Testing for the fitness benefits of natural transformation during community-embedded evolution

Macaulay Winter[1*], Klaus Harms[2], Pål Jarle Johnsen[2], Angus Buckling[3] and Michiel Vos[1]

1= European Centre for Environment and Human Health, University of Exeter Medical School, Environment and Sustainability Institute, Penryn Campus, TR10 9FE, United Kingdom

2= Microbial Pharmacology and Population Biology Research Group, Department of Pharmacy, Faculty of Health Sciences, UiT - The Arctic University of Norway, Tromsø, Norway

3= Department of Biosciences, University of Exeter, Penryn Campus, TR10 9FE, United Kingdom

*= corresponding author mw730@exeter.ac.uk

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Abstract

Natural transformation is a process where bacteria actively take up DNA from the environment and recombine it into their genome or reconvert it into extra-chromosomal genetic elements. The evolutionary benefits of transformation are still under debate. One main explanation is that foreign allele and gene uptake facilitates natural selection by increasing genetic variation, analogous to

meiotic sex. However, previous experimental evolution studies comparing fitness gains of evolved transforming- and isogenic non-transforming strains have yielded mixed support for the "sex hypothesis." Previous studies testing the sex hypothesis for natural transformation have largely ignored species interactions, which theory predicts provide conditions favourable to sex. To test for the adaptive benefits of bacterial transformation, the naturally transformable wildtype *Acinetobacter baylyi* and a transformation-deficient *ΔcomA* mutant were evolved for five weeks. To provide strong and potentially fluctuating selection, *A. baylyi* was embedded in a community of five other bacterial species. DNA from a pool of different *Acinetobacter* strains was provided as a substrate for transformation. No effect of transformation ability on the fitness of evolved populations was found, with fitness increasing non-significantly in most treatments. Populations showed fitness improvement in their respective environments, with no apparent costs of adaptation to competing species. Despite the absence of fitness effects of transformation, wildtype populations evolved variable transformation frequencies that were slightly greater than their ancestor which potentially could be caused by genetic drift.

Introduction

Natural transformation is a process whereby bacteria actively take up free DNA from the environment during a physiological state termed competence, followed by the recombination of this DNA into the recipient's genome (or its reconversion into extra-chromosomal genetic elements). Natural transformation has been demonstrated in 80+ species across divergent bacterial lineages (Johnston *et al.*, 2014) but is likely to be present in more species. Natural transformation can mediate the cell-to-cell transfer of large tracts of DNA, including virulence (Frosch and Meyer, 1992), antibiotic resistance (Blokesch, 2016; Winter *et al.*, 2021) and metabolic genes (Tumen-Velasquez *et*

46 *al.*, 2018), making it one of the main prokaryote Horizontal Gene Transfer (HGT) mechanisms. The
47 uptake of free DNA from the environment has been argued to provide three distinct (but not
48 mutually exclusive) potential benefits to cells. First, as a source of nucleotides to be used for energy
49 or building blocks (with recombination or maintenance of extrachromosomal DNA being a by-
50 product) (Redfield, 1993, 2001; Hülter *et al.*, 2017), second, to serve as templates for repairing
51 genetic damage (Michod, Wojciechowski and Hoezler, 1988; Hoelzer and Michod, 1991; Mongold,
52 1992; Steinmoen, Knutsen and Håvarstein, 2002; Guiral *et al.*, 2005; Ambur *et al.*, 2016; Hülter *et al.*,
53 2017), and third as a mechanism to create genetic variation (Vos, 2009; Ambur *et al.*, 2016).

54

55 The genetic variation or 'sex' function of natural transformation has received most attention. Unlike
56 many other HGT mechanisms, natural transformation is not mediated by Mobile Genetic Elements
57 but is solely under the control of the recipient cell (Seitz and Blokesch, 2013; Johnston *et al.*, 2014;
58 Dubnau and Blokesch, 2019) and therefore could be assumed to be adaptive. By recombining
59 adaptive alleles and genes in the same genomic background, natural transformation can result in the
60 avoidance of clonal interference, allowing populations to adapt more rapidly. Indeed, both
61 mathematical modelling (Levin and Cornejo, 2009; Engelstädter and Moradigaravand, 2013;
62 Peabody, Li and Kao, 2017) and laboratory evolution experiments (Baltrus, Guillemin and Phillips,
63 2007; Woods *et al.*, 2020; Nguyen *et al.*, 2022) have supported this hypothesis. However, there
64 remains controversy about the sex function of transformation, and not all experimental studies have
65 found that fitness of recombining wildtype cells increased after evolution compared to isogenic,
66 non-recombinogenic mutants. For instance, evolution experiments utilising the model system
67 *Acinetobacter baylyi* found transformation-mediated fitness benefits either to be present (Perron *et*
68 *al.*, 2012), equivocal (Renda *et al.*, 2015; Utnes *et al.*, 2015) or absent (Bacher, Metzgar and De
69 Crécy-Lagard, 2006; McLeman *et al.*, 2016). Multiple studies found the ability to transform was lost
70 during experimental evolution, indicating that any potential recombination-mediated fitness

71 benefits were outweighed by the cost of maintaining the molecular machinery involved (Bacher,
72 Metzgar and De Crécy-Lagard, 2006; Renda *et al.*, 2015).

