Supporting Information:

§ S1: How a Rugged Landscape Can Fail to Give a Tortoise-Hare Signal

S1.1. Potential causes:

On a rugged landscape, fitness in a structured population will increase more slowly than an unstructured population (the Tortoise initially lags behind, before overtaking, the Hare; see Fig. 2b). That is, for populations differing in structure evolving on a rugged landscape, early evolution will produce a pattern similar to that predicted under a smooth landscape (e.g., before time point 250, the pattern in Figure 2b would be hard to distinguish from the entire trajectory of Figure 2a). While the presence of a Tortoise-Hare pattern indicates ruggedness, its absence does not necessarily imply a smooth landscape.

For example, in the experiment of Kryazhimskiy *et al*. (2012) (1), the unstructured population ended the experiment with higher average fitness. This pattern is consistent with a smooth landscape, but is not inconsistent with a rugged one. As the authors themselves acknowledge, had their experiment run longer, they may have observed higher fitness under lower rates of migration (i.e., a fitness crossing).

Even when there is abundant time for evolution to take place, it is still possible that evolution on a rugged landscape will fail to yield the Tortoise-Hare pattern. For instance, it is possible that the landscape is rugged, but peaks are of a homogeneous height. This could produce the fitness pattern shown in Figure 2a.

Finally, the landscape could be rugged with heterogeneous peak heights, but the ancestor could be positioned in the domain of a single peak. Consistent with this possibility, Kryazhimskiy *et al*. started their experiment with a lab-adapted strain of yeast and evolved their populations under standard laboratory conditions. If their yeast had access to only a single domain in a rugged landscape, a Tortoise-Hare pattern would not be expected. In such a case Kryazhimskiy *et al*. would be justified in claiming that the *local* topography of such a landscape was smooth (and indeed, they restrict their claim of smoothness accordingly).

S1.2. Investigating the influence of the initial position in the landscape:

As outlined in our Methods, we introduced several deleterious mutations into our ancestor and evolved our populations under a stressful environment (in the presence of sub-lethal concentrations of the antibiotic tetracycline). Such manipulation was intended to displace our ancestral genotype from a peak, but it also may have placed it at a point where multiple domains were accessible. To address the effect of ancestor starting position, we describe additional NK simulations here. In addition to starting our ancestor at a random bit string, we

consider three other starting positions: (i) valley, (ii) pre-adapted, and (iii) "silver-spoon." For the valley simulations, we performed a "hill-plunge of steepest descent," moving downhill from a random genotype until we hit a valley genotype (a genotype from which all mutations were beneficial), which served as the ancestor. For the pre-adapted simulations, we allowed a random ancestor to evolve briefly (in an unstructured population) to produce a "pre-adapted" ancestor. For the silver-spoon simulations, all genotypes were ranked for fitness and the genotype defining the 99th fitness percentile was chosen as the ancestor.

In the random and valley starting positions, the Tortoise-Hare pattern was observed and the Structured treatment ended at significantly higher average fitness than the Unrestricted treatment (Mann-Whitney tests, $p<0.001$; Fig. S1). However, in the pre-adapted and silver-spoon starting positions, the Tortoise-Hare pattern was not seen and fitness was indistinguishable between the treatments in the long run (Mann-Whitney test, $p=0.36$ and $p=0.57$ respectively; Fig. S1). These simulations demonstrate that the starting position of a population in a landscape will influence the statistical pattern of fitness of populations differing in structure.

