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

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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

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

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

Material and Methods

Acinetobacter baylyi ADP1 constructs

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 Briefly, the *sfGFP::apraR* cassette (Charpentier and Laaberki, unpublished) and *mCherry::specR* (Cooper, Tsimring and Hasty, 2017) cassettes were amplified via PCR with 1kb flanking regions homologous to the *att*Tn*7* locus and a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

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

Donor DNA

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

Competition assays

For all replicates of the five concurrent species community treatment and the abiotic treatment, 100 evolved green-fluorescent wildtype and *ΔcomA A. baylyi* clones were picked, pooled and frozen at –80˚C before use. A mixture containing equal volumes of the green-fluorescent *A. baylyi* pool, a *ΔcomA* red-fluorescent *A. baylyi*, and members of the five species community (where appropriate) 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

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

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

Results

Natural transformation does not provide fitness benefits in a community context

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

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

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

Transformation frequency did not decrease after evolution

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

Discussion

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

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

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

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

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

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

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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	9.9337775	0.042	0.1008	ns
Abiotic	ΔcomA - Abiotic	Wildtype - Abiotic	6	6	1.0599866	7.2996805	0.323	0.4493333	ns
Abiotic	ΔcomA - Abiotic	Wildtype - Biotic	6	5	0.2247326	5.5893440	0.83	0.83	ns
Abiotic	ΔcomA - Biotic	Wildtype - Abiotic	6	6	2.5367861	7.6512206	0.036	0.1008	ns
Abiotic	ΔcomA - Biotic	Wildtype - Biotic	6	5	1.6066542	5.8561068	0.16	0.2742857	ns
Abiotic	Wildtype - Abiotic	Wildtype - Biotic	6	5	-0.6125396	8.6263379	0.556	0.6065454	ns
Biotic	ΔcomA - Abiotic	ΔcomA - Biotic	6	6	-2.9648507	8.2809824	0.017	0.084	ns
Biotic	ΔcomA - Abiotic	Wildtype - Abiotic	6	6	2.7877285	8.9297554	0.021	0.084	ns
Biotic	ΔcomA - Abiotic	Wildtype - Biotic	6	5	-0.6904628	4.9094744	0.521	0.6065454	ns
Biotic	ΔcomA - Biotic	Wildtype - Abiotic	6	6	4.8533578	9.8340302	0.00070	0.008412	**
Biotic	ΔcomA - Biotic	Wildtype - Biotic	6	5	1.0391550	6.3311292	0.337	0.4493333	ns
Biotic	Wildtype - Abiotic	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

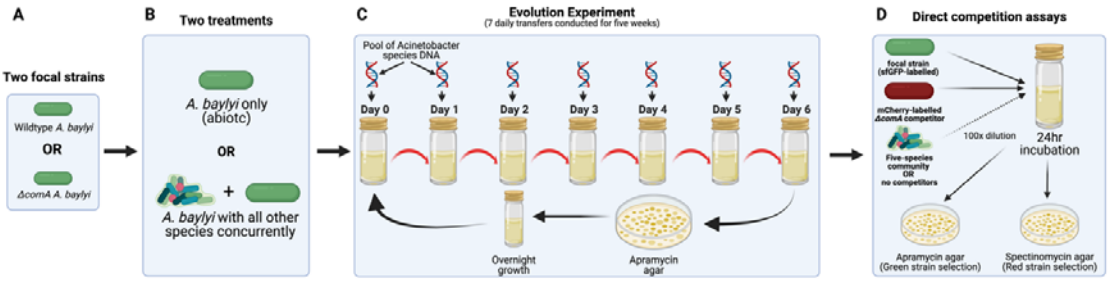
438 of evolution (Wilcoxon-test). Samples are tested using heat-killed cell lysate.

