1 **How the tortoise beats the hare: Slow and steady adaptation in structured**

2 **populations suggests a rugged fitness landscape in bacteria**

3 Joshua R. Nahum[1,2] Peter Godfrey-Smith[3], Brittany N. Harding[1,4], Joseph H. Marcus[1,4],

4 Jared Carlson-Stevermer[1,5], and Benjamin Kerr[1,4]

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6 1.   BEACON Center for the Study of Evolution in Action

7 2.   Beacon Center, Michigan State University, East Lansing, MI 48824

8 3.   Philosophy Program, The Graduate Center, City University of New York, New

9 York, NY

10 4.   Department of Biology, University of Washington, Seattle, WA 98195

11 5.   Department of Biomedical Engineering and Wisconsin Institutes for

12 Discovery, University of Wisconsin, Madison, WI

13

14 **Abstract:**

15

16 In the context of Wright's adaptive landscape, genetic epistasis can yield a multi-

17 peaked or "rugged" topography. In an unstructured population, a lineage with

18 selective access to multiple peaks is expected to rapidly fix on one, which may not be

19 the highest peak. Contrarily, beneficial mutations in a population with spatially

20 restricted migration take longer to fix, allowing distant parts of the population to

21 explore the landscape semi-independently. Such a population can simultaneous

22 discover multiple peaks and the genotype at the highest discovered peak is expected

23 to fix eventually. Thus, structured populations sacrifice initial speed of adaptation

24 for breadth of search. As in the Tortoise-Hare fable, the structured population

25 (Tortoise) starts relatively slow, but eventually surpasses the unstructured

26 population (Hare) in average fitness. In contrast, on single-peak landscapes (e.g.,

27 systems lacking epistasis), all uphill paths converge. Given such "smooth"

28 topography, breadth of search is devalued, and a structured population only lags

29 behind an unstructured population in average fitness (ultimately converging). Thus,

30 the Tortoise-Hare pattern is an indicator of ruggedness. After verifying these

31 predictions in simulated populations where ruggedness is manipulable, we then

32   explore average fitness in metapopulations of *Escherichia coli*. Consistent with a

33   rugged landscape topography, we find a Tortoise-Hare pattern. Further, we find that

34   structured populations accumulate more mutations, suggesting that distant peaks

35   are higher. This approach can be used to unveil landscape topography in other

36   systems, and we discuss its application for antibiotic resistance, engineering

37   problems, and elements of Wright's Shifting Balance Process.

38

39   **Significance Statement:**

40   Adaptive landscapes are a way of describing how mutations interact with each other

41   to produce fitness. If an adaptive landscape is rugged, organisms achieve higher

42   fitness with more difficulty because the mutations to reach high fitness genotypes

43   may not be always beneficial. By evolving populations of *Escherichia coli* with

44   different degrees of spatial structure, we identified a Tortoise-Hare pattern, where

45   structured populations were initially slower, but overtook less structured

46   populations in mean fitness. These results, combined with genetic sequencing and

47   computational simulation, indicate this bacterial adaptive landscape is rugged. Our

48   findings address one of the most enduring questions in evolutionary biology, in

49   addition to, providing insight into how evolution may influence medicine and

50   engineering.

51

52   **Keywords:** adaptive landscape, experimental evolution, NK model, landscape

53   topography, spatial structure

54

55   /body

56   **Introduction**

57

58   The adaptive landscape was introduced by Sewall Wright to visualize potential

59   constraints faced by evolving systems of genes (1). One incarnation of Wright's

60   landscape portrays the relationship between an organism's genotype and its fitness

61   as a topographical map. Imagine placing all possible genotypes of an organism

62   together on a plane, where the distance between two genotypes represents the

63   number of mutations needed to generate one genotype from the other (here we

64   focus on asexual organisms). Each genotype is assigned a height directly

65   proportional to its fitness (the third dimension). An evolving population is

66   represented as a cloud of points on the resulting landscape, where each member of

67   the population is a point. Novel genotypes arise in the population via mutations,

68   expanding the extent of the cloud. In contrast, natural selection diminishes the range

69   of the cloud, shifting its weight uphill as less fit genotypes are culled. Thus the

70   combination of mutation and selection leads to the population "climbing" adaptive

71   hills to their "peak," which is a genotype from which all mutations are deleterious. If

72   we assume strong selection and weak mutation (*SSWM*), the population cloud is

73   mostly confined to a single climbing point, where the rapid fixation of each rare

74   beneficial mutation shifts the point uphill (2, 3). Overall, a population's evolutionary

75   trajectory is taken to be sensitive to the gradients on this three-dimensional

76   landscape.

77

78   As pointed out by many authors, including Wright (4–6), the actual geometry of the

79   space of possible genotypes has extremely high dimensionality, which cannot be

80   projected into two dimensional space in a way that preserves all distances. We

81   explore an alteration of the classic representation (see (7–9)) that ensures

82   genotypes differing by a single mutation are equidistant (while the distance

83   between genotypes differing by multiple mutations is distorted). This approach

84   involves creating a network, in which nodes are genotypes and edges connect

85   genotypes differing by a single substitution. This network is embedded in two

3

86   dimensions, where genotypes are grouped along the abscissa by their distance from

87   a common genotype and along the ordinate by their fitness.

88

89   Using this representation, a mutation network without epistasis is shown in Figure

90   1a. This network would also be labeled as a "smooth landscape," as the single peak

91   is accessible (i.e., can be reached by a series of beneficial mutations) from any other

92   genotype. Evolution of populations on this landscape gives an example of mutational

93   convergence. Under *SSWM* assumptions, we show that despite three different initial

94   mutational steps, independent evolutionary trajectories converge at the peak (Fig.

95   1a) and fitness likewise converges (Fig. 1b). In contrast, Figure 1c shows a network

96   with sign epistasis, where the sign of the fitness effect of a mutation depends on the

97   background in which it occurs. Such sign epistasis is a necessary (but not sufficient)

98   condition for the existence of multiple peaks. On this "rugged" landscape, the final

99   genotype reached under three independent trajectories is contingent upon the

100   initial mutation (Fig. 1c). In this case, fitness values of different populations can

101   remain divergent over time if peaks are heterogeneous in height (Fig. 1d). Here we

102   see that a population can become trapped at a suboptimal peak in the presence of

103   epistasis.

