Title: Adaptation is influenced by the complexity of environmental change during evolution in a dynamic environment.

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Abstract

The environmental conditions of microorganisms' habitats may fluctuate in unpredictable ways, in terms of temperature, carbon source, pH, and salinity to name but a few. Such environmental heterogeneity presents a challenge for such microorganisms, as they have to adapt not only to be fit under a specific condition, but they must also be robust across many conditions *and be able to deal with the switch between conditions itself*. While experimental evolution has been used for decades to gain insight into the adaptive process, this has been largely in unvarying conditions. In cases where changing environments have been investigated, relatively little is known about how such environment influence the dynamics of the adaptive process itself, as well as the genetic and phenotypic outcomes. We designed a systematic series of evolution experiments where we used two conditions with differing time-scales of adaptation and varied the rate of switching between them. We used lineage tracking to follow adaption itself, and whole genome sequenced adaptive clones from each of the experiments. We find that the both the switch rate and the order of the conditions influences adaptation, and that switching can both speed up and slow down adaptation, depending on those parameters. We also find different adaptive outcomes, both at the genetic and phenotypic level, even if populations spent the same amount of total time in the two different conditions, but that the order and/or switch rate differed. Thus, in a variable environment adaptation depends not only on the nature of the conditions and phenotypes under selection, but also on the complexity of the manner in which those conditions are combined to result in a given dynamic environment.

Introduction

How organisms evolve is a fundamental question in biology, and how they adaptively evolve in response to *changing* environments is a question whose answer is central to rational vaccine development[1], as well as to understanding the evolution of multiple antibiotic resistance[2,3], the evolution of immune systems[4], and even heritability[5]. Specific aspects of this question include: "What are the parameters for which success in a changing, or dynamic environment can be selected for?," and, “Are there general mechanisms for adaptation to dynamic environments?” In nature, some environmental changes are predictable, and organisms can evolve responses to such predictable changes. For example, *E. coli* shows asymmetric anticipation of carbon sources, such that in the presence of lactose, *E. coli* anticipates that maltose will become available, because this is what has repeatedly experienced in the mammalian gut, and is due to lactose modestly inducing the genes required for maltose metabolism[6]. However, when wild *E. coli* are brought into the lab, which typically lacks this selective pressure, this “anticipation” is lost as the strain undergoes domestication[6]. Likewise, circadian clocks are thought to provide a fitness benefit, allowing organisms to adapt physiologically to diurnal changes in light, temperature, and humidity[7]. In Cyanobacteria, the benefit of a circadian clock can only be maintained in the lab by continued exposure to a rhythmic environment[8]. Changes in the environment may also be unpredictable or random, and whether organisms can evolve to better deal with such environmental changes is an open question.

Experimental Microbial Evolution (EME) is a prospective approach to studying adaptive evolution in the laboratory and was first developed ~140 years ago[9]. EME has been used to address fundamental evolutionary questions, such as the rate at which beneficial mutations fix[10], and the influence of both ploidy[10] and sex[11] on that rate. High-throughput sequencing has made it possible to establish at high resolution how mutations accumulate in co-evolving lineages, revealing clonal interference, with hundreds or thousands of beneficial lineages competing[12-15], sometimes even with multiple lineages persisting in a quasi-stable state for thousands of generations[16]. While EME has provided many insights into the evolutionary process[(see 17,18 for reviews)], such experiments have typically be performed in either constant environments (such as the chemostat), predictably fluctuating environments (as in serial transfer), or in some instances in environments where a variable of interest changes monotonically over time, as in a morbidistat[19], or over space, as in the mega-plate experiment[20]. However, outside of the laboratory organisms are almost never challenged to adaptively evolve in such predictable environments, but rather must cope with variability and stochasticity. To date, only a few EME studies[(see 21 for review)] have sought to determine either how microbes adapt to unpredictable changes in the environment, or what characteristics of such changes might be important in influencing adaptation. For example, *Pseudomonas fluorescens* was evolved in variable environments, switching between contrasting carbon sources (xylose and mannose), and it was found, contrary to expectation, that populations frequently evolved to be niche specialists, and became adapted to the less favorable carbon source[22]. By contrast, when evolving in a heterogeneous environment containing multiple carbon sources, adaptation converges on the most productive carbon source[23]. In another example, a recent study investigated the fitness of the yeast deletion collection under different time scales of periodic environmental change and showed that some mutants are better at dealing with the environmental switch itself, suggesting that it is possible to evolve genotypes that are adapted to change, *per se*[24]. To date, no EME studies have followed the dynamics of lineages adapting in populations as they experience such changing environments.

To fill this gap, and to improve our knowledge of how dynamic environments impact the evolutionary process, a systematic (for the same set of environments) exploration of the parameters of dynamic environments is needed, to determine how these parameters affect evolutionary dynamics, and the fitness effects of adaptive mutations across environments. Here we present a series of experiments that explore evolution during switching between two environmental conditions (glucose containing medium with fluconazole, vs. medium containing ethanol/glycerol with no drug), varying two important parameters: 1) the degree of randomness of the switches between the two conditions, and 2) the consecutive time spent in each condition. Using DNA barcode-based lineage tracking we followed the evolutionary dynamics in 8 different environmental scenarios, investigating the statistics of the evolutionary dynamics, and determining the phenotypic and genotypic characteristics of adaptive mutants arising in each. We found that the speed of adaptation could be either slowed down or sped up depending on the rate of switching between conditions, and that different switching dynamics could lead to the selection of clones with very different behaviors in each environment. Finally, we found that different environmental sequences select for different phenotypic and genotypic outcomes.