73

74 Studies to date lack interactions with multiple competitors that almost certainly characterise most
75 natural situations. This could be an important shortcoming, as for sex to remain selectively
76 advantageous it is necessary for selection to be strong and dynamic (Charlesworth, 1993; Burt,
77 2000), and interspecific competitors could greatly influence both these requirements (Otto and
78 Nuismer, 2004; Kawecki *et al.*, 2012). However, while interspecific competition can create
79 fluctuating conditions, it can also constrain evolution (Luján *et al.*, 2022). Experimental evolution
80 approaches have hitherto relied on evolving recombining clones in isolation. To study the
81 evolutionary benefits of natural transformation in the context of species interactions, we here
82 experimentally evolve a transformable *A. baylyi* wildtype and a non-transformable isogenic *ΔcomA*
83 mutant for five weeks in the presence of other species (*i.e.,* under biotic conditions) or alone (*i.e.,*
84 under abiotic conditions). Specifically, we use a system of five bacterial species which have been
85 previously shown to stably coexist (Castledine, Padfield and Buckling, 2020; Padfield *et al.*, 2020;
86 Newbury *et al.*, 2022). Our experimental evolution approach allows us to test 1) whether the
87 evolved wildtype strain will be fitter than its non-recombining counterpart, specifically after
88 evolution under biotic conditions and 2) whether transformation rate of the evolved wildtype has
89 changed in response to these treatments.

90

91 **Material and Methods**

92

93 <u>*Acinetobacter baylyi* ADP1 constructs</u>

94 Two variants of the wildtype *A. baylyi* ADP1 strain with chromosomally encoded GFP and RFP,
95 respectively were constructed using fluorescence::AMR cassettes derived from strains gifted by the
96 Charpentier lab (Claude Bernard University, Lyon), and Hasty lab (University of San Diego, California),
97 respectively. Briefly, the *sfGFP::apraR* cassette (Charpentier and Laaberki, unpublished) and
98 *mCherry::specR* (Cooper, Tsimring and Hasty, 2017) cassettes were amplified via PCR with 1kb
99 flanking regions homologous to the *att*Tn*7* locus and a putative prophage p4 region, respectively.
100 Primers used for the *sfGFP::apraR* and *mCherry::specR* cassettes were 5'3
101 AAAGCCAATCGCTGACAGATGGTGG-3', 5'-TTGGTCAGTGCCTGTCTTGCTGGTGAGCCGGTACGC-3', and
102 5'-TCACCTGCATCCACTCAAGTGTCGTTT-3', 5'-AAAGCCAATCGCTGACAGATGGTGG-3', respectively
103 (Integrated DNA Technologies, USA). PCR products were added to *A. baylyi* in LB Miller broth
104 (Formedium, England) at 37˚C and 180rpm for 24 hours at 1µg/mL to allow for chromosomal
105 recombination of PCR amplificates via natural transformation Transformants were isolated by
106 plating on LB agar containing 240µg/mL apramycin (Duchefa, The Netherlands), or 360µg/mL
107 spectinomycin (Melford, UK), respectively. Next, non-competent counterparts of each fluorescent
108 strain were generated by deletion of the *comA* gene via sequential natural transformation with
109 linearised plasmids pKHNH6 and pKHNH3. Plasmids pKHNH6 and pKHNH3 were linearised prior to
110 transformation using restriction enzymes EcoRV (Promega, USA) and KpnI (Fisher, USA), respectively.
111 pKHNH6 carried a *comA*$^{+}$::(*nptII sacB*) allele embedded in its natural flanking regions, and natural
112 transformation of the respective fluorescence-marked *A. baylyi* strains by linearized plasmid DNA
113 resulted in transformation-proficient isolates that were kanamycin-resistant and sensitive to 50g/L
114 sucrose. Transformation of those respective isolates by pKHNH3 (carrying a *ΔcomA* allele) resulted in
115 a sucrose-resistant, kanamycin-susceptible and transformation-deficient strains, respectively
116 Deletion of *comA* was verified using agar containing 50g/L sucrose, agar containing 10ug/mL
117 kanamycin, and by PCR using primers 5'-TTGGTGTGATTGGTACGGTGGCTGGTGC-3' and 5'-
118 CTTGCAGACGATTGCTTACCTCAGCACTCGG-3'. The non-competent *A. baylyi* strains were confirmed
119 to be non-transformable at the detectable limit (10^{-7}) in all (6) technical replicates.

120

121 Five-species community

122 The five-species community was composed of compost isolates belonging to the genera
123 *Pseudomonas, Achromobacter, Variovorax, Ochrobactrum* and *Stenotrophomonas* (as identified by
124 16S rRNA sequencing) (Castledine, Padfield and Buckling, 2020; Padfield *et al.*, 2020) (Table 1).

125

126 Evolution experiment

127 The apramycin resistant wildtype and *ΔcomA A. baylyi* ADP1 focal strains were separately
128 propagated in three distinct competition environments with sixfold replication. Experimental
129 treatments included competition against none, or all five of the five-species community concurrently
130 (Figure 1). Cultures were grown in 25% TSB medium at 28˚C in static conditions in glass microcosms
131 with one layer of ColiRoller glass beads (Millipore, Merck, USA) covering the base of the microcosm
132 to provide additional spatial structure. Instead of transferring a small volume to new microcosms,
133 spent nutrient broth was replaced with fresh broth in the same microcosms, resulting in
134 approximately 34-fold daily dilutions. This approach maintained spatial structure and saved on glass
135 and plastic waste (Alves *et al.*, 2021). Every 7th day, cultures were vortexed, diluted 10-fold and
136 plated on LB agar plates containing 240µg/ml apramycin to select for the green-fluorescent wildtype
137 or *ΔcomA A. baylyi* focal strain. After 24 hours of incubation at 37˚C, the bacterial lawn from each
138 individual replicate was scraped off with a sterile loop and transferred to a new microcosm for
139 overnight growth (reaching stationary phase). Overnight cultures of the five competitor species were
140 also made in LB broth at this time from -70˚C freezer stocks. Equal volumes of overnight cultures of
141 the focal *A. baylyi* strain and competitor species (where appropriate; Table 1) totalling 100uL was
142 added to a new microcosm containing 9.9mL 25% TSB, commencing the next week of transfers. DNA
143 as a substrate for transformation sourced from a pool of 20 *Acinetobacter* strains was added at the

