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POPULATION SUBDIVISION AND ADAPTATION IN ASEXUAL POPULATIONS OF *SACCHAROMYCES CEREVISIAE*

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Population subdivision limits competition between individuals, which can have a profound effect on adaptation. Subdivided populations maintain more genetic diversity at any given time compared to well-mixed populations, and thus "explore" larger parts of the genotype space. At the same time, beneficial mutations take longer to spread in such populations, and thus subdivided populations do not "exploit" discovered mutations as efficiently as well-mixed populations. Whether subdivision inhibits or promotes adaptation in a given environment depends on the relative importance of exploration versus exploitation, which in turn depends on the structure of epistasis among beneficial mutations. Here we investigate the relative importance of exploration versus exploitation for adaptation by evolving 976 independent asexual populations of budding yeast with several degrees of geographic subdivision. We find that subdivision systematically inhibits adaptation: even the luckiest demes in subdivided populations on average fail to discover genotypes that are fitter than those discovered by well-mixed populations. Thus, exploitation of discovered mutations is more important for adaptation in our system than a thorough exploration of the mutational neighborhood, and increasing subdivision slows adaptation.

KEY WORDS: **Adaptation, epistasis, fitness landscape, population structure.**

Spatial structure restricts competition between individuals, and hence can have a dramatic impact on evolution. Theoretical work has described a number of ways in which this and other forms of population subdivision affect evolution (Rousset, 2004), such as by changing the patterns of genetic variation (Charlesworth et al., 2003) or by creating opportunities for speciation (Gavrilets et al., 1998). Population subdivision has particularly important implications for adaptation; because it restricts selection to act locally rather than globally, subdivision "shields" parts of the population from competition with alleles that arise elsewhere, at least until sufficient migration occurs.

In principle, population subdivision could either promote or inhibit adaptation. On the one hand, subdivision increases the time it takes for a beneficial mutation to sweep through the population (Whitlock, 2003). Because beneficial mutations take longer to reach high frequencies in the population, further beneficial mutations tend to occur in various genetic backgrounds other than the background with the highest fitness. The subdivided population thus cannot fully "exploit" all the mutations that occur. That is, population subdivision magnifies the effect of clonal interference (Gerrish and Lenski 1998; Gordo and Campos 2006) and prevents the population from accumulating multiple mutations (Desai and Fisher, 2007), slowing adaptation. On the other hand, reducing the rate of selective sweeps allows the population to maintain genetic diversity for longer in the face of selection (Wakeley, 1998; Cherry and Wakeley, 2003) and hence "explore" more directions

in genotype space at once (Handel and Rozen, 2009; Jain et al., 2011). This makes it possible for the population to find beneficial combinations of epistatically interacting mutations which otherwise would be missed, speeding the overall rate of adaptation.

Thus, subdivided populations are better at "exploration" of the genotype space but are less good at "exploitation" of already discovered mutations compared to well-mixed populations. Whether population subdivision promotes or inhibits adaptation to a given environment depends on how important exploration is relative to exploitation, which in turn depends on the structure of epistasis between beneficial mutations (Handel and Rozen, 2009; Jain et al., 2011). If beneficial mutations act independently to increase fitness (i.e., in the absence of epistasis), the fitness landscape is "smooth" in the sense that a population can discover and substitute all beneficial mutations irrespective of order. In this case, exploitation is more important than exploration: the more and better mutations the population substitutes, the faster it adapts. In contrast, if the effect of a mutation depends strongly on the genetic background in which it occurs (i.e., in the presence of epistasis), the fitness landscape may be "rugged," in the sense that the order and the identity of mutations which a population substitutes determine which fitness it will eventually attain. In this case, exploration may be more important than exploitation. Thorough exploration of accessible mutations could allow the population to discover mutational trajectories that are initially only weakly beneficial or even neutral, but open up many adaptive opportunities later on (Burch and Chao, 2000; Blount et al., 2008; Rozen et al., 2008; Salverda et al., 2011; Woods et al., 2011). Thus, maintaining an appreciable amount of genetic diversity may be advantageous for the population in the long run.

This potential trade-off between exploration and exploitation exists in both sexual and asexual populations. However, in this article we focus exclusively on asexual populations, which allows us to examine the effect of subdivision on adaptation, without the complicating effects of recombination among diverse genotypes. In Figure 1, we illustrate the effect of population subdivision on adaptation on a smooth fitness landscape where exploitation is more important than exploration (left panel) and on a rugged fitness landscape where the converse is true (right panel). For the sake of clarity we show an artificial situation where both well-mixed and subdivided populations receive the same mutations at the same times. On a smooth landscape, subdivision slows down the spread of each beneficial mutation and retards adaptation. On a rugged landscape, population subdivision shields mutations *B* and *C* (which are only weakly beneficial) from being outcompeted by mutation *A*. The subdivided population is thus able to find the strongly beneficial *BC* double-mutant much faster than the well-mixed population (Jain et al., 2011). This evolutionary advantage of subdivision on rugged fitness landscapes is related but not identical to Sewall Wright's shifting balance theory (Wright, 1982), as we describe in more detail in the "Discussion".