104

105   Because Sewall Wright thought epistasis was pervasive (10), he was particularly

106   concerned about confinement of populations at suboptimal peaks within rugged

107   landscapes. He proposed the Shifting Balance Process (SBP) to explain how

108   populations move from lower to higher peaks. Integral to the SBP is population

109   structure. Wright envisioned a population that was distributed into semi-isolated,

110   sparsely-populated subpopulations (demes) in which genetic drift enabled some

111   subpopulations to take downward steps by fixing deleterious mutations. In this way,

112   a subset of the metapopulation is able to move from one peak's domain of attraction

113   to another, thus crossing "adaptive valleys." Therefore, Wright's SBP depends on

114   two critical assumptions: the presence of epistasis generating landscape ruggedness

115   and the presence of population structure.

116

4

117   By using one factor discussed by Wright (population structure) as an experimental

118   variable, an empirical assay can be constructed for another of Wright's factors

119   (landscape ruggedness). Upon first glance, population structure would seem to

120   hinder adaptation. In a population in which migration is not spatially restricted

121   (unstructured population), a beneficial mutant that arises can rapidly fix in what is

122   termed a selective sweep. On the other hand, a favored mutant arising in a

123   population with restricted migration (structured population) advances more slowly

124   in what might be termed a "selective creep." By reducing the rate of initial

125   adaptation, the slow competitive displacement occurring within a structured

126   population may also allow multiple semi-independent searches of the fitness

127   landscape by geographically distant regions of the population. For a smooth

128   landscape (e.g., Fig 1a), this enhanced exploration is superfluous as all selectively

129   accessible trajectories lead to the same single peak. Therefore, on smooth

130   landscapes, structure only slows adaptation. However, on a rugged landscape,

131   additional exploration may reveal alternate peaks. For instance, in Fig. 1c, while an

132   unstructured population might exclusively follow one of the colored trajectories, a

133   structured population may be able to explore them all simultaneously. In this way, a

134   structured population can survey a broader set of paths. As discovered peaks may

135   differ in height, a comparison of them enables the population to eventually reach a

136   better endpoint on average (11, 12). On a rugged landscape, fitnesses in populations

137   differing in structure emulate the classic Tortoise-Hare fable. Specifically the

138   unstructured population initially adapts faster (the Hare) but is overtaken by the

139   structured population (the Tortoise), which is a poor starter but a strong finisher.

140   Importantly, on a smooth landscape, the Tortoise never takes the lead, and the

141   crossing of average fitness trajectories is not predicted. Thus, when manipulations

142   to population structure do produce a Tortoise-Hare pattern, we have a signature of

143   ruggedness.

144

145   Before investigating this signature in a biological system in which landscape

146   topography is cryptic, we confirm the above predictions using a computational

147   system in which landscape topography is known and manipulable. Specifically, we

5

148   control landscape ruggedness by employing Kaufmann's NK model (13–15) and

149   then track evolving metapopulations of bit strings, in which the pattern of migration

150   between demes is manipulated. Following this simulation, we then turn to evolving

151   metapopulations of *Escherichia coli* under a similar experimental manipulation of

152   population structure. Discovery of a Tortoise-Hare pattern would be indicative of a

153   rugged topography.

154

155   **Results and Discussion**

156

157   *Patterns of Average Fitness*

158   In the NK model, simulated organisms are bit strings of length N, and the parameter

159   K is the number of loci affecting the fitness contribution of each locus (see Methods).

160   As K increases, the level of epistatic interaction increases, yielding more rugged

161   landscapes; hereafter, we refer to K as a "ruggedness" parameter. We explore how

162   ruggedness affects fitness trajectories in evolving metapopulations that differ in

163   population structure. We consider either metapopulations with spatial restrictions

164   to migration (hereafter, the Restricted treatment) or metapopulations where

165   migration can occur between any two demes (Unrestricted treatment). For a smooth

166   landscape topography (K=0, N=15), average fitness initially increases more rapidly

167   in the Unrestricted treatment relative to the Restricted treatment; however, both

168   trajectories converge over time (Fig. 2a). For a rugged landscape (K=8, N=15),

169   fitness in the Restricted treatment once again lags behind fitness in the Unrestricted

170   treatment at the outset. Instead of converging, however, the fitness trajectories

171   cross, yielding a higher final fitness for the spatially restricted treatment (Fig. 2b).

172   Indeed, we find significantly higher fitness in the Restricted treatment for K>3 at the

173   end of our simulation (Fig. 2c; Mann-Whitney tests with Bonferroni corrections,

174   p<0.001). The pattern in Figure 2b agrees with the Tortoise-Hare prediction, while

175   the crossing does not occur for the Unrestricted treatment in Figure 2a. More

176   generally, with sufficient ruggedness, a structured population can eventually

177   outperform an unstructured population (Fig. 2c). This pattern is not limited to our

178   specific form of population structure, as Bergman *et al.* (1995) (16) reported a

6

179   similar result using an NK model where bit strings dispersed variable distances

180   along a single dimension.

181

182   We next turned to examining fitness trajectories in evolving metapopulations of

183   *Escherichia coli*. Similar to the NK model, we propagated the bacteria under two

184   treatments differing in migration pattern: Restricted and Unrestricted (see

185   Methods). Early and late in the evolutionary run, we sampled five random isolates

186   from each metapopulation and determined their fitness relative to the common

187   ancestor. Early in the experiment (at transfer 12), fitness in the Restricted treatment

188   was significantly lower than the Unrestricted treatment (Fig. 3; Mann-Whitney test,

189   p=0.015). However, at the end of the experiment (transfer 36), the opposite pattern

190   was found, with fitness in the Restricted treatment surpassing the Unrestricted

191   treatment (Fig. 3; Mann-Whitney test, p=0.015). This pattern is consistent with a

192   rugged landscape topography.

193

194   *Patterns of Evolutionary Distance*

195   There are a few ways to account for the benefit that population structure confers on

196   rugged landscapes (Figs. 2b and 3). First, a population may have access to multiple

197   peaks that differ in height. A structured population can explore multiple domains in

198   parallel, eventually comparing the results. Thus, it will tend to attain a higher

199   endpoint; for the same reasons that the expectation of the maximum of a sample

200   increases with sample size. This effect holds when all peaks are equidistant from the

201   ancestral population. A second possibility (not mutually exclusive with the first) is

202   that peaks differ in both height and distance from the ancestral population. Suppose

203   that the initial mutations on accessible paths to the more distant and higher peaks

204   are less beneficial than mutations leading to the nearby peaks, as in (17). In this

205   case, intermediate genotypes approaching distant peaks risk being outcompeted in

206   an unstructured population (consider the first mutant on the blue path to the more

207   distant peak in Fig. 1c competing against the other first mutants). This is because

208   the slower fixation of these intermediates allows for better competitors (from

209   domains of nearer peaks) to arise. In contrast, these more distant peaks become

7

210 accessible in a structured population due to reduced competitive displacement. If

211 some of these distant peaks are also higher, then structured populations are

212 predicted to both achieve better fitness and accumulate more mutations.