144 point of nutrient replenishment each day for each treatment (see next section). At the end of the
145 final week of transfers, cultures were plated on LB agar supplemented with 240µg/ml apramycin
146 where 100 colonies were picked and pooled for overnight growth and later frozen at -70˚C in 25%
147 glycerol prior to use in subsequent competition and transformation assays (Fig. 1).

148

149 Donor DNA

150 Twenty *Acinetobacter* strains (table 1; Ray and Nielsen, 2005; Alseth et al., 2019) were cultured
151 separately in LB broth overnight. Cultures were then pooled in equal volumes and lysed following
152 the Qiagen® Genomic DNA Handbook (April 2012) protocol. Combined DNA from the eluate was
153 precipitated by adding two volumes of ice-cold ethanol and centrifuged at 26000xg for 15 minutes to
154 pellet the DNA. DNA was dissolved in TE buffer to a final concentration of 227.2ng/µL (Nanodrop
155 2000, Thermo Scientific) by heating at 50˚C and stirring for 16 hours. DNA was frozen at -20˚C in
156 single-use aliquots for addition to each daily transfer. When used during the evolution period, DNA
157 was diluted to a final concentration of 250ng/ml (the saturating concentration of genomic DNA for
158 transformation in *A. baylyi* (Overballe-Petersen *et al.*, 2013)). The raw and annotated DNA
159 sequencing data for the 19 strains were deposited at the European Nucleotide Archive under
160 BioProject accession number PRJEB55833 (Winter *et al.*, 2023).

161

162 Competition assays

163 For all replicates of the five concurrent species community treatment and the abiotic treatment, 100
164 evolved green-fluorescent wildtype and *ΔcomA A. baylyi* clones were picked, pooled and frozen at –
165 80˚C before use. A mixture containing equal volumes of the green-fluorescent *A. baylyi* pool, a
166 *ΔcomA* red-fluorescent *A. baylyi*, and members of the five species community (where appropriate)
167 was produced for each replicate. One hundred microlitres of mixed culture was immediately

168 inoculated to 9.9ml of 25% TSB and grown for 24 hours in glass microcosms at 28˚C with no
169 agitation. Each of the six replicate evolved populations per treatment were competed with the
170 differentially marked *ΔcomA* strain in conditions identical to treatments in the evolution experiment
171 (i.e., containing either all or none of the five competitor species). Samples were plated 0 hours and
172 24 hours after inoculation on LB agar supplemented with either apramycin (240µg/ml) or
173 spectinomycin (360µg/mL) to determine the densities of the evolved (green)- and *ΔcomA* (red)-
174 fluorescent *A. baylyi*, respectively (Fig. 1).

175

176 Transformation Assays

177 Red fluorescent, spectinomycin-resistance conferring marker DNA was obtained by heating
178 overnight cultures of the *A. baylyi* ADP1 *mCherry::specR* at 70˚C for 1.5 hours and centrifuging at
179 2500xg for 15 minutes and resuspending in reduced volume to produce a 50x concentration of
180 lysate. Lysate was stored at -20˚C for up to one month before downstream application. Freezer
181 stocks of 100 pooled clones for each endpoint population were inoculated in LB broth and grown
182 overnight at 37˚C. Cultures were diluted ten-fold with LB broth supplemented with cell lysate at a
183 final concentration of 2.5x maximum cell density and incubated for 3 hours at 37˚C and 180 rpm.
184 Cells were then plated on LB agar supplemented with 240µg/ml apramycin and 360µg/ml
185 spectinomycin, and non-selective LB agar and incubated for 48 hours at 28˚C. Transformation
186 frequency was calculated by dividing the CFU/mL of the transformed (doubly fluorescent and dually-
187 AMR) population by the total population CFU/mL. As a control for spontaneous spectinomycin
188 resistance mutations, we included a treatment where no *mCherry::specR* DNA was added.

189

190 Statistical Analysis

191 Normal distribution of the data was verified using Shapiro-Wilk tests. To determine the relative
192 fitness of evolved lines relative to the unevolved control in competition experiments, the selection-
193 rate constant was calculated as described in (Lenski *et al.*, 1991). Selection-rate constant estimates
194 were analysed with paired t-tests for comparisons using the mean value for each measured
195 population (treatments tested within assay conditions and grouped by genotype and evolution
196 treatment conditions). Because relative fitness values were often negative (i.e., the common
197 competitor displayed greater fitness than the evolved focal strains), analyses in this assay were
198 conducted using the selection-rate constant in lieu of the relative fitness parameter (Lenski *et al.*,
199 1991). As ancestral populations were not significantly different to each other in fitness as a function
200 of genotype (paired t-tests; biotic assay conditions, $p=0.35$, abiotic assay conditions, $p=0.74$), fitness
201 measurements of the evolved populations were standardised to ancestors by subtracting the
202 ancestral selection-rate constant (averaged for both genotypes) from that of the evolved population
203 in all analyses. Standardised selection rates significantly different to 0 demonstrate fitness change.