Results

Experimental Design

We evolved barcoded diploid yeast populations in dynamic environments built using two single environment blocks (see Fig. 1 for experimental design), varying two main parameters: i) the time spent in each particular environment, relative to the timescale of adaptation within that environment and ii) the periodicity/randomness of the switching between environments. The timescale of adaptation is the time required for a certain fraction of the population to exhibit an adaptive phenotype within a given environment: for diploid yeast evolving by serial transfer in synthetic complete (SC) medium with 2% glucose + 4 μg/ml Fluconazole (hereafter referred to as “Fluconazole”), ~20% of the population is adaptive after 48 generations, while, in SC medium with 2% glycerol and 2% ethanol (“Gly/Eth), the timescale of adaptation is much longer: ~15% of the population is adaptive after 144 generations (Humphrey, Herissant et al, in prep.). We designed 8 different evolution experiments (Fig. 1) using the Fluzonazole and Gly/Eth environments, chosen specifically because of their different timescales of adaptation. The first two experimental sequences were designed so that environmental blocks are periodically switched, with consecutive time spent in each on the order of the time scale of adaptation (periodic_adap1 and periodic_adap2): 144 consecutive generations in Gly/Eth and 48 consecutive generations in Fluconazole. The next two sequences were designed so that blocks are periodically switched at a rate that is 6-fold faster than the previous sequence; thus the consecutive time spent in each environment is 6-times shorter than the time scale of adaptation (periodic_smaller1 and periodic_smaller2): 24 consecutive generations in Gly/Eth and 8 consecutive generations in Fluconazole.

We also designed one experiment with random switching between environments, with blocks for which the duration of residence is of the magnitude of the time scale of adaptation (random_adap1), and two experiments that randomly switch between blocks of environment, for which the duration of residence in each environment is less than the time scale of adaptation (random_smaller1 and random_smaller2). A final experiment was designed that combined the two block environments, i.e. SC with 2% glycerol, 2% ethanol and 4μg/ml Fluconazole (Mix).

Eight populations of barcoded diploid yeast were then evolved for 576 generations in each of the 8 different sequences of environment. The yeast populations contain two barcodes, such that one (BC1, low diversity) encodes the evolutionary condition itself, while the second (BC2, high diversity) is used for lineage tracking within the evolution experiment, to distinguish lineages from one another. We followed the population dynamics using lineage tracking[12] for the first 192 generations, allowing us to characterize the "early" stages of adaptation in different dynamic environments. We isolated 336 clones from generation 192 of each evolution, determined their barcodes (see Methods), and pooled unique lineages for which we could recover a barcode sequence. We remeasured the fitness of all lineages in this pool in 5 environments: Fluconazole, Gly/Eth, Mix, 8 generations in Fluconazole and 24 in Gly/Eth (1:3), 8 generations in Fluconazole and 8 in Gly/Eth (1:1) (Fig 3. and SI 1,2). The rationale behind remeasurement in the 1:1 environment was to determine if there has been selection for a phenotype related to their ability to *switch between* environments, instead of fitness in one of the two environment blocks *per se*. Pooled fitness remeasurement experiments were performed in triplicate as described[25], and also included known neutral barcoded lineages, barcoded adaptive yeast from a Fluconazole only evolution and barcoded adaptive yeast from a Gly/Eth only evolution (Humphrey, Herissant et al, in prep.) as controls. Neutral lineages in our experiments are defined as lineages behaving in the 5 environments as the known neutral lineages (Fig. SI 3). Fitness was determined as described previously[26].

The dynamics of adaptation are affected by the environmental dynamics

We observed that while each sequence of environments gave rise to distinct lineage dynamics, some environmental sequences gave rise to similar lineage behaviors (Figure 2A). For example, lineage trajectories from periodic_smaller1 and periodic_smaller2 are similar, because their sequences of environment are essentially the same, except they are offset from one another by a single environmental block. Likewise, periodic_adap2 and random_adap1 display visually similar lineage trajectories, likely because the lengths of the environmental blocks are similar (on the order of timescale of adaptation). By contrast, trajectories from periodic_smaller1 and periodic_smaller2 clearly differ from those of periodic_adap2 and random_adap1. We thus classified the environments into 3 groups: strong selection, intermediate selection and weak selection, based on both the rate at which neutral lineages went extinct and the diversity of adaptive lineages after 192 generations (Figure 2B). Under strong selection (periodic_adap1, Mix (which both initially contain Fluconazole), and random_smaller1), the populations are rapidly taken over by a few, fit lineages, with neutral lineages going rapidly extinct. Indeed, after 192 generations the 100 most abundant lineages are 84%, 80%, and 80% of these populations respectively. By contrast, in the weak selection environments (periodic_smaller1, periodic_smaller2) only a few lineages increased in frequency, and after 192 generations the 100 most abundant lineages represent only 6.7% or 2.7% of the populations respectively. Under intermediate selection (periodic_adap2, random_adap1 and random_smaller2) many more lineages significantly change their frequencies, while there is still diversity in the isolated lineages: the top 100 lineages at generation 192 represent 20%, 32% and 13% of the total populations for those experiments respectively. These characterizations of strength of selection are consistent with the change in the Shannon entropy in each population (Figure 2C) – under strong selection, diversity crashes early, under intermediate selection the diversity decreases

around 160 generations, while under weak selection, the diversity stays high through the first 192 generations.

