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Mutator dynamics in fluctuating environments

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Populations with high mutation rates (mutator clones) are being found in increasing numbers of species, and a clear link is being established between the presence of mutator clones and drug resistance. Mutator clones exist despite the fact that in a constant environment most mutations are deleterious, with the spontaneous mutation rate generally held at a low value. This implies that mutator clones have an important role in the adaptation of organisms to changing environments. Our study examines how mutator dynamics vary according to the frequency of environmental fluctuations. Although recent studies have considered a single environmental switch, here we investigate mutator dynamics in a regularly varying environment, seeking to mimic conditions present, for example, under certain drug or pesticide regimes. Our model provides four significant new insights. First, the results demonstrate that mutators are most prevalent under intermediate rates of environmental change. When the environment oscillates more rapidly, mutators are unable to provide sufficient adaptability to keep pace with the frequent changes in selection pressure and, instead, a population of 'environmental generalists' dominates. Second, our findings reveal that mutator dynamics may be complex, exhibiting limit cycles and chaos. Third, we demonstrate that when each beneficial mutation provides a greater gain in fitness, mutators achieve higher densities in more rapidly fluctuating environments. Fourth, we find that mutators of intermediate strength reach higher densities than very weak or strong mutators.

Keywords: mutation; adaptation; natural selection; evolution; simulation; drug resistance

1. INTRODUCTION

Mutators are being observed in an increasing number of bacterial species including *Escherichia coli* (LeClerc *et al.* 1996; Matic *et al.* 1997), *Pseudomonas aeruginosa* (Oliver *et al.* 2000) and mouse gut bacteria (Giraud *et al.* 2001). Recent years have seen a great increase in the volume of work examining the causes and consequences of mutator clones (see the recent review by Sniegowski *et al.* (2000)). Interest in this field has been further fired by the establishment of a link between the presence of highly mutable clones and the evolution of antibiotic resistance (Oliver *et al.* 2000).

Stable environments select for low rates of mutation, constrained only by the costs of error avoidance and error-repair mechanisms (Kimura 1967; Drake 1991). This is due to the fact that in constant environments most mutations are deleterious (Kimura 1967). Theoretical studies (Leigh 1970, 1973; Gillespie 1981; Ishii *et al.* 1989) have shown that in a changing environment higher mutation rates can be favoured. Gillespie (1981) investigated the evolution of mutation rate in a randomly fluctuating environment, and showed that regions of parameter space exist in which high, intermediate and low rates of mutation are selected. This type of theoretical work is backed up by recent empirical studies that have shown that even a single change in the environment can cause an increase in the density of high mutability clones within laboratory populations of *E. coli* (Mao *et al.* 1997; Sniegowski *et al.* 1997). Model studies (Taddei *et al.* 1997; Tenaillon *et al.* 1999, 2000) also concentrate on the spread of a mutator clone through a population experiencing a single shift in its environment. These studies show that a pulse of mutator clones spreads through the population following an environmental shift, thus enabling more rapid adaptation to the novel environment. In these models, the mutator genes are shown to hitchhike on alleles that are beneficial in the new environment (e.g. Tenaillon *et al.* 1999). Once the population is well adapted to the new environment, the mutators are selected against due to the ongoing costs of deleterious mutations associated with them. Kessler & Levine (1998) used a more analytical approach to show that mutators may reach high densities when a population is far from its fitness equilibrium, but that their density is suppressed near the fitness equilibrium due to the detrimental effects of the deleterious mutations.

The potential consequences of mutator strains are still poorly understood, although the recent establishment of a link between mutator clones of *P. aeruginosa* (Oliver *et al.* 2000) and the evolution of drug resistance seems certain to generate considerable interest in this area. Taddei *et al.* (1997) use a model to demonstrate that even when mutators are present at relatively low densities they can speed up evolution, and it is this increased evolvability of mutator clones that poses the greatest concerns. Mutators may also be important in areas other than medicine: for example, their presence may enable some species to more rapidly evolve pesticide resistance.

