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The Role of Standing Genetic Variation in Adaptation of Digital Organisms to a New Environment

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Abstract

Evolutionary adaptation to a new environment depends on the availability of beneficial alleles. Beneficial alleles may appear as new mutations or may come from standing genetic variation—alleles already present in the population prior to the environmental change. Adaptation from standing genetic variation in sexually-reproducing populations is expected to be faster than from new mutations because beneficial alleles from standing genetic variation occur at a higher starting frequency and are immediately available. The distribution of fitness effects of alleles from standing genetic variation are expected to be different from that of new mutations because standing genetic variation has been 'pre-tested' by selection. Whether adaptation uses standing genetic variation or new mutations as a source of beneficial alleles is unknown. In this study, I conducted experimental evolution of digital organisms to determine the source of beneficial alleles during adaptation. I also tested the speed of adaptation and the fitness effect of alleles under these two sources of genetic variation. I found that the major source of beneficial alleles after an environmental change was standing genetic variation, but new mutations were necessary for long-term evolution. I also found that adaptation from standing genetic variation was faster than from new mutations, and the mean fitness effect of alleles from standing genetic variation were neutral, whereas new mutations were deleterious. Interestingly, I found that an important advantage of standing genetic variation was that recombination appeared to bring together beneficial combinations of alleles from standing genetic variation. These results support the hypothesis that adaptation occurs mostly from standing genetic variation and provide an additional advantage for such adaptation.

Introduction

When a population adapts to a new environment, beneficial alleles may appear as new mutations or come from standing genetic variation (Barrett and Schluter, 2008). Standing genetic variation refers to the presence of alternative alleles at each genetic locus in a population. Standing genetic variation may be maintained in a population for several reasons (Hartl and Clark, 1997); e.g., alleles with little or no effect on fitness may rise to moderate frequencies by random genetic drift. Standing genetic variation may be a major source of beneficial alleles in a new environment, with two important implications for the dynamics of adaptation. First, adaptation from standing genetic variation should be faster than adaptation from new mutations because beneficial alleles would be immediately available and would be present at higher frequencies (Barrett and Schluter, 2008). Second, the distribution of fitness effects of alleles from standing genetic variation should be different than that of new mutations because standing genetic variation has been 'pre-tested' by surviving previous generations of selection against deleterious alleles (Barrett and Schluter, 2008).

Whether standing genetic variation is an important source of beneficial alleles for adaptation is unknown. Studies have employed three main approaches to answer this question (reviewed in Barrett and Schluter (2008)): analysis of the signature of selection, presence of the beneficial allele in the ancestral population, and phylogenetic analysis for inferring the history of alleles. These methods, however, are necessarily indirect and each has their unique set of problems. Of course, the "surest way to determine the source of beneficial alleles is to locate the genes themselves and establish their histories" (Barrett and Schluter, 2008). In this study, I used digital organisms to follow individual alleles through time as populations adapted to a new environment, and I determined whether beneficial alleles appeared as new mutations or came from standing genetic variation. I also tested whether adaptation from standing genetic variation was faster than from new mutations and whether the fitness effects of standing genetic variation were different from those of new mutations.

I conducted my experiments using Avida (Ofria and Wilke, 2004), an artificial life program designed to study questions in evolution, e.g., the complexity of epistasis (Lenski et al., 1999), the effect of mutational robustness on evolvability (Elena and Sanjuán, 2008), and the genetic architecture of sexual organisms (Misevic et al., 2006). Digital organisms in Avida consist of a sequence of computer instructions that encodes their ability to replicate and perform Boolean logic operations (or 'tasks'). Variation in the efficiency of replication and in the ability to perform tasks arises via mutation and, in sexual organisms, recombination.

Organisms that are able to perform tasks are rewarded by allowing them to run more of their code per unit of time, effectively increasing their replication rate. Inheritance, variation, and differential reproduction in digital organisms allow them to evolve via natural selection and genetic drift. Thus, evolution in Avida is not simulated. The advantage of working with Avida is that one can run thousands of generations of experimental evolution in hours, perform replicate experiments with identical starting conditions, manipulate and analyze genomes easily, and record measurements like fitness with high accuracy.