Environmental switching can slow down adaptation

The fitness remeasurement data allow us to better understand the differences in evolutionary behavior between different environments, how clones evolved in one environment fare in another, and how a change in environment affects fitness and adaptation (and possibly also *evolvability*). For clones isolated from any of the environments, we observe no strong deleterious fitness effects when fitness is measured in a single environment block for either of the two conditions (Fig.3A, SI11). By contrast, there is a strong negative correlation between these two conditions when fitness is measured in the context of a switching environment, such that clones often display a fitness cost in the fluconazole portion of the environment (Fig. 3B; for full data see SI 12). In Fig. 3A, fitness is measured over 40 consecutive generations in each condition separately (see SI 1), but in Fig. 3B fitness in Fluconazole is measured over 8 generations in between 24 consecutive generations in Gly/Eth, and fitness in Gly/Eth is measured following 8 generations in Fluconazole. This change of fitness behavior results in a slower rate of adaptation in periodic_smaller1 and periodic_smaller2. The deleterious effect results from the 8 generations in Fluconazole rather than the 24 generations in Gly/Eth (see SI 13,15). Indeed, fitness in the 24 generations in Gly/Eth and 40 generations Gly/Eth is largely the same (SI 15). By contrast, fitness in the Fluconazole environment over 8 generations is not strongly correlated with fitness in the Fluconazole over 40 generations (SI 13). The effect of environment switching is also evident in the lineage abundances, as observable 'zig-zag' patterns, in both the evolutions themselves (Fig. 2, especially for the few top lineages, and lineages in bold), and the fitness remeasurement experiments (SI 1, bottom two panels). We hypothesize that at small timescales we are observing the effects of the switch rather than of the environments themselves; for example, the switch may lead to a change in lag phase, dependent on the new environmental block. Such a change then appears to slow down adaptation in these rapidly switching conditions.

Environmental switching can speed up adaptation

As shown above, sometimes environmental change can elicit a fitness cost, which can slow down adaptation, as measured by the increase in lineage abundances over time. However, in periodic_adap2 and random_smaller2, a changing environment appears to actually *increase* the rate of adaptation (Fig. 4). In both periodic_adap2 and random_smaller2, we observe little adaptation in Gly/Eth before entering the Fluconazole block, but then substantial increases in the frequencies of some lineages either at or immediately following the environment switch. These lineages show significant beneficial fitness effects in Gly/Eth (Fig. 4). It is possible that selection for lineages with modest fitness benefits in the Gly/Eth condition selects generalists that also have increased fitness in Fluconazole. The stronger selective pressure in Fluconazole than in Gly/Eth (see difference of scale in fitness SI 2 panel B) might then be the reason for this behavior, as both neutral and non-generalist lineages are then rapidly outcompeted in the face of the drug. Alternatively, some lineages may be good at switching, or instead might opportunistically take advantage of a dip in the population mean fitness due to the environmental switch.

The dynamics of the changing environment affects both the beneficial mutational spectrum and adaptive outcomes

We whole genome sequenced adaptive clones isolated from generation 192 from each evolution (7 to 51 uniquely barcoded clones per environment, for a total of 198 sequenced clones; of these, 112 had reliable fitness estimates, and 81 were considered to be non-neutral), and identified a total of 482 mutations. From these, we identified genes that were recurrent targets of mutation (Table 1). The pair of paralogous zinc finger transcription factors encoded by *PDR1* and *PDR3*, mutations in which are known to result in pleiotropic drug resistance, were frequent targets of adaptation in periodic_adap1, periodic_adap2, and Mix, likely due to selection in a "long" consecutive period in Fluconazole. Conversely, we observed frequent, heterozygous, likely loss of function mutations in *HEM3* in the periodic_smaller1 and random_smaller1 environments, which spend "short" amounts of consecutive time in Fluconazole, and for which the main fitness contribution likely comes from Gly/Eth environment. *HEM3* encodes porphobilinogen deaminase[27], which catalyzes the third step in heme synthesis[28]. Heme is as a cofactor for a wide variety of processes, including respiration and ergosterol biosynthesis. *HEM3* is essential in media lacking specific supplements, and knockout mutants both lack ergosterol and fail to respire. It is unclear why decreased heme biosynthesis might be adaptive in the respiratory conditions of the Gly/Eth environment because heme is a cofactor of cytochrome C, which is responsible for the transfer of electrons between complexes III and IV in the electron transport chain. Heme is also a co-factor for cytochrome C peroxidase, which contributes to mitochondrial detoxification of hydrogen peroxide. A decreased rate of heme biosynthesis likely benefits one or both of these respiratory processes, resulting in a fitness benefit in the presence of a non-fermentable carbon source. Strikingly, heme is also required for sterol production (e.g. [29]), and fluconazole itself inhibits ergosterol production, through the inhibition of the heme containing protein cytochrome P450, encoded by *ERG5*. It is thus possible that these *HEM3* mutants also enjoy a modest fitness benefit in the presence of fluconazole, due to the pleiotropic effects that might be expected by a change in the rate of heme biosynthesis.

In addition to the nature of the periodic environment influencing the beneficial mutational spectrum, it also influences the nature of adaptation itself, specifically in regard to the emergence of generalists (which have positive fitness in both growth conditions) vs. specialists (which are fit in only one of the growth conditions). First, we note that selection for generalists is order dependent (Fig. 3). Indeed, strong selection for Fluconazole at the beginning of periodic_adap1, followed by selection in Gly/Eth enriched the population for lineages with high fitness in Fluconazole but approximately neutral fitness in Gly/Eth. Conversely, growth in Gly/Eth, followed by growth in the presence of Fluconazole selects for generalists, that are highly fit in Gly/Eth, with even modest fitness benefits in Fluconazole; in addition, a few mutants with high fitness in Fluconazole, of a similar magnitude to those selected in periodic_adap1, also had time to be selected (Fig. SI2 panel A, 11). This kind of generalist also arose during evolution in a consistent Gly/Eth environment (Fig. SI2, 11), despite the lack of selection in the Fluconazole environment; however, the converse is not true – clones selected in fluconazole do not show fitness gains when measured in Gly/Eth (Fig. SI2, 11), suggesting the most fit clones in Fluconazole are not generalists. Finally, a group of mutants that have high fitness in both Fluconazole and Gly/Eth arose in the Mix experiment, but those strategies were rarely observed in periodic_adap1 or 2 (Fig SI2).

