probability of beneficial mutations in populations modelled by exponential and logistic growth or decline. These authors proved that the conjecture made by Kimura holds true for the populations in which the product ks is small, where k is the total number of discrete population sizes.

All the papers mentioned above assume a Poisson distribution of offspring. Although such a distribution may be a good model of reproductive success in many species, some species clearly cannot be modelled well by such a distribution (e.g. bacteria that reproduce by binary fission). Pollak (2000) studied the fixation probability of beneficial mutations in a population that changes cyclically in size, assuming a very general distribution of successful gametes, described by a mean and variance, which are functions of the population size. Assuming that a beneficial mutation first appears in a single heterozygous individual, and that such an individual has $1+s$ times as many offspring as the wild-type, Pollak proved that the result found for the Poisson-distributed offspring by Ewens (1967) and Otto & Whitlock (1997) still holds: that the fixation probability is approximately proportional to the harmonic mean of the effective population sizes in the cycle and inversely proportional to the population size when the mutation manifests.

3.2. Population bottlenecks

In an attempt to provide estimates of the fixation probability for microbial populations maintained in experimental evolution protocols, Wahl and Gerrish studied the effect of population bottlenecks on fixation. A population bottleneck is a sudden, severe reduction in population size. In experimental evolution, bottlenecks are an inherent feature of the protocol (Lenski *et al.* 1991; Lenski & Travisano 1994; Bull *et al.* 1997); the population typically grows for a fixed period of time, and then is sampled randomly such that it is reduced to its initial size. The repetition of this procedure is called 'serial passaging'.

An important point to note is that at the population bottleneck, each individual—mutant or wild-type—survives with the same probability. Thus the 'offspring' distribution of each individual at the bottleneck is the same, for either mutant or wild-type. By contrast, during growth the selective advantage of the mutant is realized. Thus the case of growth between population bottlenecks is not simply a special case of cyclic population sizes.

Wahl & Gerrish (2001) derived the probability that a beneficial mutation is lost due to population bottlenecks. For this derivation they used both a branching process approach (Haldane 1927; Fisher 1930) as well as a diffusion approximation (Wright 1945; Kimura 1957, 1962). When selection is weak, Wahl and Gerrish demonstrated that the two approaches yield the same approximation for the extinction probability X of a beneficial mutation that occurs at time t between

bottlenecks: $1-X \approx 2srt\tau\, e^{-rt}$. Here s is the selective advantage of the mutant over the wild-type strain, r is the Malthusian growth rate of the wild-type population and τ is the time at which a bottleneck is applied. It was thus found that the fixation probability, π, drops rapidly as t increases, implying that mutations that occur late in the growth phase are unlikely to survive population bottlenecks. Since this model treats only extinction due to bottlenecks, this effect is *not* due to the large wild-type population size late in the growth phase, but rather due to the fact that the beneficial mutant does not have sufficient time to found a lineage large enough to survive the bottleneck. Wahl and Gerrish also defined an effective population size given by $N_e \approx N_0 r\tau$, where N_e is the effective population size and N_0 is the population size at the beginning of each growth phase. This approximation is independent of the time of occurrence of the mutation as well as its selective advantage.

In 2002, this model was extended to include resource-limited growth (Wahl *et al.* 2002). Resource limitation was included in order to better model serial passaging protocols for bacterial populations, in which the growth phase is typically limited by a finite resource in the growth medium. For both resource-limited and time-limited growth, mutations occurring in the early stages of a growth phase were more likely to survive. Wahl *et al.* predicted that although most mutations occur at the end of growth phases, mutations that are ultimately successful occur fairly uniformly throughout the growth phase.

The two papers described above included extinction during bottlenecks, but did not include the effects of genetic drift during the growth phase, i.e. the possibility of extinction of an advantageous mutant lineage between bottlenecks. Heffernan & Wahl (2002) incorporated the latter effect, assuming a Poisson distribution of offspring during the growth phase, and using a method based on the work of Ewens (1967). This model predicted a greater than 25 per cent reduction in the fixation probability for realistic experimental protocols, compared with that predicted by Wahl & Gerrish (2001).

The method presented by Heffernan is valid for both large and small values of selective advantage, s. This was an important extension of previous results, especially given the recent reports of large selective advantages in the experimental literature (Bull *et al.* 2000). When selection is weak and the mutation occurs at the beginning of a growth phase, Heffernan and Wahl derived the approximation $\pi \approx s(k-1)$, where k is the number of generations between bottlenecks. This approximation is analogous to the classic result $\pi \approx 2s$ (Haldane 1927) but is *increased* by a factor of $(k-1)/2$.

