

1 **Local adaptation mediated niche expansion in correlation with genetic**
2 **richness**

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10

11 **Abstract**

12 As a central issue in evolution and ecology, the quantitative relationship among the
13 genome, adaptation and the niche was investigated. Local adaptation of five *Escherichia*
14 *coli* strains carrying either the wild-type genome or reduced genomes was achieved by
15 experimental evolution. A high-throughput fitness assay of the ancestor and evolved
16 populations across an environmental gradient of eight niches resulted in a total of 80
17 fitness curves generated from 2,220 growth curves. Further analyses showed that the
18 increases in both local adaptiveness and niche broadness were negatively correlated with
19 genetic richness. Local adaptation caused common niche expansion, whereas niche
20 expansion for generality or speciality was decided by genetic richness. The order of the
21 mutations accumulated stepwise was correlated with the magnitude of the fitness increase
22 attributed to mutation accumulation. Pre-adaptation probably participated in coordination
23 among genetic richness, local adaptation and niche expansion.

24
25 **Keywords:** genetic richness, local adaptation, genome reduction, niche expansion,
26 environmental gradient, growth rate, experimental evolution, fitness landscape

27

28 Introduction

29 In nature, microorganisms of various genome sizes inhabit a range of environments,
30 i.e., niches, which is the consequence of local adaptation¹ and is constrained by
31 evolutionary costs². Genome size, i.e., genetic richness, was believed to be associated
32 with the ecological niche³, which was supported by the linkages between genome
33 streaming and niche partitioning⁴, gene loss and niche shift⁵, genome reduction and
34 habitat transition⁶ or metabolic cost⁷, and genome architecture and habitat⁸ or niche-
35 directed evolution⁹. These ecological findings and genomic analyses provided strong
36 evidence of the relationship between the genome and niche established during adaptive
37 evolution in nature. However, the quantitative evaluation of this relationship is largely
38 insufficient and might require experimental demonstration in the laboratory.

39 First, the contribution of genetic richness to adaptative evolution, i.e., local adaptation,
40 remains unclear, although changes in genome size are commonly observed in nature^{10,11,12}
41 and known as the major driving force for adaptive evolution, e.g., horizontal gene
42 transfer^{13,14}. Genome size, i.e., genetic richness, was experimentally reduced^{15,16,17} to
43 achieve the minimal genetic requirement for living organisms^{18,19}. The reduced genomes
44 tended to show decreased fitness^{20,21} and increased mutagenesis²², which could both be
45 restored by experimental evolution²². These studies verified the connection between
46 genetic richness and adaptive evolution, but quantitative evaluation via comparison to the
47 wild-type genome was lacking.

48 Second, the contribution of the local adaptation achieved by evolution to niche
49 broadness was unknown. To date, experimental studies have generally focused on the
50 target component out of numerous components that comprise the environment, e.g., the
51 carbon source²³ or antibiotics²⁴, as the trigger factor for adaptative evolution. The
52 environment, either the culture medium used in the laboratory or the ecological niche in
53 the wild nature, is comprised of not only the target component but also a number of other
54 nutrients and trace elements. The participation of components other than the target
55 component in adaptative evolution was generally neglected. Machine learning predicted
56 that the priorities of the medium components were differentiated in deciding the bacterial
57 growth²⁵, indicating the varied adaptiveness in response to the individual components
58 comprised of the environment. The fitness landscape across the environmental gradients
59 of all related components (niches) was crucial for us to address the question of how local
60 adaptation contributes to niche broadness.

61 The present study addressed the questions of how genetic richness (genome reduction)
62 contributed to local adaptation and whether and how local adaptation caused changes in
63 niche broadness (Fig. 1A). Genetic richness was represented by genome reduction; local

64 adaptation was achieved by experimental evolution; and niche broadness was evaluated
65 by fitness curve fitting across the environmental gradients of the components (niches)
66 presented in the experimental evolution (Fig. 1B).

67

68 **Results**

69 *Experimental evolution-mediated local adaptation was correlated with genetic richness*

70 Local adaptation, which was achieved by experimental evolution with *E. coli* strains
71 of varied genome sizes, showed that the fitness increase was correlated with genetic
72 richness. Five laboratory *E. coli* strains carrying either the wild-type (N0) or the reduced
73 (N7, 14, 20, 28) genomes (Table S1) were subjected to experimental evolution in
74 chemically defined medium (designated C0). A gradual increase in the growth rate was
75 commonly observed in the reduced genomes during evolution for approximately 1,000
76 generations (Fig. 2A). The growth rates of the evolved populations (Evos) were all higher
77 than those of the ancestors (Ancs), indicating that local adaptation was achieved
78 regardless of genetic richness (Fig. 2B). This finding was somehow consistent with our
79 previous finding that genome reduction was correlated with a decrease in the growth rate²⁰.

80 Both the changes in growth rate and the rates of the changes in evolution were
81 significantly correlated with genome reduction (Fig. 2C), indicating coordination
82 between local adaptation and genetic richness. The correlation of genome reduction with
83 the evolutionary rate of fitness increase was supported by the previous finding of the
84 correlation between genome reduction and the spontaneous mutation rate²², a global
85 parameter representing evolvability. Additionally, the cellular redox activity, representing
86 the metabolic activity inside the cell, was found to be correlated with the growth rate, and
87 the changes in growth rate and the changes in redox activity were correlated (Fig. 2D).
88 The results further verified that local adaptation was achieved metabolically.

89

90 *Local adaptation caused niche expansion in correlation with genetic richness*

91 Whether the local adaptation caused the fitness changes across the environmental
92 gradient was analysed. The growth fitness of Ancs and Evos was precisely evaluated in
93 29 medium combinations (C0 and C1~28), which comprised eight chemical components
94 used for the evolutionary condition C0 (Fig. 3A, Table S2). A total of 2,220 growth rates
95 calculated from the corresponding growth curves were acquired (Table S5). A global
96 increase in growth rate under most medium combinations was observed in the reduced
97 genomes (Fig. 3B, Fig. S2). Fitness improvement was achieved not only under
98 evolutionary condition C0 but also across the concentration gradient. It seemed that larger
99 deletions from the genome led to larger changes in growth fitness. In comparison, the

100 growth rates of the wild-type genome (N0) were slightly changed. The results revealed
101 that whether local adaptation triggered global adaptation across the environmental
102 gradient was dependent on genetic richness.

103 According to the growth rates (Fig. 3B) in eight chemical components (niches), the
104 niche space (\mathcal{S}) was newly defined by cubic polynomial regression to the normalized
105 fitness curve, in which the maxima of both the concentration gradient and the growth rate
106 were normalized to one unit (Fig. 4A). The normalization of individual fitness curves
107 determined a fixed niche breadth available for the comparison among the varied niches and
108 genomes. The niche space (\mathcal{S}) was determined as the shadowed space under the regression
109 curve. Consequently, a total of 80 niche spaces (Fig. S3), as well as the changes in niche
110 space attributed to local adaptation (Fig. S4), were calculated. It seemed that both the
111 niche space and its change were more closely associated with genetic richness (genomes)
112 than with the niche types (chemical components). To achieve an overall evaluation of
113 niches, the niche broadness (total \mathcal{S}) was defined as the sum of the eight niche spaces (Fig.
114 4B).

115 The niche broadness was narrowed in response to genome reduction (Fig. 4C, green);
116 however, it was significantly widened due to experimental evolution (Fig. 4C, pink). The
117 local adaptation expanded the niche broadness of all Evos to a roughly equivalent level
118 (Fig. 4D, left), indicating homeostasis in niche expansion. The variation in spaces of the
119 eight niches commonly declined in the Evos (Fig. 4D, right), indicating that local
120 adaptation reduced niche divergence for balanced niche expansion. Intriguingly, the
121 changes in niche broadness were positively correlated with both genome reduction (Fig.
122 4E, left) and changes in the growth rate for local adaptation (Fig. 4E, right). These results
123 suggested that local adaptation mediated global coordination of niche expansion with
124 genetic richness for homeostatic and balanced niche broadness.

125

126 *Niche expansion for speciality or generality was dependent on the genetic richness*

127 A gradual shift from the evolutionary trade-off in niche space to global niche expansion
128 occurred in response to genome reduction (Fig. 4C). The niche broadness of Anc and Evo
129 entirely overlapped in the reduced genomes with larger deletions (N14, N20, N28) but
130 partially overlapped in the wild-type genome (N0) and the reduced genome with a
131 relatively small deletion (N7). For instance, trade-offs occurred in N0; that is, the
132 improvement in the niches of thiamin, K^+ , PO_4^+ and Fe^{2+} and the deficiency in the niches
133 of glucose, Mg^{2+} , NH_4^+ and SO_4^{2+} implied niche expansion for speciality. In contrast,
134 omnidirectional expansion of niche broadness occurred in the reduced genomes of N14,
135 N20 and N28. These results revealed that whether local adaptation triggered global

136 adaptation across the environmental gradient or the niche-dependent trade-off was
137 dependent on the genetic richness.