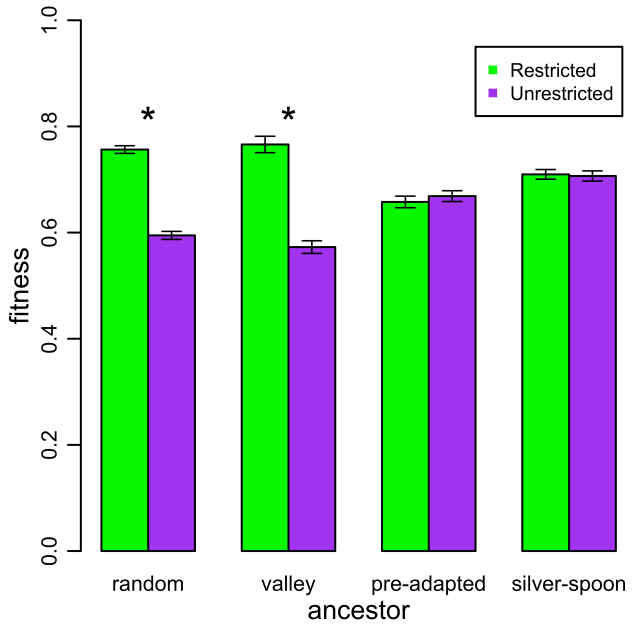

Figure S1: Metapopulations of bit strings of length N=15 evolved on a rugged landscape (K=8), where migration was either restricted or unrestricted. Average fitness in the metapopulation is shown at time point 1000 for a randomly chosen ancestor, an ancestor starting in a valley, an ancestor resulting from adaptation before the run, and an ancestor in the top percentile of fitness (the "silver-spoon" ancestor). Bars represent the mean of 40 replicates, whiskers give the standard error of the mean, and asterisks indicate significant differences.

S1.3. Additional NK simulation methods:

To study the effect of starting position in the adaptive trajectories in structured and unstructured populations, we examined starting the population with different types of ancestors. The "random" ancestor (used in the primary text) is simply a random bit string. To generate the "valley" ancestor, we start with a random bit string and substitute the worst (lowest fitness) possible mutation until no deleterious mutations are possible, and that bit string is the ancestor for the evolutionary run. To produce the "pre-adapted" ancestor, we start with a random bit string and evolve a population initialized with this bit string under unrestricted migration for 50 updates. Then bit strings are sampled from the evolved population until one is found that has a higher fitness than the starting bit string. That adapted bit string is the ancestor. To determine the "silver-spoon" ancestor, all possible bit strings (2^{15}) are ranked according to fitness and the 99th percentile genotype is the ancestor.

§ S2: A Simple Model to Illustrate the Tortoise-Hare Pattern

Here we consider an extremely simple rugged landscape. There is one valley genotype (the ancestor) connected to an infinite number of peaks. Specifically, the ancestral genotype can experience any one of an infinite number of beneficial mutations where the selective benefit of a mutation is exponentially distributed. Each beneficial mutant constitutes a peak genotype from which mutation back to the ancestor (or to any other mutant) cannot occur. Once the valley genotype is extinct, no new peak genotypes will be generated.

In the evolving metapopulation, individuals were embedded within 100 demes. Each deme contained 1000 organisms. All organisms start with the ancestral (valley) genotype. The ancestor's fitness was set to 1. When an organism with the ancestral genotype experienced a mutation (rate of 0.001 per division), the new genotype had a fitness of $1 + s$, where $s \sim \mathrm{Exp}(1)$. Selection within a deme involved the removal of a random organism, regardless of fitness, and its replacement by the birth of an organism from the same deme chosen by a fitness-weighted lottery. This death-birth process was iterated 1000 times for each deme, followed by migration between demes. For the "Limited" migration treatment, 25 random individuals from each deme were collected into a migrant pool. Then 25 random individuals from the migrant pool were added back to each deme (chosen without replacement). For the "Unlimited" migration treatment, all individuals were randomly permuted among subpopulations. There were 50 replicate lines in each treatment, and each metapopulation experienced 200 selection-migration episodes (updates).

In Figure S2, we see that the average fitness in these evolving metapopulations clearly demonstrates the Tortoise-Hare pattern.

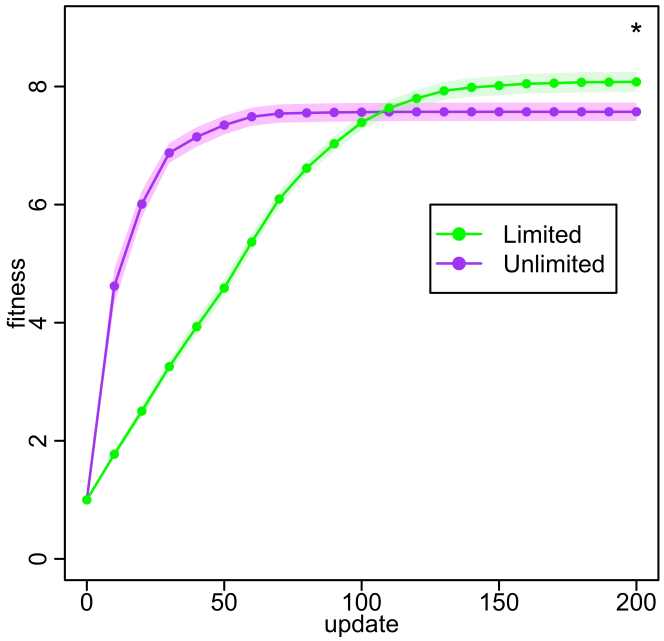

Figure S2: Metapopulations evolved on a simple rugged landscape, where migration was either limited or unlimited. The solid lines represent average fitness in both treatments over time, and shading gives the standard error of the mean for 50 replicate lines.

