Quasi-species evolution in subdivided populations favours maximally deleterious mutations

Brendan D. O’Fallon^{1,*}, Frederick R. Adler² and Stephen R. Proulx³

¹Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA

²Department of Mathematics, University of Utah, 155 South 1400 East, Salt Lake City, UT 84112, USA

³Department of Ecology, Evolution and Organismal Biology, Iowa State University, 253 Bessey Hall, Ames, IA 50011, USA

Most models of quasi-species evolution predict that populations will evolve to occupy areas of sequence space with the greatest concentration of neutral sequences, thus minimizing the deleterious mutation rate and creating mutationally ‘robust’ genomes. In contrast, empirical studies of the principal model of quasi-species evolution, RNA viruses, suggest that the effects of deleterious mutations are more severe than in similar DNA-based microbes. We demonstrate that populations divided into discrete patches connected by dispersal may favour genotypes where the deleterious effect of non-neutral mutations is maximized. This effect is especially strong in the absence of back mutation and when the amount of time spent in hosts prior to dispersal is intermediate. Our results indicate that RNA viruses that produce acute infections initiated by a small number of virions are expected to evolve fragile genetic architectures when compared with other RNA viruses.

Keywords: quasispecies; robustness; population structure

1. INTRODUCTION

Much of classical population genetics theory focuses on understanding the action of natural selection, mutation and drift at one or a few loci. Quasi-species theory, first described by Eigen (1971), explicitly incorporates mutation–selection balance at a large number of loci and is often used to describe the dynamics of species with relatively high mutation rates and large population sizes. In this view, populations consist of a ‘cloud’ of genotypes dispersed in sequence space, possibly clustered around one or a few most fit types. Because RNA viruses have both high mutation rates (Drake & Holland 1999) and large population sizes, they are often considered exemplars of quasi-species dynamics (Domingo 2006).

Many human and animal pathogens are RNA viruses, including HIV, hepatitis A and C, poliovirus and foot-and-mouth disease, and quasi-species models have led to a number of insights regarding these pathogens. The concept of the error threshold, which predicts that increasing the mutation rate beyond a critical value leads to loss of the most fit viral types, has enjoyed recent empirical support in terms of the RNA mutagen ribavirin (Crotty *et al.* 2001; Graci & Cameron 2002). Quasi-species theory has also informed research on the effectiveness (and limitations) of combined antiretroviral therapy on HIV-1 infection (Mullins & Jensen 2006). The ability of poliovirus to invade CNS tissue was shown to depend upon the presence of a diverse mutant cloud (Vignuzzi *et al.* 2006). Interestingly, this result was not due to the presence of neurotropic types in the cloud but rather due to the diversity of the cloud itself.

Several investigations have found that natural selection may operate differently in quasi-species. Instead of driving a population towards a single most fit genotype, natural selection may push populations to ‘flat’ regions of fitness landscapes, where the fraction of possible mutations that are neutral is maximized, and the resulting deleterious mutation rate minimized (Schuster & Swetina 1988; Bornberg-Bauer & Chan 1999; van Nimwegen *et al.* 1999; Wilke *et al.* 2001; Wilke & Adami 2003). Evolution to flat areas may occur even in the presence of higher, but less flat areas on the fitness landscape. This result has been termed the ‘Survival of the flattest’ (Wilke *et al.* 2001), and sequences in flat regions of sequence space are sometimes said to be mutationally ‘robust’ since the fitness consequence of a random new mutation is minimized.

Evolution of mutational robustness through quasi-species effects has not been conclusively observed in a biological population, but several studies have examined the process *in silico*. Wilke *et al.* found that digital organisms evolved more robust ‘genotypes’ at high mutation rates (Wilke *et al.* 2001), and similar results were obtained from a simulation of RNA secondary structure (Wilke 2001). More recently, the RNA viroids of chrysanthemum (*Dendranthema grandiflora*, Codoner *et al.* 2006) were found to favour a more robust type at high mutation rates. On the other hand, Holmes (2003) has noted that RNA viruses often carry features that appear to make their genomes less robust, such as overlapping genes and sequences with multiple and conflicting functions. Additionally, a recent review noted that RNA viruses exhibit a ‘hypersensitivity to deleterious mutation’ when compared with similar DNA-based organisms (Elena *et al.* 2006, p. 169). This contrast is in part due to ambiguity regarding the word ‘robustness’. The Survival of the flattest results treat robustness as minimization of the rate of deleterious mutation

* Author for correspondence (brendanofallon@fastmail.fm).