Standing Genetic Variation in Digital Organisms

To generate a well-adapted, sexual population with standing genetic variation prior to the environmental change, I initialized an empty 'world' with an organism that could replicate but could not perform any tasks. I set the world size to 10,000 cells and the environment to reward for the default nine tasks (Lenski et al., 1999). I set the copy mutation rate to 0.1 mutations per genome per generation and, to ensure homologous recombination, I fixed the length of all genomes to 200 instructions and turned off insertion and deletion mutations. I let 50 such replicate populations evolve for 500,000 updates—a measurement of time in Avida—which was about 42,000 generations. I then picked a random population in which the consensus sequence could perform all nine tasks (35 out of the 50 could perform all nine tasks), and I took a random sample of 1,000 individuals from this population to serve as the ancestral population before the environmental change.

To measure the amount of standing genetic variation in the ancestral population, I measured the heterozygosity of each locus of the population. The heterozygosity of a locus is $H = 1 - \sum_{i=1}^{k} p_i^2$, where k is the number of alleles segregating at that locus and p_i is the frequency of the ith allele (Gillespie, 2004, p. 15). Here I adopted the convention that a locus is polymorphic (i.e., has standing genetic variation) if its most common allele has a frequency < 0.95 (Hartl and Clark, 1997, p. 53). A locus that had standing genetic variation would have a minimum heterozygosity of $1 - (0.95^2 + 0.05^2) = 0.095$. Because there are 26 possible alleles (i.e., instructions) per locus in digital organisms, the maximum possible heterozygosity is approximately 0.9615.

I found substantial standing genetic variation in the ancestral population (Figure 1). Of 200 loci, 125 (62.5%) were polymorphic. The heterozygosity of each locus ranged from 0.0 to 0.8859, with a mean heterozygosity of 0.3781 (0.3334–0.4246, 95% bootstrap CI). For comparison, Stephens et al. (2001) found in humans that the heterozygosity of 313 genes ranged from 0.012 to 0.929, with a mean of 0.534. In natural populations of *E. coli*, Selander and Levin (1980) found that the heterozygosity of 20 enzyme-encoding genes ranged from 0.055 to 0.887, with a mean of 0.4718. My results demonstrate that the ancestral population exhibited levels of standing genetic variation consistent with that observed in biological populations. Furthermore, they support the claim that standing genetic variation is a ubiquitous property of evolving genetic systems (Gibson and Dworkin, 2004; Barrett and Schluter, 2008).

Source of Beneficial Alleles

Having established that the ancestral population harbored abundant standing genetic variation, I determined whether adaptation to a new environment relied on this genetic variation or on new mutations as a source of beneficial alleles. In this study, I examined beneficial alleles with fitness effects greater than 1%. With the ancestral population, I started 20 new replicate populations in a world of 1,000 cells and an environment that rewarded for 68 different tasks (the original nine tasks were not rewarded for). As a control, I also started another set of 20 replicate populations where every individual had an identical genotype (i.e., isogenic), set to the consensus sequence of the ancestral population. Although the consensus genotype did not actually exist in the ancestral population, its fitness was 1.0070 relative to the highest fit individual in the ancestral population (excluding those who could immediately perform tasks), and 1.0337 relative to the mean fitness of the ancestral population. Thus, the control population was not at a disadvantage compared to the ancestral population. All other configuration settings were identical to those used for the evolution of the ancestral population. Note that the populations that started with standing genetic variation were also allowed to get new mutations (the mutation rate was set to 0.1 mutations per genome

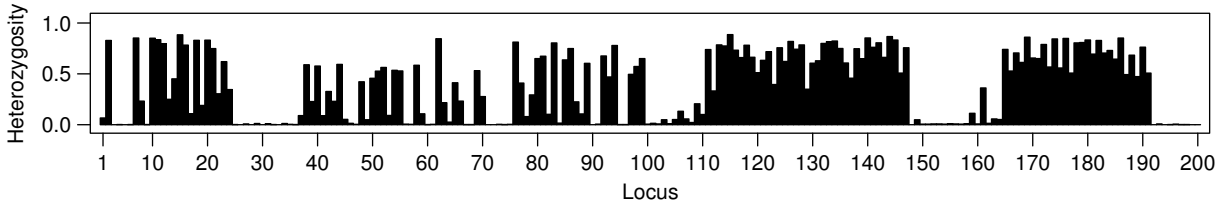

Figure 1: The heterozygosity of each locus of the population before the environmental change. Heterozygosities above 0.095 indicate the presence of standing genetic variation.

per generation). I let these replicate populations evolve for 10,000 updates ($\sim$ 850 generations), saving each population every 100 updates.