The work discussed in this section considers only the loss of beneficial mutations due to bottlenecks and genetic drift. In reality, rare beneficial mutations in asexual populations may also be lost during the growth phase due to competition between multiple new beneficial alleles (see §5.3) or quasi-species interactions (see §5.2). Most importantly, the papers described above either assume deterministic growth between bottlenecks or discrete generation times with offspring numbers that are Poisson distributed. These are not ideal simplifications for many microbial populations. Thus, the tailored life-history models described in §6 should provide a more accurate approach to these questions, although they have not, as yet, been as fully developed as the papers described here.

3.3. Dynamically changing population sizes

Three intriguing papers addressing population sizes that change dynamically, according to underlying birth and death events, appeared in 2006 and 2007.

Lambert (2006) developed an extension of the Moran (1958) model, assuming that birth events have a constant per capita rate, while death events have a per capita rate that increases with population density. Lambert addressed three model constructions: the first model considered independent continuous-state branching processes; the second model considered branching processes conditioned to produce a constant population size; and finally the third model included logistic density dependence through a density-dependent death rate.

For the first and second models at a large population limit, Lambert pointed out that the factor 2 in Haldane's result of $\pi \approx 2s$ for very small s stems from the assumption that the offspring distribution is Poisson. For near-critical branching processes, more generally, $\pi \approx 2s/\sigma$, where σ is the variance of the offspring distribution (Haccou *et al.* 2005). Thus, increased reproductive variance always reduces the fixation probability in such models.

For the third model, density dependence results in an upper asymptotic limit on the 'invasibility coefficient'; that is, the rate at which the selective advantage of the mutant increases the fixation probability. Consequently, Lambert found that Haldane's classic approximation ($\pi \approx 2s$) and Kimura's diffusion approximation (equation (2.1)) tend to underestimate the fixation probability of beneficial mutations in growing populations and overestimate it in declining populations. This result is consistent with those of Parsons & Quince (2007*a*,*b*), described below, as well as the classic predictions of Fisher (1930), Kojima & Kelleher (1962) and Kimura & Ohta (1974).

Ultimately, Lambert derived a concise expression for the fixation probability, which holds for all three models. The limitation of this approach is that it holds only when the selective advantage of the beneficial mutation is small, such that higher order terms in s are negligible.

Parsons & Quince (2007*a*) introduced stochastic population sizes in a similar way. In contrast to the work of Lambert, Parsons and Quince considered density-dependent birth rates and density-independent death rates. Another key difference is that Parsons and Quince did not assume that selection is weak. In particular, they argued based on their results that the parameter space over which the assumptions in Lambert (2006) are valid may in fact be quite limited.

In the first case considered (the 'non-neutral case'), the carrying capacities of the mutant and wild-type are not equal. For advantageous mutants, Parsons and

Quince found that stochastic fluctuations in the wild-type population do not affect the fixation probability. On the other hand, for deleterious mutants, the fixation probability is proportional to the fluctuation size of the wild-type population, but relatively insensitive to initial density.

In a second paper, Parsons & Quince (2007*b*) investigated the 'quasi-neutral' case: the carrying capacities of mutant and wild-type are identical, but the birth and death rates are different. Since the carrying capacities are determined by a ratio of the birth and death rates, this implies a life-history trade-off between these parameters. Parsons and Quince used a diffusion approximation to determine the fixation probability when the carrying capacity is large. The authors predicted an increase in fixation probability for the type with a higher birth rate in growing populations and a reduction in a shrinking population. When the population is at carrying capacity initially, the type with a higher birth rate has larger fluctuations in population size and thus a reduced fixation probability.

A shared feature of the approaches described in this section is that beneficial mutations can affect more than one life-history parameter or 'demographic trait'. Both models predict that the fixation probability depends on this mechanism of the selective advantage. This work is thus closely related to the more detailed life-history models described in §6 to follow.

4. SUBDIVIDED POPULATIONS

Pollak (1966) was the first to address the question of the fixation probability (π) in a subdivided population. Pollak considered a situation in which K subpopulations occupy their respective habitats, with the possibility of migration between subpopulations. A branching process approach was used to deduce that for symmetric migration, π in a subdivided population is the same as that in a non-subdivided population. Later, for the case of symmetric migration, Maruyama (1970, 1974, 1977) used the Moran model with a diffusion approach to show that a similar result holds.

