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The effect of spatial structure on adaptation in *Escherichia coli*

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Populations of organisms are generally organized in a given spatial structure. However, the vast majority of population genetic studies are based on populations in which every individual competes globally. Here we use experimental evolution in *Escherichia coli* to directly test a recently made prediction that spatial structure slows down adaptation and that this cost increases with the mutation rate. This was studied by comparing populations of different mutation rates adapting to a liquid (unstructured) medium with populations that evolved in a Petri dish on solid (structured) medium. We find that mutators adapt faster to both environments and that adaptation is slower if there is spatial structure. We observed no significant difference in the cost of structure between mutator and wild-type populations, which suggests that clonal interference is intense in both genetic backgrounds.

Keywords: adaptation; spatial structure; mutation rate; clonal interference

1. INTRODUCTION

Natural populations are often dispersed over a spatial environment where individuals compete locally. The effect of spatial structure on evolution and adaptation has been studied in several theoretical models (Rousset (2004) and for a recent review see Charlesworth *et al.* (2003)). A recent study has predicted that if mutation rates to new beneficial alleles are high, adaptation proceeds more slowly in a spatially structured environment than in one where competition is global (Gordo & Campos 2006). We can therefore say that there is an adaptive cost of spatial structure. The reason for this relates to an important phenomenon that limits adaptation in asexuals—the Hill–Robertson effect (1966) or clonal interference (Gerrish & Lenski 1998). In asexual organisms, clones with different beneficial mutations will compete with each other and only one will fix in the population. This leads to a reduction in the rate of adaptation and to an increase in the mean effect of fixed mutations (Campos & De Oliveira 2004). This interference is more important the higher the product of population size and beneficial mutation rate, that is, the higher the number of segregating mutations. If competition is local, the time to fixation is longer and the chance that another beneficial mutation will appear in a different individual, and compete with the first one, is higher (Gordo & Campos 2006). Therefore, adaptation in asexuals when there is spatial structure is slower than if there is no structure.

In nature, the spatial organization of individuals in a population is probably variable. Periodic changes in spatial structure have been modelled and it has been shown that these changes impose an even higher adaptive cost of spatial structure. This is so because newly arising mutations that have not yet risen in frequency at the time of the spatial rearrangement have a higher probability of being lost (Perfeito *et al.* 2006). Therefore, the biggest difference in adaptation rates should be seen between populations with a fluctuating spatial structure and populations with no spatial structure.

There are a few studies on adaptation of organisms in structured environments using experimental evolution in micro-organisms (Chao & Levin 1981; Korona *et al.* 1994; Rainey & Travisano 1998; Dionisio *et al.* 2005; Habets *et al.* 2006). However, none of these studies addressed differences in adaptation dynamics specifically.

In this work we use an experimental system with *Escherichia coli* to directly test the theoretical prediction that a structured environment has a high adaptive cost. We compare the rates of adaptation to a medium with no spatial structure and to a structured medium with periodical changes in spatial structure (Perfeito *et al.* 2006). We studied a wild-type *E. coli* strain and a mutator strain, where the impact of clonal interference is expected to be higher (Cooper 2007). We find that mutators adapt faster than wild-type populations in both environments. We also show that adaptation proceeds more slowly in a structured environment than in one where each individual competes with every other, for both mutators and wild-type. We therefore observe a cost of structure on the rate of adaptive evolution in both genetic backgrounds. This is expected theoretically when clonal interference plays a major role in the evolution of these populations.

2. MATERIAL AND METHODS

(a) *Bacterial strains*

The *E. coli* strains used were K12 MG1655 srl::Tn10 MutS$^+$ ara$^+$, K12 MG1655 srl::Tn10 MutS$^+$ Δara, K12 MG1655 srl::Tn10 MutS$^-$ ara$^+$ and K12 MG1655 srl::Tn10 MutS$^-$ Δara, which were constructed by P1 transduction (Miller 1992) from strains K12 MG1655 Δara, K12 srl::Tn10 MutS$^-$ Str^R and K12 srl::Tn10 MutS$^+$ Str^R kindly provided by I. Matic. When plated on rich medium supplemented with arabinose, the ara$^+$ strains create white colonies, while the Δara strains give rise to red colonies, so the initial and final number of each strain can be readily assessed (Levin *et al.* 1977). The mutator strain has a mutation rate which is 60-fold higher than the wild-type (Trindade *et al.* in preparation).