204

205 Generalised statements about treatment effects observed in fitness assays were made using linear
206 mixed effect models with the lme4 package v1.29 (Bolker, 2022). Model selection was achieved
207 using backward stepwise regression, using biological replicates as a random explanatory variable.
208 Model residuals were checked using the DHARMa package v0.4.5 (Hartig, 2022). All biological
209 replicates for the selection-rate constant analyses were measured in triplicate and averaged before
210 downstream analyses. Transformation frequencies were analysed non-parametrically with Wilcoxon
211 tests as the data were not normally distributed (Shapiro-Wilks tests, $p<0.001$). T-tests, Wilcoxon,
212 Kruskal-Wallis and Levene testing was conducted using the rstatix package v0.7.0 (Kassambara,
213 2021). In all analyses, a *p* value of less than 0.05 was considered significant. Multiple testing was
214 corrected for with false discovery rate (FDR) correction.

215

216 **Results**

217 Natural transformation does not provide fitness benefits in a community context

218 To test whether natural transformation favours adaptation in a biotic community context compared
219 to an abiotic environment, we evolved a recombining wildtype *A. baylyi* and an isogenic non-
220 recombining *ΔcomA* mutant supplemented with DNA extracted from a pool of conspecifics as a
221 substrate for natural transformation. This five species community has been shown to stably coexist
222 in 1/64th strength tryptic soy broth (TSB), and 28˚C with weekly passages (Padfield *et al.*, 2020), but
223 was found to also exist with *A. baylyi* stably in 25% TSB medium with daily transfers (results not
224 shown). After five weeks of evolution, changes in fitness of each evolved line relative to a single
225 unevolved, differentially marked *ΔcomA* strain were measured using pairwise competition assays.
226 Populations were assayed in both presence and absence of competitors. All populations increased in
227 fitness relative to ancestral populations (1 sample t-tests: $p<0.01$, corrected for multiple testing,
228 Figure 2), except for the wildtype strains evolved in abiotic and biotic conditions when assayed in
229 biotic conditions ($p=0.486$, and $p=0.058$, respectively; Figure 2, Table 1).

230

231 Linear mixed effects models revealed no interactions between explanatory variables: genotype,
232 evolutionary conditions, and assay conditions when predicting fitness increases ($X^2 = 4.2241$, df =4, p
233 = 0.3765; Figure 2). Genotype and evolution conditions are very significant explanatory variables for
234 model predictions ($p<0.001$ and $p<0.01$, respectively). The *ΔcomA* populations appear better
235 adapted to the experimental environments relative to the wildtype (Welch's two sample t-test,
236 $p=0.4401$; Figure 2). Adaptation to abiotic conditions occurs more effectively than to biotic
237 conditions, irrespective of evolution conditions or genotype (t-test, $p<0.0001$). Evolution in a biotic
238 environment leads to greater adaptation to biotic environments, but no greater adaptation to
239 abiotic environments (pairwise t-testing, $p<0.01$, and $p=0.113$, respectively; Figure 2). Pairwise t-

240 tests of the differences in selection-rate constants of the evolved populations (grouped by genotype
241 and evolution conditions) tested separately with respect to their assay conditions (biotic and abiotic)
242 showed no significant differences (p>0.05; Figure 2; Table 2).

243

244 Transformation frequency did not decrease after evolution

245 To test whether the lack of fitness difference of the wildtype strain stemmed from a possible loss of
246 natural transformation, we conducted transformation assays for the abiotic treatment and the five
247 species community treatment (Figure 1). No significant differences in transformation frequency
248 were found between the evolved wildtype lineages across the two evolution environments or the
249 ancestor (Wilcoxon test, p>0.05; Figure 3; Table 4). While transformation frequency did not change
250 significantly, observed transformation frequencies were higher in all evolved wildtype populations
251 than in the ancestor. A separate experiment using donor DNA acquired through chemical lysis (the
252 method used in the evolution experiment), resulted in a much lower transformation frequency for
253 all tested samples and did not show a clear significant difference in transformation frequency
254 between any test groups (p>0.05; Supplemental Figure 1; Table 5).

255

256 **Discussion**

257 Here we tested if recombination mediated by natural transformation in *A. baylyi* provided adaptive
258 benefits under biotic and abiotic experimental conditions. All replicate populations in either
259 treatment increased fitness compared to their ancestors when assayed in abiotic conditions (Figure
260 2). Populations of the non-recombining (*ΔcomA)* genotype also show increased fitness relative to
261 their ancestor when assayed under biotic conditions regardless of evolutionary conditions. This
262 contrasts with the recombining wildtype, which does not show significant increases in fitness after
263 evolution in either environment, contrary to our hypothesis that sex is advantageous in the presence

264 of other species. However, the absence of significant fitness increases observed in the wildtype
265 which was evolved and tested in biotic conditions are probably due to a single tested population
266 which is less fit than the ancestor, lowering the estimation of the mean (Figure 2). Further, no
267 significant differences in fitness between evolved linages were found when comparing across
268 genotypes and assaying in biotic conditions. Therefore, this study has not found a clear and
269 significant beneficial (or deleterious) effect of natural transformation on adaptative evolution.