Recent studies (Taddei *et al.* 1997; Tenaillon *et al.* 1999, 2000), parameterized to simulate the behaviour of *E. coli* populations, have concentrated on the dynamics of mutators in response to a single environmental shift. Many diseases (or pests) are treated by a selection of different drugs (or pesticides), and the disease-causing microbes (or pest) may frequently be exposed to a rapidly changing

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Table 1. The different mutation rates used within the model. All these rates are increased by a factor, m, in the mutator clones. Environment mutations give rise to genotypes that are better or worse adapted to the current conditions with equal frequency.

mutation type	mutation rate in wild-type
deleterious mutation	10^{-4}
environment mutation	10^{-8}
wild-type to mutator	10^{-7}
mutator to wild-type	10^{-7}

drug (or pesticide)-use regime. In this study, we are interested in understanding how mutators behave in an environment that is subject to repeated environmental oscillations. We extend the approach taken by earlier models (Taddei *et al.* 1997; Tenaillon *et al.* 1999, 2000) to explore mutator dynamics under different regimes of environmental fluctuations. Here, unlike Gillespie (1981), the environment is subject to regular fluctuations.

2. THE MODEL

The model developed here draws on recent papers that have established how mutators may spread through an asexual population adapting to a novel environment (Taddei *et al.* 1997; Tenaillon *et al.* 1999, 2000). The present study is deterministic and is parameterized using mutation rates, selective advantages and costs of deleterious mutations that are consistent with those observed in long-term experiments with *E. coli* (Kibota & Lynch 1996; Ninio 1991; table 1). All mutation rates are increased by a factor m in the mutator genotype. A genotype that is not adapted to its environment and has no deleterious alleles has a fitness of 1.0. Each deleterious mutation causes an additive loss of fitness, d. For all the results shown here $d = 0.05$.

We assume that the environment can be in one of two states (0 and 1), and that this state fluctuates regularly through time with frequency, f. At the start of the simulation, the environment is set to state 0, and we assume that the population is well adapted ($A = 0.0$ for the whole population) with no deleterious mutations. Here, it is assumed that there are 11 possible genotypes (0–10)—type 0 is perfectly adapted to environment 0, and type 10 is perfectly adapted to environment 1. Conversely, type 10 is poorly adapted to environment 0, and type 0 has low fitness in environment 1. Genotype 5 has equal fitness in both environments. Mutations can lead to genotypes that are better (or more poorly) adapted to the current environmental condition. A mutation results in a shift of plus or minus one to the genotype with equal probability: thus mutations occurring to genotype 3, increase the density of genotypes 2 and 4. Ten mutations are required for a shift from a genotype that is perfectly adapted to one environment to a genotype that is ideally suited to the other environment. Each mutation that increases the degree to which a genotype is adapted to a particular environment results in an increase in fitness, a. Similarly, each mutation that results in a genotype being less adapted to the current environment reduces fitness by a. Unless stated otherwise, $a = 0.05$.

3. RESULTS

(a) *Mutator density and the frequency of environmental fluctuations*

Three distinct types of dynamics are observed as the frequency of environmental oscillations increases (figure 1*a*). Type I dynamics occur when the frequency of environmental fluctuations is low; here, the results are similar to those obtained in previous studies (Taddei *et al.* 1997). Mutators spread through the population following each environmental fluctuation, allowing the population to adapt more rapidly to the new environment than if they were not present. Once the population is well adapted, the mutators are selected against, due to the higher rate at which they suffer from deleterious mutations (figure 1*b*). Type II and type III dynamics are novel and significant. Type II dynamics occur when the environment oscillates more rapidly, resulting in the mutator density not returning to a mutation–indirect-selection equilibrium between each fluctuation. Instead, the mutators can be present at high densities within the population throughout time (figure 1*c*). Type III dynamics occur when the environment fluctuates even more rapidly between the two states. During this stage, the mutators are only present at a low density within the population, with their concentration increasing only marginally when the environment changes (figure 1*d*). With these rapid environmental oscillations, mutator genotypes are unable to provide enough genetic variability to keep track with the changing selective pressures and, instead, a non-mutator genotype that is reasonably adapted to both environments prevails, in this case genotype 5, due to it having the highest geometric mean fitness.