At the end of the runs, I found that the populations that started with standing genetic variation increased in mean fitness to 8.31 (7.74–8.87, 95% bootstrap CI) relative to the ancestral population in the new environment (i.e., the evolved populations were 8.31 times more fit in the new environment than the ancestral population). These populations were able to perform an average of 7.9 tasks, with a range of 5 to 10. The mean number of fixed, derived alleles—defined as having a frequency > 0.95 in the evolved population but < 0.95 in the ancestral population—was 56.25, ranging from 38 to 70. Figure 2 shows the history of two allele fixation events, one from standing genetic variation and the other from a new mutation, that occurred in the first replicate population. Of the 56.25 fixed, derived alleles, 47.8 (85%) existed as standing genetic variation in the ancestral population. In the control populations, mean fitness increased to 7.18 (6.62–7.76, 95% bootstrap CI) relative to the ancestral population. The control populations were able to perform an average of 6.7 tasks, with a range of 5 to 9. The mean number of fixed, derived alleles in the control populations was 5.15, ranging from 2 to 9. It was surprising that the populations that started with standing genetic variation fixed 10 times more alleles than the control populations, despite both sets of populations having similar final fitnesses and number of tasks performed.

The finding that 85% of fixed, derived alleles in the populations that started with the ancestral population existed as standing genetic variation may indicate that most beneficial alleles came from standing genetic variation. It is not clear, however, whether they were fixed by neutral genetic drift, natural selection, or genetic linkage and hitchhiking with beneficial alleles. For example, genetic hitchhiking in Avida can occur when alleles nearby a highly beneficial allele rise in frequency along with the beneficial allele. Hitchhiking occurs because the beneficial allele and nearby (i.e., genetically linked) alleles spread faster than recombination can break them apart. It is also not clear at what frequency the derived alleles first became beneficial. Therefore, I developed a method to systematically measure the fitness of individual alleles through time and determine the frequency at which they became beneficial.

First, for each fixed, derived allele at the end of each run, I calculated both the allele's frequency and fitness effect every 100 updates, starting at the first update. To calculate the fitness effect of an allele at the current update, I first selected from the population the individual with the highest fitness who had the allele. I then created a clone of the individual and substituted the allele with an alternative allele drawn randomly from the standing genetic variation at that locus. I then calculated the fitness of the individual with the allele relative to the fitness of the individual without it. If this relative fitness was greater than 1.01, then the fitness effect of the allele ($> 1\%$) was beneficial at the current update. While testing this method, I found some cases where the fitness effect of the allele was considered beneficial only because the individual with the alternative allele had unusually low fitness. To reduce the frequency of such cases, I also required that the allele be beneficial for the individual with the second highest fitness. I stopped analyzing further updates as soon as I found the allele to be beneficial or if it became fixed.

In populations that started with standing genetic variation, I found that out of the mean 56.25 alleles that fixed, a mean of 31.9 became beneficial at some point in their history. I found that only 13.4% of these beneficial alleles became beneficial at a frequency < 0.05 (Figure 3, lower horizontal red line); the remaining 86.6% became beneficial at a frequency > 0.05. Supposing standing genetic variation comprises alleles with frequencies > 0.05, these results indicate that the majority of beneficial alleles came from standing genetic variation. In the control populations, I found that out of the mean 5.15 alleles that fixed, a mean of 5.1 became beneficial at some point in their history. I found that 77.3% of these beneficial alleles became beneficial at a frequency < 0.05 (Figure 3, upper horizontal red line); the remaining 22.7% became beneficial at a frequency > 0.05. Therefore, in contrast to populations that started with standing genetic