138 A niche-specific correlation of the changes in niche space to local adaptation and
139 genome reduction was observed (Fig. 5). Significant correlations of niche expansion (i.e.,
140 changes in niche space) with local adaptation (i.e., changes in growth rate in C0) (Fig.
141 5A) and genetic richness (i.e., genome reduction) (Fig. 5B) were commonly found in the
142 niches of glucose, SO_4^{2+} and Mg^{2+} . The co-correlation of the local adaptation and the
143 genetic richness to niche expansion was consistent with the correlation between the
144 growth change and genome reduction (Fig. 2C). Note that the trade-off in niche expansion
145 of N0 reduced the niche space of glucose, SO_4^{2+} and Mg^{2+} (Fig. 4C), in which niche
146 expansion was significantly correlated with genome reduction (Fig. 5B). This implied
147 that the genetic richness was highly sensitive to these three niches with regard to carbon,
148 sulphate and magnesium.

149

150 *Stepwise mutation accumulation was associated with an additive increase in fitness*
151 *regardless of genetic richness*

152 To discover the genetic mechanism participating in local adaptation-associated niche
153 expansion, genome mutation analysis was performed. An approximately equivalent
154 number of gene mutations were detected in the Evos, regardless of the genome (Fig. 6A,
155 Table S3). Temporal changes in the allele frequency of mutants showed that the mutations
156 accumulated serially and were fixed in a stepwise manner. In addition, only a few gene
157 mutations were able to compensate for the large genomic deficiency (Fig. 6A), which was
158 somehow consistent with the finding that the mutations caused the metabolic rewiring of
159 a reduced genome due to experimental evolution²⁶. Note that the changes in transposons
160 were ignored, and the mutations fixed in Evos were identified in the reduced genomes but
161 not in the wild-type genome. The mutated genes were related to transporters and
162 regulators (Table S3), which indicated that resource diffusion for utilization and global
163 gene regulation contributed to local adaptation-associated niche expansion.

164 How the stepwise accumulation of the mutations contributed to local adaptation was
165 further investigated. The colonies/mutants carrying the mutations in the order of
166 evolutionary accumulation were acquired and subjected to a growth fitness assay. Note
167 that the mutants with the second mutation (*rbsR* and *fliE/fliF*) in N7 failed to be acquired,
168 indicating the co-fixation of these two mutations during evolution. A gradual increase in
169 the growth rate of the colonies in the order of mutation accumulation was commonly
170 observed, except for a transient decrease caused by the second mutation that occurred in
171 N14 (Fig. 6B). The mutation accumulation-associated increase in growth rates was

172 commonly observed in all the reduced genomes. This demonstrated that the mutations
173 were beneficial and contributed to local adaptation in an additive manner, which was
174 independent of genetic richness. In addition, an intriguing power law for the contribution
175 of the gene mutations to the local adaptation was observed; that is, lower growth rates
176 prior to mutation fixation led to larger changes in growth rate after the mutation was fixed
177 (Fig. 6C). The first mutations were more likely to improve the growth fitness than the
178 mutations that were fixed later, although the statistical significance was weak because
179 there were too few mutations (Fig. S5). The negative correlation and the order of the
180 fitness contribution of the mutations indicated the pre-adaptation proposed for the
181 evolution of diversity²⁷ and the predictivity of the mutation-mediated fitness landscape²⁸.

182

183 *Hypothesis of pre-adaptation in local adaptation-associated niche expansion*

184 The mechanism linking the fitness landscape, which was commonly applied for
185 explaining evolutionary adaptation²⁹, to pre-adaptation for niche expansion was proposed
186 as follows (Fig. 7A). **i)** Growth fitness was correlated with genome reduction²⁰. Larger
187 deletions led to a greater distance from the fitness peak. Not only the gene function but
188 also the genetic richness might have played a role in the fitness landscape. Increasing the
189 genome size might have been easier than evolving the well-regulated gene function. **ii)**
190 Evolution improved the local adaptation to C_0 in correlation with genetic richness. The
191 greater the distance from the fitness peak was, the larger change it was to reach the
192 equivalent adaptiveness (fitness). This was supported by the increase in growth rate
193 caused by experimental evolution and the correlation between genetic richness and
194 changes in growth rate (Fig. 2). The mutations were fixed in a stepwise manner during
195 evolution, leading to an additive fitness increase (Fig. 6). **iii)** In alternative environments,
196 genetic richness determines whether there is global adaptation for niche generalization or
197 a trade-off for niche specialization, which is supported by the correlation between niche
198 broadness and genome reduction (Fig. 4). The location in the initial fitness landscape (e.g.,
199 C_0) likely determined the fitness in the alternative environmental gradient (e.g., C_N). That
200 is, there was a higher probability of trade-off closer to the local adaptive peak, whereas
201 farther from the local adaptive peak, there was more opportunity for global adaptation,
202 which was consistent with the pleiotropic costs for carbon utilization found in the
203 experimental population³⁰. The locational bias must have contributed to the pre-
204 adaptation in evolution.

205 The pre-adaptation in the participation of the local adaptation-associated niche
206 expansion was supported by the weak but statistically significant correlation of the niche
207 spaces between Ancs and Evos (Fig. 7B). In particular, the changes in niche space were

208 highly significantly correlated with the niche spaces of Ancs but not with those of Evos
209 (Fig. 7C), which was confirmed even if evolutionary generation was taken into account
210 (Fig. S6). The magnitude of the changes was dependent on the Ancs, that is, the smaller
211 niche space evolved for the larger changes in niche space, which was consistent with the
212 genetic richness-correlated niche expansion (Fig. 4). The correlations identified in the
213 niche space and its changes, as well as the fitness contribution of the mutations, indicated
214 pre-adaptation in evolution.

215

216 **Discussion**

217 Larger genomic deletion resulted in not only lower fitness²⁰ but also faster evolution
218 (Fig. 2). Rapid adaptation occurred for the reduced genomes, which carried fewer
219 nonessential genes, in comparison to the wild-type genome, which contained a full set of
220 genes. The rapid adaptation of the reduced genome was achieved by a few mutations (Fig.
221 6), indicating that the large genomic deletions were compensated by a single mutation or
222 a few mutations. Abundant genetic information could be substituted with certain gene
223 functions for equivalent adaptiveness, providing intriguing insight into genetic
224 essentiality. The fitness landscape was employed and preliminarily explained the finding,
225 according to the assumption that both the large deletion and the single mutation were
226 located on the identical fitness landscape (Fig. 7A). Fitness landscape analysis^{31,32} is
227 generally applied to explain mutation occurrence^{33,34} and the resultant mutant, with the
228 changed distance to the fitness peak^{35,36} as evolutionary constraint^{33,37}. As genome
229 evolution occurred not only via mutations associated with gene function but also due to
230 large fluctuations in genome size, e.g., horizontal gene transfer³⁸ and streamlining^{11,39},
231 introducing the fitness landscape to genome reduction, i.e., changes in genome size as
232 well as changes in gene size, was reasonable. The fitness landscape was consistent with
233 the finding that both the single mutations and the large deletions contributed to the fitness
234 in an additive manner.

235 Owing to the direct comparison of the wild-type and reduced genomes in adaptive
236 evolution, a genetic richness-dependent evolutionary strategy was first observed. The
237 shift from the adaptive trade-off to global adaption was dependent on genetic richness
238 (Fig. 4). As local adaptation often results in maladaptation to alternative
239 environments^{23,40,41}, ecological niche speciation is often explained by adaptive trade-
240 offs^{40,42,43,44}. Environmental homogeneity is considered one of the deterministic factors
241 for trade-offs⁴⁵, and environmental fluctuations during evolution are thought to be crucial
242 for global adaptation⁴⁶. Since serial transfer was performed to maintain continuous
243 growth in the early exponential phase (Fig. S1), the evolution was supposed to occur in a

244 steady environment with sufficient resources. Nevertheless, global adaptation instead of
245 the trade-off occurred in the reduced genomes. If it was the deleted genes (gene functions)
246 that had been specifically responsible for the adaptive trade-off, the deleted genomic
247 length would never be correlated with the fitness increase. Thus, it was the genetic
248 richness, i.e., the amount of genomic information, but not the particular gene function,
249 that determined the strategies for trade-off or global adaptation to the environment, e.g.,
250 the niche or habitat.