Populations structured into discrete demes were also studied by Lande (1979) and Slatkin (1981) among others. Lande (1979) demonstrated the elegant result that if a population is subdivided into demes, the net rate of evolution is the same as the rate of evolution in a single deme, where the rate of evolution is given by the probability of fixation of a single mutant multiplied by the number of mutations per generation in one deme. This result relies on the assumption that a mutation fixed in one deme can spread through the whole population only by random extinction and colonization. Slatkin (1981) then showed that for a given pressure of selection in each local population, the fixation probability of a mutant allele is bounded below by the appropriate fixation probability in an unstructured population of the same total size and above by the fixation probability obtained by assuming independent fixation in each deme. Slatkin found that the fixation probability is higher in the low-migration limit than in the high-migration limit when a heterozygote mutant has a fitness that is less than the arithmetic mean fitness of the two homozygote states (underdominance). The reverse was found to be true when the heterozygote was more fit than the average homozygote fitness (overdominance). This stands to reason: high migration increases the fixation probability in the overdominant case and decreases the fixation probability in the underdominant case.

Barton & Rouhani (1991) further investigated the fixation probability in a subdivided population, exploring the limiting case when migration is much larger than selection, so that the difference in gene frequency between adjacent demes is very small. In a model with two demes, π was greatly reduced by migration in this model. This observation, however, did not extend to a large array of demes. Clarifying Slatkin's prediction that underdominance reduces the fixation probability, Barton and Rouhani showed that the chance of fixation is considerable despite free gene flow and moderate selection *against* heterozygotes, as long as the neighbourhood is small and the homozygote has a substantial advantage.

In contrast to Lande's result, Barton and Rouhani concluded that even though the fixation probability for any one mutation may be very low, the overall rate of fixation of any particular novel allele may be very high. This is because mutations can arise in any of a very large number of individuals; any mutation that is fixed in a large enough area has high probability of spreading through the entire population.

Like previous models, Barton and Rouhani assumed that migration is symmetric. Relaxing this assumption, Tachida & Iizuka (1991) considered asymmetric migration under the condition of strong selection and found that spatial subdivision increases π. This observation was consistent with the numerical results of Pollak (1972). However, the model by Tachida and Iizuka considered only a two-patch population. Lundy & Possingham (1998) extended the two-patch models of previous authors to investigate π in three- and four-patch systems. When migration is asymmetric, Lundy and Possingham found that the influence of a patch on the overall fixation probability depends largely on two factors: the population size of the patch and the net gene flow out of the patch.

More recently, Gavrilets & Gibson (2002) have studied the fixation probabilities in a population that experiences heterogeneous selection in distinct spatial patches, and in which the total population size is constant. In this model, each allele is advantageous in one patch and deleterious in the other. The results in this contribution are in agreement with the arguments of Ohta (1972) and Eldredge (1995, 2003) that, depending on exactly how migration rates change with population size, selection can be more important in small populations than large populations.

In a model of distinct patches, which focuses on extinctions and recolonizations, Cherry (2003) found that these two effects always reduce the fixation probability of a beneficial allele. Cherry's conclusion is consistent with Barton's (1993) observation for a favoured allele in an infinite population, but applies more generally. Cherry derived both an effective population size and an effective selection coefficient, for beneficial

alleles in this model, such that established results for unstructured populations can be applied to structured populations. In his exposition, Cherry (2004) assumed that an extinct patch can be recolonized by only one founding allele. The author goes on to explore the case of more than one founding allele after extinction, confirming that extinction and recolonization reduce the fixation probability for beneficial alleles.

Whitlock (2003) relaxed some of the assumptions in previous structured population models to study the fixation of alleles that confer either beneficial or deleterious effects, with arbitrary dominance. Whitlock constructed a model that allows for an arbitrary distribution of reproductive success among demes, although selection is still homogeneous. He found that in a 'differentially productive environment', the effective population size is reduced relative to the census size and thus the probability of fixation of deleterious alleles is enhanced, while that of beneficial alleles is decreased. In a further paper, Whitlock & Gomulkiewicz (2005) examined the question of fixation probability in a metapopulation when selection is heterogeneous among demes. In contrast to the metapopulations with homogeneous selection, Whitlock and Gomulkiewicz concluded that the heterogeneity in selection *never* reduced (and sometimes substantially enhanced) the fixation probability of a new allele. They found that the probability of fixation is bounded below and above by approximations based on high- and low-migration limits, respectively.