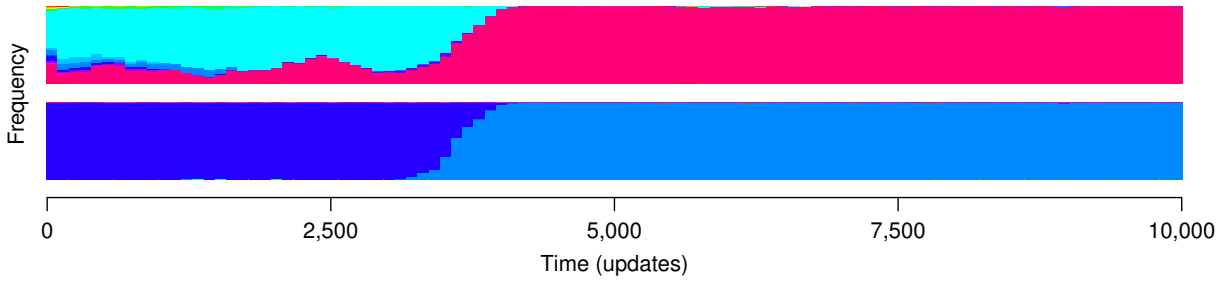

Figure 2: The frequencies of alleles through time for two loci in which an allele became beneficial and subsequently fixed. In the top plot, the beneficial allele came from standing genetic variation, and in the bottom plot, the beneficial allele appeared as a new mutation. Different alleles are represented by different colors. The y-axis in each plot ranges from 0.0 to 1.0.

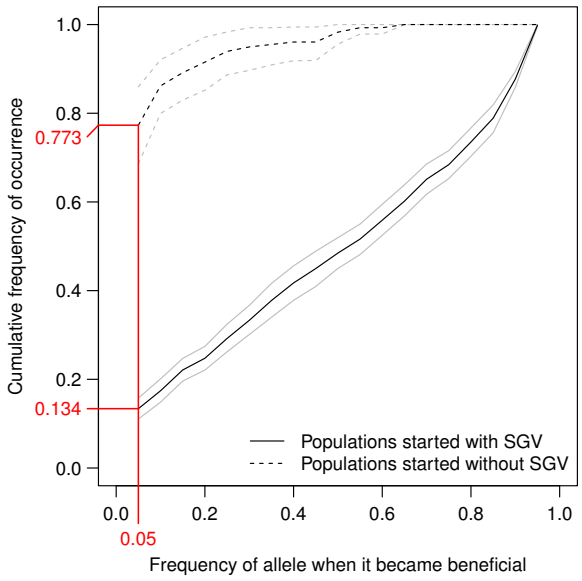

Figure 3: The cumulative frequency of fixed alleles that became beneficial at a specific frequency (0.05 bin size) for populations that started with standing genetic variation (solid lines) and for control, isogenic populations (dashed lines). The gray lines indicate the 95% bootstrap confidence interval around the mean of 20 replicate populations. The red vertical line indicates the frequency below which alleles were considered to appear as new mutations. The red horizontal lines indicate the proportions of alleles that came from new mutations for either type of population.

variation, the control, isogenic populations adapted mostly from new mutations, although almost a quarter of beneficial alleles came from standing genetic variation that arose as populations accumulated genetic polymorphism over time. Interestingly, the mean absolute (not percentage) number of new mutations per replicate for each treatment was about the same: 4.15 (3.40–4.85, 95% bootstrap CI) for populations started with standing genetic variation and 3.75 (3.3–4.2) for isogenic populations. This indicates that standing genetic variation did not inhibit new mutations from being selected.

One potential concern with the above method is that I identified beneficial alleles based on only two genotypes that had the allele, relative to two genotypes with alternative alleles. Yet the presumed beneficial alleles as well as the alternative alleles may not have the same fitness effect on other genetic backgrounds. Thus, I implemented a second method to identify beneficial alleles that considered more genotypes when measuring fitness effects. The key difference between this method and the previous is that in this method I selected all individuals who had the allele. Then, for each of these individuals I substituted the allele with an alternative allele drawn randomly from the standing genetic variation at that locus. Finally, I calculated the mean fitness of all individuals with the allele relative to the mean fitness of all individuals with the allele replaced. If this relative fitness was greater than 1.01, then I considered the allele as beneficial. Using this method, I found that in populations that started with standing genetic variation, 11.5% of alleles became beneficial at a frequency < 0.05; the remaining 88.5% became beneficial at a frequency > 0.05. In the isogenic populations, I found that 79.4% of alleles became beneficial at a frequency < 0.05; the remaining 20.6% became beneficial at a frequency > 0.05. These results are very similar to those I found with the previous method, showing that the previous method was robust to the number of genotypes considered when identifying beneficial alleles.