251 Local adaptation was first considered to be achieved in response to all components of
252 the environment. Evaluation of the fitness landscapes across all components of a wide
253 concentration gradient allowed us to find the common niche expansion related to the
254 adaptive evolution (Fig. 1). The niche expansion was attributed to the fluctuation in the
255 concentration of the components accompanied by bacterial growth, as mentioned
256 previously⁴⁷. The niche divergence (Figs. 4, 5) somehow represented the genetic
257 sensitivity to the components, i.e., the chemical types in the niche. The correlation
258 between the niche space and the genetic richness was significant in the niches of glucose,
259 NH_4^+ and SO_4^{2+} , whereas the local adaptation cancelled this correlation for NH_4^+ and
260 SO_4^{2+} and changed the correlation from negative to positive for glucose (Fig. S7). The
261 mutations that occurred for local adaptation largely compensated for the genomic
262 deficiency in using these resources, and this was reasonable because carbon, nitrogen and
263 sulphur are the essential major elements for living organisms on Earth⁴⁸. The genetic
264 richness-correlated changes in niche spaces were decided prior to local adaptation in the
265 niches of glucose and SO_4^{2+} (Fig. S7), indicating that pre-adaptation more likely occurred
266 in the niches of carbon and sulphate.

267 The local adaptation caused the large omnidirectional expansion of the niche broadness
268 for the largely deficient genomes in comparison to the small directional expansion of the
269 niche broadness for the complete and few deficient genomes (Fig. 4). Genetic richness
270 was probably associated with omnidirectional fitness across the entire niches of the
271 habitat, which was well supported by the finding that the niche broadness of the ancestor
272 determined the evolved niche broadness (Fig. 7B, C). The habitat, composed of multiple
273 niches, might decide the maximum of the overall niche broadness accessible for evolution.
274 In other words, the overall niche broadness of a defined environment seemed to be
275 homeostatic for a defined species, as the niche expanded until comparable broadness was
276 reached (Fig. 4). Note that the homeostasis of niche space was not biased by
277 normalization. As normalization to one unit was performed individually, the maxima of
278 the overall niche spaces could be differentiated in the respective genomes. Niche
279 expansion might reflect the evolutionary direction for generalists or specialists^{2,49}.

280 Deficient and sufficient genetic richness evolved for generality and speciality,
281 respectively, indicating a fundamental principle for genome evolution adaptive to
282 ecological niches.

283 In summary, the present study provided experimental evidence showing that local
284 adaption mediated niche expansion in correlation with the genome through the
285 combination of genomic and environmental gradients. Despite adaptive evolution in
286 steady environments, deficient genomes evolved in a jack-of-all-trades-and-master-of-all
287 manner, which was theoretically proposed as one of three mechanisms for specialism that
288 is widespread in nature⁵⁰, in comparison to the wild-type genome, which adopted a trade-
289 off mechanism, which was generally explained by constraints in phenotypic space^{51,52}. In
290 nature, the trade-off strategy might be more frequent and reasonable for costless
291 adaptation and niche expansion during eco-evolution^{2,53,54}. As both the genome and the
292 environment participate in ecological evolution, the coordination among genetic richness,
293 adaptiveness and niche broadness revealed a quantitative linkage of adaptive evolution to
294 ecology.

295

296 **Materials and methods**

297 *E. coli* strains

298 A total of five *E. coli* strains with either the wild-type or the reduced genome were used,
299 which were selected from the KHK collection¹⁷, an *E. coli* collection of reduced genomes
300 (from National BioResource Project, National Institute of Genetics, Shizuoka, Japan).
301 The wild-type and four reduced genomes were derived from *E. coli* W3110 and were
302 assigned as N0 and N7, 14, 20, 28, respectively (Table S1), according to previous studies
303 ^{20,22}.

304

305 *Media combinations*

306 The minimal medium M63, equivalent to C0, was used for the experimental evolution
307 for local adaptation. Its chemical composition was described in detail previously^{20,55}. The
308 concentration gradient of the components of the M63 medium was prepared just before
309 the fitness assay by mixing the stock solutions of individual chemical compounds, which
310 resulted in 28 alternative medium combinations (C1~28). The stock solutions, that is, 1
311 M glucose, 0.615 M K₂HPO₄, 0.382 M KH₂PO₄, 0.203 M MgSO₄, 0.0152 M thiamin/HCl,
312 0.0018 M FeSO₄, and 0.766 M (NH₄)₂SO₄, were sterilized using a sterile syringe filter
313 with a 0.22- μ m pore size hydrophilic PVDF membrane (Merck). The concentrations of
314 most chemical compounds were altered one-by-one on a logarithmic scale to achieve a
315 wide range of environmental gradients, as described previously²⁵, which led to a total of

316 28 combinations (Fig. 3A, Table S2). Both the medium used in the evolution (C0) and the
317 alternative medium combinations (C1~28) were used for the fitness assay. The resultant
318 concentrations of individual components in the ionic form are summarized in Table S2.

319

320 *Experimental evolution*

321 The experimental evolution of the five *E. coli* strains was performed within the early
322 exponential phase by serial transfer (Fig. S1), which was performed with 24-well
323 microplates specific for microbe culture (IWAKI) as previously described²². The *E. coli*
324 cells were cultured in eight wells, and eight tenfold serial dilutions, i.e., $10^1\sim 10^8$, were
325 prepared with fresh medium. The microplates were incubated overnight in a microplate
326 bioshaker (Deep Well Maximizer, Taitec) at 37°C, with rotation at 500 rpm. Serial transfer
327 was performed at 12- or 24-h intervals, according to the growth rate. Only one of the eight
328 wells (dilutions) showing growth in the early exponential phase ($OD_{600} = 0.01\text{-}0.1$) was
329 selected and diluted into eight wells of a new microplate using eight dilution ratios. The
330 cell culture selected daily for the following serial transfer was mixed with glycerol (15%
331 v/v) and stored at -80°C for future analyses. Serial transfer was repeatedly performed for
332 approximately 50 days. The evolutionary generation was calculated according to the
333 following equation (Eq. 1).

$$334 \quad \text{gen} = \log_2(C_i/C_j) \quad (\text{Eq. 1})$$

335 Here, C_i and C_j represent the OD_{600} of the cell culture that was used for serial transfer and
336 the theoretical OD_{600} of the cell culture at the start of incubation. C_j was calculated by
337 dividing the OD_{600} that was used in the last transfer by the dilution rate. To benefit
338 experimental replication, the cell cultures stored for the following assays were dispensed
339 into 20 microtubes in small aliquots (100 μL per tube), which were used once, and the
340 remainder was discarded, as previously described⁵⁵.

341

342 *Growth fitness assay*

343 The fitness was determined as the maximal growth rate, as previously reported²⁰. In
344 brief, the cell culture stocks were diluted 1,000-fold in fresh media (C0, C1~28) and were
345 subsequently loaded into a 96-well microplate (Costar) in six wells at varied locations.
346 The 96-well microplate was incubated in a plate reader (Epoch2, BioTek) with a rotation
347 rate of 567 rpm at 37°C. The temporal growth of the *E. coli* cells was detected by
348 measuring the absorbance at 600 nm, and readings were obtained at 30-min intervals for
349 48 h. The maximal growth rate was calculated according to the following equation (Eq.
350 2).

$$351 \quad \mu = LN(C_{i+1}/C_i)/(t_{i+1} - t_i) \quad (\text{Eq. 2})$$

352 Here, C_i and C_{i+1} represent the two reads of OD₆₀₀ values at two consecutive time points
353 of t_i and t_{i+1} . The growth fitness was the average of the five continuous growth rates that
354 exhibited the largest mean and the smallest standard deviation during the temporal
355 changes in growth rate, as previously reported²⁰. A total of 2,220 growth curves were
356 acquired, and the corresponding growth rates were calculated for the analysis (Table S5).
357

358 *Redox activity assay*

359 A cell culture in the exponential phase of growth (OD₆₀₀ = 0.01 ~ 0.3) was used for the
360 assay. The cell culture was diluted with fresh medium at twelve dilution ratios from 1.75⁰
361 to 1.75¹¹ in a final volume of 2 mL. Every 100 μL of the diluted cell culture was
362 transferred to multiple wells in a 96-well microplate (Costar), in which 20 μL of CellTiter
363 96[®] Aqueous One Solution Reagent (Promega) was added. The reduction of the
364 tetrazolium compound in the reagents was measured with a microplate reader (Epoch2,
365 BioTek) by determining the OD₄₉₀ every 2 min for 30 min. The rate of reduction was
366 calculated by linear regression of the temporal changes in OD₄₉₀, i.e., the slope of the
367 increase in OD₄₉₀ over time (min). The redox activity was determined by dividing the rate
368 of reduction by the OD₆₀₀ of the cell culture. The mean of the multiple measurements
369 (N=5) was used for the analysis.
370

371 *Niche space evaluation*

372 The fitness dynamics (i.e., the fitness curve) across the concentration gradient of each
373 chemical component were evaluated by curve fitting of a cubic polynomial with the
374 following equation (Eq. 3).

$$375 \quad \mu(x) = ax^3 + bx^2 + cx + d \quad (\text{Eq. 3})$$

376 Here, x and $\mu(x)$ represent the concentration gradient of each chemical component and
377 the growth rate under the corresponding conditions, respectively. a , b , c and d are the
378 constants. The area under the fitted curve was calculated according to the following
379 equation (Eq. 4).

$$380 \quad \text{Area} = \int_{x_{min}}^{x_{max}} ax^3 + bx^2 + cx + d \quad (\text{Eq. 4})$$

381 Here, x_{min} and x_{max} represent the minimum and maximum concentrations of each chemical
382 component, respectively. The niche space (S) was evaluated by normalizing both the
383 height and the width of the fitness curve with the following equation (Eq. 5).

$$384 \quad S = \text{Area} \times \mu_{max}^{-1} \times (x_{max} - x_{min})^{-1} \quad (\text{Eq. 5})$$

385 Here, μ_{max} is the maximal growth rate across the concentration gradient. The niche
386 broadness (S_T) of the individual genome was determined as the sum of the niche spaces

387 of the eight chemical components as follows (Eq. 6).

$$388 \quad S_T = \sum_{i=1}^n S_i \quad (\text{Eq. 6})$$

389 Here, S_i and n indicate the niche space of each chemical component and the total number
390 of chemical components, respectively.