An alternative realization of a spatially structured model was studied by Gordo & Campos (2006) who determined the rate of fixation of beneficial mutations in a population inhabiting a two-dimensional lattice. Under the assumption that deleterious mutations are absent and that all beneficial mutations have equal quantitative effect, Gordo and Campos found that the imposition of spatial structure did not change the fixation probability of a single, segregating beneficial mutation, relative to an unstructured haploid population (in agreement with the findings of Maruyama 1970). However, interestingly, spatial structure reduced the substitution rate of beneficial mutations if either deleterious mutations or clonal interference (more than one beneficial mutation segregating simultaneously) were added to the model. In an elegant example of experimental and theoretical interactions, the conclusions of Gordo and Campos were experimentally substantiated by Perfeito *et al.* (2008) who studied bacterial adaptation in either unstructured (liquid) or structured (solid) environments.

From the overview above, it is clear that an extremely rich literature surrounding the fixation probability in subdivided populations has been developed. In particular, Whitlock's recent work has relaxed a large number of the limiting assumptions in earlier papers, encompassing beneficial or deleterious mutations, arbitrary dominance, heterogeneous selection and asymmetric mutation. As argued by Whitlock & Golmulkiewicz (2005), some intriguing questions remain. For example, it seems likely that multiple alleles could be simultaneously segregating in different demes; this case has not yet been treated in a subdivided population, although it is related to §5 below.

5. MULTIPLE SEGREGATING ALLELES

In §4 above we have discussed the fixation probability in populations that are spatially subdivided (i.e. spatially heterogeneous populations). In analogy, here we consider populations that are divided into a variety of genetic rather than geographical backgrounds. This genetic heterogeneity can occur when multiple alleles are segregating simultaneously at the same locus or when contributions from other linked loci are considered. In general, the literature surrounding these questions suggests numerous possibilities for new work.

5.1. Effects of linked and deleterious alleles

The effects of linked loci on the fixation probability of a beneficial mutation have been extensively studied, beginning with the ideas of Fisher (1922) and Hill & Robertson (1966). Peck (1994), in particular, focused on the fixation probability of a beneficial mutation in the presence of linked deleterious mutations, finding that deleterious mutations greatly reduce the fixation probability in asexual, but not sexual, populations. A more detailed model is presented by Charlesworth (1994) who derived expected substitution rates and fixation probabilities for beneficial alleles when the deleterious alleles are at completely linked loci. A key result of this work is that deleterious linked loci reduce the effective population size, by a factor given by the frequency of mutation-free gametes.

Barton (1994, 1995) derived a more comprehensive method for computing the fixation probability of a favourable allele in different genetic backgrounds. For a single large heterogeneous population, Barton found that loosely linked loci reduce fixation probability through a reduction in the effective population size, by a factor that depends on the additive genetic variance. At tightly linked loci, however, Barton demonstrated that deleterious mutations, substitutions and fluctuating polymorphisms each reduce the fixation probability in a way that cannot be simply captured by an effective population size.

The study of linked loci was extended by Johnson & Barton (2002) who estimated the fixation probability of a beneficial mutation in an asexual population of fixed size, in which recurrent deleterious mutations occur at a constant rate at linked loci. Johnson and Barton assumed that each deleterious mutation reduces the fitness of the carrier by a factor of $(1-s_d)$ (i.e. any deleterious mutation has the same quantitative effect on fitness). Furthermore, it is assumed that the beneficial mutation increases the fitness of an individual carrier by a factor of $(1+s_b)$ regardless of the number of deleterious mutations present in the carrier. Thus, the relative fitness of an individual with a beneficial mutation and i deleterious mutations is $w_i=(1+s_b)(1-s_d)^i$. Johnson and Barton estimated the fixation probability by summing f_iP_i, where f_i is the probability that a beneficial mutation arises in an individual with i deleterious mutations and P_i, given by the solution of simultaneous equations, is the probability that a beneficial mutation arising in such an individual is not ultimately lost. Johnson and Barton

were thus able to quantify the reduction in the fixation probability of a beneficial mutation due to interference from segregating deleterious mutations at linked loci. Interestingly, this result is then used to determine the expected rate of increase in population fitness and the mutation rate that maximizes this fitness increase.