Speed of Adaptation

Adaptation from standing genetic variation should be faster than adaptation from new mutations because beneficial alleles would be immediately available and would be present at higher frequencies (Barrett and Schluter, 2008). To test this prediction, I compared the speed of adaptation between populations that started with standing genetic variation and those that started with isogenic individuals. I re-evolved both types of populations at the additional mutation rates (U) of 0.01 and 0.0 (no new mutations) per genome per generation (the original populations were run at a mutation rate of 0.1). I added these new treatments because, given that the only source of mutations for the isogenic populations were new mutations, the mutation rate would be an important variable on the rate of adaptation. Population size would also be an important variable on the rate of adaptation, but I did not investigate its effects in this study.

I found that at the 0.1 mutation rate, the rate of adaptation for populations that started with standing genetic variation was significantly greater for most of the first four thousand updates than isogenic populations, then became less significantly so for the rest of the run (Figure 4A). At the 0.01 mutation rate, however, the rate of adaptation was significantly greater for the entire run (Figure 4B). Interestingly, at the 0.0 mutation rate, populations with standing genetic variation continued to adapt for several thousand updates, but, as expected, isogenic populations could not evolve (Figure 4C). These results clearly demonstrate that adaptation from standing genetic variation was faster than from new mutations. Yet new mutations were necessary for long-term evolution, as shown by the fact that adaptation from standing genetic variation without new mutations stopped after several thousand updates.

Fitness Effect of Random Alleles From Different Sources of Variation

The distribution of fitness effects of alleles from standing genetic variation should be different than that of new mutations because standing genetic variation has been ‘pre-tested’ by

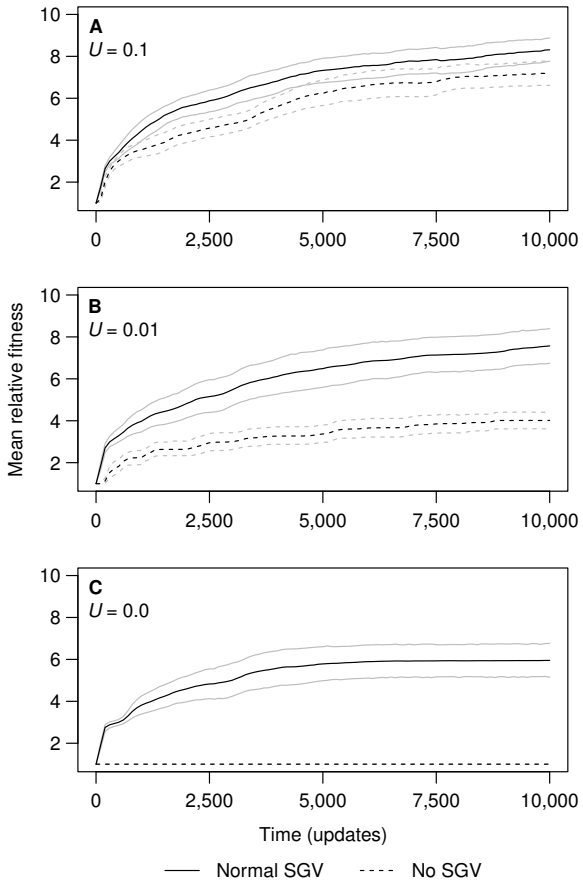

Figure 4: The mean fitnesses (relative to the ancestor) of populations evolved after an environmental change at (**A**) 0.1, (**B**) 0.01, and (**C**) 0.0 mutations per genome per generation (U). Populations evolved starting either with the ancestral population (solid line), which contained standing genetic variation (SGV) or with an isogenic population based on the consensus sequence of the ancestral population (dashed line). Gray lines represent the 95% bootstrap confidence intervals around the mean.

selection (Barrett and Schluter, 2008). To test this prediction, I generated the fitness effect distribution of alleles coming from either standing genetic variation or new mutations, measured in the new environment. First, I sampled 1,000 random (but viable) individuals from the ancestral population and mutated a single, random locus of each individual to an allele drawn randomly from the standing genetic variation (if there was any variation at that locus). I also sampled another set of 1,000 individuals from the ancestral population and mutated a single locus of each individual to an allele drawn randomly from all 25 possible alternative alleles. To prevent the possibility that these random mutations were more deleterious only because they disrupted fixed alleles, I ensured that the loci were drawn from the same pool of loci that had standing genetic variation. Finally, I measured the