391

392 *Genome resequencing and mutation analysis*

393 The stored cell culture was inoculated into 4 mL of fresh M63 medium in a test tube
394 and grown at 37°C with shaking at 200 rpm. Once cell growth reached the stationary
395 phase ($\text{OD}_{600} > 1.0$), rifampicin was added to the culture at a final concentration of 300
396 $\mu\text{g/mL}$ to stop genome replication initiation. After 3 h of culture with rifampicin, the cells
397 were collected as previously reported⁵⁶. Genomic DNA was extracted using a Wizard
398 Genomic DNA Purification Kit (Promega) in accordance with the manufacturer's
399 instructions. The sequencing libraries were prepared using the Nextera XT DNA Sample
400 Prep Kit (Illumina), and paired-end sequencing (300 bp \times 2) was performed with the
401 Illumina MiSeq platform. The sequencing reads were aligned to the *E. coli* W3110
402 reference genome (AP009048.1, GenBank), and the genome mutations were analysed
403 with the Breseq pipeline (version 0.30.1)⁵⁷. The fixed mutations (Table S3) were
404 subsequently analysed for the temporal order of accumulation during evolution. The raw
405 data set was deposited in the DDBJ Sequence Read Archive under accession number
406 DRA011629.

407

408 *Sanger sequencing and single-colony isolation*

409 The genomic region of approximately 300-600 kb centred on the position of the
410 mutation was amplified by PCR with PrimeSTAR HS DNA Polymerase (TaKaRa Bio)
411 and the corresponding primers (Table S4). Amplicons were purified using a MinElute
412 PCR Purification Kit (Qiagen), and Sanger sequencing was conducted by Eurofins
413 Genomics K. K. (Tokyo, Japan). The resulting electropherogram was analysed using
414 Sequence Scanner Software v2.0 (Thermo Fisher Scientific), and the ratio of the mutants
415 within the cell population was calculated according to the peak values, as described
416 previously⁵⁸. Stored cell cultures with an interval of ~ 100 generations were analysed to
417 identify the heterogeneity of the cell population. Single-colony isolation was performed
418 from the heterogeneous population to isolate the homogeneous mutants. The cell culture
419 was spread on LB agar plates, and 10-30 single colonies per plate were subjected to
420 Sanger sequencing. The colonies of the homogeneous mutant were stored for the fitness
421 assay as described above.

422

423 **Authors' contributions**

424 MK and IN performed the experiments, MK and BWY analysed the data and drafted
425 the manuscript, BWY conceived the research and rewrote the paper, and all authors
426 approved the final manuscript.

427

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432

433 **Competing interests**

434 The authors declare that there are no competing interests.

435

436 **References**

- 437 1. Kawecki TJ, Ebert D. Conceptual issues in local adaptation. *Ecology letters* **7**,
438 1225-1241 (2004).
- 439 2. Bono LM, Draghi JA, Turner PE. Evolvability Costs of Niche Expansion. *Trends*
440 *in genetics : TIG* **36**, 14-23 (2020).
- 441 3. Alneberg J, *et al.* Ecosystem-wide metagenomic binning enables prediction of
442 ecological niches from genomes. *Commun Biol* **3**, 119 (2020).
- 443 4. Graham ED, Tully BJ. Marine Dada bacteria exhibit genome streamlining and
444 phototrophy-driven niche partitioning. *The ISME journal* **15**, 1248-1256 (2021).
- 445 5. Chu X, Li S, Wang S, Luo D, Luo H. Gene loss through pseudogenization
446 contributes to the ecological diversification of a generalist Roseobacter lineage.
447 *The ISME journal* **15**, 489-502 (2021).
- 448 6. Salcher MM, Schaeffle D, Kaspar M, Neuenschwander SM, Ghai R. Evolution in
449 action: habitat transition from sediment to the pelagial leads to genome
450 streamlining in Methylophilaceae. *The ISME journal* **13**, 2764-2777 (2019).
- 451 7. Ankrah NYD, Chouaia B, Douglas AE. The Cost of Metabolic Interactions in
452 Symbioses between Insects and Bacteria with Reduced Genomes. *mBio* **9**,
453 (2018).
- 454 8. Getz EW, Tithi SS, Zhang L, Aylward FO. Parallel Evolution of Genome
455 Streamlining and Cellular Bioenergetics across the Marine Radiation of a
456 Bacterial Phylum. *mBio* **9**, (2018).
- 457 9. Andrei AS, Salcher MM, Mehrshad M, Rychtecky P, Znachor P, Ghai R. Niche-
458 directed evolution modulates genome architecture in freshwater Planctomycetes.

- 459 *The ISME journal* **13**, 1056-1071 (2019).
- 460 10. Kuo CH, Ochman H. Deletional bias across the three domains of life. *Genome*
461 *biology and evolution* **1**, 145-152 (2009).
- 462 11. Batut B, Knibbe C, Marais G, Daubin V. Reductive genome evolution at both ends
463 of the bacterial population size spectrum. *Nat Rev Microbiol* **12**, 841-850 (2014).
- 464 12. Maistrenko OM, *et al.* Disentangling the impact of environmental and
465 phylogenetic constraints on prokaryotic within-species diversity. *The ISME*
466 *journal* **14**, 1247-1259 (2020).
- 467 13. Keeling PJ, Palmer JD. Horizontal gene transfer in eukaryotic evolution. *Nature*
468 *reviews Genetics* **9**, 605-618 (2008).
- 469 14. Daubin V, Szöllősi GJ. Horizontal Gene Transfer and the History of Life. *Cold*
470 *Spring Harbor Perspectives in Biology* **8**, a018036 (2016).
- 471 15. Posfai G, *et al.* Emergent properties of reduced-genome *Escherichia coli*. *Science*
472 **312**, 1044-1046 (2006).
- 473 16. Kato J, Hashimoto M. Construction of consecutive deletions of the *Escherichia*
474 *coli* chromosome. *Molecular systems biology* **3**, 132 (2007).
- 475 17. Mizoguchi H, Sawano Y, Kato J, Mori H. Superpositioning of deletions promotes
476 growth of *Escherichia coli* with a reduced genome. *DNA research* **15**, 277-284
477 (2008).
- 478 18. Rees-Garbutt J, Chalkley O, Landon S, Purcell O, Marucci L, Grierson C.
479 Designing minimal genomes using whole-cell models. *Nature communications* **11**,
480 836 (2020).
- 481 19. Xavier JC, Patil KR, Rocha I. Systems biology perspectives on minimal and
482 simpler cells. *Microbiol Mol Biol Rev* **78**, 487-509 (2014).
- 483 20. Kurokawa M, Seno S, Matsuda H, Ying BW. Correlation between genome
484 reduction and bacterial growth. *DNA research* **23**, 517-525 (2016).
- 485 21. Karcagi I, *et al.* Indispensability of Horizontally Transferred Genes and Its Impact
486 on Bacterial Genome Streamlining. *Molecular biology and evolution* **33**, 1257-
487 1269 (2016).
- 488 22. Nishimura I, Kurokawa M, Liu L, Ying BW. Coordinated Changes in Mutation
489 and Growth Rates Induced by Genome Reduction. *mBio* **8**, (2017).
- 490 23. Satterwhite RS, Cooper TF. Constraints on adaptation of *Escherichia coli* to
491 mixed-resource environments increase over time. *Evolution* **69**, 2067-2078 (2015).
- 492 24. Baym M, *et al.* Spatiotemporal microbial evolution on antibiotic landscapes.
493 *Science* **353**, 1147-1151 (2016).
- 494 25. Ashino K, Sugano K, Amagasa T, Ying BW. Predicting the decision making

