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

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1. INTRODUCTION

Natural populations 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 with *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

2. MATERIAL AND METHODS

(a) **Bacterial strains**

The *E. coli* strains used were K12 MG1655 srl::Tn10 MutS\(^+\) ara\(^-\), K12 MG1655 srl::Tn10 MutS\(^-\) ara\(^+\), K12 MG1655 srl::Tn10 MutS\(^-\) ara\(^-\) and K12 MG1655 srl::Tn10 MutS\(^+\) ara\(^+\). The mutator strain has a mutation rate which is 60-fold higher than the wild-type (Trindade 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 Unitor.
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 (Punchain 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 \( U_a \sim 10^{-6} \) (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 interference has a major impact during adaptation (Gordo & Campos 2006).
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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