Different sequences of environment select for different phenotypes

To further understand how the sequence of environments affects the types of fitness benefits that are selected, we performed Principal Components Analysis on the fitness remeasurement data for all of the isolated mutants in the 5 remeasurement conditions; the first two principal components explain 89% of the variance (Fig. 5A). Based on their fitness profiles, we defined seven clusters of clones using Euclidian distance (SI 4, SI5 and 6 for threshold dependence), and examined the fitness of the clones in each cluster in each condition (Fig. 5B). Cluster 3 contains clones that are somewhat fitter in all remeasurement conditions, while Cluster 5 contains clones with extreme beneficial fitness in all the remeasurement environments. By contrast, clones in cluster 7 have very high fitness in fluconazole but generally neutral fitness in Gly/Eth. Cluster 4 clones shows fitness benefits in the mixed environment, and more modest fitness in the switching environments (1:1, 1:3), while clones in cluster 6 show extreme fitness gains in the switching environments (1:1, 1:3), high fitness in the Gly/Eth environment, but small/average fitness in the others. Finally, cluster 1 clones only show fitness benefits in Fluconazole, with strong trade-offs in the switching environments and the Gly/Eth environment. Clones from a given evolving environment map to one or occasionally two clusters (Fig.5 C), while some evolving environments share some cluster usage. For example, strong initial selection for a “long” time in Fluconazole in both the Mix and periodic_adap1 environments selects for similar phenotypes in cluster7. By contrast, a “long” time in Gly/Eth followed by a “long” time in Fluconazole may explain the similar usage of cluster 3 for clones from Periodic_adap2, Random_adap1 and random_smaller2. Finally, cluster membership for clones from both periodic_smaller1 and periodic_smaller2 is similar (clusters 3 and 4), and shares some properties with cluster membership of clones from random_smaller1, another sequence built with blocks of 8 generations in Fluconazole.

Discussion

Our data demonstrate that how a population adapts to a changing environment depends on how the environment varies in the types of selection presented as well as in their order and tempo. We defined our environmental sequences using two parameters: residence time in each environment and periodicity/randomness of the switches between environments. In doing so, we shed light on how those parameters influence the outcome of adaptation in dynamic environments. These two parameters are obviously not an exhaustive set: for example, we did not explicitly test how the abruptness of environmental change impacts adaptation. To explore such a parameter, we could have asked if the anticorrelation of fitness between small blocks of environments was a by-product of switching abruptly between environments, and if it might have been modulated by slower transitions.

Adaptation in a varying environment will also be influenced by the joint distribution of fitness effects for adaptive mutations in each of those environments – that is, the fitness effects of all beneficial mutations from any given environment as measured across the other environment(s). If there is strong antagonistic pleiotropy between two environments, then the most fit mutations in the first environment will be strongly selected against in the second environment. The evolutionary outcome thus will likely depend on the time scale of adaptation relative to the switching frequency – if sufficient time is spent in the first environment for adaptive mutations to reach high frequency, the second environment is likely to select for compensatory mutations on top of them. Conversely, if a short time is spent in the first environment relative to the time scale of adaptation, the second environment will likely instead cause such mutants to go extinct. In both cases, adaptation is likely to slow down. The joint distribution of fitness effects will depend

on the nature of the specific environments – correlated, or even uncorrelated environments may not greatly constrain adaptation, while anticorrelated environments will.

Our study also highlights the importance of environmental order in determining evolutionary outcomes. Our design explores the simplest of ordering possibilities – with only 2 environments, one ordering is simply a shift of the alternate order. Even so, we detect a strong influence (1 then 2 or 2 then 1, i.e. periodic adap1 and 2) at small time scales, probably driven by the difference of fitness scale between the two blocks in periodic_adap1 and 2 (Fig. SI2). The fact that we do not see any fit clones for Gly/Eth in periodic adap1 might stem from the fact that many lineages are at high frequency after the fluconazole environment: under such conditions it becomes hard to capture the rise of mutants of small to medium fitness effect. In periodic_adap2, we observe the opposite: at the end of the first environment, Gly/Eth, lineages had not reached high frequencies, and then encountered a new environment, Fluconazole, for which mutations with a much higher fitness effect could be selected. Nonetheless, we were unable really to infer *how* environment order influences adaptation, as it might become more relevant over a longer time scale, incorporating more switches between environments: to be adapted to a well-defined repetitive sequence of environments, the population should likely face this repeated sequence many times. Indeed, to fully understand the influence of dynamic environments on adaptation, the time scales that might be required are likely orders of magnitude longer than needed for non-switching environments. This has two main consequences for our experiment:

First, as our experimental approach relied on having barcode diversity remaining in the population, both for lineage tracking to follow the trajectories, and fitness remeasurement (we require that lineages have different barcodes to be able to remeasure their fitness in pooled fashion), by necessity we had to focus on the first 192 generations of each evolution. Indeed, barcode sequencing of generation 576 clones showed that only one or two lineages remained at the end of each experiment. Furthermore, those lineages were already the most abundant at generation 192 (Fig. 1A), meaning that lineage tracking beyond generation 192 has limited power to observe ongoing adaptation (Fig. SI31). Moreover, those lineages that fix (or nearly so) by generation 576 are already “special” by generation 192, in that they are somewhat distinct from the clusters to which they belong in the PCA projection (larger points on the PCA plot).

Second, it is challenging to measure fitness from the lineage trajectories during the evolution itself, and therefor to estimate the DFE. In our experiments one full environment cycle is 192 generations and because the environment changes, directly using Maximum Likelihood inference on the lineage tracking data is less informative than would be the case in a consistent environment. In our case, four models (instead of two), determining the fitness effects across the sum of the two environments, have to be considered, capturing the behavior of each lineage as: neutral in both, neutral then adaptive, adaptive then neutral, or adaptive then further adaptive. Distinguishing between these models is challenging, as the number of data points available to reconstruct the distribution is low. Even more challenging is the uncertainty on the identity of the remeasured clone (is it representative of the lineage from which it comes?) and for which the fitness is assessed by Maximum Likelihood inference: is it the original mutant, a second mutant rising on the background of the first, a different background mutant that was in the population before the change of environment etc. We developed an algorithm for Maximum Likelihood inference of dynamics in our changing environment data that highlight our capacity to analyze

the data that way. The power and flaws of such algorithm are depicted on simulated data (Fig. SI 17-25) and applied to our data (Fig.SI 26-30).