- 495 chemicals used for bacterial growth. *Sci Rep* **9**, 7251 (2019).
- 496 26. Choe D, *et al.* Adaptive laboratory evolution of a genome-reduced *Escherichia*
497 *coli*. *Nature communications* **10**, 935 (2019).
- 498 27. Padfield D, Vujakovic A, Paterson S, Griffiths R, Buckling A, Hesse E. Evolution
499 of diversity explains the impact of pre-adaptation of a focal species on the
500 structure of a natural microbial community. *The ISME journal* **14**, 2877-2889
501 (2020).
- 502 28. Fragata I, Blanckaert A, Dias Louro MA, Liberles DA, Bank C. Evolution in the
503 light of fitness landscape theory. *Trends in ecology & evolution* **34**, 69-82 (2019).
- 504 29. de Visser JA, Krug J. Empirical fitness landscapes and the predictability of
505 evolution. *Nature reviews Genetics* **15**, 480-490 (2014).
- 506 30. Jasmin JN, Zeyl C. Evolution of pleiotropic costs in experimental populations.
507 *Journal of evolutionary biology* **26**, 1363-1369 (2013).
- 508 31. Tenaillon O. The Utility of Fisher's Geometric Model in Evolutionary Genetics.
509 *Annu Rev Ecol Evol Syst* **45**, 179-201 (2014).
- 510 32. Martin G, Lenormand T. The fitness effect of mutations across environments:
511 Fisher's geometrical model with multiple optima. *Evolution; international journal*
512 *of organic evolution* **69**, 1433-1447 (2015).
- 513 33. Nahum JR, Godfrey-Smith P, Harding BN, Marcus JH, Carlson-Stevermer J, Kerr
514 B. A tortoise-hare pattern seen in adapting structured and unstructured populations
515 suggests a rugged fitness landscape in bacteria. *Proceedings of the National*
516 *Academy of Sciences of the United States of America* **112**, 7530-7535 (2015).
- 517 34. Bajic D, Vila JCC, Blount ZD, Sanchez A. On the deformability of an empirical
518 fitness landscape by microbial evolution. *Proceedings of the National Academy of*
519 *Sciences of the United States of America* **115**, 11286-11291 (2018).
- 520 35. Barrick JE, Kauth MR, Streliaoff CC, Lenski RE. *Escherichia coli* *rpoB* mutants
521 have increased evolvability in proportion to their fitness defects. *Molecular*
522 *biology and evolution* **27**, 1338-1347 (2010).
- 523 36. Schick A, Bailey SF, Kassen R. Evolution of Fitness Trade-Offs in Locally
524 Adapted Populations of *Pseudomonas fluorescens*. *The American naturalist* **186**
525 **Suppl 1**, S48-59 (2015).
- 526 37. Szamecz B, *et al.* The genomic landscape of compensatory evolution. *PLoS*
527 *biology* **12**, e1001935 (2014).
- 528 38. Soucy SM, Huang J, Gogarten JP. Horizontal gene transfer: building the web of
529 life. *Nature reviews Genetics* **16**, 472-482 (2015).
- 530 39. Martinez-Cano DJ, *et al.* Evolution of small prokaryotic genomes. *Frontiers in*

- 531 *microbiology* **5**, 742 (2014).
- 532 40. Goddard MR, Bradford MA. The adaptive response of a natural microbial
533 population to carbon- and nitrogen-limitation. *Ecology letters* **6**, 594-598 (2003).
- 534 41. Rodriguez-Verdugo A, Carrillo-Cisneros D, Gonzalez-Gonzalez A, Gaut BS,
535 Bennett AF. Different tradeoffs result from alternate genetic adaptations to a
536 common environment. *Proceedings of the National Academy of Sciences of the*
537 *United States of America* **111**, 12121-12126 (2014).
- 538 42. Chavhan Y, Malusare S, Dey S. Larger bacterial populations evolve heavier fitness
539 trade-offs and undergo greater ecological specialization. *Heredity* **124**, 726-736
540 (2020).
- 541 43. Sexton JP, Montiel J, Shay JE, Stephens MR, Slatyer RA. Evolution of Ecological
542 Niche Breadth. *Annu Rev Ecol Evol S* **48**, 183-206 (2017).
- 543 44. Cooper VS, Lenski RE. The population genetics of ecological specialization in
544 evolving *Escherichia coli* populations. *Nature* **407**, 736-739 (2000).
- 545 45. Bono LM, Smith LB, Jr., Pfennig DW, Burch CL. The emergence of performance
546 trade-offs during local adaptation: insights from experimental evolution.
547 *Molecular ecology* **26**, 1720-1733 (2017).
- 548 46. Wang S, Dai L. Evolving generalists in switching rugged landscapes. *PLoS*
549 *computational biology* **15**, e1007320 (2019).
- 550 47. Cooper VS. Long-term experimental evolution in *Escherichia coli*. X. Quantifying
551 the fundamental and realized niche. *BMC evolutionary biology* **2**, 12 (2002).
- 552 48. Wackett LP, Dodge AG, Ellis LB. Microbial genomics and the periodic table.
553 *Applied and environmental microbiology* **70**, 647-655 (2004).
- 554 49. Kassen R. The experimental evolution of specialists, generalists, and the
555 maintenance of diversity. *Journal of evolutionary biology* **15**, 173-190 (2002).
- 556 50. Remold S. Understanding specialism when the jack of all trades can be the master
557 of all. *P Roy Soc B-Biol Sci* **279**, 4861-4869 (2012).
- 558 51. Shoal O, *et al.* Evolutionary trade-offs, Pareto optimality, and the geometry of
559 phenotype space. *Science* **336**, 1157-1160 (2012).
- 560 52. Fraebel DT, Mickalide H, Schnitkey D, Merritt J, Kuhlman TE, Kuehn S.
561 Environment determines evolutionary trajectory in a constrained phenotypic
562 space. *Elife* **6**, (2017).
- 563 53. Farahpour F, Saeedghalati M, Brauer VS, Hoffmann D. Trade-off shapes diversity
564 in eco-evolutionary dynamics. *Elife* **7**, (2018).
- 565 54. Ferenci T. Trade-off Mechanisms Shaping the Diversity of Bacteria. *Trends in*
566 *microbiology* **24**, 209-223 (2016).

- 567 55. Kurokawa M, Ying BW. Precise, High-throughput Analysis of Bacterial Growth.
568 *J Vis Exp*, (2017).
- 569 56. Kishimoto T, *et al.* Transition from positive to neutral in mutation fixation along
570 with continuing rising fitness in thermal adaptive evolution. *PLoS genetics* **6**,
571 e1001164 (2010).
- 572 57. Barrick JE, *et al.* Identifying structural variation in haploid microbial genomes
573 from short-read resequencing data using breseq. *BMC genomics* **15**, 1039 (2014).
- 574 58. Kishimoto T, *et al.* Molecular Clock of Neutral Mutations in a Fitness-Increasing
575 Evolutionary Process. *PLoS genetics* **11**, e1005392 (2015).
- 576

577 **Figure legends**

578 **Figure 1 Conceptual illustration of the study. A.** Fitness landscapes of the reduced
579 genomes in the environmental gradient. Environment. The evolution of the genomes
580 (open circles), from Anc to Evo, leads to not only local adaptation to the environment for
581 evolution (C0, white broken line) but also changes in fitness landscapes across the
582 environmental gradient. The colour gradation from red to blue indicates the fitness from
583 high to low. **B.** Overview of the experiments and analyses performed in the present study.
584 Five genomes (strains) are shown as circles. Black arrows indicate the experimental
585 and/or analytical studies. The keywords newly defined in the present study are underlined.
586 The colour variation and gradation represent the difference in the chemical components
587 (niches) and the concentration gradient, respectively.

588

589 **Figure 2 Correlation between local adaptation and genome reduction. A.** Temporal
590 changes in growth rates during the experimental evolution. The five genomes are
591 indicated. **B.** Growth rates in the medium for evolution. Open and closed bars represent
592 the growth rates of Ancs and Evos in the medium C0, respectively. Standard errors of
593 biological replications ($n > 6$) are indicated. Asterisks indicate the statistical significance
594 of the two-tailed Student's t-test ($p < 0.01$). **C.** Correlation between growth changes and
595 genome reduction. The lengths of the genomic deletions are plotted against the ratio of
596 the growth rates of Anc and Evo (left) and the ratio per generation (right). The Spearman
597 rank correlation coefficients and statistical significance are indicated. The red line
598 indicates the logarithmic regression. **D.** Correlation between the growth rate and cellular
599 redox activity. The left and right panels show the relationships between the growth rate
600 and the redox activity (NADH) and the changes in both, respectively. Open and closed
601 circles represent Ancs and Evos, respectively. The Spearman rank correlation coefficients
602 and statistical significance are indicated.