5.2. Quasi-species fixation

Quasi-species theory describes the evolution of a very large asexually reproducing population that has a high mutation rate (Eigen & Schuster 1979; Eigen *et al.* 1988, 1989; Domingo *et al.* 2001). This theory is often cited in describing the evolution of RNA viruses (Domingo *et al.* 2001; Wilke 2003; Manrubia *et al.* 2005; Jain & Krug 2007). Several authors have questioned the relevance of quasi-species theory to viral evolution (Moya *et al.* 2000; Jenkins *et al.* 2001; Holmes & Moya 2002), arguing that the mutation rates necessary to sustain a quasi-species are unrealistically high. In contrast, however, Wilke (2005) reviewed related literature and argued that quasi-species theory is the appropriate model for the population genetics of many haploid, asexually reproducing organisms.

In typical models of population genetics, it is assumed that mutations are rare events, such that an invading mutant strain will not mutate again before fixation or extinction occurs. In contrast, in quasi-species models, the offspring of a mutated individual are very likely to mutate before fixation. Consequently, the fitness of an invading quasi-species is not solely determined by the fitness of the initial/parent mutant, but depends on the average fitness of the 'cloud' of offspring mutants related to that parent, continually introduced by mutation, and removed through selection (the 'mutation–selection balance'). In quasi-species theory, therefore, the fixation of a mutant is defined to be its establishment as a common ancestor of the whole population; since the population is never genetically identical, the standard definition does not apply.

Wilke (2003) first investigated the fixation probability of an advantageous mutant in a viral quasi-species. This contribution uses multitype branching processes to derive an expression for the fixation probability in an arbitrary fitness landscape. Wilke initially assumed that mutations that are capable of forming a new invading quasi-species are rare. Thus, while mutations within the quasi-species are abundant, only one quasi-species will be segregating from the wild-type quasi-species at any given time. Under this assumption, the fixation probability was determined for fixation events that increase the average fitness of the population (situations where the average fitness is reduced or left unchanged were not addressed). If π_i denotes the probability of fixation of sequence i, that is, the probability that the cascade of offspring spawned by sequence i does not go extinct, and M_{ij} gives the expected number of offspring of type j from sequences of type i in one generation, Wilke demonstrated that the vector of fixation probabilities $\hat{\pi}$ satisfies $\hat{\pi} = 1 - e^{-M\hat{\pi}}$ (with the convention $e^{\hat{x}} = (e^{x_1}, e^{x_2}, \ldots)^{\mathrm{T}}$). This implies

$$\pi_i \approx s_i + \sqrt{s_i^2 + 2\sum_{k \neq i} M_{ik}\pi_k}, \tag{5.1}$$

where $s_i = M_{ii} - 1$. In this model, if the invading sequence gets no support from its mutational neighbours (i.e. the off-diagonal elements of matrix M are zero) and $s_i > 0$, then equation (5.1) reduces to Haldane's celebrated result, $\pi_i = 2s_i$. If the off-diagonal elements of M are non-zero, π_i is increased even if some $s_i < 0$, so long as the spectral radius of M is greater than one.

As discussed more fully in §6, estimates of the fixation probability are extremely sensitive to assumptions regarding the life history of the organism. Wilke's elegant result is a generalization of Haldane's approach, retaining the assumptions of discrete, non-overlapping generations and Poisson-distributed offspring. As these assumptions are not particularly well suited for the life history of viruses, it remains unclear which conclusions of this study would hold in viral populations.

5.3. Clonal interference

In a genetically homogeneous asexual population, two or more beneficial mutations may occur independently in different individuals of the population. Clonal interference refers to the competition that ensues between the lineages of these independent mutations thereby, potentially, altering the fate of the lineages. The idea that competing beneficial mutations may hinder a beneficial mutation's progress to fixation was formulated by Muller (1932, 1964) in his discussions on the evolutionary advantage of sex. Since that time, numerous studies have been conducted on the subject of clonal interference; in the last decade a rich literature, both experimentally and theoretically, has developed, sparked by renewed interest in the adaptation of asexual populations in laboratory settings.