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

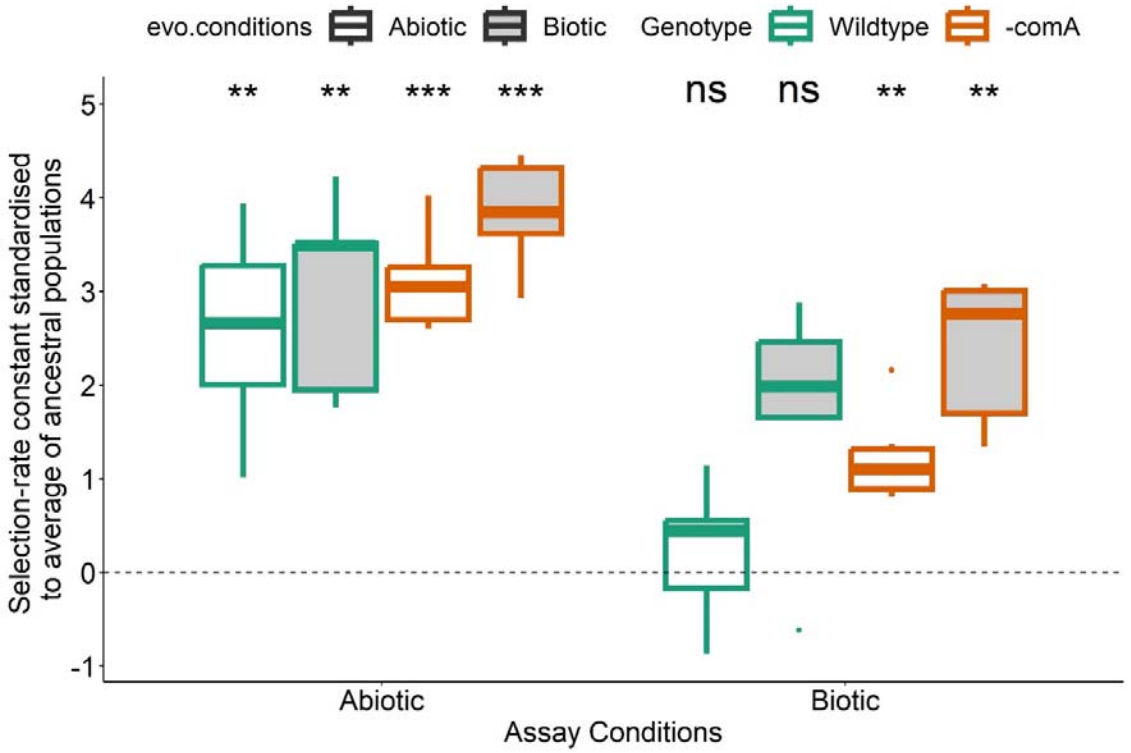
440 of evolution (Wilcoxon-test). Samples are tested using chemically acquired cell lysate.

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



441

442 Figure 1. Illustration of the evolution experiment and direct competition assays. A: Two focal strains
443 (competent wildtype and isogenic non-competent counterpart) were cultured separately in their
444 respective treatment conditions (B). B: Focal strains were subjected to two treatment conditions:
445 monoculture (abiotic) or co-culture with five competitor species. C: Cultures were propagated by
446 replacing spent broth with fresh media resulting in an approximately 34-fold dilution each passage.
447 After the 6th passage, all species except the focal strain are killed off using LB agar amended with
448 apramycin. The focal strain and freezer stocks of the competitors were allowed to grow to maximum
449 density in LB broth before being inoculated together for another week of passaging. D: After five full
450 weeks of passaging, the focal strains are selected for again with use of apramycin agar and frozen in
451 25% glycerol at -70˚C until tested against a common competitor to measure relative fitness. Figure
452 created with BioRender.com.



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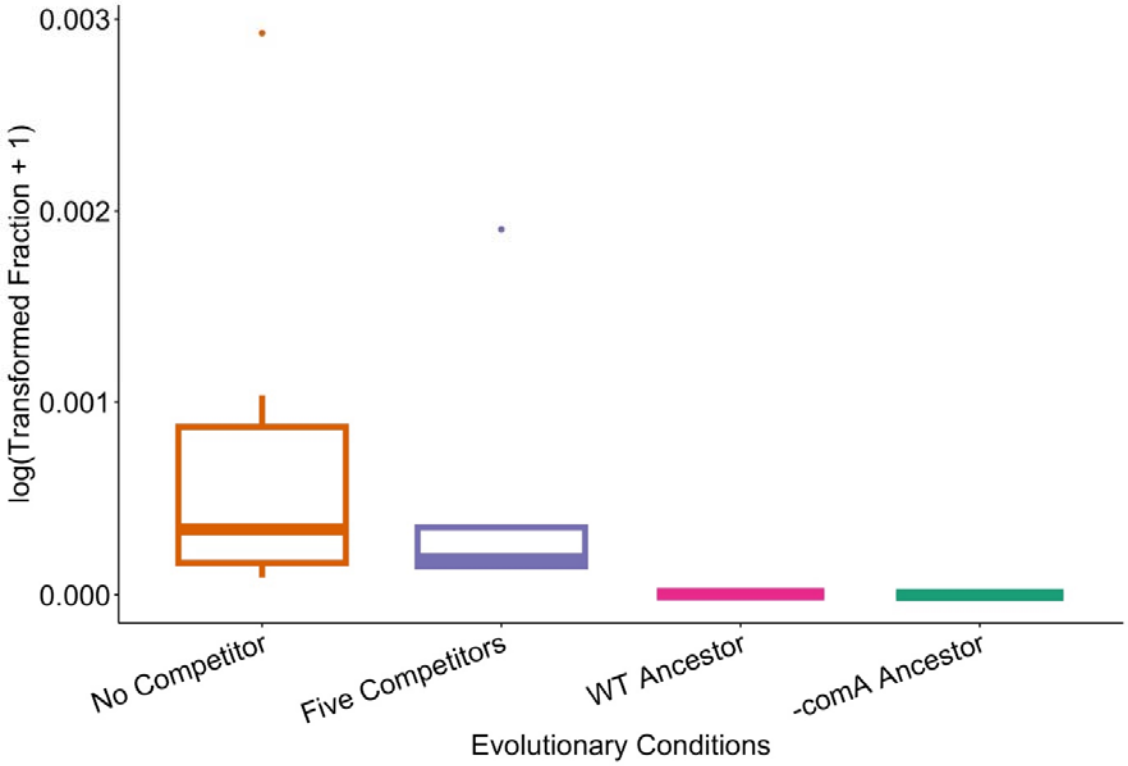
454