(b) *Experimental evolution*

All populations were derived from single clones (either mutator or non-mutator) which were grown from stocks in 50 ml tubes with 10 ml of M9 minimal medium supplemented with 5% glucose (MM), at 37°C. Every day, the cultures were diluted and grown in a 60 mm diameter Petri dish with MM supplemented with agar (structured environment where the structure was randomized every day) or in a tube with liquid MM incubated at 230 RPM in an Infors HT Unitron

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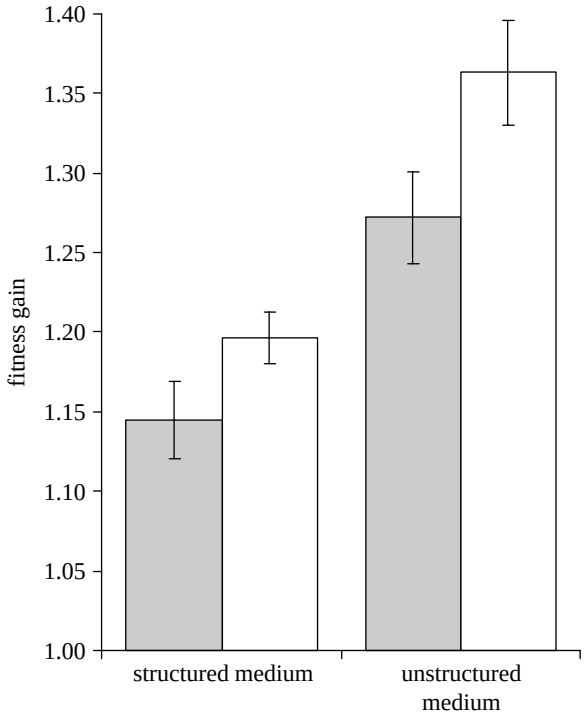

Figure 1. Mean fitness relative to the ancestral after 275 generations of evolution in either structured (solid) or unstructured (liquid) media. Grey bars represent the wild-type results and white bars represent the mutator populations. The error bars show twice the standard error.

Shaker (unstructured environment) so that approximately 6×10^6 bacteria were allowed to propagate for approximately 11 generations, reaching a final number of colony-forming units (CFUs) of 10^{10}. The final CFU number was the same on either the Petri dishes or the tubes (Student's *t*-test: $t_{90}=0.43$, $p=0.67$). The effective population size is $N_e=B\cdot G$ (Haldane 1927; Wahl *et al.* 2002), where B is the initial number of bacteria and G is the number of generations between bottlenecks. N_e was approximately 7×10^7 in both environments and the evolution occurred during approximately 275 generations. In the structured environment, five independent wild-type populations and six independent mutator populations were allowed to evolve. In the case of the unstructured environment, six independent populations of both wild-type and mutator were evolved. There was no significant difference either in liquid or solid medium in the adaptation rate of ara^+ and Δara (Student's *t*-tests: $p>0.05$) strains, so they were grouped and analysed together. After 25 days (approx. 275 generations), all populations were competed with the ancestor in the same environment used in the evolution experiment to assess their fitness increase (see electronic supplementary material).

3. RESULTS

Replicate populations of both mutator and wild-type strains were allowed to adapt to either structured (liquid) or unstructured (solid) media and after 275 generations their fitnesses were measured. The results are summarized in figure 1. As expected, in both environments, the fitness was higher for the mutator strains than for the wild-type. This agrees with previous experiments in unstructured environments (de Visser *et al.* 1999) and we show here that the same is true for a structured environment (two-way ANOVA, $p<0.001$). Owing to their high mutation rate, the probability that a mutator will have a beneficial mutation is higher than the wild-type. Mutators also suffer from an increased probability of accumulating deleterious mutations (Funchain *et al.* 2000; Trindade *et al.* in preparation). Although the accumulation of deleterious mutations in our experiment is more likely in mutators, given our large population size, empirical estimates of deleterious mutation rate and effect and the mutator strength, these are probably purged in our populations (Crow & Kimura 1970).