603

604 **Figure 3 Growth fitness across the environmental gradient. A.** Heatmap of the
605 concentration gradient. The chemical components of the media are indicated. The
606 concentrations are shown on the logarithmic scale. C0 is the environment for evolution.
607 Both C0 and C1~C28 were used for the fitness assay. **B.** Growth fitness across the
608 concentration gradient of individual chemical components. The mean growth rates in the
609 29 medium combinations are shown. The concentrations of the chemical components are
610 shown on a logarithmic scale. The wild-type and reduced genomes are indicated as N0
611 and N7~N28, respectively. Purple and green represent Evo and Anc, respectively.
612 Standard errors of biological replications ($n > 6$) are indicated.

613

614 **Figure 4 Niche broadness. A.** Definition of the niche space. The left and right panels
615 indicate the fitness curve of N7 across the concentration gradient of glucose and the B-
616 spline regression of the normalized fitness curve, in which both the concentration gradient
617 and the growth rates are rescaled within one unit, respectively. The area in shadow was
618 determined as the niche space (S) of N7 in the niche of glucose. **B.** Radar chart of the
619 eight niche spaces. The niche names, i.e., the eight chemical components, and the scale
620 of the niche space are illustrated in the monotone radar chart. **C.** Evolutionary changes in
621 niche broadness. The five genomes are shown separately. Purple and green represent Evo
622 and Anc, respectively. **D.** Niche broadness and divergence. The sum of the eight niche
623 spaces is defined as the total niche broadness (left). The standard deviation of the eight
624 niche spaces is defined as the niche divergence (right). **E.** Correlation of niche broadness
625 to local adaptation. The changes in niche broadness between Ancs and Evos are plotted
626 against the lengths of the genomic deletion (left) and the changes in growth rates of the
627 local adaptation (right). The Spearman rank correlation coefficients and statistical
628 significance are indicated.

629

630 **Figure 5 Niche-differentiated changes in the niche space caused by local adaptation.**
631 **A.** Relationships between the changes in niche space and the changes in growth rate. The
632 changes in niche space of the five genomes are plotted against the changes in growth rate
633 in the medium C0 with respect to the eight chemical components (niches). **B.**
634 Relationships between the changes in niche space and the lengths of genomic deletion.
635 The chemical components (niches) and the statistical significance of the Spearman rank
636 correlation are indicated. Boldfaces associated with asterisks represent statistical
637 significance (*, $p < 0.05$; **, $p < 0.01$).

638

639 **Figure 6 Fitness increase attributed to the mutations. A.** Stepwise accumulation of
640 genome mutations. Mutation during the evolution. The temporal changes in the mutations
641 fixed during the evolution are shown. The four reduced genomes and the gene names of
642 mutants are indicated. **B.** Additive increase in the growth rates of the mutants in the
643 medium for evolution. Gradation from white to dark blue indicates the mutants with
644 respect to those shown in **A**. Standard errors of biological replications ($n > 6$) are indicated.
645 Asterisks indicate the statistical significance of the two-tailed Student's t-test (*, $p < 0.05$;
646 **, $p < 0.01$). **C.** Correlation between mutation accumulation and changes in growth rates.
647 A total of 12 mutants of a single accumulated mutation are shown. The Spearman rank
648 correlation coefficients and statistical significance are indicated.

649

650 **Figure 7 Mechanism for pre-adaptation.** **A.** Illustration of the proposed mechanism.
651 The fitness landscapes in environment C_0 for the evolution and the alternative
652 environments C_N are shown as the planes in blue. The red cross and the contour lines
653 indicate the fitness peak (maximum) and the fitness gradient, respectively. The open and
654 closed circles indicate the ancestor and evolved genomes, respectively. The size of the
655 circles indicates the genome size. **B.** Correlated niche spaces of Ancs and Evos. The
656 colour variation from white to dark red represents the five different genomes of $N_0 \sim N_{28}$.
657 The niche spaces of Ancs and Evos are named S_{Anc} and S_{Evo} , respectively. **C.**
658 Relationships of S_{Anc} and S_{Evo} to the changes in niche spaces. The changes in the niche
659 space caused by the evolution are plotted against S_{Anc} and S_{Evo} in the left and right panels,
660 respectively. The Spearman rank correlation coefficients and statistical significance are
661 indicated.