270

271 There is clear trade-off asymmetry displayed by evolving the wildtype *A. baylyi* in the presence or
272 absence of the five competitor species (biotic and abiotic conditions, respectively). Evolution of the
273 wildtype under biotic conditions did not constrain adaptation to an abiotic environment, but the
274 converse is not true (Figure 2). This is because the factors in abiotic conditions (growth media,
275 temperature, oxygen availability, spatial structure) were also consistent in the biotic condition and
276 are therefore selected for in the biotic environment. However, there may be some adaptive
277 mutations that arise under biotic conditions which are not advantageous in the abiotic environment,
278 and therefore were not selected for during evolution in abiotic conditions (Gomez *et al.*, 2022). Our
279 data show that evolution in a biotic environment best prepares a population for a biotic
280 environment, and does not constrain adaptation to an abiotic environment, regardless of the
281 population's ability to naturally transform.

282

283 Interestingly, mean transformation frequencies of all tested populations increased relative to the
284 transformable ancestor, although this was not statistically significant. This contrasts with previous
285 studies where transformation frequencies declined markedly after experimental evolution (Bacher,
286 Metzgar and De Crécy-Lagard, 2006; Utnes *et al.*, 2015). The maintenance, and potential increase of
287 transformability in all evolved populations suggests that the ability to transform was not selected

288 against and may have increased because of genetic drift, given that it served no apparent adaptive
289 benefit. The increase of transformation frequency at varying rates per population (Figure 4) suggests
290 that significant differences both within and between treatment groups may occur after a more
291 prolonged evolution period.

292

293 Taken together, these findings show no significant evolutionary costs or benefits of transformation
294 in *A. baylyi*. This does not mean that no bacterial species use transformation to facilitate faster
295 adaptation, as the physiological contexts and molecular mechanisms of natural transformation vary
296 across species (Johnston *et al.*, 2014). Specifically, there is convincing evidence supporting the sex
297 hypothesis for transformation in *H. pylori* (Baltrus, Guillemin and Phillips, 2007; Woods *et al.*, 2020;
298 Nguyen *et al.*, 2022). There is no doubt that transformation can give an immediate adaptive benefit
299 to *A. baylyi* such as in the contexts of antimicrobial resistance acquisition (Domingues *et al.*, 2012;
300 Perron *et al.*, 2012; Hülter *et al.*, 2017; Mantilla-Calderon *et al.*, 2019), but its long-term benefits
301 remain uncertain. It is possible that different growth conditions, interactions with live DNA donors,
302 and reduced doubling rates of cells (limiting the frequency and contribution of mutation for
303 adaptation), can better demonstrate the adaptive benefit of natural transformation in *A. baylyi* than
304 what has been shown in this study.

305

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313

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315

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428

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430 Table 1. List of strains used in this study.

Strain	*Fluorescence label*	*Naturally Competent?*	*Antibiotic resistance marker*	*Used as*
wildtype A. baylyi ADP1	sfGFP (green)	Yes	Apramycin (480ug/mL)	Focal strain in evolution experiment
ΔcomA A. baylyi ADP1	sfGFP (green)	No	Apramycin (480ug/mL)	Focal strain in evolution experiment
ΔcomA A. baylyi ADP1	mCherry (red)	No	Spectinomycin (600ug/mL)	Common competitor for fitness assays
Achromobacter	None	No	N/A	Community member
Ochrobactrum	None	No	N/A	Community member
Pseudomonas	None	No	N/A	Community member
Stenotrophomonas	None	No	N/A	Community member
Variovorax	None	No	N/A	Community member

A. sp. 01B0, KmR isolate 2.	None	No	N/A	DNA Donor
A. sp. 26B2, isol. 3.	None	Yes	N/A	DNA Donor
A. sp. 423D, isol. 3.	None	Yes	N/A	DNA Donor
A. sp. 48A1, isol. 3.	None	No	N/A	DNA Donor
A. sp. 511B, isol. 5.	None	No	N/A	DNA Donor
A. sp. 56A1, isol. 3.	None	No	N/A	DNA Donor
A. sp. 62A1, isol. 3.	None	Yes	N/A	DNA Donor
A. sp. 63A1, isol. 7.	None	Yes	N/A	DNA Donor
A. sp. 66A1, isol. 1.	None	No	N/A	DNA Donor
A. sp. 71A1, isol. 4.	None	No	N/A	DNA Donor

A. sp. 81A1, isol. 2.	None	No	N/A	DNA Donor
A. sp. 85A1, isol.3.	None	Yes	N/A	DNA Donor
A. sp. A06, isol. 7.	None	Yes	N/A	DNA Donor
A. sp. A3-6, isol. 3.	None	No	N/A	DNA Donor
A. sp. AD512A, isol. 4.	None	No	N/A	DNA Donor
A. baumannii AZR3410, isol. 1.	None	No	N/A	DNA Donor
A. sp. AZR54, isol. 8.	None	Yes	N/A	DNA Donor
A. calcoaceticus AZR583, isol. 9.	None	Yes	N/A	DNA Donor
A. sp. P1-6, isol. 3.	None	Yes	N/A	DNA Donor

A. baumannii clinical isolate FZ21	None	Unknown	N/A	DNA Donor

431

432

433 Table 2. Pairwise comparisons of evolved populations' fitness gains after 5 weeks of evolution

434 compared to a differentially marked *ΔcomA* A. baylyi strain (t-test).