§ S3: Diversity in Evolving Populations

S3.1. Diversity in digital and bacterial populations:

A structured population performs a broader search on the adaptive landscape, as the rate of competitive displacement is lower. Consequently, the standing genetic diversity of a structured population is expected to be greater than diversity in an unstructured population. In Figure S3a, we see that this pattern does not depend on landscape ruggedness (Mann-Whitney tests, $p<0.01$ for $K=0$ and $K=8$). Thus, despite landscape topography, we predict to find higher genetic diversity in a structured population, and this is what we find in our bacterial metapopulations (Mann-Whitney test, $p=0.015$; Fig. S3b).

S3.2. Diversity methods:

Consider a sample of G genotypes (bit strings or nucleotide sequences). We use the diversity index of Nei and Li (1979) (2):

$$\pi = \frac{2}{G^2} \sum_{i=2}^{G} \sum_{j=1}^{i-1} \pi_{ij} ,$$

where π_{ij} is average number of differences (in bits or bases) per site between genotype i and genotype j. We refer to π as bit diversity (in the NK model) or nucleotide diversity (for our bacterial system).

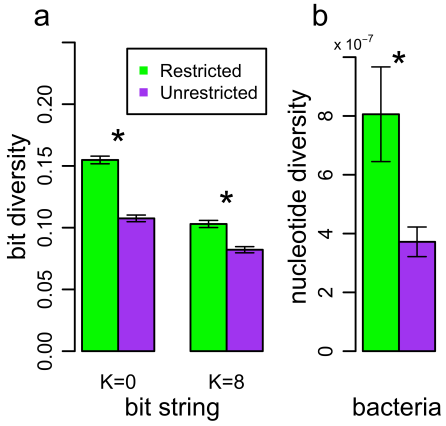

Figure S3: Genetic diversity in the digital and bacterial populations. (a) Average bit diversity of a sample of eight evolved bit strings from time point 1000 in the NK model simulations. Whether the landscape is smooth (K=0) or rugged (K=8), diversity is significantly greater in the Restricted treatment than the Unrestricted treatment. Bars represent the mean of 40 replicates. (b) Average nucleotide diversity within bacterial metapopulations at the final transfer of the experiment (T=36). For each metapopulation, full genome sequences from each of five isolates was used to compute the diversity index. Nucleotide diversity is significantly greater in the Restricted treatment than the Unrestricted treatment. Bars represent the mean of 5 replicates. In both parts of the figure, whiskers give the standard error and asterisks indicate significant differences.

S3.3. Violation of strong-selection-weak-mutation (SSWM) assumptions

The relatively high degree of diversity in the evolving bacterial populations demonstrates a violation of strict SSWM assumptions. Isolates from the same metapopulation (in both treatments) were often not single mutant neighbors (see Figure 5 and Supplemental Table 1); thus, the diversity does not simply represent a mixture of the genotype fixing and the genotype being displaced in the midst of selective sweep. While divergent genotypes are expected in the structured population (Restricted Migration), they also appear in our less structured treatment (Unrestricted Migration). Most likely, the diversity results partly from the presence of small fitness differences between genotypes, such that new beneficial mutations may arise before old beneficial mutations fix (leading to a form of clonal interference). We also note that the Unrestricted treatment possessed some degree of population structure (i.e., this was not a "well-mixed" population), which could also contribute to diversity. Such diversity may enable metapopulations in the Unrestricted treatment to explore multiple domains simultaneously. However, the main effects of structure outlined in this paper still apply. If the landscape is multi-peaked, metapopulations in the Restricted treatment are expected to sample *a greater number of domains* (and indeed, the Restricted metapopulations had significantly greater diversity). We

emphasize that the same violations of SSWM assumptions occur in the NK model (see Supplemental Figure 1 for diversity profiles), in which the same migration treatments were used, and we note that the Tortoise-Hare pattern is observed for sufficiently rugged landscapes (Figure 2). Thus, even in populations violating strict SSWM assumptions, the greater parallel search that comes with greater population structure is predicted to lead to better long-term adaptation in rugged landscapes with heterogeneity in peak height.