A review of this growing literature would be substantial, and is outside the scope of this contribution, relating more closely to adaptation and adaptation rates than to fixation and extinction probabilities, narrowly defined. However, we give a brief overview of the standard means of estimating fixation probabilities under clonal interference, and refer the reader to other recent contributions (Campos & de Oliveira 2004; Campos *et al.* 2004, 2008; Rosas *et al.* 2005; De Visser & Rozen 2006).

Gerrish & Lenski (1998) published the first discussion of fixation probabilities under clonal interference. Gerrish and Lenski considered the possibility that while an initial beneficial mutation is not yet fixed, it is possible for a set of other mutations to emerge in the population. If at least some of these mutations survive extinction when rare (for example, due to genetic drift), a competition ensues between the focal mutation and the subsequent mutations. Assuming that the probability density for the selective advantage of beneficial mutations is given by $\alpha e^{-\alpha s}$, Gerrish and Lenski stated that the probability the focal mutation fixes will be $\alpha \int_0^\infty \pi(s)\exp(-\lambda(s) - \alpha s)\mathrm{d}s$. The function $\pi(s)$ gives the probability that a given beneficial mutation

is not lost through drift when rare, while the function $\lambda(s)$ gives the mean number of mutations that: occur before the focal mutation fixes; have a higher s than the focal mutation; and survive drift. We note that $\lambda(s)$ is also a function of the population size, the mutation rate and α. Under the assumption that mutations appear spontaneously at a constant rate, $e^{-\lambda(s)}$ then gives the probability that zero superior mutations occur, and survive drift, before the focal mutation fixes. This basic structure for the fixation probability during clonal interference has been augmented in subsequent contributions (Campos & de Oliveira 2004; Campos *et al.* 2004). The most interesting prediction of this work is that at high mutation rates, clonal interference imposes a 'speed limit' on the rate of adaptation.

There is a small conceptual flaw in this derivation (P. Gerrish 2000, personal communication), which is that the possibility that other beneficial mutations were segregating *before* the initial appearance of the focal individual was neglected. If many mutations are segregating simultaneously, the focal beneficial mutation is likely to have arisen on the background of a previously segregating beneficial mutation. Thus mutations may sweep in groups, the 'multiple mutation' regime. Conceptually, the multiple mutation regime lies on a continuum between clonal interference as described by Gerrish & Lenski (1998) and quasi-species dynamics.

The dynamics of adaptation in the multiple mutation regime have been recently described in some detail (Desai & Fisher 2007; Desai *et al.* 2007). In contrast to the work of Gerrish & Lenski (1998), these authors predicted that clonal interference may not always reduce adaptation rates. Like Gerrish and Lenski, this approach depends on the underlying probability that a beneficial mutation escapes extinction through drift when rare, and assumes that this probability is proportional to s.

6. LIFE-HISTORY MODELS

In almost every contribution discussed so far, beneficial mutations are assumed to increase the average number of offspring: so-called 'fecundity mutants'. For many organisms, however, a mutant may have the same average number of offspring as the wild-type, but may produce these offspring in a shorter generation time: 'generation time mutants'. An example here is bacterial fission in the presence of antibiotics: many antibiotics reduce cell growth and thus mutations conferring resistance have a reduced generation time.

This issue was first addressed by Wahl & DeHaan (2004) who approximated the fixation probability for beneficial generation time mutants (π_G), in a population of constant size or a population that grows between periodic bottlenecks. The approach is closely related to that of Pollak (2000). In a model with the Poisson offspring distribution with mean 2 and weak selection, it was found that $\pi_G \approx s/\ln(2)$ for a constant population size, while $\pi_G \approx \tau s/2\ln(2)$, when τ, the number of generations between population bottlenecks, is moderately large. For a mutation that increases fecundity, the analogous approximation is $\pi \approx 2s$ in a constant population size (Haldane 1927), while an estimate of $\pi \approx \tau s$ was obtained for a population with a moderately large τ (Heffernan & Wahl 2002). Thus, assuming that all mutations confer a fecundity advantage leads to an overestimate of the order $2\ln(2) \sim 1.4$ for generation time mutations.

These results emphasize the sensitivity of fixation probabilities to the underlying life history of the organism being modelled, and to the specific effect of the beneficial mutation on this life history. Based on these results, Hubbarde and co-authors studied the fixation probability of beneficial mutations in a 'burst–death model' (Hubbarde *et al.* 2007; Hubbarde & Wahl 2008). This model is based on the well-known continuous-time branching process called the birth–death process, in which each individual faces a constant probability of death, and a constant probability of undergoing a birth event, in any short interval of time. Thus, the generation time or lifetime of each individual is exponentially distributed.