Several recent experimental studies have investigated the effects of spatial structure on maintaining genetic diversity (Kerr et al., 2002, 2006; Ponciano et al., 2009), promoting adaptive radiation (Korona et al., 1994; Rainey and Travisano, 1998), and slowing or speeding up the rate of adaptation (Habets et al., 2006, 2007; Perron et al., 2007, 2008; Perfeito et al., 2008; Rozen et al., 2008). However, most of these studies did not entirely separate the effects of spatial structure on the rate of adaptation from other effects such as potential differences in selection pressures acting in populations with different spatial structures (but see Habets et al., 2007, for an exception).

In this article, we introduce an experimental system that allows us to tune the degree of geographic structure in a population of budding yeast adapting to a laboratory environment while keeping all other experimental parameters exactly identical. We consider populations with the simplest possible geographic structure, the island model, in which the population is subdivided into a number of partially isolated subpopulations, or "demes," each of which exchanges an equal number of migrants with all other demes (Wright, 1943; Maruyama, 1970). By precisely controlling migration rates between demes we control the amount of time it takes for beneficial mutations to sweep and, consequently, the amount of genetic diversity that a population maintains. In this way we systematically explore how the degree of population structure across a range of migration rates influences the rate of adaptation in close to a thousand parallel populations.

Materials and Methods

STRAINS

All long-term evolution was conducted using haploid *Saccharomyces cerevisiae* strain DBY15104 (Lang et al., 2011). DBY15104 is derived from the W303 background with genotype *MAT***a**, *ade2-1, CAN1, his3-11, leu2-3,112, trp1-1, URA3, bar1*Δ*::ADE2, hml*αΔ*::LEU2,* and carries a ClonNat^R-marked *GPA1* allele derived from RM11-1a. All fitness assays were conducted as competitions between the evolved population and an mCherry-marked reference strain, DBY15108, as described in Lang et al. (2011).

LONG-TERM EVOLUTION

We propagated a total of 3520 individual wells in eleven 384-well plates (Greiner), where each well served as an individual deme, in batch culture for 550 generations without shaking at 30°C. All cultures were grown in 64-μL YPD (1% yeast extract, 2% peptone, 2% dextrose) per well and were serially diluted $1:2^{10}$ every 24 h, which corresponds to 10 generations