Despite the fact that fitness increased in all cases, the augment was bigger in the unstructured environment, for both mutators and wild-type populations (two-way ANOVA, $p<0.001$ for both strains). So, as predicted theoretically, there is a cost on the speed of adaptation when there is spatial structure (Gordo & Campos 2006; Perfeito *et al.* 2006).

It was also predicted that the adaptive cost of structure (measured by the difference between the rate of adaptation in the unstructured and the structured environments, normalized by the rate in the unstructured environment) should increase with the rate to advantageous mutations (U_a) but have a limit for high U_a, in conditions where clonal interference has a major impact during adaptation (Gordo & Campos 2006). This limit is also observed when we consider a spatial structure that fluctuates in time (Perfeito *et al.* 2006; electronic supplementary material). We observe that after 275 generations of adaptation, the cost for the wild-type was 10% and for mutators it was 12%. Since this difference is not significant (two-way ANOVA, $p=0.42$), this suggests that the beneficial mutation rate is so large that further increases in the mutation rate do not lead to higher costs of structure. Nevertheless, the adaptation rate still increases with the beneficial mutation rate, which is expected under models that postulate intense clonal interference (Desai *et al.* 2007). In fact, a high U_a is compatible with recent estimates for *E. coli* on the order of 10^{-5} beneficial mutations per genome per generation (Perfeito *et al.* 2007).

To estimate the rate and effects of beneficial mutations that best describe the data, we used stochastic simulations of the process of adaptation in the unstructured environment during 275 generations (further details of the simulations are available on the electronic supplementary material). We find that a $U_a\sim10^{-6}$ (for the wild-type strain), and a mean selective effect of 2% can explain the observed fitness increases in the unstructured environment. Given an effective population size of 10^7 and an estimated U_a of 10^{-6}, this implies that the effect of clonal interference is strong which can explain the observed cost of structure in both the genetic backgrounds, provided the beneficial mutation rates are similar in both environments.

4. DISCUSSION

We study the adaptation of *E. coli* in structured and unstructured environments using strains with different mutation rates. Our results can be compared with recent predictions of theoretical models (Perfeito *et al.* 2006), which studied adaptation in fluctuating spatial environments. We show that mutator populations adapt faster than wild-type populations in both environments and that, independently of the mutation rate, the increase in fitness in the structured environment is smaller than in the unstructured one. This can be explained because, on one hand the effect of clonal

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interference is higher when there is structure (Gordo & Campos 2006), and on the other hand, the effect of genetic drift is higher when the spatial structure is not stable (Perfeito *et al.* 2006). We do not observe a significantly higher cost of structure for the mutators, which is expected if the adaptive mutation rate is very large in both genetic backgrounds.

Previously, Miralles *et al.* (1999) found that increasing population structure reduced the rate of adaptation in an RNA virus. Recently, Habets *et al.* (2006) used *E. coli* evolving in a structured environment with the aim of studying how spatial structure affects the emergence and maintenance of diversity in an ecological perspective. For this purpose, they analysed diversity parameters by comparing single clones from the evolved populations with the ancestor. In particular, they found that in an environment with a fixed spatial structure, frequency-dependent selection is very common, whereas in a mixed structure environment, as the one we use, frequency dependence is not detected. Importantly, they observed that adaptation is slower when there is no spatial structure, which is opposite to what we found. The discrepancy between the results may be due to a difference in methodology, because we measured fitness of a large sample of the evolved populations whereas these authors measured fitness of single clones (for more details, see electronic supplementary material).

In natural environments, there will always be some spatial structure. In particular, for bacterial communities, individuals are likely to compete locally and the spatial structure is likely to change in time. Bacteria biofilms are one such example. Biofilms show a high capacity to develop virulence and antibiotic resistance (Costerton *et al.* 1981). Although there may be a selective advantage in growing as groups in a spatial structure, there is also a cost due to a slower adaptation rate. Understanding the dynamics of adaptation in such structured environments might shed some light into how these structures evolve and what might be the best way to fight them.

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