Both of those limitations inherent to exploring long time scales of adaptation using barcoding approaches would likely be mitigated by using an approach that allows periodic introduction of additional barcodes, to maintain barcode diversity over time within the evolving populations, and measuring fitness of isolated clones in each of the environments at each environmental switch.

Conclusions

We characterized the impacts that dynamic environments can have on adaptation and found that switching between conditions with different dynamics can influence adaptation at multiple levels. We found that the rate of adaptation itself is influenced by switching, and that adaptation could speed up or slow down, depending on the rate of switching. When switching was fast relative to the timescale of adaptation in either of the two conditions alone, adaptation was generally slowed down, while a slower switching rate *could* speed up adaption. We also found that the order of conditions influenced the adaptive outcome, that is, conditions are not commutative, similar to the idea of priority effects in the field of ecology, such that it matters what happens first. Specifically, we found that the order could influence whether generalists were selected over specialists. Finally, different targets of adaptation were selected in different dynamic environments (even when the same amount of time had been spent in each of the different conditions), necessarily resulting in different phenotypic outcomes.

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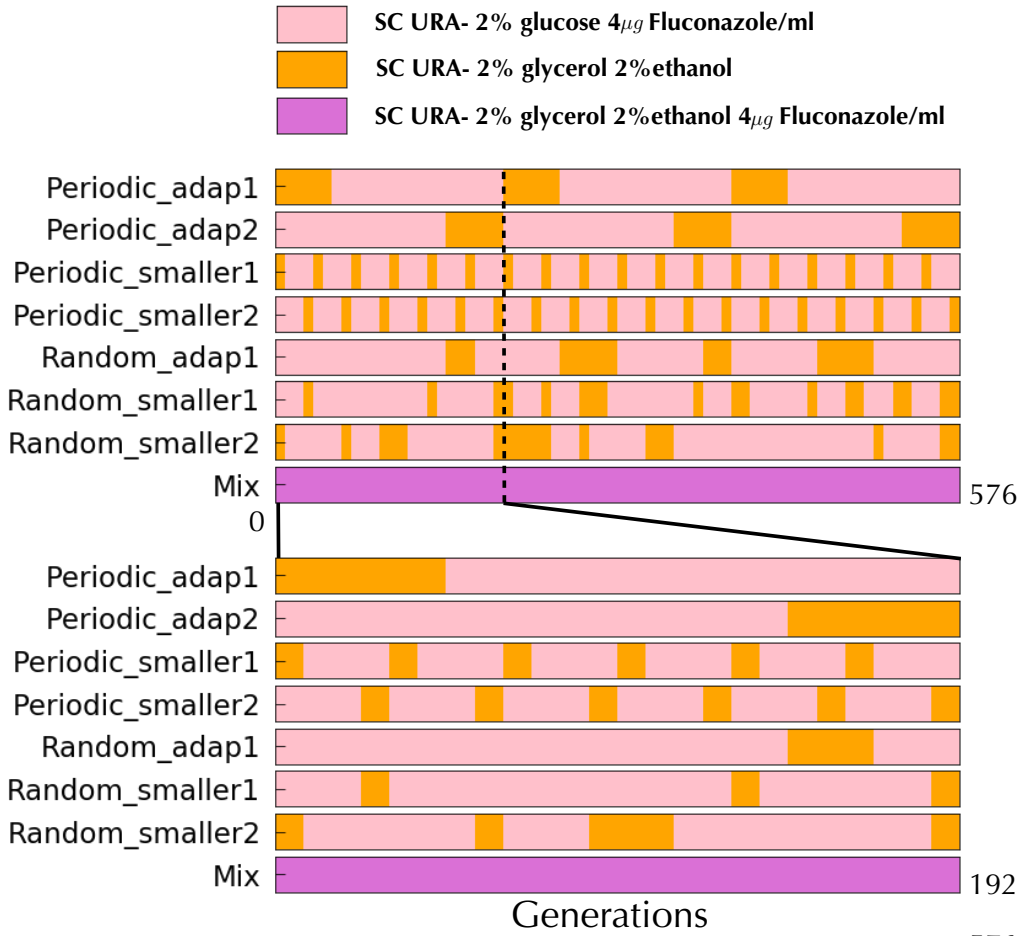

Figure 1: Experimental design. Each experiment was constructed using blocks of 8 generations in Fluconazole and 24 generations in Gly/Eth. At the end of 576 generations each population has spent the same amount of time in Fluconazole (144 generations) and Gly/Eth (432 generations) but in different sequences. Note, between generations 0 and 192, the total time spent in Fluconazole is 48 generations and 144 generation in Gly/Eth, for periodic_adap1, periodic_adap2, periodic_smaller1, periodic_smaller2 and random_smaller2. By contrast, in random_adap1 and random_smaller1 the total time spent in Fluconazole is 24 generations, with 168 generations in Gly/Eth.