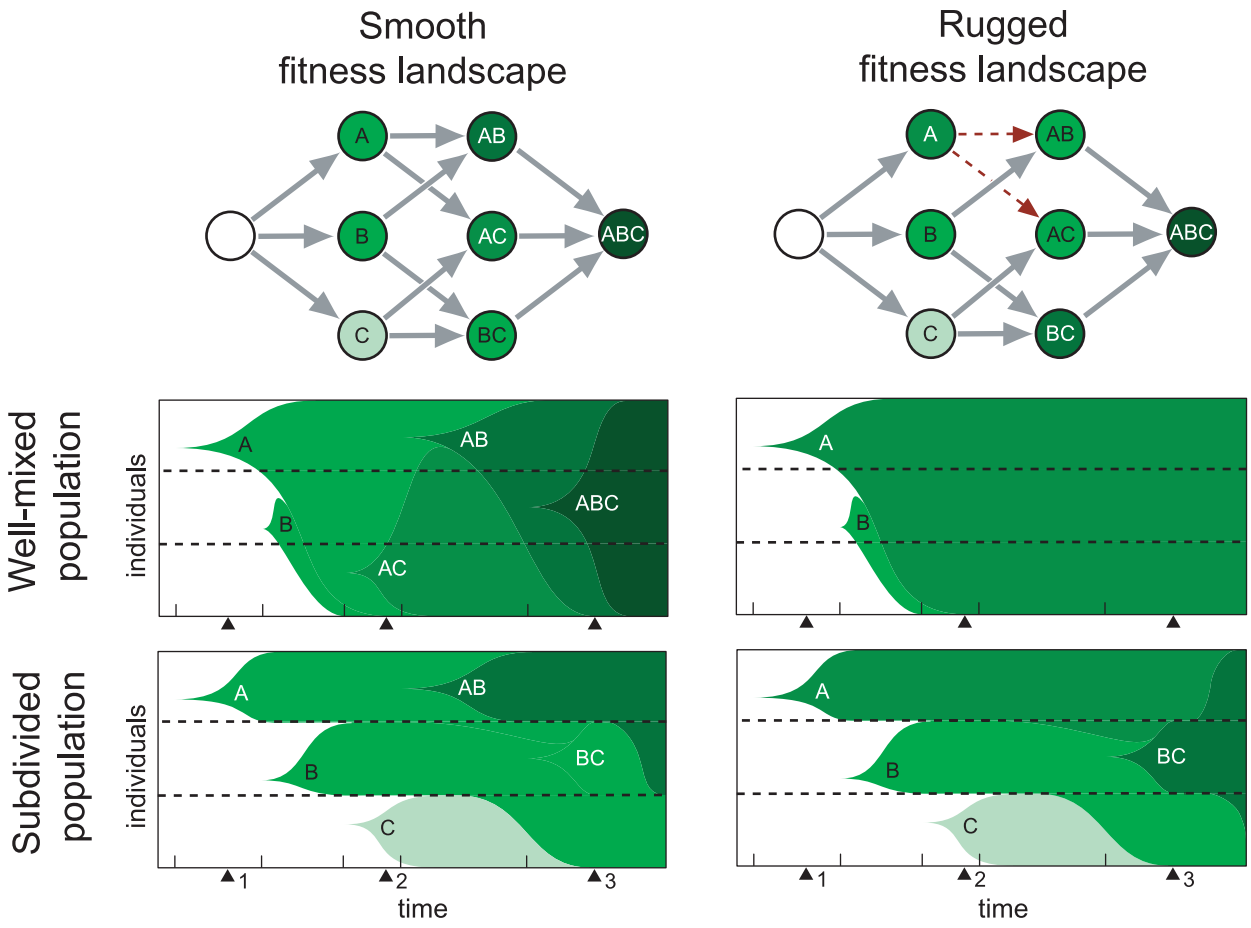

Figure 1. **Illustration of the evolution of well-mixed and subdivided populations on different fitness landscapes. Left and right columns show adaptation on a smooth and rugged fitness landscape, respectively. Top row illustrates mutational trajectories from the wild-type to an advantageous triple-mutant. The fitness of each genotype is denoted by the color: darker shade of green represents higher fitness. Beneficial and deleterious mutations are indicated with gray and red arrows, respectively. On the rugged landscape, mutation *A* is the best single-mutant but makes all further mutations deleterious. Middle and bottom rows show the dynamics by which mutations occur and spread in well-mixed and subdivided populations on smooth and rugged landscapes. In all four depicted time courses the same mutations are assumed to occur at exactly the same times; the only difference is that in subdivided populations mutations spread across demes more slowly. Arrows denote the three timescales described in the text. Note that on the smooth landscape, the mean fitness of the well-mixed population always exceeds that of the subdivided population and it also exceeds the mean fitness of the champion deme at longer timescales (arrow 3). On the rugged landscape, the mean fitness of the well-mixed population exceeds that of the subdivided population until the *BC* mutant spreads in the latter, but it never exceeds the mean fitness of the champion deme.**

of growth per day. All media was supplemented with ampicillin (100μg/mL) and tetracyclin (25μg/mL) to prevent bacterial contamination. Each plate contained a unique pattern of 64 blank wells to detect contamination and cross-contamination events and to prevent plate misidentification. Cultures were frozen in our growth media supplemented with 15% glycerol at −80°C every 50 generations.

These 3520 demes were divided into hundreds of independent populations. We implemented two different population sizes. In our small populations, five demes comprised a single population, whereas in our large populations 20 demes comprised a population. At both population sizes, we implemented three different types of population structures: well-mixed populations, spatially structured populations, and unmixed populations.

Different population structures were implemented via different mixing schemes during each of the dilutions, as illustrated in Figure 2. In well-mixed populations, each deme making up the population was completely mixed with all the others prior to dilution, as illustrated in Figure 2B. These populations thus have almost no spatial structure—they are completely mixed at every daily dilution cycle and are divided into separate wells only to ensure that they experience the growth environment identical to that of all other populations. In subdivided populations, at each dilution a sample from each deme was transferred only to