213

214 To explore the number of mutations accrued by evolving populations, we first

215 return to the NK model. We define evolutionary distance to be the number of

216 mutational differences between an evolved isolate and its ancestor. In the NK model,

217 this is the Hamming distance (18). As ruggedness increases, the degree of

218 population structure affects final evolutionary distance from the ancestor; we find a

219 significantly higher distance in the Restricted treatment for K>3 at the end of our

220 simulation (Fig. 4; Mann-Whitney tests with Bonferroni corrections, p<0.001). Thus,

221 on a rugged landscape, a population with restricted migration moves both higher

222 (Fig. 2c) and further (Fig. 4) than a less structured population.

223

224 Evolutionary distance was explored similarly in the *E. coli* metapopulations. In

225 addition to assessing fitness on the isolates from the end of the experiment (transfer

226 36), we sequenced their genomes to determine their evolutionary distance from the

227 common ancestor. The locations of all identified mutations in all sequenced isolates

228 are shown in Figure 5. The number of mutations accumulated in each isolate is the

229 evolutionary distance (the points to the right of the table in Fig. 5). We find that

230 isolates have moved a significantly greater distance in the Restricted treatment

231 (Mann-Whitney test, p=0.045). The increased distance traversed by digital and

232 bacterial populations is consistent with rugged landscapes in which more distant

233 peaks are being reached by structured populations.

234

235 The ability of the structured populations to move higher (in average fitness) and

236 further (in evolutionary distance) is engendered by the capacity for parallel search.

237 The presence of simultaneous selective creeps should increase the standing

238 diversity within a structured population relative to an unstructured one. In line with

239 this prediction, the metapopulations in the Restricted treatment had significantly

240 higher genotypic diversity than the Unrestricted treatment (Mann-Whitney test on

8

241   the nucleotide diversity index **p**, p=0.016). We note that greater diversity in

242   structured populations is expected regardless of the topography of the landscape

243   (see Supplemental Figure 1), however, such diversity is only advantageous when the

244   landscape is multi-peaked.

245

246   *Previous Empirical Work*

247   An extreme form of population structure involves a set of completely isolated

248   populations. If the landscape contains multiple peaks of different heights, these

249   populations can diverge in fitness, genotype and phenotype. Korona *et al.* (19) and

250   Melnyk & Kassen (20) measured phenotypic diversity within, and diversity in

251   fitness among, a set of replicate evolving microbial populations. Under some growth

252   conditions (growth on agar surfaces for *Rastonia eutropha* (19) and growth in

253   minimal xylose for *Pseudomonas fluorescens* (20)) these authors found that both

254   forms of diversity remained high at the end of the experiment, which they took as

255   evidence for a rugged landscape. Under other growth conditions (growth in liquid

256   for *R. eutropha* (19) and growth in minimal glucose for *P. fluorescens* (20)) they

257   found lower diversity, consistent with a smoother landscape. Like the present study,

258   these earlier studies used statistical patterns to infer topographical properties of

259   landscapes.

260

261   This statistical approach was also used in a recent study by Kryazhimskiy *et al.* (21).

262   They propagated metapopulations of asexual *Saccharomyces cerevisiae* and varied

263   the rate (as opposed to pattern) of migration. In contrast to the results we report,

264   they find yeast from treatments with higher rates of migration evolved higher

265   fitness over the course of their experiment. This led the authors to conclude that

266   epistasis was weak and the landscape was smooth. While it is entirely possible that

267   their yeast and our bacteria differ in landscape topography, we discuss some

268   alternative interpretations of their results in the Supplement.

269

270   Rather than inferring landscape topography from population-level statistical

271   patterns, an alternative approach involves fully characterizing the fitnesses of

272   genotypes in a small section of the landscape (22–30). This approach often involves

273   considering two genotypes differing by M mutations, engineering all 2^M possible

274   combinations, and assessing the fitness of each constructed genotype. Epistasis can

275   be gauged directly by measuring the influence of genetic background on the fitness

276   effect of a mutation. Some studies using this approach have uncovered instances of

277   sign epistasis (22, 24, 29), while other studies have found only magnitude epistasis

278   (23, 26). Some studies have reported multiple peaks (24, 29), while others have

279   found only a single peak (22, 26). As with the inferential approach described above,

280   this engineering approach has revealed a potential diversity of landscape

281   topography. We suggest that combining the (top-down) inferential approach and

282   (bottom-up) engineering approach is a promising direction for exploring the nature

283   of adaptation.

284

285   *Wright's Shifting Balance Process*

286   During the Modern Synthesis, two divergent views on adaptation were debated. One

287   view considers adaptation as the sequential fixation of beneficial mutations. This

288   perspective (often associated with Fisher) does not highlight epistatic contingency,

289   focusing instead on selection and mutation as the major processes of evolution (31).

290   A second perspective (often linked to Wright) recognizes epistatic interaction as

291   constraining adaptation. Based on his empirical observations, Wright felt that such

292   epistasis was pervasive, leading, within his adaptive landscape metaphor, to

293   multiple peaks (2). In this view, evolving populations can become trapped in

294   suboptimal positions. To illustrate how escape from the domains of suboptimal

295   peaks was possible, Wright introduced the Shifting Balance Process (SBP). He

296   assumed that adapting populations were spatially structured as a metapopulation of

297   semi-isolated demes. The SBP is heuristically divided into three phases. In Phase I,

298   the demes, which Wright assumed were small, drift in genotype space, enabling

299   movement into other domains. In Phase II, selection within demes produces a set of

300   semi-independent hill-climbing episodes. In Phase III, the resulting genotypes are

301   exchanged among demes and the best competitors spread through the

302   metapopulation at large. Thus, Wright's view of adaptation, in contrast to Fisher's,

303   invokes a complex combination of processes, specifically brought together to solve a

304   problem generated by epistatic contingency.