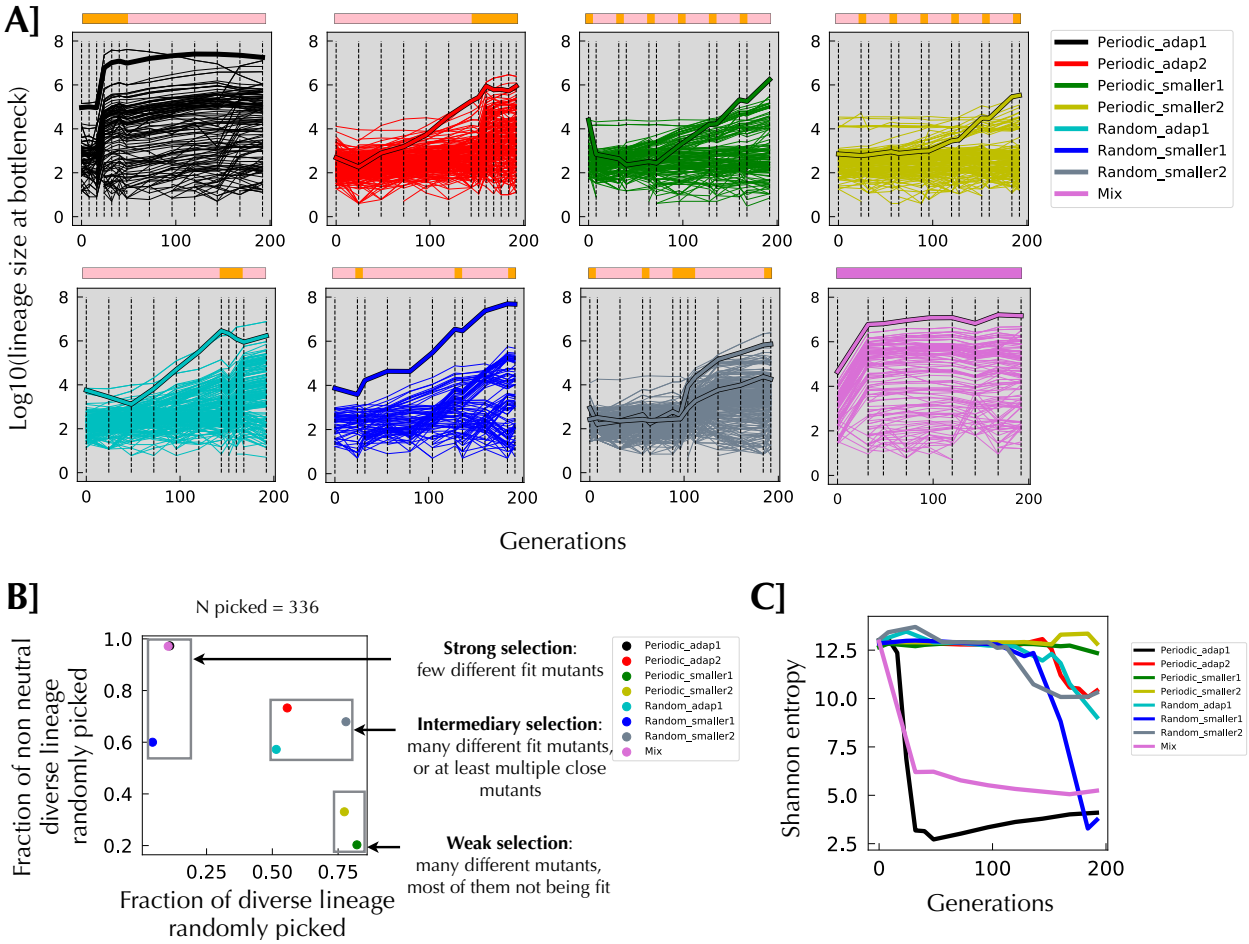

Figure 2: A] Lineage tracking of a subsample of lineages for each experiment over the course of 192 generations. The lineages shown are for which single colonies were later isolated. Thicker lines indicate lineages that were fixed at the end of the experiment (generation 576). Here fixed lineages denote the only lineages found in a sample of 384 randomly picked clones at generation 576. Dashed lines represent sampled time points. Environments are indicated by the color strips above each graph, with colors as described in Figure 1. **B] Strength of selection.** Barcode diversity and the adaptive fraction for 336 lineages randomly picked from each experiment at generation 192. **C] Shannon entropy of all lineages in each experiment during the first 192 generations.** Shannon entropy was calculated from the lineage tracking data.