Genotype	*Evolution conditions*	*Assay conditions*	*n*	*t statistic*	*df*	*p*	*p.adj*	*p.adj.signif*
ΔcomA	Abiotic	Abiotic	6	14.3936022	5	0.0000292	0.0001168	***
ΔcomA	Biotic	Abiotic	6	16.4278834	5	0.0000153	0.0001168	***
Wildtype	Abiotic	Abiotic	6	5.92225311	5	0.00196	0.003136	**
Wildtype	Biotic	Abiotic	5	6.20760288	4	0.00343	0.00457333	**
ΔcomA	Abiotic	Biotic	6	6.00144	5	0.00184	0.003136	**
ΔcomA	Biotic	Biotic	6	7.14547314	5	0.000834	0.002224	**
Wildtype	Abiotic	Biotic	6	0.78449068	5	0.468	0.468	ns
Wildtype	Biotic	Biotic	5	2.7510881	4	0.0513	0.05862857	ns

435 Table 3. Pairwise comparisons of evolved populations' fitness gains after 5 weeks of evolution (t-
436 test).

Assay conditions	*group1*	*group2*	*n 1*	*n 2*	*statistic*	*df*	*p*	*p.adj*	*p.adj.signif*
Abiotic	ΔcomA - Abiotic	ΔcomA - Biotic	6	6	-2.3280544	9.93372775	0.042	0.1008	ns
Abiotic	ΔcomA - Abiotic	Wildtype - Abiotic	6	6	1.05998667	7.29968058	0.323	0.44933333	ns
Abiotic	ΔcomA - Abiotic	Wildtype - Biotic	6	5	0.22473268	5.58934404	0.83	0.83	ns
Abiotic	ΔcomA - Biotic	Wildtype - Abiotic	6	6	2.53678612	7.65122067	0.036	0.1008	ns
Abiotic	ΔcomA - Biotic	Wildtype - Biotic	6	5	1.60665424	5.85610684	0.16	0.27428571	ns
Abiotic	Wildtype - Abiotic	Wildtype - Biotic	6	5	-0.6125396	8.62633793	0.556	0.60654545	ns
Biotic	ΔcomA - Abiotic	ΔcomA - Biotic	6	6	-2.9648507	8.28098244	0.017	0.084	ns
Biotic	ΔcomA - Abiotic	Wildtype - Abiotic	6	6	2.78772855	8.92975545	0.021	0.084	ns
Biotic	ΔcomA - Abiotic	Wildtype - Biotic	6	5	-0.6904628	4.90947446	0.521	0.60654545	ns
Biotic	ΔcomA - Biotic	Wildtype - Abiotic	6	6	4.85335787	9.83403025	0.000701	0.008412	**
Biotic	ΔcomA - Biotic	Wildtype - Biotic	6	5	1.03915502	6.33112928	0.337	0.44933333	ns
Biotic	Wildtype -	Wildtype - Biotic	6	5	-	5.8342106	0.078	0.156	ns

Abiotic	2.1351918	9

437 Table 4. Pairwise comparisons of evolved populations' transformation frequency gains after 5 weeks

438 of evolution (Wilcoxon-test). Samples are tested using heat-killed cell lysate.

group1	*group2*	*n1*	*n2*	*statistic*	*p*	*p.adj*	*p.adj.signif*
ΔcomA Ancestor	Abiotic Culture	1	6	0	0.286	0.4995	ns
ΔcomA Ancestor	Community	1	5	0	0.333	0.4995	ns
ΔcomA Ancestor	WT Ancestor	1	1	0	1	1	ns
Abiotic Culture	Community	6	5	16	0.931	1	ns
Abiotic Culture	WT Ancestor	6	1	6	0.286	0.4995	ns
Community	WT Ancestor	5	1	5	0.333	0.4995	ns

439 Table 5. Pairwise comparisons of evolved populations' transformation frequency gains after 5 weeks

440 of evolution (Wilcoxon-test). Samples are tested using chemically acquired cell lysate.

group1	*group2*	*n1*	*n2*	*statistic*	*p*	*p.adj*	*p.adj.signif*
ΔcomA Ancestor	Abiotic Culture	1	6	0.5	0.313	0.666	ns
ΔcomA Ancestor	Community	1	5	0	0.333	0.666	ns
ΔcomA Ancestor	WT Ancestor	1	1	0	1	1	ns
Abiotic Culture	Community	6	5	11	0.537	0.8055	ns
Abiotic Culture	WT Ancestor	6	1	4	0.857	1	ns
Community	WT Ancestor	5	1	5	0.333	0.666	ns