305

306   While there have been various theoretical explorations of the plausibility of SBP (16,

307   32–35), Wright's ideas have been criticized because they demand a delicate balance

308   of various evolutionary processes. For instance, populations need be small enough

309   for effective drift (Phase I), but large enough for effective selection within demes

310   (Phase II). Migration should be sufficiently restricted for drift and selection within

311   demes (Phase I and II), but sufficiently unrestricted for effective exchange of

312   genotypes among demes (Phase III). However, if we abandon Wright's goal of

313   explaining how populations cross valleys, many of these conflicts vanish. Both

314   Fisher and Wright acknowledged that environmental change could alter the

315   landscape, and, in the process, reposition the peaks. Imagine that a population

316   experiences such a change and subsequently resides somewhere on the new

317   landscape with access to multiple domains. In our experiment, our ancestor

318   contained deleterious mutations and evolved in a stressful environment (see

319   Methods), which potentially yielded access to multiple peaks. In this case, demes

320   need not be small for the discovery of multiple peaks (and indeed, our experimental

321   demes were large). With large subpopulations, selection within demes will proceed

322   efficiently; however, limitations to migration between demes will still allow for

323   parallel exploration of a rugged landscape. Thus, Phases II and III can jointly yield

324   adapted populations even if Phase I is absent. If landscapes are indeed rugged,

325   population structure can retain the critical role Wright foresaw, even if all the

326   details of the SBP are not present.

327

328   *Applications*

329   One case where populations are potentially poised in multiple domains on a

330   landscape involves the evolution of microbes exposed to antibiotics. When a

331   bacterial population experiences a sufficiently high concentration of an antibiotic,

332   susceptible genotypes are replaced by resistant mutants. When the drug is removed,

333   these mutants tend to carry fitness costs relative to their susceptible progenitors.

11

334   The cost can be alleviated by a mutation resulting in reversion to susceptibility or a

335   mutation that compensates for the impairment without loss of resistance (36, 37).

336   There is some evidence that reversion and compensation constitute distinct peaks

337   in a rugged landscape (38, 39). Thus, we see that a changing environment (exposure

338   and removal of a drug) may position a microbial population at a landscape position

339   where multiple peaks are accessible (7). It is at such a position that population

340   structure may influence the evolutionary trajectory. Björkman *et al.* (38) and

341   Nagaev *et al.* (39) serially passaged *Salmonella typhimurium* and *Staphylococcus*

342   *aureus* resistant to fusidic acid either in well-mixed flasks or within murids (mice or

343   rats). These authors found that the bacteria more often reverted when grown *in vivo*

344   than *in vitro*. They explain these results by noting that the flask and murid

345   environments differ markedly and may consequently place different selective

346   pressures on revertants and compensated strains (indeed, they present data to this

347   effect). In our terminology, the landscape in a flask and a mouse may be different.

348   However, even if the landscape was identical (but rugged) in both, the results might

349   not be unexpected because a murid environment is highly structured and a shaking

350   flask is not. Thus, if the "reversion peak" is higher than most to all of the

351   "compensation peaks" (the authors present data consistent with this ordering) then

352   evolution in a structured environment is predicted to revert at higher frequency. In

353   this way, the structure that pathogenic bacteria experience (including in the bodies

354   of human hosts) can potentially influence the course of antibiotic resistance

355   evolution.

356

357   Not only can the ideas in this paper apply in a medical context, but also they may

358   address practical engineering problems. Evolutionary principles have been utilized

359   to find solutions to computational problems, a discipline known as evolutionary

360   computation. In this field, putative solutions constitute a population, new solutions

361   are generated by mutation and recombination, and better solutions can outcompete

362   their contemporaries. One defining feature of a difficult problem is the presence of

363   multiple optima in the map from the specification of a solution (i.e., genotype) to its

364   quality (i.e., fitness). As early as 1967, Bossert (40) suggested that dividing a

365   population of solutions into subpopulations could yield better evolutionary

366   outcomes. Subsequently, the inclusion of population subdivision in evolutionary

367   algorithms has produced better solutions in a variety of applications, including

368   analogue circuit design (41), financial trading models (42), and multi-objective

369   scheduling (43). Besides the efficiency in networked computational resources that

370   accompanies population subdivision, a deeper exploration of the landscape of

371   solutions is predicted to occur when multiple domains can be semi-independently

372   searched (44, 45).

373

374   *Synthesis*

375   Contingency in evolution is affected by the underlying topography of adaptive

376   landscapes. When no epistasis is present, a smooth landscape results, and ultimately

377   evolution converges to the single peak. Contingency requires (sign) epistasis,

378   specifically of a kind generating multi-peaked, or rugged, landscapes. (We again note

379   that sign epistasis is not sufficient for multi-peaked landscapes and indeed can

380   constrain evolution to a subset of paths to a single peak; see Weinreich *et al.* (22) for

381   an example.) In the case of heterogeneous peak heights, population structure may

382   enable the simultaneous exploration of multiple domains and ultimately lead to the

383   discovery of higher peaks than would be possible in an unstructured population.

384   Thus, using population structure as an experimental variable, we have a signal for

385   this kind of ruggedness. In the case of our bacterial populations, we have presented

386   a pattern consistent with ruggedness. Additionally, it appears that structured

387   populations move further in the landscape, suggesting that the most accessible

388   peaks may not be the highest. Ultimately it is an empirical issue whether other

389   biological systems posses such ruggedness. However, statistical patterns in fitness

390   and evolutionary distance may help to distinguish landscape topography.

391   Specifically, a rugged landscape topography can be inferred by comparing

392   structured (Tortoise) and unstructured (Hare) populations and assessing whether

393   slow and steady adaption "wins the race."

394

395 **Methods**

396 *Ancestral strain*

397 The bacterial ancestor was derived from a K-12 strain of *E. coli* (BZB1011) by

398 selecting for resistance to colicin E2, then colicin D, and then phage T6. Both

399 resistance to colicin E2 and phage T6 are known to be individually costly (46, 47).

400 The initiation of the experiment with an unfit strain was intentional (see

401 Supplement for rationale).

402

403 *Experimental treatments*

404 Each metapopulation was comprised of 96 subpopulations (the 96 wells of an 8×12

405 microtiter plate). The metapopulation was initiated with the ancestral strain in each

406 well. These subpopulations grew for 12 hours in 200 mL of lysogeny broth (LB-

407 Miller) supplemented with a sub-inhibitory concentration of tetracycline (0.25

408 mg/mL). After growth, each well in the metapopulation was diluted 40-fold into

409 fresh growth medium using a 96 slot-pin multi-blot replicator (5 µL into 200 µL).

