Changes in natural transformation after salt adaptation

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Keywords: horizontal gene transfer, natural transformation, evolution of evolvability, salt adaptation, experimental evolution

Abstract

The exchange of genes between potentially unrelated bacteria is termed horizontal gene transfer (HGT) and is a driving force in bacterial evolution. Natural transformation is one mechanism of HGT where extracellular DNA (eDNA) from the environment is recombined into a host genome. The widespread conservation of transformation in bacterial lineages implies there is a fitness benefit. However, the nature of these benefits and the evolutionary origins of transformation are still unknown. Here, I examine how ~330 generations or 100 days of serial passage in either constant or increasing salinities impacts the growth rate and transformation efficiency of *Pseudomonas stutzeri*. While the growth rate generally improved in response to serial transfer, the transformation efficiency of the evolved lineages varied extensively, with only 39-64% of populations undergoing transformation at the end of adaptive evolution. In comparison, 100% of the ancestral populations were able to undergo natural transformation. I also found that evolving *P. stutzeri* with different cell lysates (or populations of dead cells) minimally affected the growth rate and transformation efficiency, especially in comparison to the pervasiveness with which transformation capacity was lost across the evolved populations. Taken together, I show that the efficiency of eDNA uptake changes over relatively rapid timescales, suggesting that transformation is an adaptive and selectable trait that could be lost in environments where it is not beneficial.

Introduction

Natural transformation is a mechanism of horizontal gene transfer whereby bacteria acquire extracellular DNA (eDNA) from the environment and recombine it into their genomes. Transformation plays a key role in bacterial evolution (Ambur et al. 2016; Koonin 2016); however, the fitness benefits of transformation remain unknown, despite extensive study. While it is generally accepted that transformation

can facilitate adaptation through genetic recombination, the consequences of this genetic exchange can be both beneficial and costly (Baltrus 2013). The theoretical benefits of transformation are similar to meiotic sex and include speeding up adaptation, combining beneficial genes into one genome, and separating beneficial mutations from deleterious loads (Kim and Orr 2005; Cooper 2007; Mell and Redfield 2014; Takeuchi et al. 2014; Koonin 2016). However, extracellular DNA from dead bacteria can also carry an increased mutational load or promote the spread of selfish genes (Redfield 1988; Redfield et al. 1997). Consequently, the fitness advantages of transformation and the environmental conditions in which they are conferred have been difficult to quantify experimentally (see Table 1).

There are several potential explanations for the evolutionary maintenance of transformation [reviewed in 42]. Several of them posit that transformation evolved as a byproduct of acquiring DNA for nutrients (Redfield 1993; Claverys et al. 2006; Moradigaravand and Engelsta 2013; Sinha et al. 2013) or genome repair (Michod et al. 1988; Charpentier et al. 2012; Johnston et al. 2014). However, the presence of cellular machinery dedicated to protecting extracellular DNA (eDNA) from degradation inside the cell, suggests that eDNA is not acquired purely for the nutrient benefit (Seitz and Blokesch 2013; Johnston et al. 2014). In addition, many bacterial taxa preferentially kill and transform eDNA from close relatives, a process somewhat analogous to the exchange of DNA in eukaryotic sex (Guiral et al. 2005; Veening and Blokesch 2017).

Transformation is also similar to eukaryotic sex in that it is primarily beneficial in stressful or continuously changing environments. Population genetic models (Lynch et al. 1993; Takeuchi et al. 2014; Koonin 2016) and experimental evolution studies (Baltrus et al. 2007; Engelmoer et al. 2013) have shown that transformation is beneficial in rapidly fluctuating or stochastic environments where transformable cells can outcompete non-transformers (Palmer and Cartwright 2018; Carvalho et al. 2019). Theoretically, this is because transformation can increase genetic variation, thereby increasing the efficiency of natural selection (Barton and Charlesworth 1998). Transformation does not always provide a fitness benefit in stressful environments though, as Bacher et al. (2006) found that competent lineages of *Acinetobacter baylyi* did not adapt to novel laboratory conditions faster than their non-competent competitors, and repeatedly lost the ability to transform eDNA.

While several other studies have shown that transformation is beneficial in stressful environments (see Table 1), it is still unclear how the availability of beneficial genes might alter transformation-mediated fitness effects. For instance, antibiotic resistance only evolved via transformation when antibiotic resistance genes were provided, while phage resistance evolved in the presence of phage-sensitive or -resistant DNA (Perron et al. 2012; Mcleman et al. 2016). Since sequence similarity improves the efficiency of homologous recombination, it is generally accepted that transformation is most prevalent between closely related organisms (Soucy et al. 2015). However, sharing genes with close relatives could limit the acquisition of novel gene combinations and ultimately limit adaptation.

Here, I aim to better understand the evolutionary benefits of transformation by

evolving *Pseudomonas stutzeri* – a highly transformable soil bacterium – in either constant or increasing salt concentrations for 100 days, while supplying different sources of eDNA (cell lysates or dead cells). At the end of the experiment, I quantify the growth rate, population size, and transformation efficiency (transformants/µg eDNA) of the evolved populations and compare this to the same measurements in the starting isolate or ancestor. I specifically address the following questions: 1) Does the transformation efficiency increase in response to evolving in a variable – relative to a constant environment (increasing salinity vs. constant low salinity)? 2) Does evolving with dead halophiles or dead *Pseudomonad* relatives better facilitate adaptation to high salt concentrations?