(b) *Complex mutator dynamics in fluctuating environments*

Interesting mutator dynamics occur when the environment fluctuates at intermediate frequencies (figure 2). Stable limit cycles (figure 2*a*,*b*) occur under a relatively wide range of conditions. Chaotic dynamics (figure 2*c*) are also observed, albeit less frequently. As the density of mutators within the population varies, so too does the ability of the population to adapt to changing conditions. Thus, in the region where chaotic mutator dynamics are observed, the population's rate of evolution changes chaotically through time. Complex dynamics are not observed either when the environment fluctuates very rapidly or when it fluctuates at a very low frequency.

(c) *Varying the fitness benefit of becoming better adapted*

The relative gain in fitness provided by each well-adapted allele determines the frequency of fluctuations under which mutators are sustained at high densities. As the gain in fitness increases, the mutators achieve higher densities in more rapidly fluctuating environments (figure 3). Alleles that provide resistance to drugs will frequently confer great fitness benefits, so we might expect mutators to reach high densities in populations adapting to different drug regimes, even when the frequency of variation in drug use is rapid.

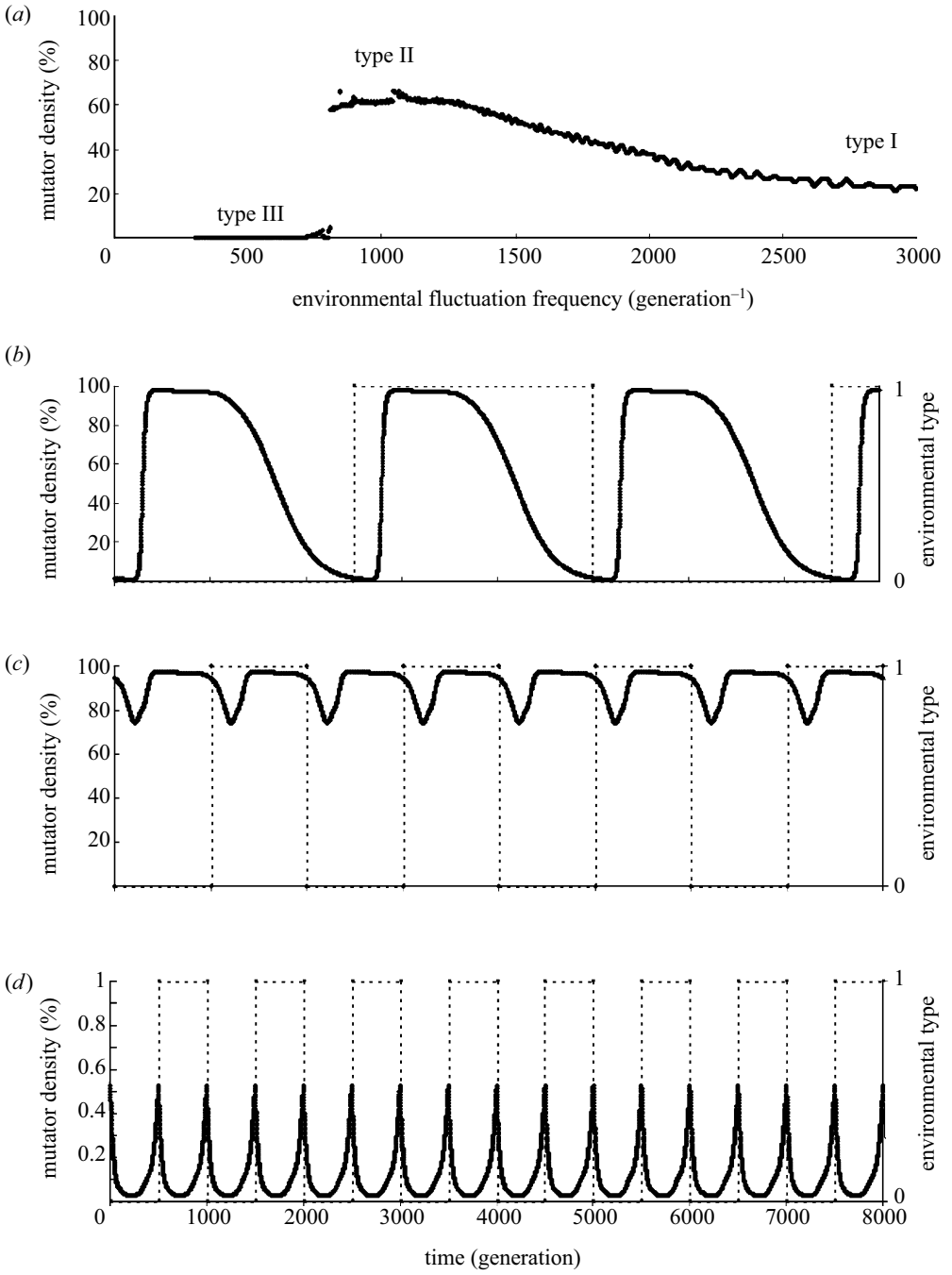