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 $p<0.05$; **, $p<0.01$; ***, $p<0.001$).



461
462 Figure 3. Treatment-level transformation frequencies of ancestral and evolved populations using
463 heat-killed cell lysate. Transformation frequencies were measured in triplicate per biological
464 replicate. The No Competitor, Five Competitor, WT ancestor, and *ΔcomA* ancestor treatments had 6,
465 5, 1, and 1 biological replicates, respectively.

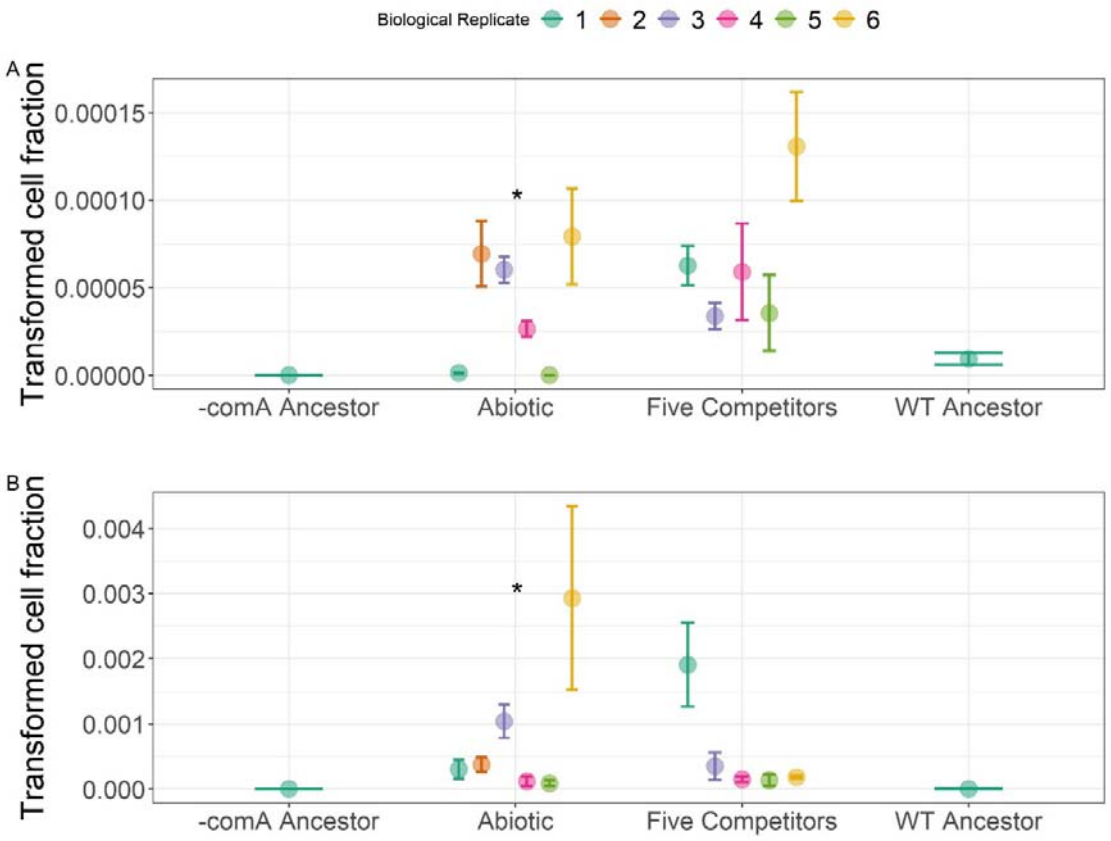


Figure 4. Variation of transformation frequencies of biological replicates within evolutionary treatment groups for (A) chemically acquired and (B) heat-killed cell lysate. Transformation frequencies were measured in triplicate per biological replicate. Points are biological replicates and bars are means ± 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.