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(by increasing the genomic fraction of neutral mutations) and ignore the effects of those mutations that are deleterious. However, robustness has also been used to describe the effects of deleterious mutations while ignoring neutral mutations (e.g. Krakauer & Plotkin 2002) and is sometimes used to characterize the entire distribution of mutational effects (e.g. Elena *et al.* 2006). In the analysis we present below, we partition the effects of mutation into two components, one describing the probability that a new mutation is deleterious and the other describing the effect on fitness of the deleterious mutations.

Models of quasi-species evolution have almost uniformly assumed that individuals exist in a large, well-mixed population. Although two studies have examined the rate of adaptation in subdivided populations of RNA viruses linked by migration (Miralles *et al.* 1999; Cuevas *et al.* 2003), the studies did not address the evolution of mutational robustness. The assumption of panmixis simplifies mathematical analysis but ignores the fact that RNA virus populations are subdivided into hosts. Subdivision is known to have important consequences for a number of population genetic processes (Wright 1931; Wilson 1980; Whitlock 2002, 2003; Cherry 2003), but few authors have examined the interplay between population structure and evolution of the fitness landscape. A first hint that population subdivision may affect the evolution of robustness came from Hadany *et al.* (2006) who found that an allele that amplified the deleterious effect of an allele at a different locus was favoured in a subdivided population.

In this paper we demonstrate that, in a subdivided population, natural selection may favour sequences where the negative fitness consequences of deleterious mutations are maximized. We first examine a simple mathematical model that elucidates the relative effects of deleterious mutation rate, the number of dispersers and the amount of time between dispersal bouts on the preferred degree of deleteriousness of mutations. We then generalize the model using individual-based simulations and examine more complex fitness functions, finite population size and realistic dispersal structure. We discuss the implications of these findings for RNA virus evolution and the evolution of genetic architecture.

2. MODELS AND RESULTS

(a) Analytic model

We begin by examining a simple analytic model that assumes deterministic dynamics within hosts, an infinite number of hosts, and only two mutational classes, wild-type and mutant (Bull *et al.* 2005 examined a similar model, but did not include subdivision or back mutation). We track the frequency of two competing strains, labelled A and B. The abundance of the wild-type and mutant types are denoted A_0 and A_1 , respectively, and similarly for strain B. Each wild-type grows at exponential rate b and some fraction μ of these offspring mutate to become the mutant individuals. Therefore, μ represents the deleterious mutation rate, and not the overall genomic mutation rate. The mutant class grows at exponential rate $b(1-s)$. The parameter s describes the mean effect on fitness of all deleterious mutations, but does not incorporate the effects of neutral or beneficial mutations nor the probability that a

given mutation is neutral or deleterious (which is reflected in μ). Mutants revert to the wild-type class at rate μ_2 . We allow each strain to have a different μ and s , denoted μ_A and s_A for strain A and μ_B and s_B for B, but for simplicity assume both strains have equal rates of back mutation. Differential equations describing the growth of strain A are then

$$\frac{dA_0}{dt} = b(1 - \mu_A)A_0 + b\mu_2A_1 \quad \text{and}, \quad (2.1)$$

$$\frac{dA_1}{dt} = b\mu_A A_0 + b(1 - s_A)(1 - \mu_2)A_1. \quad (2.2)$$

The growth of B is governed by similar expressions. In the analysis below, we assume that the rate of back mutation is small compared with the forward mutation rate, and that selection is strong enough to balance mutation and maintain presence of the wild-type. This requires that $\mu_2 < \mu < s$ for both strains.