§ S4: Pilot Experiment

S4.1. Pilot experiment overview:

The main bacterial experiment described in the text was preceded by a pilot experiment, which differed in a few important ways. First, the bacterial ancestor was derived from a strain of *E. coli* B (REL606) by selecting for resistance to the antibiotic rifampicin. (In contrast, the ancestor in the main experiment was derived from a strain of *E. coli* K-12 by selecting for resistance to colicin E2, colicin D, and phage T6.) Second, the bacterial population in the pilot experiment was propagated as a biofilm on the surface of an agar-filled Petri dish. (In contrast, the bacterial population in the main experiment was propagated as a metapopulation distributed into the 96 wells of a microtiter plate.) Two runs of the pilot experiment were conducted, each with an independently isolated rifampicin-resistant mutant. As in the main experiment, each pilot run had two treatments differing in population structure. In the first, which we label "Static," the biofilm is transferred by pressing the fully grown Petri dish on a replica plating platform with velveteen cloth and then pressing a fresh Petri dish on the same cloth. This transfer protocol ensures a dilution of the biofilm from the exhausted dish is deposited on the fresh dish in a way that preserves spatial relationships. In the second treatment, which we label "Mixed," the fully grown dish was pressed multiple times on the velvet-covered platform, rotating at different random angles, before the fresh dish was pressed to acquire the spatially-mixed sample. The Static and Mixed treatments roughly map to the Restricted and Unrestricted treatments, respectively, of the main experiment.

S4.2. Pilot protocol:

Each independently derived rifampicin-resistant ancestor was grown in 5 mL of DM1000 over 24 hours, shaking at 37°C. A 100μL aliquot was spread over an MG agar-filled Petri dish (without rifampicin) with glass beads to initiate each population. Because all pilot runs were conducted in triplicate, each ancestor was spread onto six Petri dishes (to initiate three Static populations and three Mixed populations). These dishes were incubated over 24 hours at 37°C. In the Static treatment, the fully grown dish was pressed lightly on a sterile velveteen cloth stretched over a replica plating tool. A fresh Petri dish was then carefully pressed onto the same cloth to obtain a spatially structured dilution of the original population. A second fresh Petri dish was also pressed onto the same cloth.

Both dishes were incubated over 24 hours at 37°C. The first dish was used for the next transfer, while the second dish was scraped into saline, vortexed, and a 1 mL aliquot was frozen at −80°C. The Mixed treatment proceeded identically to the Static treatment, except at each transfer, the fully-grown dish was (1) pressed lightly on the velvet, (2) turned clockwise at a random angle and pressed a second time, (3) turned counter-clockwise at another random angle and pressed a third time, and (4) turned clockwise at another random angle and pressed a fourth time. The fresh dishes were then pressed on the velvet to initiate the spatially mixed sample. Each replicate population was transferred 33 times. Four or five isolates (derived by picking random colonies after plating dilutions of the frozen population samples) were obtained for every population for transfer 9 (early in the experiment) and transfer 33 (at the end of the experiment).

S4.3. Pilot Fitness Assay:

Fitness was assayed on the surface of Petri dishes (the relevant environment for evolution). The ancestor was the common competitor for every competition, and was marked neutrally with the ability to use arabinose. The evolved isolate and marked ancestor were grown separately in 10 mL of DM1000 over 24 hours, shaking at 37°C. After growth, 100μL of each culture was spread on a separate MG Petri dish using glass beads; the dishes were incubated over 24 hours at 37°C. The dish with the marked ancestor and the dish with the evolved isolate were each pressed onto the same sterile velveteen cloth on a replica platform to initiate the spatial competition. Immediately, ½ of the competition dish was scraped into 10mL of saline, vortexed, diluted and plated on TA agar plates (resulting colony counts gave the initial densities of each competitor; note the ancestor formed pink colonies while the evolved isolate formed red colonies). The competition dish containing the other (unscraped) half of the co-culture was placed at 37°C for 24 hours. After incubation, the remaining half of the co-culture was scraped into 10mL of saline, vortexed, diluted and plated on TA agar plates (resulting colony counts gave the final densities of each competitor). If E_t and A_t are the densities of evolved and ancestral cells at time t, respectively, then the fitness of the evolved isolate relative to its ancestor is given by:

$$w(E,A) = \frac{\log\left(\frac{E_{24}}{E_0}\right)}{\log\left(\frac{A_{24}}{A_0}\right)}.$$