In contrast to a birth–death model, however, a burst event can add more than one offspring to the population simultaneously (a burst of two might model bacterial fission; a burst of 100 might model a lytic virus). The burst–death model explored by Hubbarde *et al.* treats populations in which the expected size is constant (i.e. the death rate balances the burst rate), and populations that grow between periodic bottlenecks. Hubbarde *et al.* computed the fixation probability for mutations that confer an advantage by increasing either the burst size or the burst rate. This work was extended by Alexander & Wahl (2008) who compared the fixation probability of mutations with equivalent effects on the long-term growth rate, i.e. equally 'fit' mutations. The latter paper demonstrates that mutations that decrease the death rate (increasing survival) are most likely to fix, followed by mutations that increase the burst rate. Mutations that increase the burst size are least likely to fix in the burst–death model.

The important departure in the burst–death model from previous work is that a beneficial mutation may affect a number of life-history traits independently. Thus, the mean number of offspring can change independently of p_0, the probability of having zero offspring. While the mean largely determines the long-term growth rate, or Malthusian fitness, of the mutant, the fixation probability is sensitive to short-term processes, particularly p_0.

By contrast, when generation times are fixed and offspring numbers are Poisson distributed, the *only* way for a mutation to be beneficial is for it to increase the mean number of offspring, by a factor typically denoted $(1+s)$. The probability of leaving zero offspring is completely constrained by this mean, and this ultimately implies that fixation probabilities, while perhaps not equal to $2s$, are at least proportional to s under these classic assumptions.

This simple proportionality no longer holds when more complicated, and thus more realistic, life histories are considered. The overall conclusion here is that for many real populations, estimates of the fixation probability should take into account both the life-history details of the organism and the mechanism by which the mutation confers a reproductive advantage.

7. FROM THEORY TO EXPERIMENT

The experimental study of evolution has been recently accelerated through the study of rapidly evolving organisms, such as bacteria, viruses and protozoa (Lenski *et al.* 1991; Lenski & Travisano 1994; Papadopoulos *et al.* 1999). These organisms adapt to laboratory conditions on experimentally feasible time scales, making them ideal candidates for the real-time study of evolution. These experiments have generated tremendous interest in evolutionary biology, allowing for experimental tests of some of the most basic features of adaptation.

To date, however, the fixation probability of a specific beneficial mutation has never been experimentally measured. With the advent of serial passaging techniques that allow for experimental designs with very high numbers of replicates (e.g. 96-well plates), we argue that an experimental estimate of the fixation probability is finally within reach. After 80 or 90 years of theory, the possibility of experimental validation is fascinating.

On the other hand, the models developed to date are probably not sufficiently tailored to the life histories of the organisms that could be used in such experiments. Neither bacteria nor viruses are well modelled by discrete, non-overlapping generations, nor by a Poisson distribution of offspring. Recent contributions by Parsons & Quince (2007*a*,*b*) and Lambert (2006), as well as work from our own group (Hubbarde *et al.* 2007; Alexander & Wahl 2008) have highlighted the extreme sensitivity of fixation probabilities to such assumptions.

For experiments involving bacteria, we suggest that theoretical predictions of the fixation probability must be based specifically on bacterial fission. A beneficial mutation might reduce the generation time, for example, or increase the probability that one or both of the daughter cells survive to reproductive maturity. For experiments involving viruses, theoretical predictions must likewise be tailored to include the processes of viral attachment, the eclipse time and then the release of new viral particles through budding or lysis. Other microbial systems will present their own life histories and their own modelling challenges. In addition, population bottlenecks, washout from a chemostat or limited resources must be imposed in experimental systems to prevent unbounded microbial growth.

A final note is that very often, in estimating the fixation probability, it is assumed that selection is weak. This phrase means for example that the selective advantage s is sufficiently small that terms of order s^2 are negligible. This assumption has been widely, and very usefully, employed in population genetics over decades, and is still considered to be relevant to most natural populations. Recent evidence from the experimental evolution of microbial populations, however, has indicated that some beneficial mutations exert extremely high selection pressures, with s of the order of 10 or more (Bull *et al.* 2000). Thus, a further challenge for theoreticians is to design organism- and protocol-specific models that retain accuracy and tractability, even for very strong selective effects.

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