Figure 1. Mutator density in populations at varying environmental fluctuation frequencies with a mutator strength of 100. (*a*) Time-averaged mutator density over a range of environmental fluctuation frequencies from 0 to 3000 generation^{-1}. Type I, II and III behaviours are marked. (*b–d*) Mutator density (solid line) over time with varying fluctuation frequencies. The environmental type is illustrated by a dotted line. (*b*) Example of type I with fluctuation of 2500 generation^{-1}. (*c*) Example of type II with fluctuation of 1000 generation^{-1}. (*d*) Example of type III with fluctuation of 500 generation^{-1}. The mutator density scale is from 0 to 1%. In all these examples, $a = d = 0.05$.

(**d**) *Varying mutator strength*

Highest mutator densities are achieved when the mutators are of intermediate strength (figure 4). When mutator strength is low, mutators never reach a high density and thus have little impact on the evolution of the population to a changing environment. Intermediate strength mutators can reach high density. These mutators cause enough genetic variability to enable the population to adapt to a change in the environment. Hitchhiking on the well-adapted alleles, they reach a high density within the population. At higher mutator strengths, mutators do not reach such a high density. These powerful mutators rapidly generate genotypes with optimum values of the quantitative trait. Once all beneficial mutations have been acquired, indirect selection against these mutators is especially intense due to the greatly increased rate of deleterious mutations associated with them. Here, the indirect benefits of a mutator genotype are too short lived for mutators to hitchhike to a high density within the population. However, Taddei *et al.* (1997) have shown