We model subdivision as follows. Individuals grow in an infinite number of patches for τ time units, at which time each patch contributes equally to a large migrant pool. Each patch in the next generation is colonized by m founders drawn at random from the migrant pool. Thus, in contrast to the deterministic within-host dynamics, patch colonization is a stochastic process. We first assume that mutants colonize new patches with probability equal to that of wild-types. We then demonstrate that the analytic condition for invasion in this case is qualitatively similar to, but more complicated than, the case where mutants do not colonize new patches, and we analyse the simplified case.

If mutant types colonize patches as efficiently as wild-types, then the condition for invasion of strain A into strain B is given by

$$m \sum_{i=0}^{m-1} [g(1, 0, m-1-i, i) + \rho g(0, 1, m-1-i, i)] \times \binom{m-1}{i} \rho^i > (1 + \rho)^{m-1}, \quad (2.3)$$

where $g(i, j, k, l)$ denotes the frequency of A_0 types in a patch founded by i A_0 s, j A_1 s, k B_0 s and l B_1 s τ time units after colonization and ρ is the fraction of mutants that exist at mutation-selection equilibrium within a given strain across all hosts. If mutants cannot colonize new patches, then the condition for invasion reduces to

$$R_B(\tau)[mA_0(\tau) - (m-1)B_0(\tau)] - B_0(\tau)R_A(\tau) > 0, \quad (2.4)$$

where $R_A(\tau)$ and $R_B(\tau)$ describe the total within-patch abundance of the A and B strains τ rounds after colonization, respectively, and $A_0(\tau)$ and $B_0(\tau)$ are the abundances of the wild-types alone (see mathematical appendix in the electronic supplementary online for derivation of equations (2.3) and (2.4)). Extensive numerical testing indicates that differences in the value of μ_A^* produced by numerical solution of (2.3) and (2.4) for the equilibrium μ are typically less than 1 and 5% in the worst cases, respectively (results not shown). Because analysis of condition (2.4) is more straightforward than that of (2.3), we restrict our consideration to the former, realizing that results obtained in this case are only an approximation of the more general case.

In the simple case where both strains have equal mutation rates and there is no back mutation, both wild-type classes grow at the same rate and all $A_0(\tau)$ and $B_0(\tau)$ terms factor out of condition (2.4). Rearranging (2.4) leaves the invasion condition $B_1(\tau) > A_1(\tau)$ indicating that the strain with the lower mutant growth rate may invade the strain whose mutants grow more quickly. Because mutants grow at rate $b(1-s)$, strains with larger s are favoured.

Including back mutation and allowing strains to have different deleterious mutation rates result in a more complicated invasion condition. Not only are selection coefficients and mutation rates important but also are the number of migrants that found each patch (m) and the number of rounds spent within a patch before dispersal (τ). Figure 1 illustrates parameter combinations that allow for invasion of strain A into strain B (with $s_B = 0.1$ and $\mu_B = 0.01$), by numerically solving the invasion condition (2.4) for μ_A . Parameter combinations below the curve allow for invasion, while those above the curve fail. In figure 1a invasion curves for several combinations of s_A and s_B are plotted as functions of the amount of time spent within hosts (τ). For s close to μ , the size of the parameter space that allows for invasion is larger, such that for a greater range of mutation rates and τ , strains with larger s will be favoured. figure 1a also demonstrates that there is a region of intermediate τ compatible with invasion by strains with large s . If τ is too small, then not enough growth occurs within patches before dispersal, the population becomes effectively unstructured and invasion cannot proceed. If τ is large, then within-patch dynamics dominate, each patch is essentially an independent panmictic population and the benefit of large s is lost.

figure 1b examines the effect of increasing levels of back mutation (μ_2). Increasing back mutation shrinks the parameter space compatible with invasion by strains with larger s . If μ_2 is large enough, then for large τ a large s strain may be unable to invade even if it has a mutation rate equal to that of the resident strain. For example, if the rate of back mutation is equal to 5% of the forward mutation rate, $s_A = 0.15$ and $s_B = 0.1$, then strain A is favoured only if $\tau < 450$.

(b) Simulations

The above analysis involves a number of simplifications, including synchronized dispersal and recolonization of patches, only a few mutational classes and infinite numbers of individuals and patches. To investigate more realistic dispersal dynamics, fitness landscapes and finite population size, we turn to individual-based simulations.