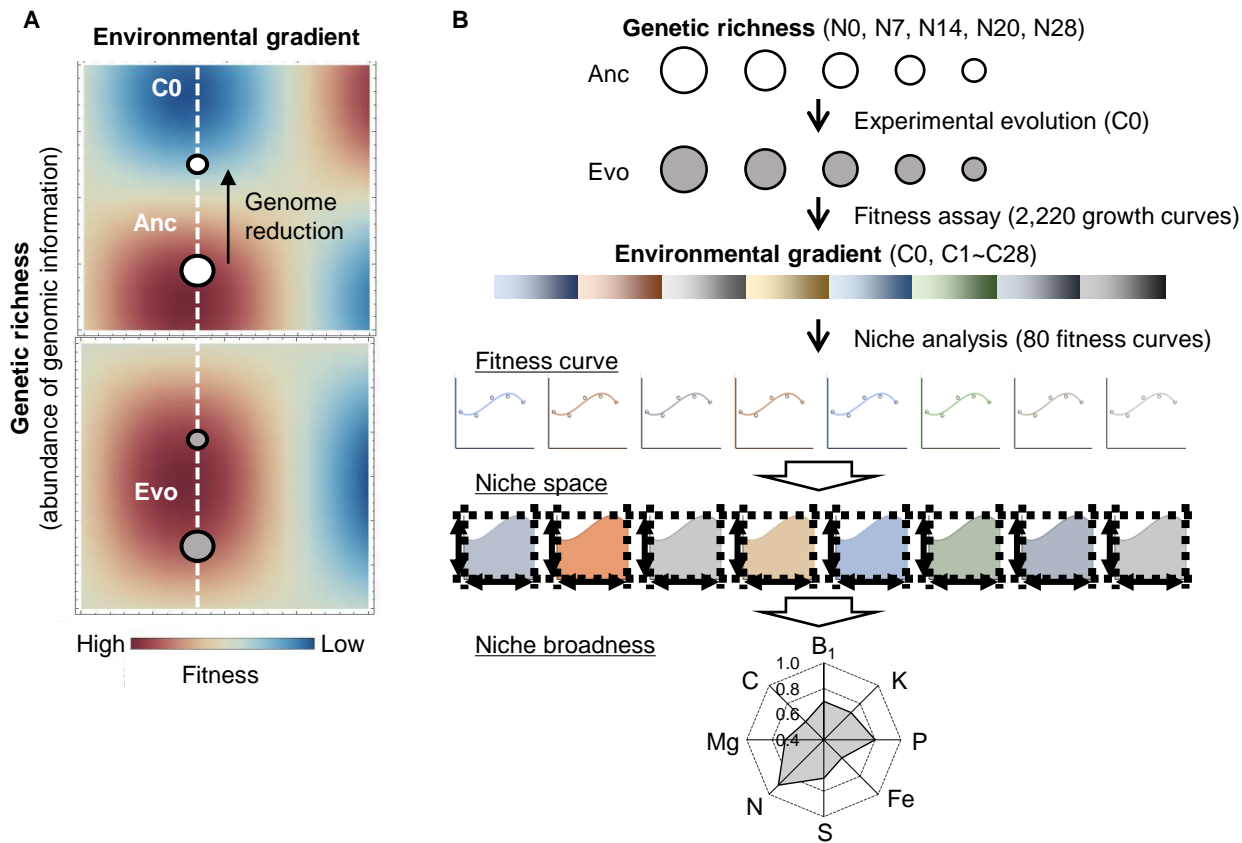


Figure 1

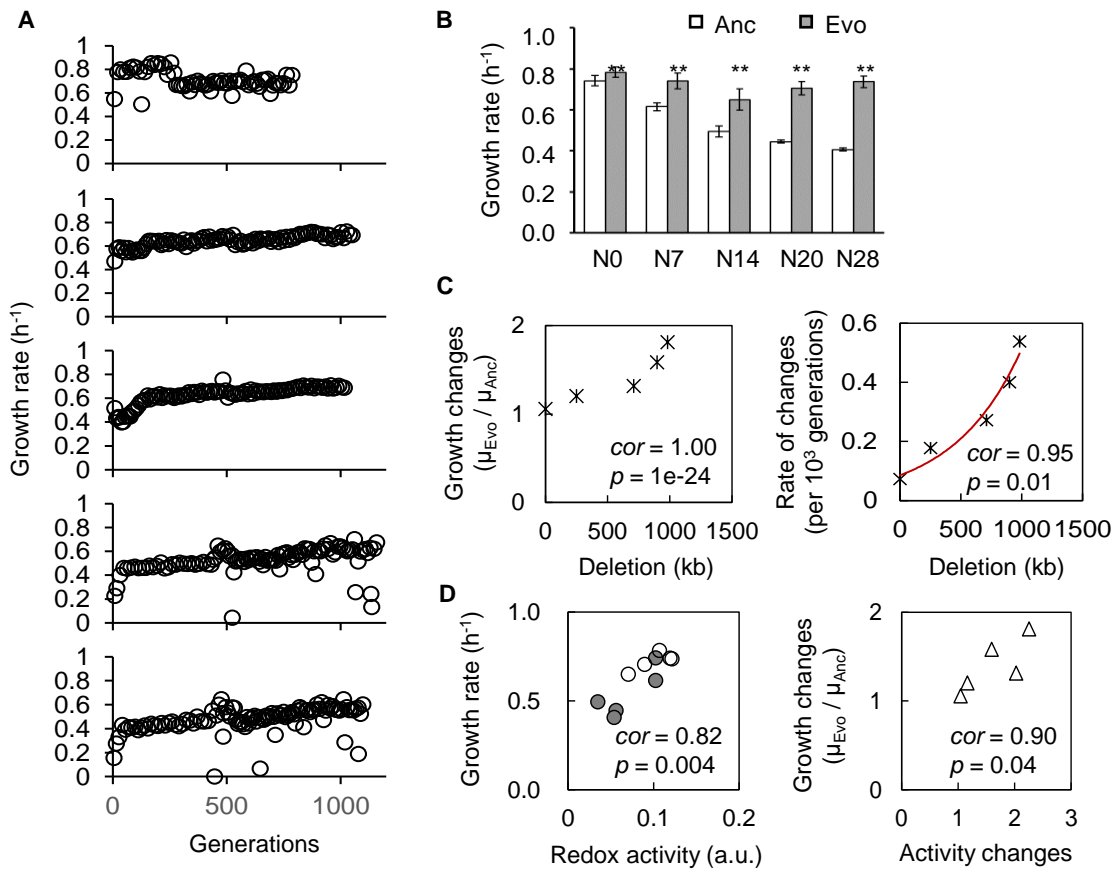


Figure 2

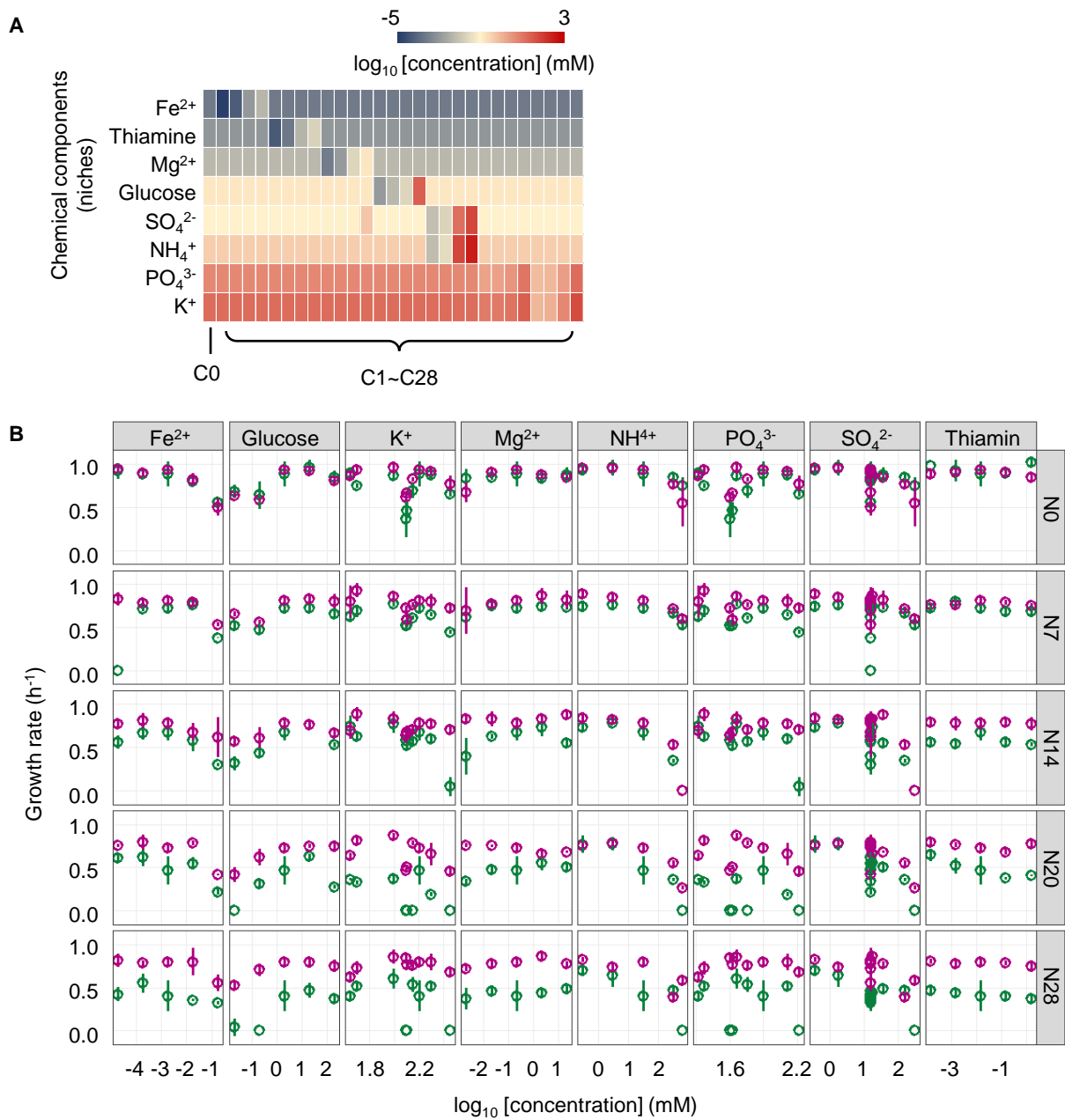


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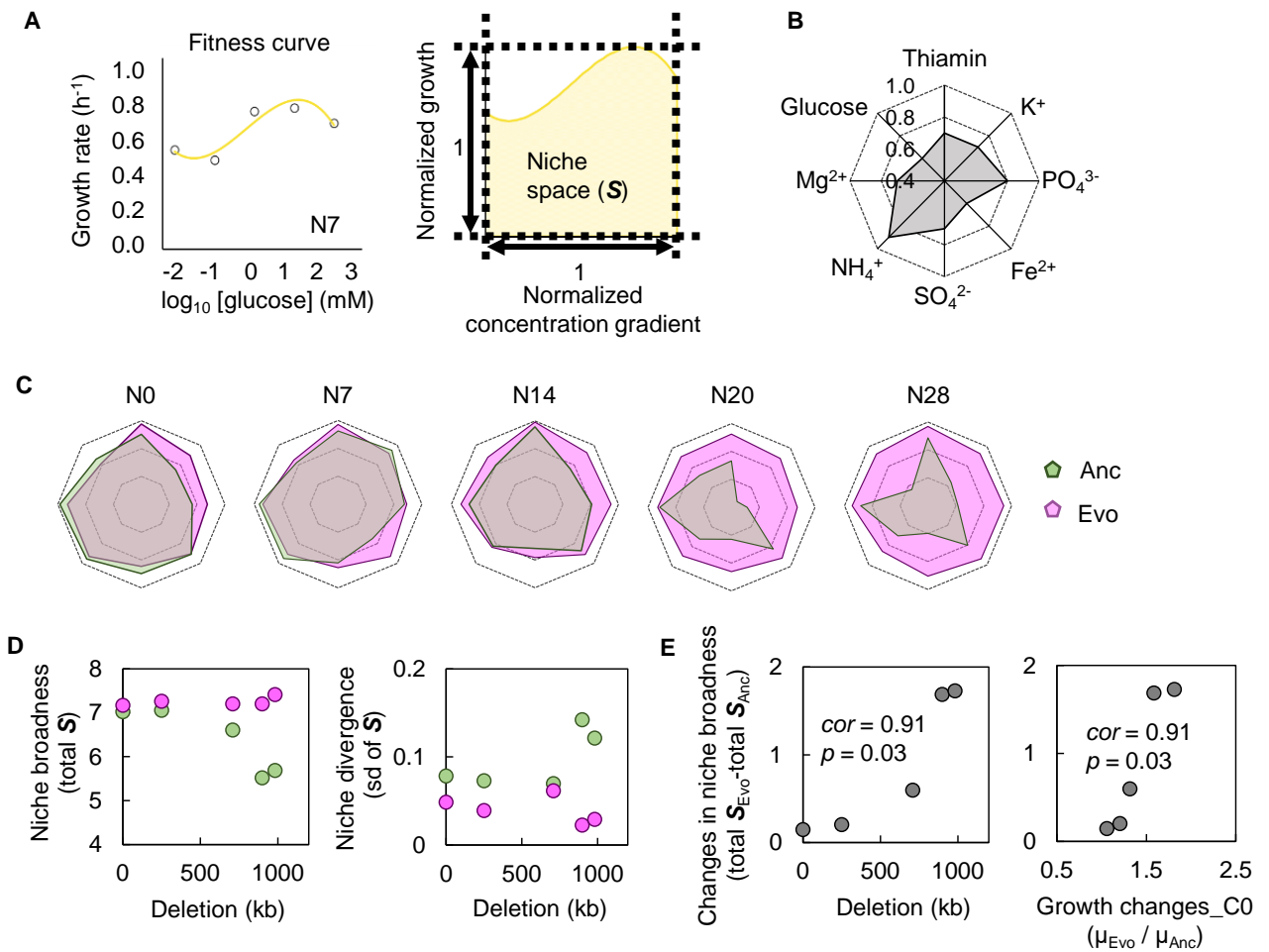