S4.3. Pilot results:

The results for the two strains are shown in Figure S4. Fitness in the Mixed treatment isolates is greater than the Static treatment isolates early, but the relationship is reversed late. Thus, a Tortoise-Hare pattern is observed for each strain. The differences are not significant, but we note that only three replicates of each treatment were run. These trends motivated our full experiment in which the number of replicates, the opportunity for adaptation (via compensation to, or

reversion of, more costly markers, and the presence of an antibiotic stress), and the control over population structure (by using defined migration patterns within a metapopulation) were all increased.

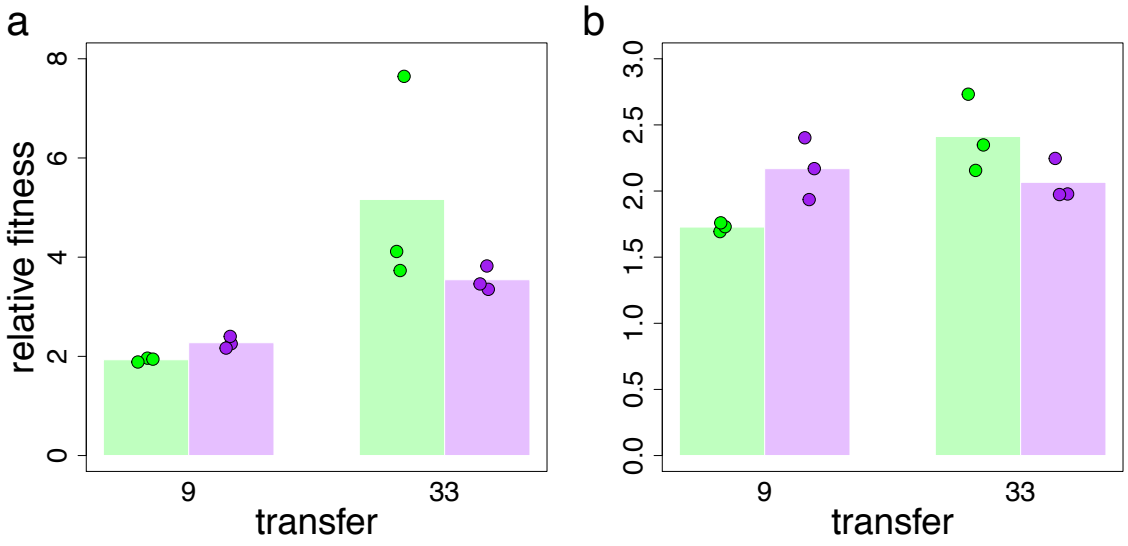

Figure S4: The results of a pilot experiment for ancestral strain *a* (part a) and ancestral strain *b* (part b). The average fitness of four or five isolates (relative to their ancestor) from each replicate evolving population is shown as a point, where the Static treatment is in green and the Mixed treatment is in purple. Points are jittered for detection. The height of each bar gives the average of the average fitnesses across the three replicates within the relevant treatment at the relevant transfer. There are no significant differences, except for ancestral strain *a* at transfer 9 ($p=0.02758$, Welch's two sample t-test), but the trend for both strains is consistent with a Tortoise-Hare pattern and motivated the full experiment described in the text.

§ S5: Fitness Relative to Ancestor Predicts Fitness Relative to a New Strain

Throughout this manuscript, we have measured fitness for our evolved isolates by performing competitions against their common ancestor. How well does fitness against this ancestral competitor predict fitness against other competitors? If differences in fitness are primarily due to differences in growth rate, then relative fitness against one competitor should be positively correlated with relative fitness against another. On the other hand, if competitive outcomes are affected by social interactions (e.g., toxic inhibition or cross-feeding), then fitness relative to one competitor may not predict fitness relative to another (i.e., if such social interactions change with the genotype of the competitor). To explore the predictive power of our fitness metric, we performed additional competitions.