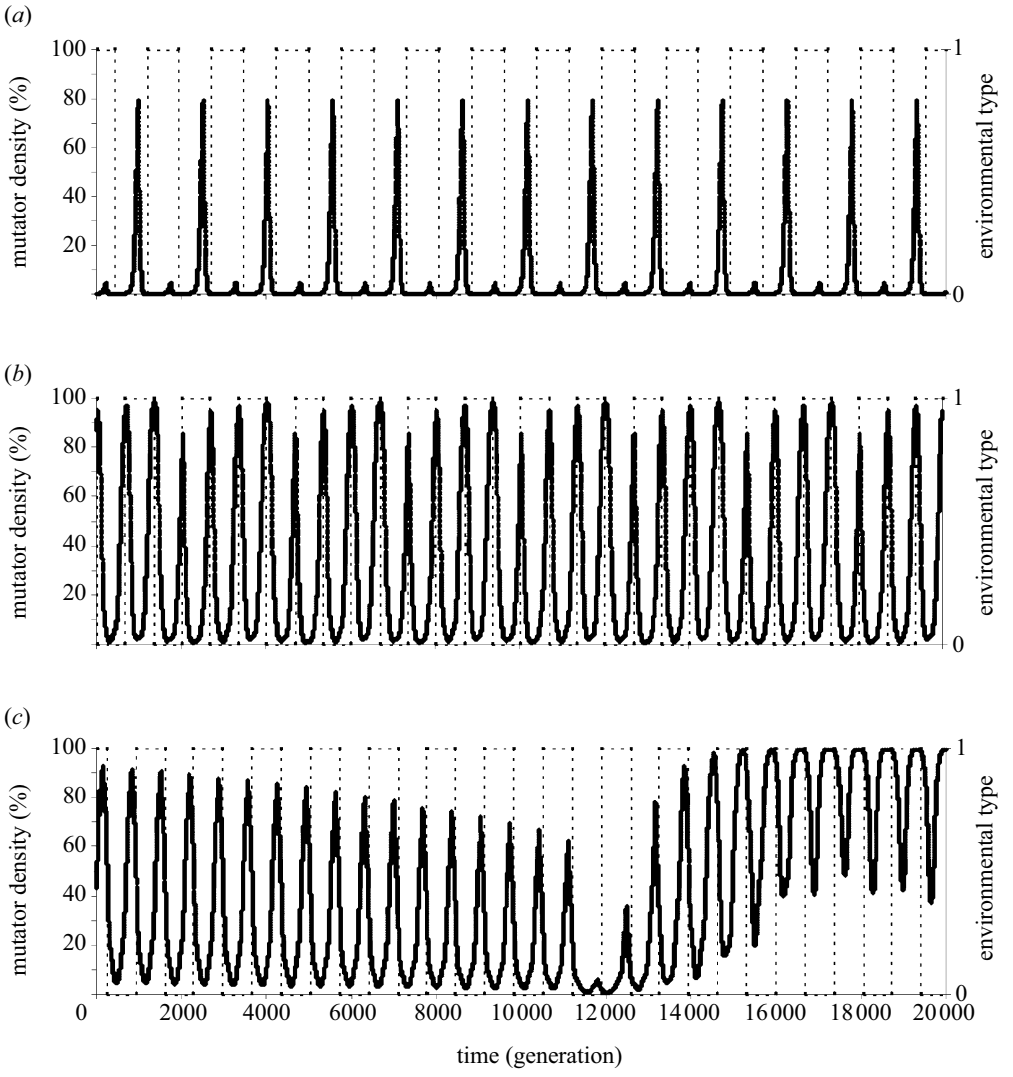

Figure 2. Complex dynamics illustrated by plotting mutator density (solid line) against time. The environmental type is shown by a dotted line. (*a*) Two-point limit cycle exhibited at a mutator strength of 75 and at a frequency of fluctuation of 764 generation^{-1}. (*b*) Four-point limit cycle exhibited at a mutator strength of 25 and at a frequency of fluctuation of 667 generation^{-1}. (*c*) Chaos exhibited at a mutator strength of 10 and at a frequency of fluctuation of 685 generation^{-1}.

that even though they may remain at a low density, high-strength mutator genes can have a large impact on the adaptation of an organism to a novel environment.

4. DISCUSSION

(a) *Mutator density and the frequency of environmental fluctuations*

Our results demonstrate that the relationship between mutator density and the rate of environmental fluctuations is unlikely to be simple. Populations that experience environments that oscillate with intermediate frequencies will have the highest time-averaged mutator density, whereas populations living in the most variable environments may, somewhat unexpectedly, have the lowest. This finding may have consequences for microbial ecology, medicine and pest control.

The role of mutators in microbial evolutionary ecology is a potentially exciting area, but is entirely unexplored. A better understanding of environmental factors that promote the presence of mutators should help to identify those natural environments in which mutator clones may be most likely to be found and, thus, the results presented here are a vital step towards improved comprehension of the role of mutators in evolutionary ecology. As further progress is made in describing the presence of mutators within a greater number of species, studies of the ecological consequences of these mutators for microbial population and community dynamics will become possible. Such studies should provide significant new insights into the role of mutators within the natural world.

The drug that is used to treat a particular disease, or the pesticide used to control a particular species, frequently varies with time (e.g. Brabin *et al.* 1997; Henry *et al.* 1998). The results from our model suggest that the frequency at which drug or pesticide use changes may determine how abundant mutators become within populations. If the environmental conditions drive mutators to high densities, then the evolution of drug or pesticide resistance is likely to proceed more rapidly than would otherwise be the case. The work of Oliver *et al.* (2000) presents, to our knowledge, the first evidence linking mutators to the evolution of drug resistance, and further examples seem certain to follow. Our study indicates that