410 Immediately following dilution, migrations among wells occurred. Migration was

411 either restricted to occur between subpopulations adjacent to each other or was

412 unrestricted. In both treatments, every well had 1/3 probability of experiencing an

413 immigration event from one random well in its neighborhood. In the Restricted

414 treatment, this neighborhood included the wells directly north, east, south or west

415 of the focal well (using periodic boundary conditions to eliminate edge-effects). In

416 the Unrestricted treatment, the neighborhood included all wells minus the focal

417 well. All migration events were executed by a BioRobot 8000 liquid-handling robot

418 (Qiagen), which transferred 5 µL from the source well within the plate from the

419 previous transfer into the destination well within the plate from the current

420 transfer. Between transfers, plates were incubated (37 °C) and shaken (350 rpm

421 using a microtiter shaker). Each metapopulation was propagated for a total of 36

422 transfers and each treatment contained five replicates.

423

424

425

426 *Competition assay*

427 We chose five random isolates from the last transfer of each metapopulation (here

428 we denote any one of these strains as *E*). We marked our ancestor with resistance to

429 phage T5 (we denote this marked ancestor as *A*). Before the competition, *E* and *A*

430 were grown separately in 200 μL of growth medium for two 12-hour cycles (with

431 40-fold dilution at transfer). After this acclimation phase, we added 5 μL of *E* and 5

432 μL of *A* to a well containing 200 μL of fresh growth medium. The titer of each strain

433 was assessed, through selective plating with and without phage T5, immediately

434 after the competition was initiated and again after 12 hours. If $X(t)$ is the titer of

435 strain X at time t, then the fitness of the evolved strain relative to its ancestor is

436 given by:

437

438
$$w(E,A) = \frac{\ln(E(12)/E(0))}{\ln(A(12)/A(0))}.$$

439

440 *Whole Genome Resequencing*

441 Using the same isolates from the last transfer, we performed chromosomal DNA

442 extractions using Qiagen Mini DNA Kits. Each sample was barcoded and multiplexed

443 to 24 samples per lane with Illumina TruSeq. Whole genome resequencing

444 (University of Washington High Throughput Genomics Unit) was performed with

445 single-end 36-bp unpaired reads on Illumina HiSeq to an average of 30X coverage.

446 Illumina reads were aligned for mutational discovery by Breseq 0.19 (J. E. Barrick,

447 unpublished algorithm) against *E. coli W311* [GenBank: AP009048]. Alignments

448 were considered only if they covered 95% of a read. For every isolate, Sanger

449 sequencing (Genewiz) of several loci (*fimE*, *marR*, *ompF*, and *stfR*) was used to

450 confirm putative mutations.

451

452 *NK Model*

453 For the simulations, individuals were embedded within 96 demes in an 8 x 12 array.

454 Each deme contained 1000 organisms. Each organism's genotype was a bit string

455   (fixed length ordered list of 0's and 1's) of length N=15. The fitness of an organism

456   was the sum of the fitness contributions of each of the 15 loci divided by the number

457   of loci. The contribution of each locus was determined by its allelic state and the

458   allelic states of the subsequent K loci (wrapping to the beginning of the bit string as

459   needed). For each locus, 2^{K+1} random numbers (uniformly distributed between 0

460   and 1) described all possible fitness contributions of that locus (given any possible

461   combination of alleles at relevant loci). Thus a mutation at a single locus affected the

462   fitness contribution of the mutated locus and K other loci. Selection within a deme

463   involved the removal of a random organism, regardless of fitness, and its

464   replacement by the birth of an organism from the same deme chosen by a fitness-

465   weighted lottery. Upon birth, the offspring bit string differed from its parent at a

466   random locus with probability 0.1 (the mutation rate). This Moran death-birth

467   process was iterated 1000 times for each deme, followed by migration between

468   demes. During each migration event, 25 individuals were chosen at random and

469   removed from one deme (the destination), and then replaced by copies of 25

470   individuals chosen at random from the other deme (the source). Each deme

471   experienced an immigration event with probability 1/3. Migration was either

472   restricted or unrestricted in precisely the same manner as the bacterial experiment

473   above. Each replicate run of the Unrestricted treatment was paired with a replicate

474   of the Restricted treatment, where each member of the pair shared the same NK

475   landscape as well as the same ancestor (a random bit string used to populate the

476   entire metapopulation). In the figures, one selection-migration episode is termed an

477   "update."

478

479   **Acknowledgments**

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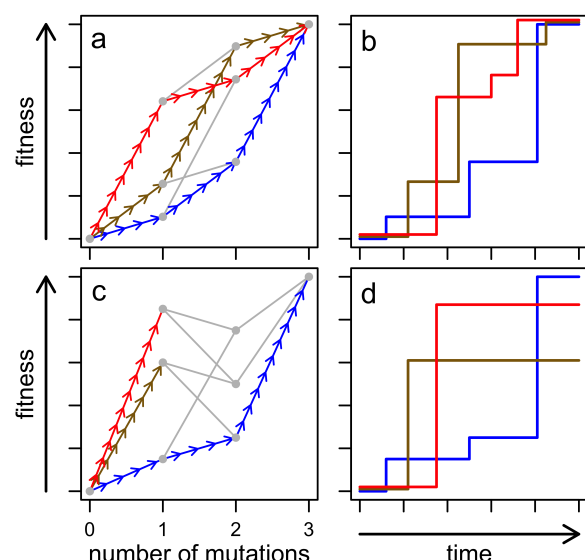602

603

604   **Figure Legends:**

605

606   **Figure 1. Adaptive paths in hypothetical landscapes**. Here we consider a simple

607   biallelic three locus system. (a) The adaptive landscape can be visualized by plotting

608   genotype fitness as a function of the number of mutations on a wild type

609   background. Each of the $2^3=8$ genotypes is given by a gray point and edges (arrows

610   or gray lines) connect genotypes differing by a single mutation. An adaptive peak is

611   a genotype from which all mutations are detrimental. A hypothetical landscape with

612   a single peak (the triple mutant) is shown here. A selectively accessible path exists

613   between two genotypes if a series of beneficial mutations connects the less fit

614   genotype to the more fit genotype. On this "smooth" landscape, all of the 3!=6 paths

615   between the wild type (lacking mutations) and the triple mutant are selectively

616   accessible; three of these paths are shown by the arrows in different colors. (b)

617   Average fitness over time is shown for three possible populations following the

618   paths in part a. If we assume that selection is strong and mutation is weak, we can

619   represent the fixation of each beneficial mutation as a step up in the fitness

620   trajectory. All trajectories converge on the same final fitness value. (c) A

621   hypothetical landscape with multiple peaks. Starting with the wild type, selection

622   can take the population to different adaptive peaks on this "rugged" landscape, as

623   illustrated by the different colored trajectories. (d) Average fitness over time is

23

624   shown again for three possible populations following the paths in part c. The final