Each individual in the simulation is clonal and contains a genome consisting of L loci, where each locus may be in one of two states, labelled 0 or 1. In a manner similar to the analytic analysis, the L loci do not incorporate beneficial or neutral sites, and the genomic fraction of mutations with deleterious effects is assumed to be constant. In each simulation round, loci mutate independently and patches go extinct with probability $1/\tau$. Extinct patches are immediately recolonized by m founders drawn at random from all patches. Following Wilke & Adami (2001), we assume that the fitness of a sequence is given by $W = \exp[-sx^\gamma]$, where x is the total number of mutations separating the sequence from wild-type (the Hamming distance from wild-type), s again characterizes

Figure 1. The condition for invasion of strain A into strain B (numeric solution of equation (2.3) for μ_A). Area below curves indicates parameter combinations that allow for invasion by strain A. Strain B has constant mutation rate (μ_B) of 0.01. (a) $\mu_2 = 5 \times 10^{-4}$, s as indicated. (b) $s_A = 0.15$ and $s_B = 0.1$, μ_2 as indicated.

the mean effect of each deleterious mutation and γ the type of epistasis. If $\gamma > 1$, mutations interact synergistically and fitness declines more quickly than expected if effects were independent. If $\gamma < 1$ mutational effects are antagonistic and fitness declines more slowly as additional mutations accumulate. The simulations were conducted with 100 patches and 100 individuals per patch. Simulations are initiated with equal numbers of strains A and B and run until one strain is extirpated.

In figure 2a, the parameters for strain B are held constant ($s = 0.1$ and $\gamma = 1$), while parameter s for strain A is varied from 0.04 to 0.15. For each parameter combination, 100 simulation replicates are run. On the left half of figure 2a, the fitness function for strain A has a shallower slope than for strain B, indicating that the effect of deleterious mutation is less severe than strain A. On this portion of the figure, strain A outcompetes B only under population panmixis; A loses to B if the population is subdivided. In contrast, on the right half of figure 2a, strain A has s larger than B, and A outcompetes B under subdivision and loses under panmixis. These results are consistent with the mathematical analysis above; strains with relatively large s are preferred if the population is subdivided and the opposite is true if the population is panmictic.

The fitness of a given sequence is not only affected by the average deleteriousness of each mutation but also through their epistatic interactions. Figure 2b explores the effect of different levels and types of epistatic interactions by altering the parameter γ . Both strains have constant $s = 0.1$ and

Figure 2. Simulation results for competition between strain A and B. Strain B has constant landscape parameters $s = 0.1$ and $\gamma = 1.0$. (a) Strain A parameter s varied from 0.04 to 0.16, $\gamma = 1.0$. (b) Strain A parameter γ varied from 0.2 to 2.0, $s = 0.1$.

strain B has no epistasis ($\gamma_B = 1$). Parameter γ_A is varied from 0.2 to 2.0, encompassing both antagonistic ($\gamma < 1$) and synergistic ($\gamma > 1$) interactions. Under panmixis, γ has no detectable effect and strain A wins approximately half of the simulation runs in all trials. In subdivided populations, however, strain A outcompetes B under synergistic epistasis but loses to B under antagonistic epistasis (where mutations ameliorate each other's deleterious effects). This result also supports our conclusion that subdivision favours maximally deleterious mutants, but here deleteriousness is produced by epistatic interactions.

3. DISCUSSION

Models of the survival of the flattest have examined the probability that a given new mutation is deleterious or neutral and have concluded that maximum neutrality may be favoured even at the expense of immediate reproductive rate. Our model, in contrast, examines the effects of those mutations that are deleterious and demonstrates that population subdivision may favour maximal deleteriousness of these non-neutral mutations. This perhaps counter-intuitive result is due to the increase in the fraction of wild-types within a patch enjoyed by strains with large s . All strains present in a patch will benefit from the presence of a strain with large s , since that strain's less-fit mutant progeny

will not compete as effectively for dispersal. If there is enough variance in the distribution of strains among patches, then individuals of the large s type will more often benefit from each other's company, and the global frequency of steep wild-types will increase. This process may operate concurrently with the survival of the flattest effect that favours maximum neutrality (low rate of deleterious mutation). If both processes operate efficiently, resulting mutational landscapes may appear to be flat but steep-sided, and the associated distribution of mutational effects may have an increased variance, with many lethal and neutral mutations, but few of intermediate effect.