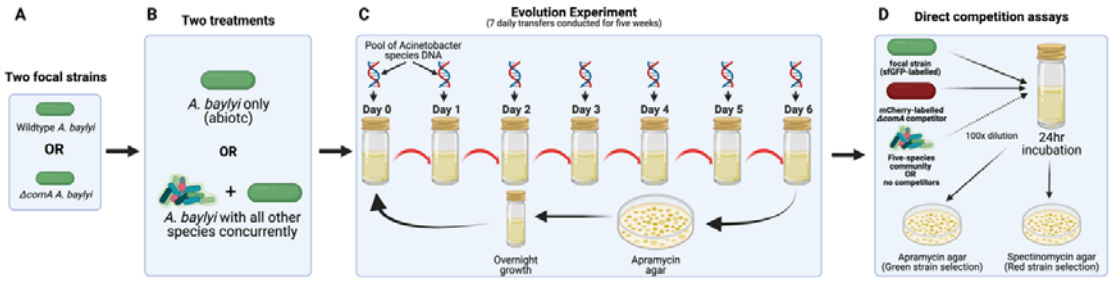
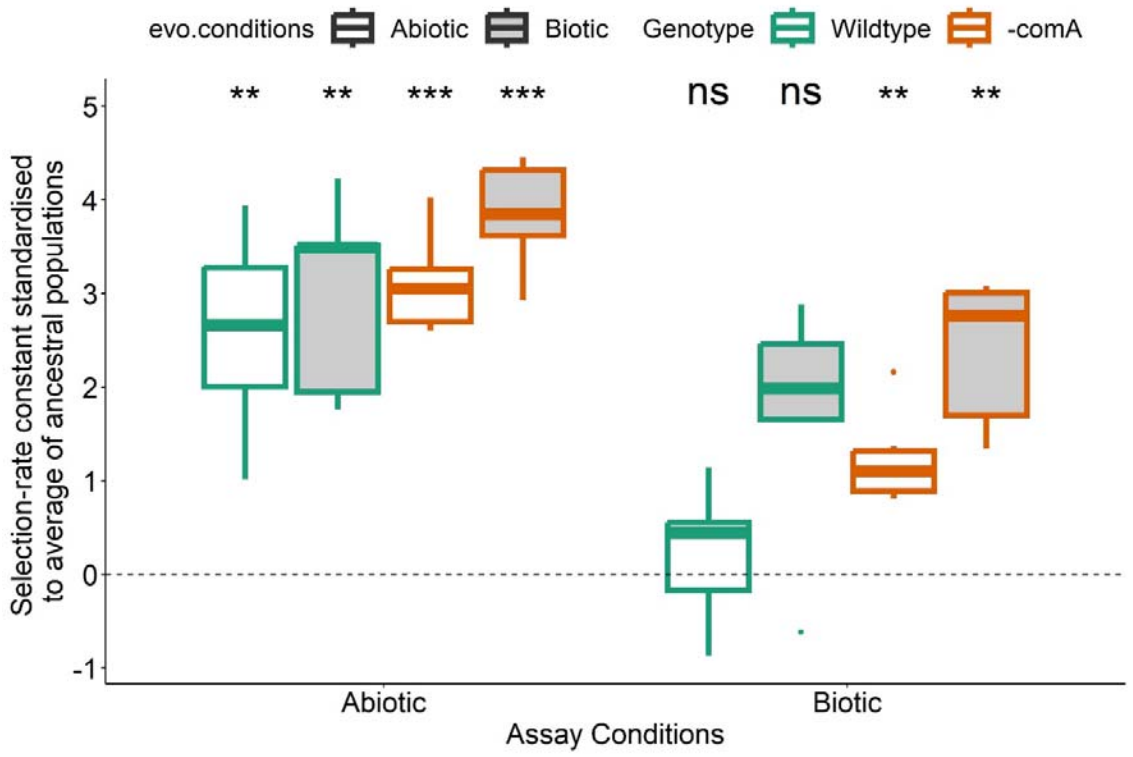


Figure 1. Illustration of the evolution experiment and direct competition assays. A: Two focal strains (competent wildtype and isogenic non-competent counterpart) were cultured separately in their respective treatment conditions (B). B: Focal strains were subjected to two treatment conditions: monoculture (abiotic) or co-culture with five competitor species. C: Cultures were propagated by replacing spent broth with fresh media resulting in an approximately 34-fold dilution each passage. After the 6th passage, all species except the focal strain are killed off using LB agar amended with apramycin. The focal strain and freezer stocks of the competitors were allowed to grow to maximum density in LB broth before being inoculated together for another week of passaging. D: After five full weeks of passaging, the focal strains are selected for again with use of apramycin agar and frozen in 25% glycerol at -70˚C until tested against a common competitor to measure relative fitness. Figure created with BioRender.com.



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455 Figure 2. Selection-rate constants of evolved populations standardised to ancestral populations in
456 respective assay conditions (y=0). Bracketed asterisks describe significant differences (t-test)
457 between evolved populations within the same assay conditions. Non-bracketed asterisks describe
458 significant differences (t-test) between evolved populations' and 0. Fitness differences of each of the
459 six biological replicates per treatment were measured in triplicate. (ns, no significant difference; *,
460 p<0.05; **, p<0.01; ***, p<0.001).