625   fitness of different evolving populations can vary.
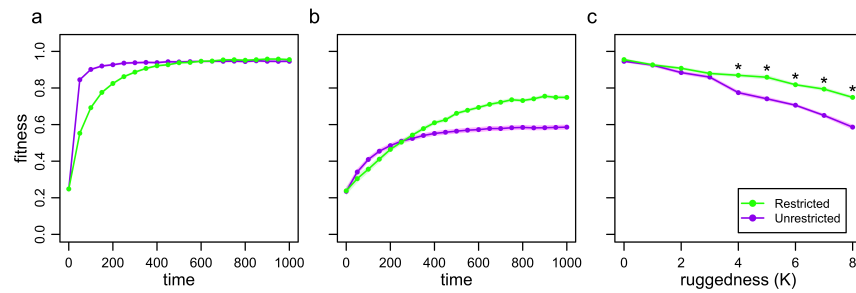
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Figure 2. Fitness in the NK model. Metapopulations of bit strings of length N=15 evolved where either migration was restricted to occur between neighboring demes or migration was unrestricted (occurring between any two demes). (a) Average fitness in the metapopulation is shown over time on a smooth landscape (K=0) or (b) a rugged landscapes (K=8). (c) Average fitness at time point 1000 is shown as a function of the ruggedness parameter, K. Note the values at K=0 and K=8 in part c correspond to the values at time point 1000 in parts a and b, respectively. In all plots, points represent the mean of 50 replicates, shading gives the standard error of the mean and asterisks indicate significant differences.

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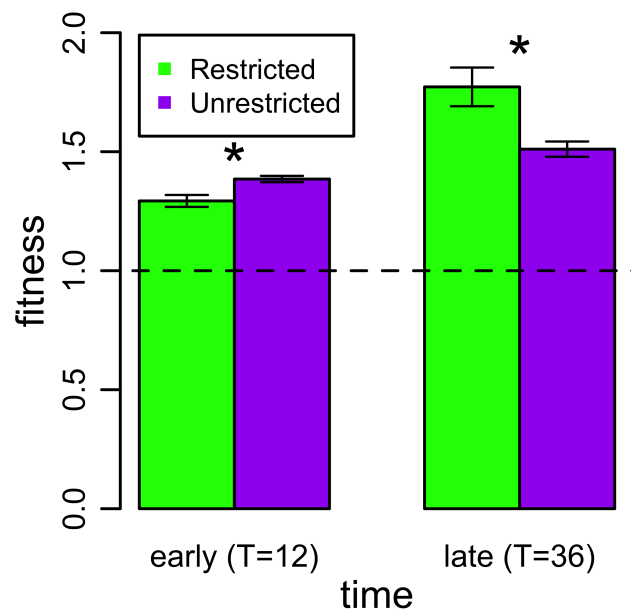
Figure 3. Bacterial fitness. Metapopulations of bacteria evolved where migration was spatially restricted or unrestricted. Average relative fitness of five isolates randomly sampled from the metapopulation is shown early in the experiment (at transfer T=12) and late in the experiment (at transfer T=36). As in Figure 2b, the ordering of fitnesses for the two treatments flips over time. Bars represent the mean of 5 replicate metapopulations, whiskers give the standard error, and asterisks denote significant differences.

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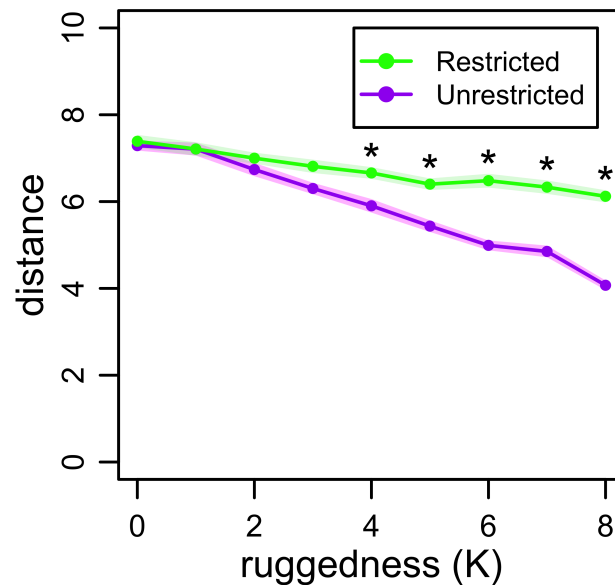
Figure 4. Distance in the NK model. Metapopulations of bit strings of length N=15 evolved where migration was spatially restricted or unrestricted. Evolutionary distance is the number of bits differing between an evolved isolate and its ancestor (the Hamming distance). Average distance at time point 1000 is shown as a function of the ruggedness parameter, K. Points represent the mean of 50 replicates, shading gives the standard error of the mean, and asterisks denote significant differences.

657
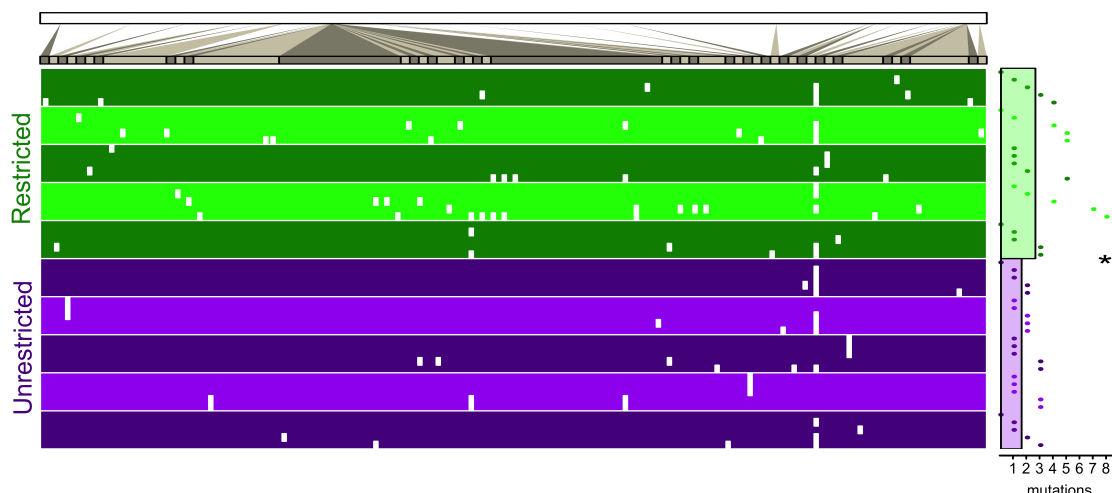
Figure 5. Bacterial distance. Metapopulations of bacteria evolved where migration was spatially restricted or unrestricted. At the end of the experiment, five isolates from each metapopulation were sequenced at the genome level. The top bar represents the genome of *Escherichia coli*. Genome regions with mutations are magnified for the table. Each isolate is a single row in the table and the location of each of its mutations is indicated by a white mark. The five isolates from each of the five replicate metapopulations are grouped by alternating shades of green (for the Restricted treatment) or purple (for the Unrestricted treatment). The horizontal distance of the point to the right of the table denotes the number of mutations in the isolate (its evolutionary distance). The horizontal distance of the green bar and the purple bar to the right of the table gives the average distance (the average of replicate averages) of isolates from the Restricted and Unrestricted treatments, respectively. The asterisk denotes a significant difference between treatments. More information regarding each mutation can be found in the Supplement.