	Source of mutation	
Fitness effect	SGV	Random
Lethal	0	58
Strongly deleterious	3	5
Mildly deleterious	186	345
Nearly neutral	729	520
Mildly beneficial	81	67
Strongly beneficial	1	5

Table 1: The number of single mutants (out of 1,000), categorized by the mutation's source and fitness effect (w): lethal ($w = 0$), strongly deleterious ($0 < w \leq 0.99$), mildly deleterious ($0.99 < w \leq 0.999$), neutral or nearly neutral ($0.999 < w \leq 1.001$), mildly beneficial ($1.001 < w \leq 1.01$), and strongly beneficial ($w > 1.01$).

fitness of these mutants relative to the original, unmutated individual.

I found that the mean fitness of mutants with mutations from standing genetic variation was 0.9994 (0.9969–1.0023, 95% bootstrap CI). The mean fitness of mutants with random mutations was 0.9496 (0.9326–0.9665, 95% bootstrap CI). Clearly, mutations from standing genetic variation did not have, on average, as strong deleterious effects as random mutations. To examine more closely the fitness effects of mutations from the two sources, I categorized each mutation based on the mutant's relative fitness (Table 1). Alleles from standing genetic variation were mostly neutral, whereas new mutations were more likely to be lethal or deleterious. Interestingly, new mutations were also more likely to be strongly beneficial than alleles from standing genetic variation, yet in the analysis where I determined the source of beneficial alleles, I found that most beneficial alleles came from standing genetic variation. This discrepancy may indicate that although alleles from standing genetic variation were not beneficial alone, combinations of these alleles brought together by recombination provided the benefits. The finding that alleles from standing genetic variation were less deleterious on average than random mutations support the hypothesis that standing genetic variation has been pre-tested by selection.

The above analysis was based on randomly generated mutants of the ancestral genotypes (i.e., at the beginning of the experiments), but it would also be interesting to know the fitness effect of beneficial alleles that actually fixed. This information was already calculated as part of determining the moment at which alleles became beneficial because it was used to determine whether alleles had achieved a fitness > 1.01 (using the first method). For populations that had evolved under standing genetic variation, the mean fitness of a genotype with a beneficial allele at the moment at which it became beneficial (relative to a genotype without the beneficial allele) was 1.54 (1.48–1.60, 95% bootstrap CI). For isogenic populations, this mean fitness was 1.47

(1.37–1.59, 95% bootstrap CI). Although the mean fitness effect of beneficial alleles for the standing genetic variation treatment was slightly higher than the isogenic treatment, they were not significantly different. The maximum relative fitness for a genotype with a beneficial allele for the standing genetic variation treatment (7.05) was higher than that for the isogenic treatment (4.50).

Discussion

I have shown that in populations of digital organisms adapting to a new environment, the major source of beneficial alleles was standing genetic variation, not new mutations. My findings are supported by selection experiments and observational studies of biological populations. Selection experiments have shown that adaptation can occur by changes in allele frequencies of standing genetic variation in the initial populations (e.g., Feder et al., 1997; Scarcelli and Kover, 2009; Teotónio et al., 2009). Observational studies of natural populations have found that alleles correlated with adaptive traits were also present in the ancestral population (e.g., Colosimo et al., 2005; Myles et al., 2005). In biological organisms, however, it is very difficult to measure the fitness effects of individual alleles, which is necessary to determine whether an allele fixed due to selection. Another problem, specific to studies of natural populations, is that the ancestral population is unavailable—the closest one can get is the extant population from which a subpopulation founded a new environment—and therefore it is often unknown whether a beneficial allele existed as standing genetic variation. The use of digital organisms allowed me to track individual alleles through time and determine the frequency at which they became beneficial.

When alleles from standing genetic variation became beneficial, their starting frequency ranged from the minimum of 5% to the maximum of 95% (Figure 3). In experimental studies of biological organisms, high starting frequencies ($> 50\%$) are not uncommon (e.g., Feder et al., 1997; Scarcelli and Kover, 2009). In natural populations, however, starting frequencies have tended to be much smaller, such as in the study by Colosimo et al. (2005), where the starting frequency of an adaptive allele was between 0.2% and 3.8% in the ancestral population. One possible reason for this discrepancy is that natural populations may be under stronger selective pressures than experimental populations (Ellegren and Sheldon, 2008), so the fitness effects of alleles in natural populations tend to be more deleterious and therefore maintained at low frequencies. Of course, allele frequency data for adaptive alleles in natural populations is scarce, so more research in natural populations should determine the frequencies at which alleles from standing genetic variation become beneficial.