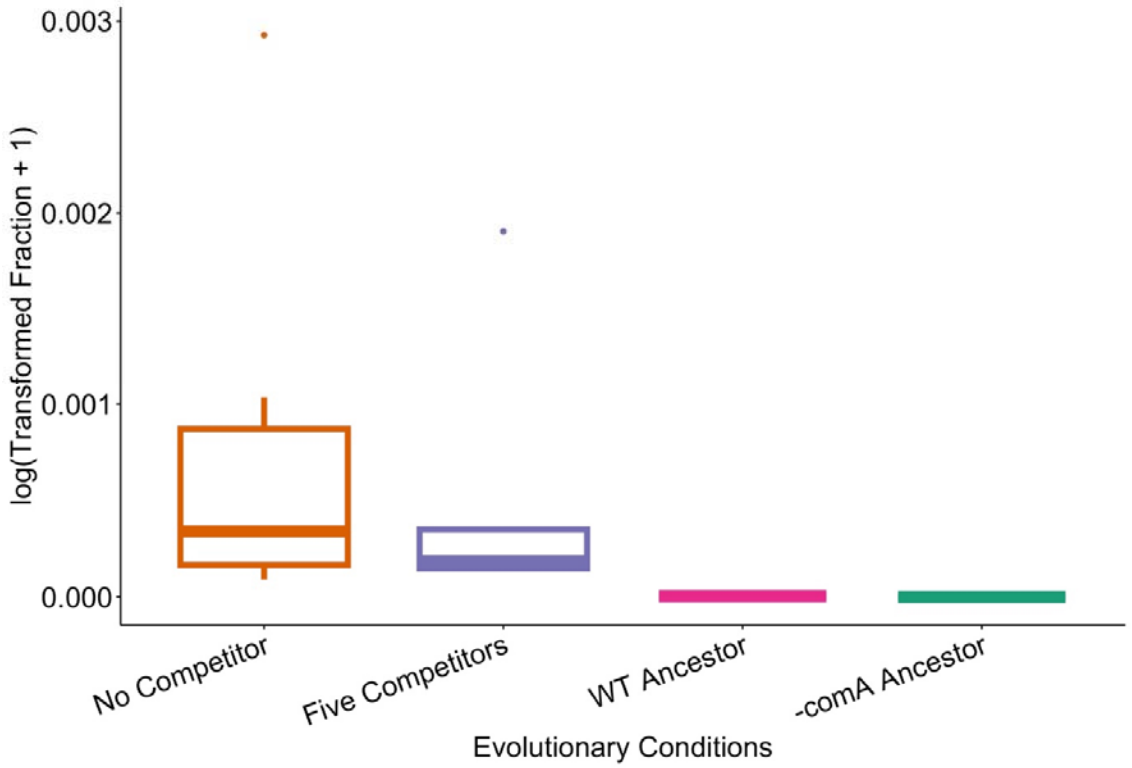

Figure 3. Treatment-level transformation frequencies of ancestral and evolved populations using heat-killed cell lysate. Transformation frequencies were measured in triplicate per biological replicate. The No Competitor, Five Competitor, WT ancestor, and *ΔcomA* ancestor treatments had 6, 5, 1, and 1 biological replicates, respectively.

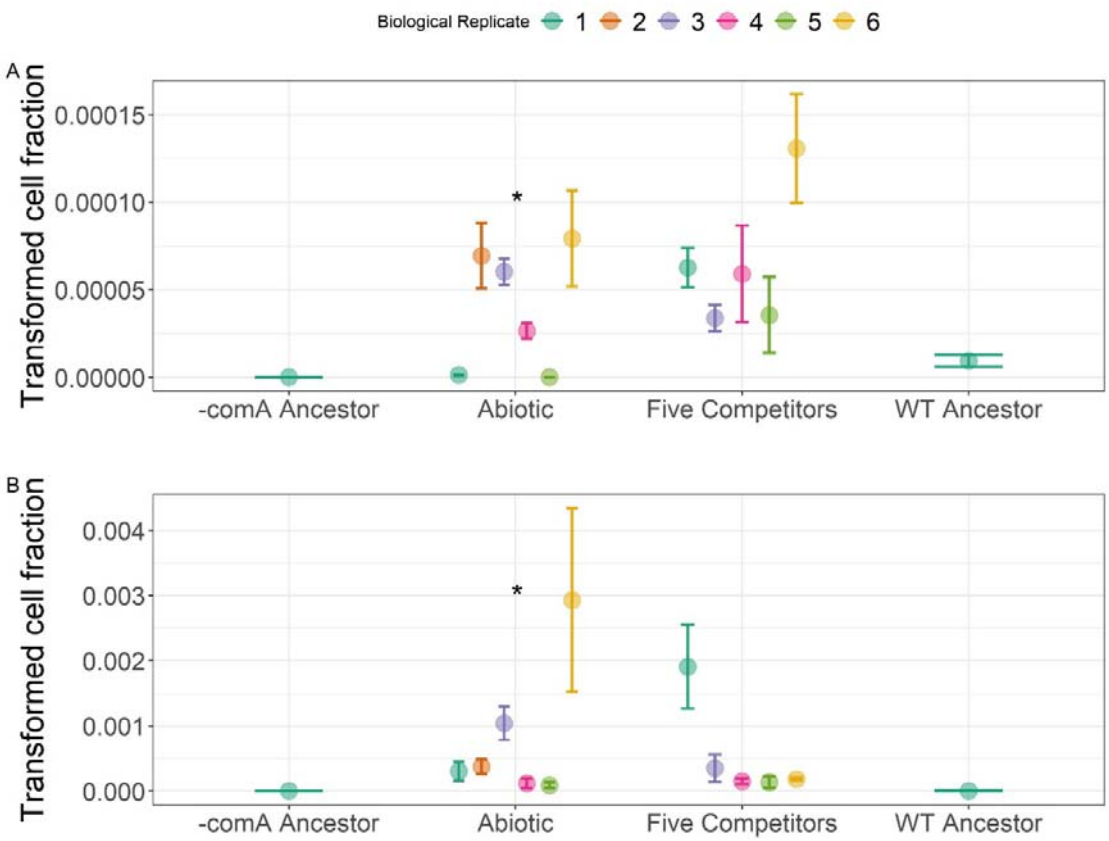

Figure 4. Variation of transformation frequencies of biological replicates within evolutionary treatment groups for (A) chemically acquired and (B) heat-killed cell lysate. Transformation frequencies were measured in triplicate per biological replicate. Points are biological replicates and bars are means ± one standard error. Asterisks denote significantly different transformation frequencies between intra-treatment biological replicates (Kruskal-Wallis, $p<0.05$).

Footnote

One treatment sample containing the wildtype strain in the presence of five concurrent competitors was lost near the end of the experimental evolution period. In the interest of ensuring all samples were directly comparable by experiencing identical conditions, we decided to not replace the lost

477 sample by evolving it independently to the other samples. This explains why the replication level for

478 one treatment is 5 instead of 6.