Figure 4

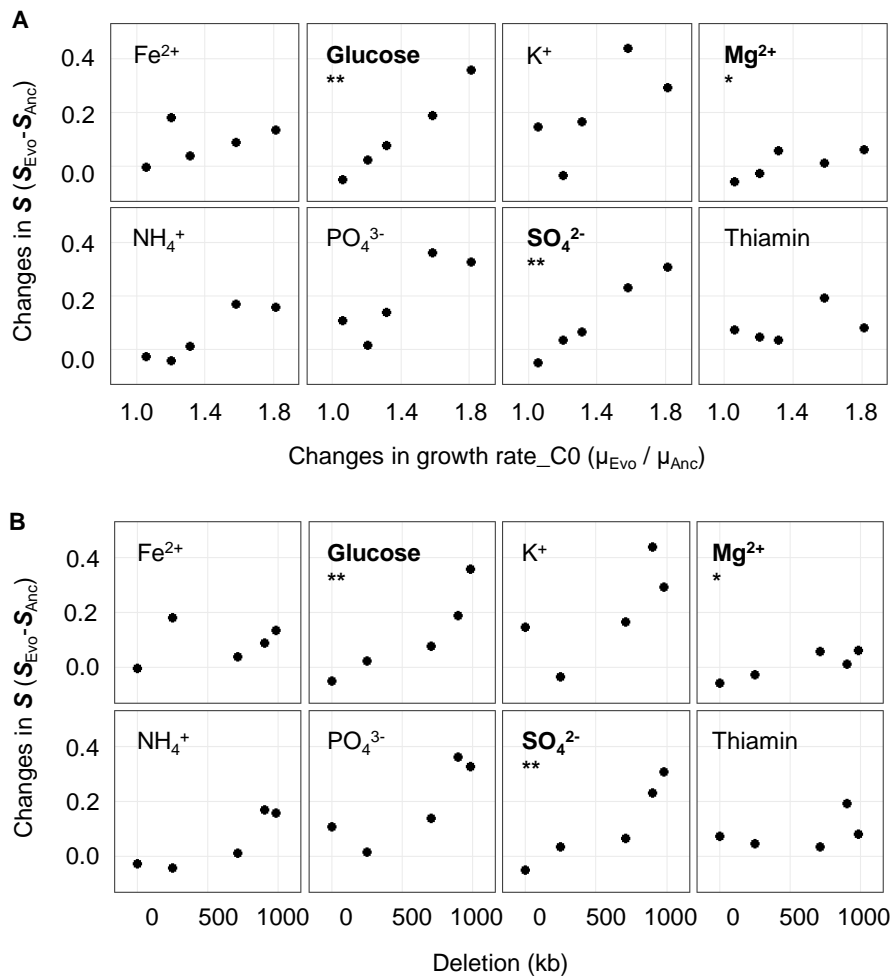


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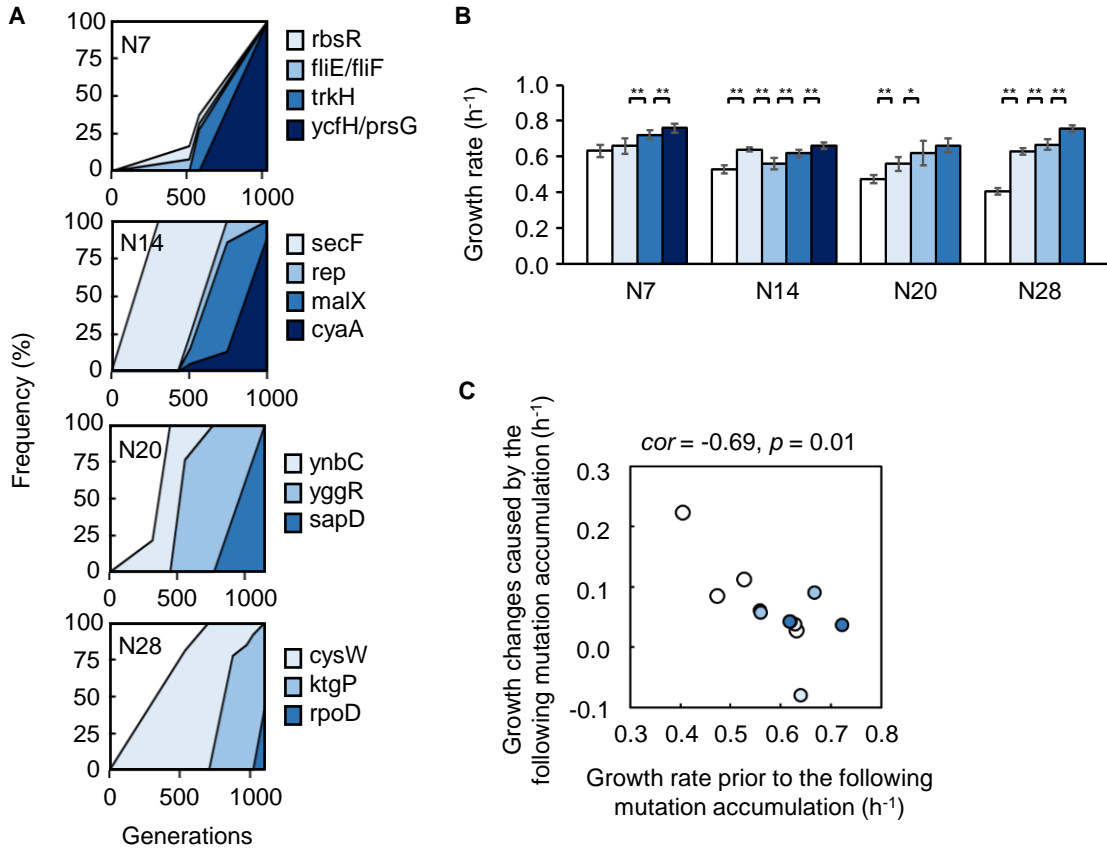


Figure 6

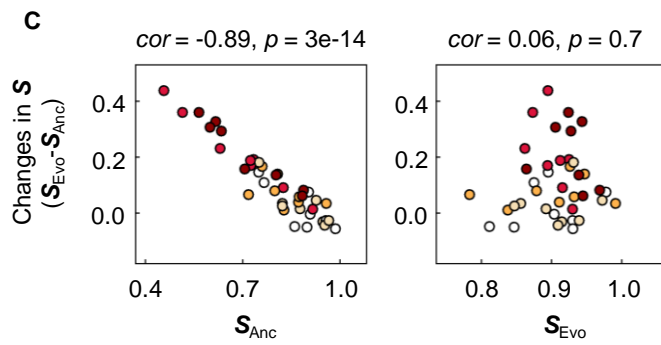
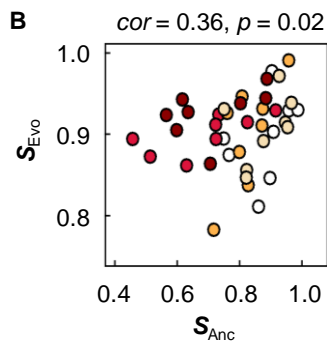
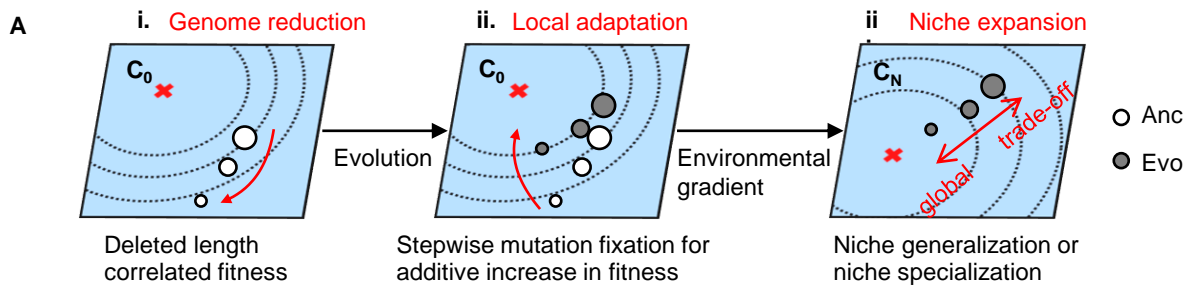


Figure 7