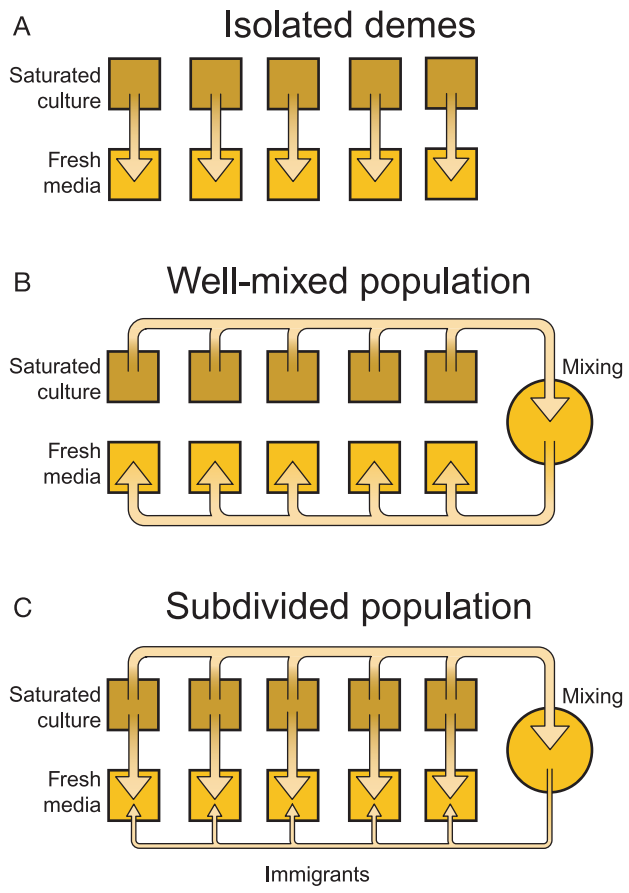

Figure 2. Conceptual scheme of dilution and mixing implemented in the experiment. Each deme is a well in a 384-well plate (squares). Mixing was done in 96-well plates (circles). (A) Isolated demes. Saturated culture from each deme is serially transferred into fresh media every 24 h. (B) Well-mixed population. Saturated culture from all wells comprising a population is mixed, diluted and distributed into the corresponding wells with fresh media every 24 h. (C) Subdivided population. Saturated culture from each deme is serially transferred into fresh media every 24 h. During migration events, which are implemented every 48 h, culture from all wells comprising the population is mixed, diluted, and distributed into all demes.

the corresponding deme in the new plate. In addition, at every other dilution a small number of migrants from each deme were transferred to all demes in the population, as illustrated in Figure 2C. We implemented three different migration rates, such that 0.7%, 2.8%, or 11.7% of individuals immigrated into each deme at each migration event (Table 1). Finally, we also implemented isolated populations as the unmixed, no-migration limiting case. In these isolated populations, at each dilution a sample from each deme was transferred only to the corresponding deme in the new plate, and no migrants were ever transferred, as illustrated in Figure 2A. Note that because there is no mixing between demes in these populations, the division of demes into populations is arbitrary. We maintained a total of 640 isolated demes; we can think of this either as 128 populations of five demes each or alternatively as 32 populations of 20 demes each. We treat them in both ways in our analysis, but it is important to remember that all the data come from the same set of 640 demes.

Our experimental design ensures that populations of all sizes and with all migration regimes evolve in the same medium and in exactly the same conditions imposed by the dilution schedule and the well geometry. The only difference between populations at different migration rates is a different number of migrants per deme at each transfer, and the only difference between populations maintained at different sizes is the number of demes from which (and to which) migration occurs.

Fitness Assays

To measure the mean fitness of evolved populations, we compared each to a reference strain (DBY15108), labeled with an mCherry fluorescent reporter, such that fitness is always measured against the same standard. We were careful to begin the fitness assay only after both strains were growing stably in the same conditions as in the evolution experiment. Thus to measure fitness, we thawed our experimental plates as well as plates of the mCherry-labeled reference strain. Each plate was acclimated by growing for 48 h, including $1:2^5$ dilutions every 24 h. Thereafter each deme in the evolved populations was mixed with the reference strain in proportion 1 : 2. Mixed cultures were propagated for 72 h in the isolated deme regime (see above, Fig. 2A), and the relative frequencies of evolved and references strains were measured using flow cytometry (Breslow et al., 2008) 24 and 72 h after mixing. Mean fitness increase of the evolved population relative to the reference was then calculated as

$$F = \frac{1}{t_2 - t_1} \log\left(\frac{n(t_2)}{n_r(t_2)} \Big/ \frac{n(t_1)}{n_r(t_1)}\right),$$

where $n(t)$ and $n_r(t)$ are the cell counts for the evolved and the reference strains at generation t after mixing, respectively. In our measurement, $t_1 = 10$ and $t_2 = 30$ generations.

We made three independent measurements of the mean fitness of each isolated deme and of each deme in each subdivided population. The mean fitness of all well-mixed populations were measured independently in each well that constituted the population, thus, yielding either five or 20 replicate measurements per well-mixed population. In addition, we measured the fitness of the ancestral population in 384 independent replicates. The fitness of the unmarked ancestral population was $0.46 \pm 0.07\%$ relative to the reference, indicating a slight but detectable selective disadvantage of 0.46% conferred by the mCherry marker. We calculated the fitness increase of evolved strains relative to the ancestor by subtracting 0.46% from all fitness measurements.

Table 1. Population sizes and migration rates implemented in the experiment.

Size	Small					Large				
Migration	N	W	M	S	W-M	N	W	M	S	W-M
Bottleneck	5000	5000	5000	5000	5000	20000	20000	20000	20000	20000
Demes	5	5	5	5	N/A	20	20	20	20	N/A
Immigrants	0	7	28	117	N/A	0	7	28	117	N/A
Replicates	128[1]	64	64	64	64	32[1]	16	16	16	32

"Migration" refers to different migration rates: no ("N"), weak ("W"), moderate ("M"), strong ("S"), and well-mixed ("W-M"). "Bottleneck" indicates the approximate number of cells transferred per entire population during each serial dilution. Note that each deme has the identical bottleneck size of 1000 individuals. "Demes" indicate the number of demes in the population. "Immigrants" indicate the number of immigrants per deme per migration event. "Replicates" indicate the number of independent replicate populations.

[1]Note that the 128 small populations with no migration and the 32 large populations with no migration refer to the same 640 independent demes, as described in "Materials and Methods."