Rates of forward and backward mutation play a central role in these results. If both strains have equal mutation rates and there is no back mutation, then strains with maximally deleterious mutational effects can always invade strains whose deleterious mutations have intermediate effects. However, if moderate degrees of back mutation are included, then strains with large s are only favoured if the amount of time spent within a patch before dispersal (τ) is below a certain level. The precise value of τ at which this transition occurs also depends on the mean fraction of mutants maintained in the population, itself a function of the number of patch founders (m) and s . In general, large s and smaller m decrease the importance of back mutation and increase the critical τ , thereby creating conditions more favourable to invasion by strains with ever larger s . Conversely, small s allows persistence of more mutants, which increases the impact of back mutation and lowers the critical τ , thus favouring invasion by strains with yet smaller s . The above reasoning holds only if both strains have equal rates of forward and back mutations. If small s comes at the cost of replication fidelity, for instance, then the dynamics are likely to be influenced primarily by the form of the trade-off between mutational effects and mutation rate.

Have RNA viruses evolved to have maximally deleterious mutational effects? Our work suggests that RNA viruses that produce acute infections (such that τ is not too large) and that have little back mutation are the most likely candidates. Because recombination is similar to back mutation (both create less mutated types), viruses with non-segmented genomes may be more likely to favour maximized deleteriousness than viruses with segmented genomes. By converting estimates of genomic deleterious mutation probabilities and the mean effects of those mutations to continuous time rates in our analytic model, solving the invasion condition (equation (2.4)) for τ and converting back to an approximate number of doublings, we find that viruses that are replicated less than approximately 30 times prior to infecting new hosts are most susceptible to the effects described above. These considerations suggest that viruses such as measles and rhinovirus, which produce relatively short-lived infections and contain non-segmented genomes, may be more likely than other RNA viruses to demonstrate increased deleteriousness of mutations.

Several studies have begun to characterize the mutational neighbourhood of RNA viruses, including vesicular stomatitis virus (VSV, Sanjuan *et al.* 2004a,b) and bacteriophage $\phi 6$ (Burch *et al.* 2007). Sanjuan *et al.* (2004a) found that roughly 40% of randomly selected point mutations were lethal, 29% were deleterious and 4% were beneficial, and that, among deleterious mutations,

the mean effect on fitness was -67.9%. In bacteriophage $\phi 6$, the mean deleterious effect of 52 random mutations was -9.3% (Burch *et al.* 2007). If phage populations are less structured than that of VSV, then these results are consistent with the predictions of our model, since deleterious mutations in the structured population have a more severe effect. In more general terms, one review has suggested that the mean effect of mutations in RNA viruses is roughly fivefold more deleterious than in DNA-based organisms (Elena *et al.* 2006). Studies such as Sanjuan *et al.* (2004a,b) and Burch *et al.* (2007) demonstrate that accurate characterization of the mutational neighbourhood of RNA viruses is feasible and similar techniques may be useful in testing the results outlined here.

Some studies of genetic architecture claim that robustness is ubiquitous and a necessary feature of biological organization (Wagner 2000, 2005; Kitano 2004). Likewise, most studies of the evolution of canalization have sought to identify the circumstances in which canalization is favoured and view decanalization as a relatively minor phenomenon (Wagner *et al.* 1997; Rice 1998; de Visser *et al.* 2003; Hansen 2006; Hansen *et al.* 2006; Liberman & Feldman 2006). We agree that robustness, both environmental and genetic, is an adaptive feature of many organisms. However, this study, and others like it (e.g. Krakauer & Plotkin 2002; Hadany *et al.* 2006; Elena *et al.* 2007), demonstrates that the evolution may not always seek to minimize deleterious mutational effects. The study of the evolution of genetic architecture may be hindered if we seek only to explain how the expression of certain characters or behaviours becomes less sensitive to perturbation.

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