In addition to the competition with their common ancestor, each of the 50 isolates from transfer 36 was competed against two extra strains. The first strain was an isolate from a Restricted metapopulation at transfer 36, and the second strain was an isolate from an Unrestricted metapopulation at transfer 36. The results of

these 100 competitions are shown in Figure S5. There are several things to note. First and foremost, fitness relative to the ancestor is a good predictor of fitness relative to the evolved competitors (R^2=0.966 for the Restricted competitor, and R^2=0.965 for the Unrestricted competitor). Second, because the linear relationship is positive, the ordering of the mean fitness across treatments (green and purple dashed lines) remains the same across competitor genotypes. Third, fitness of the strains relative to the Restricted isolate (y values in Fig S5a) is lower than fitness of the same strains relative to the Unrestricted isolate (y values in Fig. S5b). This is expected because the fitness of isolates from the Restricted treatment are generally higher than isolates from the Unrestricted treatment. Overall, these patterns are consistent with a situation in which the differences in fitness are due to differences in growth rate.

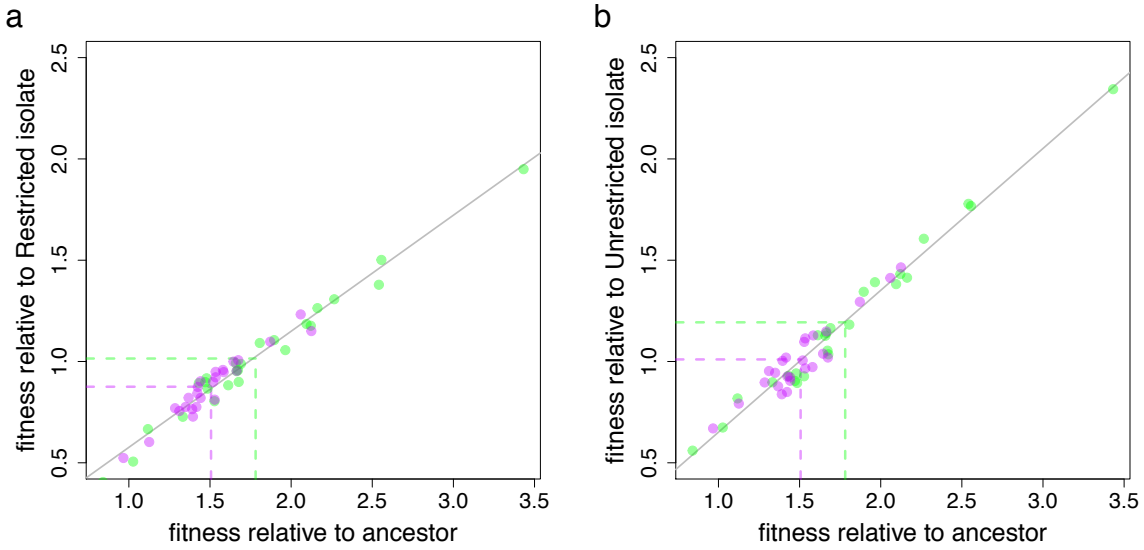

Figure S5: Fitness of 50 evolved isolates from transfer 36 relative to different competitors. Isolates from the Restricted treatment (the 25 green points) and isolates from the Unrestricted treatment (the 25 purple points) were competed against their common ancestor (relative fitness on the x-axis) and against another evolved isolate from the same transfer (relative fitness on the y-axis). This evolved competitor was either from the Restricted treatment (a), or from the Unrestricted treatment (b). For each experimental treatment, mean fitness values against each competitor are given by dashed lines (vertical lines, ancestral competitor; and horizontal lines, evolved competitor).

Table S1:

Migration Type	Population	Isolate	Position	Mutation Type	Change	Gene Left	Gene Right
Restricted	1	2	1431461	SNP	C	stfR	stfR
Restricted	1	2	4371271	DEL	2	dcuA	aspA
Restricted	1	2	4547236	SNP	C	fimE	fimE
Restricted	1	3	1621052	DEL	3	marR	marR
Restricted	1	3	4371271	DEL	2	dcuA	aspA
Restricted	1	5	100788	SNP	G	murC	murC
Restricted	1	5	987361	SNP	T	ompF	ompF
Restricted	1	5	4371271	DEL	2	dcuA	aspA
Restricted	1	5	4547667	DEL	1	fimE	fimA
Restricted	1	6	4547156	SNP	T	fimE	fimE
Restricted	1	8	NA	NONE	NA	NA	NA
Restricted	2	1	986920	DEL	2	ompF	ompF
Restricted	2	4	1197797	SNP	T	icd	icd
Restricted	2	4	1197809	SNP	T	icd	icd
Restricted	2	4	2611074	SNP	T	hyfR	hyfR
Restricted	2	4	4371271	DEL	2	dcuA	aspA
Restricted	2	4	4611002	INS	G	yjjZ	rsmC
Restricted	2	6	NA	NONE	NA	NA	NA
Restricted	2	7	1430874	SNP	G	stfR	stfR
Restricted	2	7	1431302	SNP	T	stfR	stfR
Restricted	2	7	1431530	SNP	T	stfR	stfR
Restricted	2	7	4371271	DEL	2	dcuA	aspA
Restricted	2	8	1429268	SNP	A	lomR	lomR
Restricted	2	8	1429270	SNP	T	lomR	lomR
Restricted	2	8	1431048	DEL	3	stfR	stfR
Restricted	2	8	3613480	DEL	16	tatC	tatC
Restricted	2	8	4371271	DEL	2	dcuA	aspA
Restricted	3	1	987157	SNP	T	ompF	ompF
Restricted	3	1	4371271	DEL	2	dcuA	aspA
Restricted	3	2	1431494	SNP	G	stfR	stfR
Restricted	3	2	1431497	SNP	A	stfR	stfR
Restricted	3	2	1431500	SNP	G	stfR	stfR
Restricted	3	2	1431530	SNP	T	stfR	stfR
Restricted	3	2	4546978	SNP	T	fimE	fimE
Restricted	3	4	1098012	SNP	T	ycdU	ycdW
Restricted	3	6	4477340	SNP	T	pyrL	yjgH
Restricted	4	1	4371271	DEL	2	dcuA	aspA
Restricted	4	2	1413888	SNP	A	intR	intR

Restricted	4	2	1430581	SNP	G	lomR	lomR
Restricted	4	2	1430639	SNP	T	lomR	lomR
Restricted	4	2	1431015	SNP	T	stfR	stfR
Restricted	4	3	1431138	SNP	A	stfR	stfR
Restricted	4	3	1431581	SNP	C	stfR	stfR
Restricted	4	3	1638633	SNP	G	ynfO	ynfO
Restricted	4	3	1638637	SUB	2	ynfO	ynfO
Restricted	4	3	1638691	SNP	T	ynfO	ynfO
Restricted	4	3	4371271	DEL	2	dcuA	aspA
Restricted	4	3	4547359	SNP	A	fimE	fimA
Restricted	4	4	1429200	SNP	C	lomR	lomR
Restricted	4	4	1430798	SUB	2	stfR	stfR
Restricted	4	4	1431356	SNP	T	stfR	stfR
Restricted	4	4	1431461	SNP	C	stfR	stfR
Restricted	4	4	1431494	SNP	G	stfR	stfR
Restricted	4	4	1431497	SNP	A	stfR	stfR
Restricted	4	4	1431581	SNP	C	stfR	stfR
Restricted	4	4	4546907	DEL	309	fimE	fimE
Restricted	4	6	1383618	SNP	G	ymjB	ompG
Restricted	4	6	4371271	DEL	2	dcuA	aspA
Restricted	5	2	1431356	SNP	T	stfR	stfR
Restricted	5	4	1431356	SNP	T	stfR	stfR
Restricted	5	4	4072544	SNP	T	glgA	glgA
Restricted	5	4	4371271	DEL	2	dcuA	aspA
Restricted	5	5	NA	NONE	NA	NA	NA
Restricted	5	6	455889	SNP	T	tig	clpP
Restricted	5	6	1637898	SNP	T	nohA	nohA
Restricted	5	6	4371271	DEL	2	dcuA	aspA
Restricted	5	7	4523920	DEL	22905	insB	yjhU
Unrestricted	1	3	4371271	DEL	2	dcuA	aspA
Unrestricted	1	3	4547370	DEL	1	fimE	fimA
Unrestricted	1	4	4211306	SNP	C	yrdA	yjaA
Unrestricted	1	4	4371271	DEL	2	dcuA	aspA
Unrestricted	1	5	NA	NONE	NA	NA	NA
Unrestricted	1	6	4371271	DEL	2	dcuA	aspA
Unrestricted	1	7	4371271	DEL	2	dcuA	aspA
Unrestricted	2	1	1621223	AMP	2	marR	marR
Unrestricted	2	1	4371271	DEL	2	dcuA	aspA
Unrestricted	2	3	4103874	SNP	A	ompR	ompR
Unrestricted	2	3	4371271	DEL	2	dcuA	aspA
Unrestricted	2	4	723898	SNP	A	kdpD	kdpD