Adaptation should be faster if most beneficial alleles came from standing genetic variation than if they came from new mutations (Barrett and Schluter, 2008). I found this to be the case in digital organisms if the mutation rate was low enough (Figure 4). In fact, when no new mutations were allowed, adaptation by standing genetic variation continued for several hundred generations, whereas no adaptation occurred in isogenic populations. Still, the importance of new mutations for long-term evolution was shown by the fact that adaptation stopped eventually when no new mutations were allowed. Although there are no empirical studies testing the speeds of adaptation, where beneficial alleles may come from either standing genetic variation or new mutations, my results are supported theoretically (Hermisson and Pennings, 2005). There are two reasons that adaptation from standing genetic variation should be faster than adaptation from new mutations: beneficial alleles are both readily available and present at higher frequencies than alleles from new mutations (Barrett and Schluter, 2008), which must overcome drift because they start at lower frequencies. Future experiments should be able to quantify the relative contribution of these two causes.

Although not examined in detail in this study, the population size and mutation rate can affect the relative contributions of standing genetic variation and new mutations during adaptation. For example, a sudden decrease in population size (i.e., a bottleneck) will reduce both the amount of standing genetic variation and the number of new mutations that appear each generation. In this case, standing genetic variation will still have an advantage over new mutations—especially for alleles of weak fitness effect—because weak effect alleles introduced by new mutations are easily lost due to genetic drift (Hermisson and Pennings, 2005). For large effect alleles, standing genetic variation will have a reduced advantage because large effect alleles are less likely to be lost even if they are introduced as new mutations (Hermisson and Pennings, 2005). In my experiments, mutations that allowed organisms to perform new tasks were of large effect (the default configuration in Avida), but future studies should experiment with weaker beneficial alleles. In a large population or high mutation rate, new mutations would become more important because large-effect mutations would appear more frequently.

Because alleles from standing genetic variation have had a potentially long history in an evolving population, their fitness effects in a new environment have been predicted to be less deleterious than random mutations (Barrett and Schluter, 2008). On average, I found that standing genetic variation was effectively neutral (fitness effect of 0.0006), whereas random mutations were strongly deleterious (fitness effect of 0.0504). Alleles from standing genetic variation can therefore linger in a population, increasing the chance for them to become beneficial after an environmental or genetic change. Random mutations, on the other hand, are on average deleterious and are thus more easily eliminated by selection. In biological populations, the mean fitness effect of random mutations was found to be 0.48 in RNA viruses

(Sanjuán et al., 2004), 0.12 in *C. elegans* (Vassilieva et al., 2000), and 0.22 in yeast (Zeyl and DeVisser, 2001). There are no measurements of the fitness effects of alleles from standing genetic variation in a biological population in a new environment.

For strongly beneficial mutations (i.e., fitness effect $> 1\%$), I found that random mutations were more likely to be beneficial than alleles from standing genetic variation in the new environment (Table 1). It may thus seem counter-intuitive that most beneficial alleles during adaptation came from standing genetic variation. I hypothesize that it was the combination of many alleles from standing genetic variation that provided the benefits, and together these epistatically related alleles rose to fixation. Adaptation that requires many alleles working together is known as 'polygenic adaptation' (Pritchard and Di Rienzo, 2010), although fixation of alleles is not always necessary. In fact, Pritchard and Di Rienzo (2010) hypothesize that if adaptation occurs from standing genetic variation, polygenic adaptation is likely.

In summary, this study has shown the importance of standing genetic variation in populations of digital organisms adapting to a new environment. That is, (1) most beneficial alleles came from standing genetic variation rather than from new mutations, (2) populations that started with standing genetic variation adapted faster than populations that started with identical genotypes, and (3) the fitness effects of alleles from standing genetic variation were less harmful than new mutations. Because digital organisms evolve by the same processes of natural selection and genetic drift that biological populations also experience, I suspect that the above points are also true for biological populations. A hypothesis that arose from this study was that standing genetic variation together with recombination may give rise to combinations of alleles that together are beneficial. Future work should test whether this additional advantage is true, thereby highlighting the importance of sexual recombination and standing genetic variation in evolving populations.

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