Results

To determine how the effect of population structure on the exploration/exploitation balance influences the rate of adaptation in an asexual population, we set out to tune the degree of geographic subdivision in adapting populations of haploid *S. cerevisiae,* while keeping all other experimental parameters constant. To do so, each population was subdivided into a number of demes. Each deme was maintained and serially propagated in a separate but identical well of a 384-well plate in YPD, a standard rich laboratory media. Each well was inoculated daily with approximately 1000 individuals and grown to saturation for 24 h before the next serial dilution (see "Materials and Methods" for details). We did not subject our populations to specific selection pressures other than those that are naturally imposed by our propagation regime.

The degree of population subdivision and the total population size were controlled by mixing individuals from all demes that comprise the same population, according to the scheme depicted in Figure 2 (see "Materials and Methods" for details). We implemented populations of two sizes: small populations comprised of five demes and large populations comprised of 20 demes. At both population sizes, we varied the migration rate between demes across five values: the control case of no migration, three intermediate values of migration rate, and the opposite control of a well-mixed population. We maintained between 16 and 128 independent replicate populations for each population size and each migration rate. The experimental design is summarized in Table 1. Note that the no migration case represents a singular case—because all demes are independent, it is arbitrary how we group them to construct populations of multiple demes. Thus, we maintained a total of 640 independent isolated demes; we treat these both as 128 independent populations of five demes each for the purposes of comparison with small populations and as 32 populations of 20 demes each for comparing with large populations.

This experimental design has two essential features. First, all demes of all populations are maintained on exactly the same serial dilution schedule in exactly the same environment. Larger populations simply exchange migrants with a larger total number of demes, and differences in migration rate are implemented without affecting dilution rates or amounts. Thus, all populations experience precisely the same selective environment. In contrast to previous experimental systems, this allows us to focus exclusively on the effects of the geographic subdivision on the evolutionary dynamics of adaptation. Second, all populations that have the same census size receive mutations at the same rate. The only difference between populations with different degrees of subdivision is in which mutations they explore. Therefore, differences in the rate of adaptation (if any) can stem only from the differences in the exploration/exploitation balance but not from differences in the mutation supply.

POPULATION SUBDIVISION INHIBITS ADAPTATION

We propagated the experimental populations by daily serial transfer in YPD for 550 generations and measured the fitness of all demes of all populations at the end of the experiment (see "Materials and Methods"). Over the course of evolution, populations with both sizes and all degrees of subdivision adapted relative to the ancestor. As expected, populations with the large census size reached on average higher fitness than populations with the small census size, which in turn reached higher fitness than isolated demes, although the effect of population size was not always statistically significant. These results are summarized in Tables 2 and S1 and Figure 3; full data are available in Table S4.

We next examined the effect of population subdivision on the rate of adaptation in both small and large populations. We found that the extent of fitness increase depended strongly on the degree of subdivision (Tables 2 and S1, Fig. 3). Among populations with the same census size, populations with higher migration rates achieved on average higher fitness than populations with lower migration rates. This suggests that the potential advantage of

Table 2. Summary of results.

	Isolated demes[1]	Small				Large			
Statistic	N	W	M	S	W-M	W	M	S	W-M
Mean fitness increase,[2] %	0.5	2.2	2.7	4.3	5.6	3.9	3.0	4.7	9.9
Median fitness increase,[2] %	0.3	2.3	2.8	3.8	5.0	3.7	3.2	4.5	8.6
Number of populations	640	64	64	64	64	16	16	16	32
Number adapted[3]	149	53	53	62	63	16	16	15	32
Fraction adapted,[3] %	23	83	83	97	98	100	100	94	100
Improvement over champions,[4] %	N/A	0.0 (ns)	0.6*	1.7*	2.8*	0.4 (ns)	−0.1 (ns)	1.2*	5.3*

[1]Data reported under category "Isolated demes" is for 640 isolated demes treated as individual independent populations.

[2]Mean and median fitness increase, measured in percent relative to the ancestor.

[3]Number and percent of replicate populations whose final fitness was higher than that of 95% of the ancestor fitness distribution.

[4]Difference between the median of the distribution of observed fitness and the median of the distribution of champions, as defined in the "Results," measured in percent relative to the ancestor (see also Tables S2 and S3).

*Significance at 0.001 level; "ns" denotes "not significant" ($P > 0.05$); significance is assessed by a two-tailed permutation test (10^4 permutations).

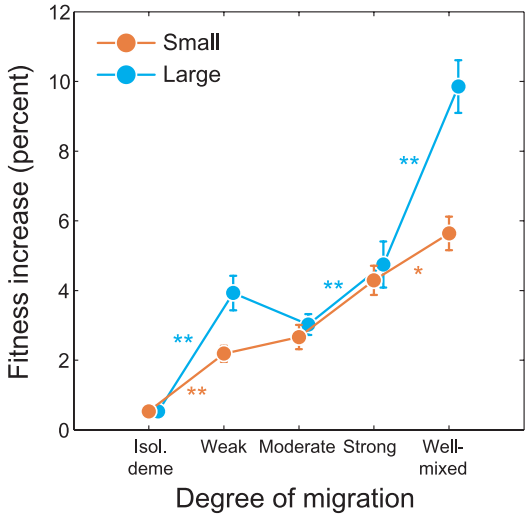

Figure 3. Increase in mean population fitness after 550 generations of evolution. The increase in mean population fitness relative to the ancestor averaged over replicate populations is shown. Error bars show ±1 standard error of the mean. Asterisk (double asterisk) shows that the difference in mean fitness increase is statistically significant at 0.05 (0.01) level (two-tailed permutation test, 10^4 permutations). See Table S1 for all pairwise comparisons.

exploration is small compared to the disadvantage of strengthening clonal interference.