672

673

674   **Supplementary Information**:

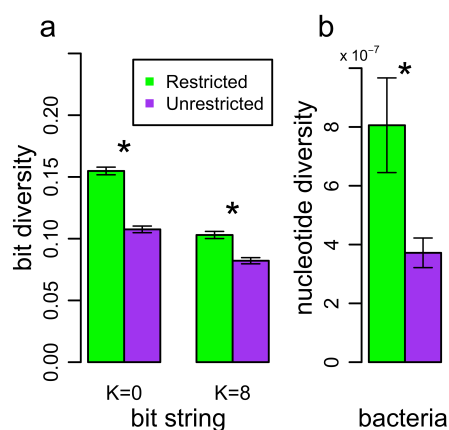675   *Diversity in digital and bacterial populations*

676   A structured population performs a broader search on the adaptive landscape, as

677   the rate of competitive displacement is lower. Consequently, the standing genetic

678   diversity of a structured population is expected to be greater than diversity in an

679   unstructured population. In Supplemental Figure 1a, we see that this pattern does

680   not depend on landscape ruggedness (Mann-Whitney tests, p<0.01 for K=0 and K=8).

681   Thus, despite landscape topography, we predict to find higher genetic diversity in a

682   structured population, and this is what we find in our bacterial metapopulations

683   (Mann-Whitney test, p=0.015; Supp. Fig. 1b).

684

685   *Diversity methods*

686   Consider a sample of G genotypes (bit strings or nucleotide sequences). We use the

687   diversity index of Nei and Li (1979) (1):

688
$$\pi = \frac{2}{G^2} \sum_{i=2}^{G} \sum_{j=1}^{i-1} \pi_{ij} \, ,$$

689   where π_{ij} is average number of differences (in bits or bases) per site between

690   genotype i and genotype j. We refer to π as bit diversity (in the NK model) or

691   nucleotide diversity (for our bacterial system).

692

693

694

695   **Supplemental Figure 1**: Genetic diversity in the digital and bacterial populations. (a)

696   Average bit diversity of a sample of eight evolved bit strings from time point 1000 in

697   the NK model simulations. Whether the landscape is smooth (K=0) or rugged (K=8),

698   diversity is significantly greater in the Restricted treatment than the Unrestricted

699   treatment. Bars represent the mean of 40 replicates. (b) Average nucleotide diversity

700   within bacterial metapopulations at the final transfer of the experiment (T=36). For

701   each metapopulation, full genome sequences from each of five isolates was used to

702   compute the diversity index. Nucleotide diversity is significantly greater in the

703   Restricted treatment than the Unrestricted treatment. Bars represent the mean of 5

704   replicates. In both parts of the figure, whiskers give the standard error and asterisks

705   indicate significant differences.

706

707

708

709   *How a rugged landscape can fail to give a Tortoise-Hare signal*

710   On a rugged landscape, fitness in a structured population will increase more slowly

711   than an unstructured population (the Tortoise initially lags behind, before overtaking,

712   the Hare; see Fig. 2b). That is, for populations differing in structure evolving on a

713   rugged landscape, early evolution will produce a pattern similar to that predicted

714   under a smooth landscape (e.g., before time point 250, the pattern in Figure 2b

715   would be hard to distinguish from the entire trajectory of Figure 2a). While the

716   presence of a Tortoise-Hare pattern indicates ruggedness, its absence does not

717   necessarily imply a smooth landscape.

718

719   For example, in the experiment of Kryazhimskiy *et al*. (2012) (2), the unstructured

720   population ended the experiment with higher average fitness. This pattern is

721   consistent with a smooth landscape, but is not inconsistent with a rugged one. As

722   the authors themselves acknowledge, had their experiment run longer, they may

723   have observed higher fitness under lower rates of migration (i.e., a fitness crossing).

724

725   Even when there is abundant time for evolution to take place, it is still possible that

726   evolution on a rugged landscape will fail to yield the Tortoise-Hare pattern.

727   For instance, it is possible that the landscape is rugged, but peaks are of a

728   homogeneous height. This could produce the fitness pattern shown in Figure 2a.

729

730   Finally, the landscape could be rugged with heterogeneous peak heights, but the

731   ancestor could be positioned in the domain of a single peak. Consistent with this

732   possibility, Kryazhimskiy *et al*. started their experiment with a lab-adapted strain of

733   yeast and evolved their populations under standard laboratory conditions. If their

734   yeast had access to only a single domain in a rugged landscape, a Tortoise-Hare

735   pattern would not be expected. In such a case Kryazhimskiy *et al*. would be justified

736   in claiming that the *local* topography of such a landscape was smooth (and indeed,

737   they restrict their claim of smoothness accordingly).

738

739   As outlined in our Methods, we introduced several deleterious mutations into our

740   ancestor and evolved our populations under a stressful environment (in the presence

741   of sub-lethal concentrations of the antibiotic tetracycline). Such manipulation was

31

742   intended to displace our ancestral genotype from a peak, but it also may have

743   placed it at a point where multiple domains were accessible. To address the effect of

744   ancestor starting position, we describe additional NK simulations here. In addition to

745   starting our ancestor at a random bit string, we consider three other starting

746   positions: (i) valley, (ii) pre-adapted, and (iii) "silver-spoon." For the valley

747   simulations, we performed a "hill-plunge of steepest descent," moving downhill from

748   a random genotype until we hit a valley genotype (a genotype from which all

749   mutations were beneficial), which served as the ancestor. For the pre-adapted

750   simulations, we allowed a random ancestor to evolve briefly (in an unstructured

751   population) to produce a "pre-adapted" ancestor. For the silver-spoon simulations, all

752   genotypes were ranked for fitness and the genotype defining the 99th fitness

753   percentile was chosen as the ancestor.