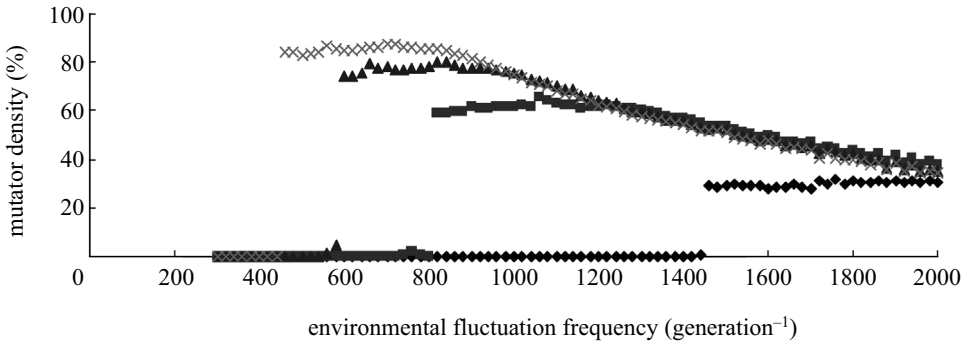

Figure 3. Effect of relative gain in fitness, *a*, provided by each well-adapted allele on mutator density over a range of environmental fluctuation frequencies. Gain in fitness: diamonds, 0.03; squares, 0.05; triangles, 0.07; crosses, 0.09.

by strategically controlling the frequency at which different drugs (or pesticides) are alternated, it might be possible to reduce the density of muators within a population. This in turn might limit the ability of the population to evolve resistance. Conversely, alternating between different control agents at the wrong frequency can increase the density of mutators, with the potential consequence of accelerating the evolution of resistance.

(b) *Mutator dynamics in fluctuating environments*

Our results demonstrate that under most model scenarios mutator density varies with time. This is true unless the environmental fluctuations are too rapid for mutators to become established within the population, in which case their density remains very close to zero throughout time. This has important implications for the search for mutator clones within natural populations. If a population is sampled and mutators are not found, it does not imply that they are never present (or important) for that population. An alternative explanation is that the mutators are temporarily at a low density. Figure 2*a* shows that, under certain scenarios, mutators can be at a low density for a high proportion of the time, but can still be highly important in allowing the population to adapt rapidly to a change in the environment.

The temporal dynamics of mutators can be complex. Although we had, to some extent, anticipated that mutators might reach highest time-averaged densities at intermediate frequency fluctuations, the complex dynamics were a surprise. Limit cycles and chaotic dynamics are a common feature of population models in ecology (e.g. Hastings *et al.* 1993; Rohani & Earn 1997; Earn *et al.* 1998) and may also be a feature of some epidemics, such as measles (Grenfell *et al.* 1995*a*,*b*). Our finding that mutators may also exhibit chaotic dynamics, driven here by the fluctuating environment, provides another example of the theoretical possibility of chaotic dynamics within biological systems. Future studies relaxing the assumption of deterministic dynamics that we make here, and in particular models that incorporate genetic drift, should reveal just how robust this result is. One exciting implication of the results presented here is that the rate of evolution within a population may vary in a complex fashion, perhaps in some instances exhibiting chaos. It has been recognized that the molecular clock does not necessarily tick at a constant rate (Ohta & Kimura 1971; Gillespie 1989; Ayala 2000); thus making estimates of the timing of evolutionary events, such as the divergence of plants and animals, problematic. Our results suggest that the existence of mutator strains within certain groups of organisms may provide an explanation for non-constant rates of molecular change.

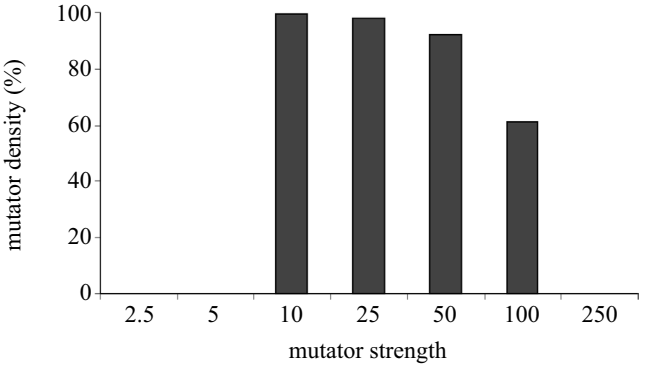

Figure 4. The density that mutators reach depends on the strength of the mutator. Highest densities are reached when the mutator has an intermediate strength. Here, the frequency of fluctuation is 1000 generation^{-1}, $a = d = 0.05$.