WELL-MIXED POPULATIONS OUTCOMPETE CHAMPION DEMES

The observation that the mean fitness of populations with less migration are lower than the mean fitness of populations with more migration suggests that the fitness landscape in our system might be smooth, and exploitation is more important than exploration. However, it is important to keep in mind the limited timescale of our experiment. It is possible that populations with less migration did in fact discover more fit genotypes than populations with more migration, but our experimental timescale was too short to observe the spread of such genotypes over the entire population. Such a situation is illustrated in Figure 1 (right panel), which shows the evolutionary advantage of subdivided populations on a rugged fitness landscape. Note that, although the subdivided population discovers the more fit genotype *BC* faster than the well-mixed population, the mean fitness of the well-mixed population would be higher than that of the subdivided population if the experiment ended, for example, at the time point indicated by arrow 3 (Fig. 1, right panel). This is because beneficial mutations take longer to spread in subdivided populations, so we may not see the beneficial effect of subdivision at the level of the whole population on short timescales.

By measuring the fitness of individual demes, we can detect whether our experimental timescale was too short to observe the benefit of exploration at the whole population level, if such benefit is indeed present. To do so, we need to understand how the fitness of individual demes of a subdivided population would change over time relative to the fitness of a well-mixed population. On sufficiently short timescales, at most one beneficial mutation will have established in both well-mixed and subdivided populations, and it will still be infrequent relative to the size of a deme (Fig. 1, arrow 1). On this timescale, the spread of beneficial mutations is not yet restricted by subdivision, and the expected mean fitness of a random deme is the same as that of the well-mixed population. Consequently, on short timescales the mean fitness of the *fittest deme* in the subdivided population (which we call the "champion deme") will be greater than the mean fitness of the well-mixed population. On somewhat longer timescales, the fittest beneficial mutation will have swept through the entire

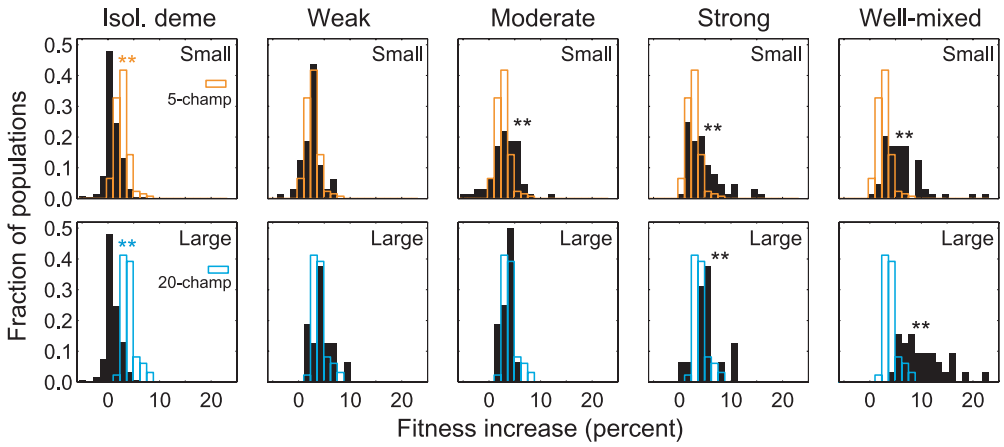

Figure 4. **Distributions of mean population fitness across replicates after 550 generations of evolution. Panels in the top row show data for small populations, panels in the bottom row show data for large populations (see Table 1). Panels in each row show data for different migration rates, as indicated by the titles. Black bars show observed distributions. Transparent orange and blue bars show the distribution of fitness of five and 20 champions, respectively (see text for details). Asterisk (double asterisk) shows that the distributions are significantly different at 0.05 (0.01) level (two-tailed permutation test, 10^4 permutations). Asterisks are colored (black) if the champions are more (less) fit than the observed populations (see Tables S2 and S3).**

well-mixed population, driving all others extinct (Gerrish and Lenski, 1998; Rozen et al., 2002). At the same time, in the subdivided population the fittest beneficial mutation will have swept through the deme where it arose, but not the entire population (Fig. 1, arrow 2). Thus, the mean fitness of the well-mixed population would be equal to that of the champion deme of the subdivided population. On even longer timescales, double-mutants will have appeared in both well-mixed and subdivided populations (Fig. 1, arrow 3). In the well-mixed population, the secondary mutations occur in the background of the fittest primary mutation because this mutation has already swept. On the other hand, secondary mutations can occur in a variety of backgrounds in the genetically diverse subdivided population. If exploration is sufficiently important, then by definition the fittest single-mutant will not produce the fittest double-mutant. Thus, the fittest deme in the subdivided population will be as fit or fitter than the well-mixed population as a whole.