Unrestricted	2	6	723898	SNP	A	kdpD	kdpD
Unrestricted	2	7	723898	SNP	A	kdpD	kdpD
Unrestricted	2	7	4371271	DEL	2	dcuA	aspA
Unrestricted	3	1	4546900	DEL	1	fimE	fimE
Unrestricted	3	3	1660526	SNP	T	ynfE	ynfE
Unrestricted	3	3	4104699	SNP	T	envZ	envZ
Unrestricted	3	3	4371271	DEL	2	dcuA	aspA
Unrestricted	3	4	4546900	DEL	1	fimE	fimE
Unrestricted	3	8	1431015	SNP	T	stfR	stfR
Unrestricted	3	8	1431050	SNP	G	stfR	stfR
Unrestricted	3	8	1637898	SNP	T	nohA	nohA
Unrestricted	4	1	2865304	SNP	A	rpoS	rpoS
Unrestricted	4	4	1429253	SNP	G	lomR	lomR
Unrestricted	4	4	1431356	SNP	T	stfR	stfR
Unrestricted	4	4	1431530	SNP	T	stfR	stfR
Unrestricted	4	5	2865304	SNP	A	rpoS	rpoS
Unrestricted	4	7	2865304	SNP	A	rpoS	rpoS
Unrestricted	5	2	1430556	SNP	G	lomR	lomR
Unrestricted	5	2	4371271	DEL	2	dcuA	aspA
Unrestricted	5	3	1430581	SNP	G	lomR	lomR
Unrestricted	5	3	2060392	SNP	G	yeeO	yeeO
Unrestricted	5	3	4371271	DEL	2	dcuA	aspA
Unrestricted	5	5	4371271	DEL	2	dcuA	aspA
Unrestricted	5	6	4546903	INS	A	fimE	fimE
Unrestricted	5	7	NA	NONE	NA	NA	NA

Table S1. Each row denotes a mutation discovered in the evolved (transfer 36) isolates that was not present in the ancestor. The columns are as follows: Migration Type (the pattern of migration defining the treatment of the isolate), Population (the identifier for the replicate metapopulation (1-5) of the isolate), Isolate (the identifier for the mutation's isolate (1-8)), Position (the genomic location of the mutation according to *E. coli W311* [GenBank: AP009048]), Mutation Type (Single Nucleotide Polymorphism (SNP), Deletion (DEL), Insertion (INS), Substitution (SUB) or Amplification (AMP)), Change (the length of a mutation for DEL, or AMP, or the new nucleotide state for SNP and INS), Gene Left (the nearest open reading frame prior to the mutation), Gene Right (the nearest open reading frame after the mutation). Note that if Gene Left and Gene Right are the same, the mutation falls within that gene. All SNP's were nonsynoymous when they occurred within an open reading frame. Note that a total of 8 random isolates from each metapopulation were initially sent for genome sequencing. However, the coverage for a sizeable fraction of the isolates was below 30x. The maximum number of isolates with sufficient coverage for any metapopulation was five. For metapopulations with less than five high-coverage isolates, we sent in a second round of sequencing composed of enough isolates to ensure every metapopulation would have five sequenced isolates. These additional isolates were chosen at random from the set isolates with insufficient coverage from the first round. For the second round, coverage exceeded 30x on all isolates and thus we could analyze genomes for exactly five isolates per metapopulation. For purpose of direct comparison, we restricted our analysis of mean fitness (see Figure 3) to the isolates that were fully genome sequenced.

Full Data, Simulation Code, and Statistical Scripts are available on the Kerr Lab Wiki: http://kerrlab.org/Public/RugLand

Supplementary References:

1. Kryazhimskiy S, Rice DP, Desai MM (2012) Population Subdivision and Adaptation in Asexual Populations of Saccharomyces Cerevisiae. *Evolution* 66(6):1931–1941.

2. Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci U S A* 76(10):5269–5273.