(c) *Varying mutator strength and the fitness advantages of being better adapted*

When greater fitness advantages are associated with mutations that are favourable to a particular environment, mutators are able to spread through the population at more rapid environmental fluctuations and are likely to reach higher densities. For populations undergoing the intense selection caused by drugs or pesticides, the fitness advantages gained from the acquisition of resistance are likely to be very high. The implication of this might be that even if drug or pesticide use is changed rapidly, mutators may be able to invade the population, thus enabling the more rapid acquisition of resistance to one (or possibly multiple) drugs or pesticides.

Mutators reach the highest density at intermediate mutator strengths. This result indicates that weak mutators may never become established within populations, and thus may pose few problems within areas such as drug and pesticide resistance, as long as the drug or pesticide being used is changed occasionally. Intermediate mutators are most likely to be at high densities, and thus are the ones that studies are most likely to find. The model suggests that under certain conditions, more than 90% of the

population may be made up of mutators. These intermediate strength mutators can bring about accelerated adaptation to changing environments. The role of these intermediate strength mutators may have important implications for medicine and pest control. Stronger mutators can also bring about rapid adaptation, with similar implications. However, the model indicates that these genotypes are less likely to reach high densities within the population and that any rise in their density is for a shorter period than for weaker mutators. This may make finding empirical examples of the most powerful mutators problematic.

(d) *High mutability in other organisms*

In this paper, we have concentrated on mutators in bacteria. High mutability is also observed in viruses (Mansky & Cunningham 2000) and also in tumours in prokaryotes (Tomlinson *et al.* 1996). Some of the results presented here may be of importance in both these areas, and again there may be important medical consequences of modelling mutator dynamics for the design of drug-use regimes. However, it would be unwise to expect the mutator dynamics exhibited in this model, parameterized for *E. coli*, to be directly applicable to either tumours or viruses. Clearly, further studies of this type, parameterized specifically for these different systems, are needed.

(e) *Future directions*

Further work is required to understand how environmental fluctuations might affect mutator dynamics in other organisms. Our model has been parameterized for *E. coli*, due both to the ease with which we could obtain the parameter values, as well as making our study comparable with the work we were extending (Taddei *et al.* 1997; Tenaillon *et al.* 1999, 2000). It will be important for future studies to tailor their parameters to fit other organisms. It will be of particular interest to model mutator dynamics for those organisms in which mutators are linked to antibiotic resistance, although currently it may be hard to obtain the necessary parameter values for many such bacterial species. Many organisms have higher levels of genetic exchange. In general, it is expected that higher levels of exchange make it less likely that selection will favour highly mutable individuals (e.g. Tenaillon *et al.* 2000), but only extensions to this type of study will allow us to ascertain whether certain environmental conditions exist in which mutators can occur even when recombination is frequent.

One of the assumptions of the current model is that the fitness effects of having poorly adapted genes for the two environments are symmetrical. Frequently, this will not be true: individuals that are exposed to drugs (or pesticides) will suffer greater reductions in fitness if they are poorly adapted to that environment compared with individuals that are well adapted to resist drugs (or pesticides) and who live in a drug-free (or pesticide-free) environment. Future work in this area might prove valuable; for example, the recent results showing the prevalence of mutators, and their association with drug resistance within hypermutable *P. aeruginosa* (Oliver *et al.* 2000), have highlighted the medical need for further studies into mutator dynamics.

5. CONCLUDING REMARKS

The work presented in this paper has clearly demonstrated the need to consider the role that environmental variability has in the establishment of strains with high mutator density. This work has considered only regularly fluctuating environments and the model was parameterized for one particular species, *E. coli*. There is great scope for further studies in this area, and the results to come out of this line of investigation may have important consequences for how frequently drug (or pesticide) regimes are altered in the future.

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