Therefore, if exploration is more important than exploitation, at no point will the expected fitness of the champion deme in a more subdivided population fall below the mean fitness of a less subdivided or well-mixed population. On the other hand, if exploitation is more important than exploration, the fact that beneficial mutations occur in fitter genetic backgrounds in well-mixed populations means that the mean fitness of well-mixed populations will eventually outstrip the fitness of the champion deme of the subdivided populations. Thus, if we observe that the champion deme in a population with less migration is on average at least as fit as a population with more migration as a whole, we would conclude that exploration might be important, but the timescale of our experiment is too short to observe the evolutionary advantage of subdivision. In contrast, if we observe that the champion deme in a population with less migration is on average less fit than a population with more migration as a whole, we would conclude that adaptation by means of exploitation is more rapid than adaptation by means of exploration.

To test this hypothesis, we randomly grouped the 640 isolated demes of the no-migration control into 128 control populations of five demes each (for comparison with the small populations) and also into 32 control populations of 20 demes each (for comparison with large populations). This grouping ensures that the total census size and hence the mutation supply rate in these control populations is exactly the same as in the corresponding subdivided or well-mixed populations. Within each control population and within each subdivided population, we found the deme that had the highest fitness at the end of our evolution experiment. We refer to the deme with the highest fitness among K demes as the "K-champion;" here we looked at 5- and 20-champions.

In Figure 4, we show the distributions of mean fitness across replicate populations for both population sizes and all migration rates and compare them with the distributions of fitness of 5- and 20-champions of control populations. We see that populations with weak and moderate migration rates attained on average the same fitness as the corresponding 5- or 20-champions (Table 2 and Fig. 4). On the other hand, populations with strong migration and well-mixed populations attained on average fitness that significantly exceed those of K-champions. In general, populations with more migration attained higher fitness on average than the champion demes of populations with less migration (Fig. S1 and Tables S2, S3). We therefore conclude that secondary mutations that occur in the background of the fittest primary mutation usually confer higher fitness than secondary mutations that occur in other genetic backgrounds. This indicates that our fitness

landscape is "smooth," and the benefits of spatial structure in enhancing exploration are outweighed by the costs of slowing exploitation.

Discussion

By making competition between individuals local rather than global, population subdivision reduces the immediate efficiency of natural selection in driving beneficial mutations to high frequency. This limits the rate at which the population can exploit the beneficial mutations that it generates, but also means that more genetic diversity can be maintained in the face of selection. This effect of enhancing "exploration" means that, somewhat paradoxically, population subdivision could in principle speed the overall rate of adaptation despite limiting the efficiency of natural selection.

We have experimentally shown that subdivided asexual populations adapt more slowly than well-mixed populations (Figure 3). Other experimental studies similarly have found an inhibiting effect of spatial structure on the rate of adaptation (Habets et al., 2007; Perfeito et al., 2008), whereas one study has shown a more complex relationship (Rozen et al., 2008). However, to our knowledge, our experiment is the first to demonstrate how varying degrees of population structure affect adaptation in isolation from other factors.

The central result of our work is the dependence of adaptation on migration rate (Figs. 3, 4, and S1; Tables 2, S2, and S3). We find that after a period of adaptation even the fittest ("champion") demes were, on average, significantly less fit than populations with strong migration or well-mixed populations. We note that, despite this difference in means, the distributions of fitness between champion demes and mean fitness of well-mixed populations overlap substantially (Fig. 4). This overlap is a consequence of the fact that isolated demes will occasionally reach higher fitness than well-mixed populations, simply due to the intrinsic randomness of the evolutionary process. This would be true on any fitness landscape, including a perfectly "smooth" nonepistatic landscape where the benefit of exploration is entirely absent. By observing the entire distribution of fitness attained by populations with different degrees of migration, we can conclude that subdivision inhibits adaptation because populations with less migration discover *on average* less fit types than do populations with more migration. Thus, in our system, maintaining more genetic diversity to find beneficial epistatic combinations of mutations is not worth strengthening the effects of clonal interference.

This conclusion means that in some sense epistasis in our system is weak and the fitness landscape is "smooth." This is consistent with several previous experimental studies (Wichman et al., 2005; Desai et al., 2007; Barrick et al., 2009; Miller et al., 2011; Rokyta et al., 2011; Lang et al., 2011). However, other studies have suggested that epistasis is common and fitness landscapes are rugged (Korona et al., 1994; Burch and Chao, 2000; Silander et al., 2007; Schoustra et al., 2009; Kvitek and Sherlock, 2011; Salverda et al., 2011). This discrepancy points to potentially important differences between fitness landscapes imposed by different experimental environments and different genetic architectures. A systematic survey of fitness landscapes across evolving systems and experimental conditions will be necessary to resolve this discrepancy. It has been suggested, for example, that patterns of epistasis are different between viruses, prokaryotes, and eukaryotes (Sanjuán and Elena, 2006) and among mutations within versus between proteins (Chou et al., 2011).