754

755   In the random and valley starting positions, the Tortoise-Hare pattern was observed

756   and the Structured treatment ended at significantly higher average fitness than the

757   Unrestricted treatment (Mann-Whitney tests, $p<0.001$; Supp. Fig. 1b). However, in

758   the pre-adapted and silver-spoon starting positions, the Tortoise-Hare pattern was

759   not seen and fitness was indistinguishable between the treatments in the long run

760   (Mann-Whitney test, $p=0.36$ and $p=0.57$ respectively). These simulations

761   demonstrate that the starting position of a population in a landscape will influence

762   the statistical pattern of fitness of populations differing in structure.

763

764   *Additional NK simulation methods*

765   To study the effect of starting position in the adaptive trajectories in structured and

766   unstructured populations, we examined starting the population with different types of

767   ancestors. The "random" ancestor (used in the primary text) is simply a random bit

768   string. To generate the "valley" ancestor, we start with a random bit string and

769   substitutes the worst (lowest fitness) possible mutation until no deleterious mutations

770   are possible, and that bit string is the ancestor for the evolutionary run. To produce

771   the "pre-adapted" ancestor, we start with a random bit string and evolve a population

772   initialized with this bit string under unrestricted migration for 50 updates. Then bit

773   strings are sampled from the evolved population until one is found that has a higher

774   fitness than the starting bit string. That adapted bit string is the ancestor. To

775   determine the "silver-spoon" ancestor, all possible bit strings (2^{15}) are ranked

776   according to fitness and the 99th percentile genotype is the ancestor.

777

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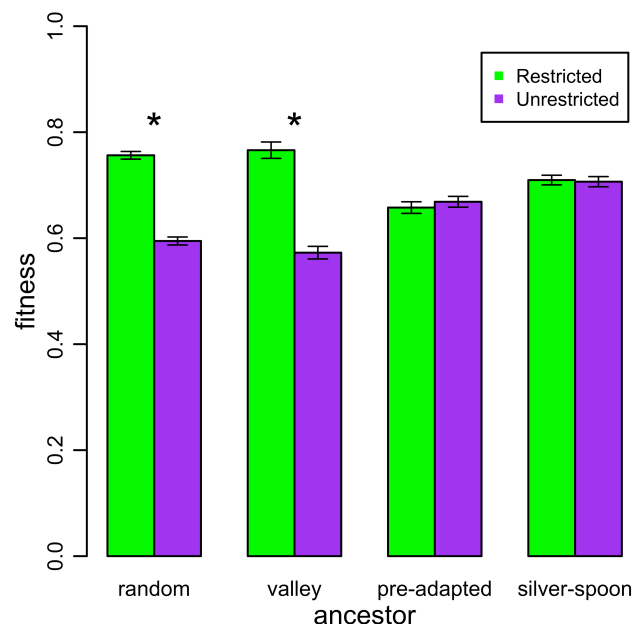
780   **Supplemental Figure 2**: Metapopulations of bit strings of length N=15 evolved on a

781   rugged landscape (K=8), where migration was either restricted or unrestricted.

782   Average fitness in the metapopulation is shown at time point 1000 for a randomly

783   chosen ancestor, an ancestor starting in a valley, an ancestor resulting from

784   adaptation before the run, and an ancestor in the top percentile of fitness (the "silver-

785   spoon" ancestor). Bars represent the mean of 40 replicates, whiskers give the

786   standard error of the mean, and asterisks indicate significant differences.

787

788 **Supplemental Table 1**:

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

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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

789

790   Each row denotes a mutation discovered in the evolved (transfer 36) isolates that

791   was not present in the ancestor. The columns are as follows: Migration Type (the

792   pattern of migration defining the treatment of the isolate), Population (the identifier

793   for the replicate metapopulation (1-5) of the isolate), Isolate (the identifier for the

794   mutation's isolate (1-8)), Position (the genomic location of the mutation according to

795   *E. coli W311* [GenBank: AP009048]), Mutation Type (Single Nucleotide

796   Polymorphism (SNP), Deletion (DEL), Insertion (INS), Substitution (SUB) or

797   Amplification (AMP)), Change (the length of a mutation for DEL, or AMP, or the new

798   nucleotide state for SNP and INS), Gene Left (the nearest open reading frame to the

799   prior to the mutation), Gene Right (the nearest open reading frame after the

800   mutation). Note that if Gene Left and Gene Right are the same, the mutation falls

801   within that gene.

802

803   *Violation of Strong-Selection-Weak-Mutation (SSWM) Assumptions*

804

805   The relatively high degree of diversity in the evolving bacterial populations

806   demonstrates a violation of strict SSWM assumptions. Isolates from the same

807   metapopulation (in both treatments) were often not single mutant neighbors (see

808   Figure 5 and Supplemental Table 1); thus, the diversity does not simply represent a

809   mixture of the genotype fixing and the genotype being displaced in the midst of

810   selective sweep. While divergent genotypes are expected in the structured

811   population (Restricted Migration), they also appear in our less structured treatment

812   (Unrestricted Migration). Most likely, the diversity results partly from the presence of

813   small fitness differences between genotypes, such that new beneficial mutations

814   may arise before old beneficial mutations fix (leading to a form of clonal

815   interference). We also note that the Unrestricted treatment possessed some degree

816   of population structure (i.e., this was not a "well-mixed" population), which could also

817   contribute to diversity. Such diversity may enable metapopulations in the

818   Unrestricted treatment to explore multiple domains simultaneously. However, the

819   main effects of structure outlined in this paper still apply. If the landscape is multi-

820   peaked, metapopulations in the Restricted treatment are expected to sample *a*

821   *greater number of domains* (and indeed, the Restricted metapopulations had

822   significantly greater diversity). We emphasize that the same violations of SSWM

823   assumptions occur in the NK model (see Supplemental Figure 1 for diversity

824   profiles), in which the same migration treatments were used, and we note that the

825   Tortoise-Hare pattern is observed for sufficiently rugged landscapes (Figure 2).

826   Thus, even in populations violating strict SSWM assumptions, the greater parallel

827   search that comes with greater population structure is predicted to lead to better

828   long-term adaptation in rugged landscapes with heterogeneity in peak height.

829

830   **Full Data, Simulation Code, and Statistical Scripts are available on the Kerr Lab**

831   **Wiki: http://kerrlab.org/Public/RugLand**

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834 **Supplementary References**:

835

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837   of restriction endonucleases. *Proc Natl Acad Sci U S A* 76:5269–5273.

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