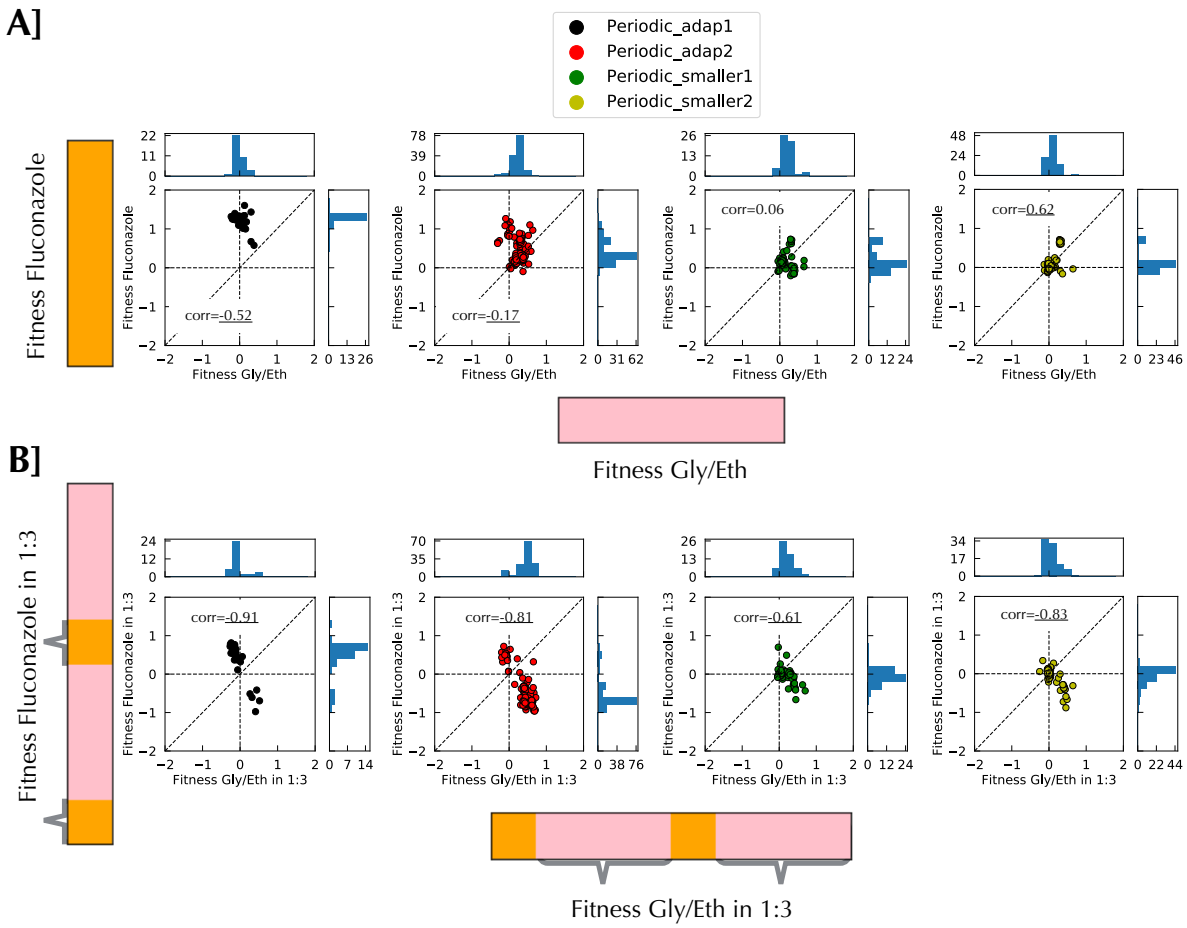

Figure 3: Fitness remeasurement for Gly/Eth and Fluconazole shows two different type of interaction between those environments according to their time scale. A] Fitness measurement for Gly/Eth and Fluconazole for 40 generations straight shows no special correlation between environments**. B]** When measurement is performed on smaller timescale and in the context of switching environment there is a net negative correlation between the two environments ,which might explain the slowest adaptive regime noted before. In this panel grey braces indicates in where the fitness was measured. In this particular switching environment Fluconazole 1:3 is the selective pressure not behaving like Fluconazole whereas Gly/Eth 1:3 and Gly/Eth are similar (SI).

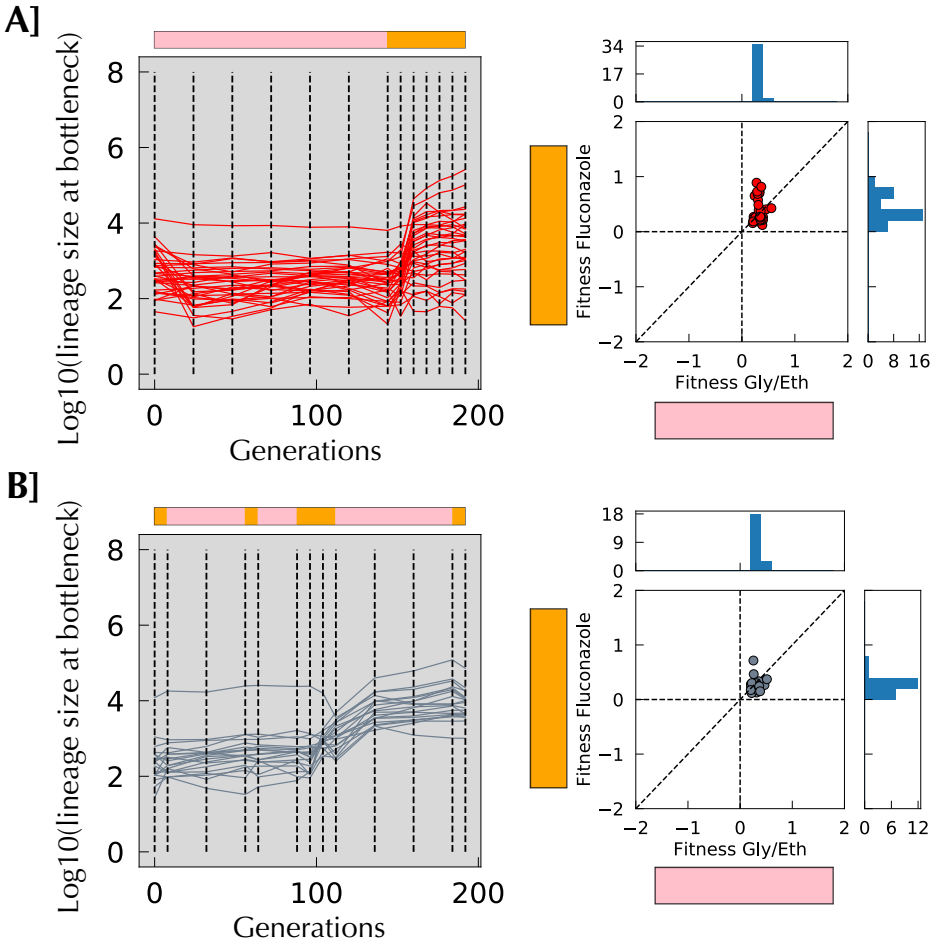

Figure 4: Change in the selective pressure helped to select for Gly/Eth fit lineage. **A]** Lineages selected in Periodic_adap2 having an average slope per cycle smaller than 0.08 in Gly/Eth environment, but still have a fitness per cycle remeasured in Gly/Eth > 0.2. Those fit Gly/Eth mutants were not able to emerge in 144 generations but considerably increased their frequency (enough to be randomly picked) after 48 generations in Fluconazole. This is probably due to two factors: reinitialization of the mean fitness and selective advantage in Fluconazole which is a more stringent selective environment. **B]** Same can be seen in Random_smaller2. The Fluconazole episode has reshuffled frequencies: some frequent lineages decrease, while others increase. When the population goes back to Gly/Eth, a large increase in frequency can be seen.