A number of recent studies have found extensive sign epistasis among beneficial mutations by genetically reconstructing intermediate combinations of mutations that fix during the course of adaptive evolution (Weinreich et al., 2006; Salverda et al., 2011; Kvitek and Sherlock, 2011). These studies have reinforced the view that sign epistasis is common, at least in some adapting systems, and that it impedes adaptation because it makes certain mutational trajectories inaccessible to natural selection (Weinreich et al., 2005; Poelwijk et al., 2007). However, sign epistasis among a small set of mutations that fix during the course of adaptation does not necessarily imply that the fitness landscape is rugged in a way that would provide an advantage to exploration. In particular, it is possible that epistasis only affects the fitness effects of individual mutations but leaves the *distribution* of fitness effects unchanged. For example, mutation A might change the sign of the fitness effect of mutation C from beneficial to deleterious while at the same time changing the sign of another mutation B in the reverse direction. Mutation A followed by B might then fix during adaptation, and the genetic reconstruction experiment would reveal sign epistasis between them. Yet, the rate of adaptation on a fitness landscape with this type of sign epistasis would not be slower than on a landscape with no epistasis, because such epistasis does not open up or close down the opportunities for adaptation. Thus, this type of sign epistasis would not imply an advantage of exploration versus exploitation. Thus, there remains a possibility that empirical fitness landscapes are smooth in the sense of providing no advantage for exploration, despite extensive epistasis between mutations. If this is the case, the phenotypic or fitness outcome of adaptation may be much more predictable than the genotypic outcome, a conjecture that has been previously proposed in the literature (Fisher, 1930; Ibarra et al., 2002; MacLean et al., 2010; Nguyen et al., 2011).

Although our data imply that epistasis in our system is either weak or that it does not strongly impede adaptation, we are currently unable to quantify this observation beyond showing that our results are inconsistent with the most extreme form of epistasis—the "uncorrelated" fitness landscape (see Appendix S1

and Fig. S2) which has been extensively studied in the theoretical literature (Kingman, 1978; Kauffman and Levin, 1987; Macken and Perelson, 1989; Flyvbjerg and Lautrup, 1992; Jain and Krug, 2007; Park and Krug, 2008). For example, we cannot exclude the possibility that there are multiple fitness peaks in the underlying fitness landscape, such that higher peaks can only be attained via multiple large-effect mutations, whereas low peaks can be attained via small-effect mutations. A major obstacle to quantifying the degree and type of epistasis is that there is no general convenient way to parameterize it. Several possibilities have been proposed in the literature (Kauffman and Levin, 1987; Perelson and Macken, 1995; Aita et al., 2000), but they are more suitable for theoretical investigations than for fitting experimental data. A general parametric model of genotype- or fitness-dependent distribution of fitness effects may be a promising way forward (Kryazhimskiy et al., 2009). Furthermore, even if a suitable parameterization of epistasis were proposed, it is currently unclear precisely how epistasis would affect the course of adaptation in a spatially structured population. The selective sweep dynamics in spatially structured populations have only recently begun to be investigated (Ralph and Coop 2010; Martens and Hallatschek 2011). Coupling these dynamics with the structure of epistasis remains an important goal for future research. Such work would make it possible to place precise quantitative bounds on the degree and type of epistasis in a given system from experimental results such as ours.

It is important to note that our conclusions about the relative importance of exploitation versus exploration and the degree of epistasis in the underlying fitness landscape hold only for the "local" mutational neighborhood of the initial genotype. The degree and type of epistasis might dramatically differ from one genotype to another, and the balance between exploitation and exploration might shift in one or another direction as mutations accumulate in a population.

Finally, we note that our results bear some consequences for the long-standing debate on Sewall Wright's shifting balance theory (Wright, 1982; Coyne et al., 1997; Wade and Goodnight, 1998). One critical difference between our conceptual framework and the shifting balance theory is that the latter relies on genetic drift to help individual demes cross fitness valleys, whereas we argue that subdivided populations can have an evolutionary advantage on some fitness landscapes even without any fixation of deleterious alleles in individual demes. Subdivision simply protects the genetic diversity within demes from selective sweeps. Nevertheless, both frameworks predict that population subdivision should be advantageous on sufficiently rugged fitness landscapes. Despite significant effort (Katz and Young, 1975; Wade, 1976; Wade and Goodnight, 1991), no unambiguous experimental evidence of evolutionary advantage of subdivided populations had yet been demonstrated.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Adaptation of well-mixed and subdivided populations on an uncorrelated fitness landscape.
Table S1. Comparison of mean-fitness distributions across populations with different sizes and structures.
Table S2. Comparison of fitness of small populations and 5-champions.
Table S3. Comparison of fitness of large populations and 20-champions.
Table S4. Flow cytometry counts.
Figure S1. Comparison of mean population fitness with the fitness of champions.
Figure S2. Mean fitness trajectories on uncorrelated fitness landscapes.

Supporting Information may be found in the online version of this article.