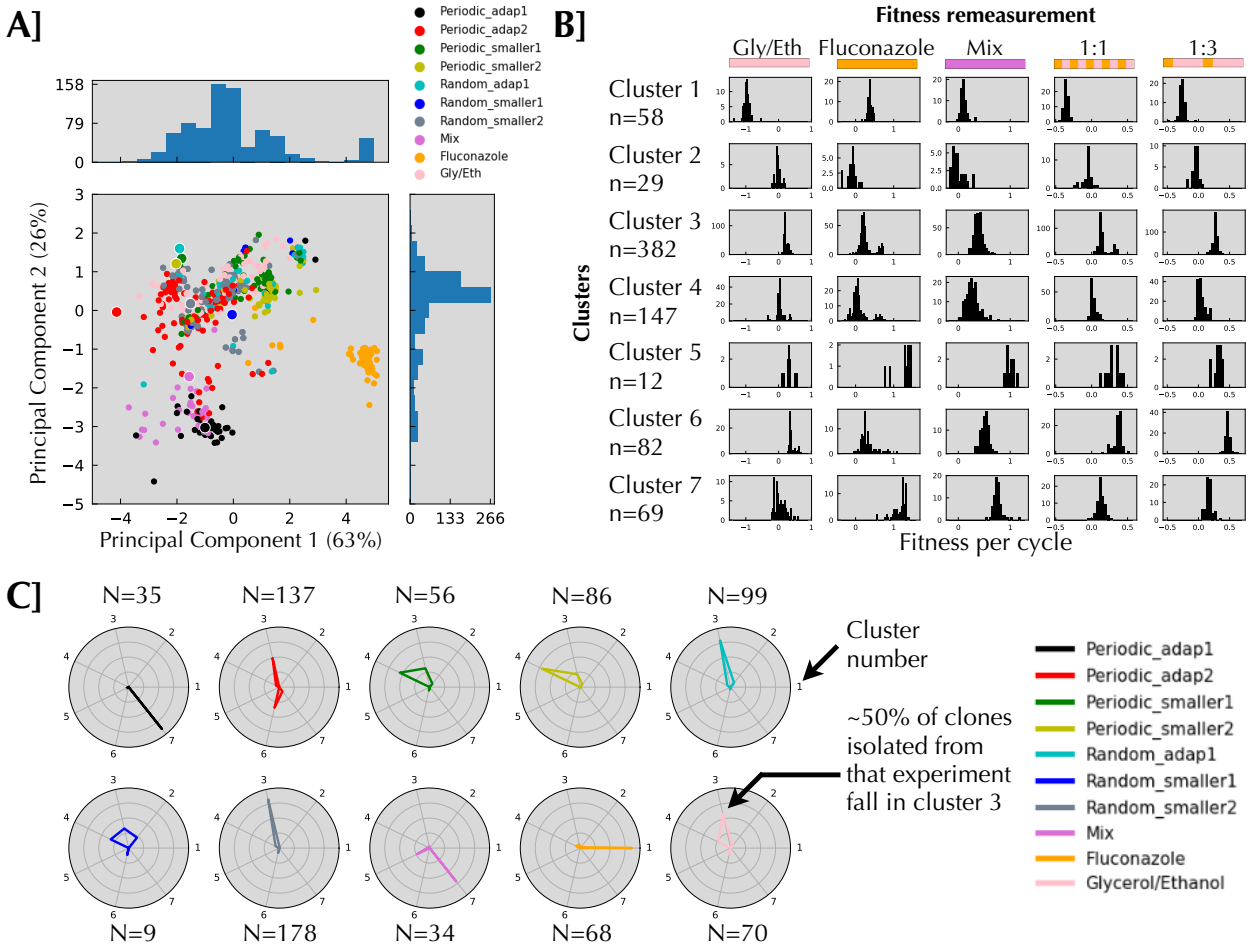

Figure 5: Fitness clusters. A] PCA analysis of combined fitness data. Each clone is represented by a five-dimensional vector corresponding to the five fitness remeasurements. This five-dimensional space is then projected onto a 2D space using PCA. Clones overrepresented at generation 576 are represented by bigger white circled points: they are quite scattered around the plots meaning that at least at generation 196 those lineages were quite different. In this representation clones that resemble each other in term of fitness from the 5 remeasurement are close together. Clones from the same experiment tend to stay together or form group that stay together (periodic_adap2, random_smaller2, random_adap1 or fluconazole). **B]** Distribution of fitness effects in the different remeasurement experiment for the different clusters. n corresponds to the number of clones within the cluster. **C]** Spider plot of cluster usage in different outcome of adaptation from different sequence of environment. N is the total number of clones picked from each sequence of environments.

	Periodic adap1	Periodic adap2	Periodic smaller1	Periodic smaller2	Random adap	Random smaller1	Random smaller2	Mix
PDR1	T817K, A763E, L878S, C756S, F769L, Q274R, G280V, P298L, Y270S, S753C, L1056P, R310W, T243A, S814Y, L537F	N234K, K253E						T817K, P870L, N234K
HEM3	L318M		D218E, K267(FRS), P108L, G133R, G250(FRS)			E65K, A181D, V132M		
PDR3	V219A, H964P	L708F						K272N, V954F, G948S
TMN2	P522T			V633M				
SGD1	D284Y, M641V							
SPT23		E122K	N348K					
MNE1		Q284L					P121S	
LAP3		D421N		H247Y				
KAP114			I107L			I335L		
MGM1			D841Y, L626V					
VID27			D737A	R62K				
SAN1				R183K, R185K(FRS)				
YHR028W-A				S73C, S73A				
DYN1			P3506S, A2554T					
RPO31						P507A, E516Q		

Table 1: Genes targeted at least twice with 2 different non-synonymous mutations (FRS= frame_shift).