Methods

Serial dilution experiment

We serially transferred *Pseudomonas stutzeri*, strain 28a24 for ~330 generations (100 days) in 96-well microtiter plates [see 101 for whole genome sequence]. Cultures were serially transferred every 24hrs at a 1:10 dilution and maintained at 26°C. For the first 50 days (~170 generations) of the experiment, all populations were transferred as one treatment in a constant salt media (1.5% salinity; 10g/L tryptone, 5g/L yeast extract, and 15g/L NaCl). After 50 days (~170 generations), the experiment was shut down due to the global covid-19 pandemic, and populations preserved in 40% glycerol at 20°C. Four weeks later, populations were revived and serially passed at 1.5% salinity for 4 days before the experiment was 're-started' on day 51 (~170 generations). At this point, we split the experiment into two treatments. The original treatment was maintained at a low constant salinity for the remainder of the experiment (1.5% salt media from day 1 to 100). The new treatment, which we refer to as the increasing salinity treatment was transferred to a 2% salt media (20g/L NaCl), where it was serially passed for 100 generations. Then on day 81, we increased the salt concentration to 2.5%, were it stayed for 67 generations until Day 100 (see Fig. 1 for the serial transfer conditions). The constant low and increasing salinity treatments had 96 replicates each. In addition, during each transfer (every 24hrs), populations were supplemented with eDNA via whole populations of dead bacteria – which equated to 5ng of genomic eDNA each transfer. We refer to these as cell lysates as they contain DNA and other cellular components (see Table S1 for a detailed list of the cell lysate sources).

Preparation of cell lysates

Individual cell lysates were prepared in 100mL batch cultures in liquid LB on a shaker table at 120rpm and 30°C (10g/L tryptone, 5g/L yeast extract, and 5g/L NaCl). After 48hrs, each culture was plated to confirm there was no contamination (10 µl replicate dots plated 3x). Each culture was then heat shocked at 90°C for 1hr. After heat shock, each culture was plated to confirm all the cells were dead. If bacterial strains still had viable colonies, these cultures went through another round of heat shock at 100-110°C, which was sufficient to kill the remaining cells. The heat shocked cultures were then spun down and resuspended in sterile nanopore water. The lysates were filtered through a 0.22µm filter and standardized to a concentration of 1ng DNA/µl using a Qubit 2.0 fluorometer (Life Technologies, USA). There were 12 cell lysate treatments with 8 replicates each. See Table S1 for expanded list of cell lysates.

Growth rate determination

All assays were conducted on the ancestral population and populations that evolved for 100 days (~330 generations). Strains of *P. stutzeri* were revived from 40% glycerol storage (-80°C) and diluted 1:10 in liquid LB media (0.5% salinity: 10g/L tryptone, 5g/L yeast extract, and 5g/L NaCl) in 250-µl microwell plates. After revival, the populations were transferred every 24hrs at a 1:10 dilution in 0.5% salinity for 4 transfers. After the fourth transfer, we moved the populations to two separate salt environments in 250-µl microwell plates, to quantify the growth rate and transformation capacity. In the constant low salinity treatment, 10 of the 96 populations never revived. In the increasing salinity treatment, 1 population never revived and 1 was contaminated, these are not reported in the results (see Table S2 for a full list).

Each 24hr assay was conducted at low salinity (1.5% salinity: 10g/L tryptone, 5g/L yeast extract, and 15g/L NaCl) and high salinity – a novel and stressful environment for *P. stutzeri* (3% salinity: 10g/L tryptone, 5g/L yeast extract, and 30g/L NaCl). For each population, we monitored absorbance at 600nm for 24hrs using a Biotek Synergy microplate reader (Winooski, VT). The growth curve data was fit to a standard form of the logistic equation using the Growthcurver package in R studio. We used the logistic equation to describe the population size N_t at time t:

$$N_t = \frac{K}{1 + (\frac{K - N_0}{N_0})e^{-rt}}$$

Transformation efficiency and frequency

Cultures were revived following the same protocol used for growth rate determination. After revival, we quantified the transformation efficiency by tracking the acquisition of gentamicin resistance into the evolved and ancestral *P. stutzeri* populations which were gentamicin susceptible. The eDNA encoding gentamicin resistance was prepared from a mutant strain of *P. stutzeri*, strain 28a24, which carries a gentamycin resistance gene and LacZ gene fused to a miniTn7 transposon (Tn7 transposition of pUC18-mini-Tn7T-Gm-lacZ). To begin the assay, we transferred 20µl from each evolved and ancestral population into 180µl of fresh LB media containing 1.5% or 3% salinity. I added genomic extracellular DNA (eDNA) resuspended in nanopore water to each population and incubated at 30°C. After 24hrs we performed a serial dilution and titers were determined on selective media (LB + gentamycin [50 µg/ml] + Xgal [40 µg/ml]) and non-selective media (LB) using triplicate 10µl dots. Population level transformation efficiency was determined by dividing the average number of transformants in a population by the µg of eDNA (0.02µg). We also report population level transformation frequencies by dividing the average number of transformants by the total number of cells or the population size.

Statistical analyses

Prior to analysis, we checked that data met assumptions of normality and homogeneity of variance. We corrected for increased homogeneity of variance across population sizes using a log transformation. We analyzed bacterial growth rates and populations sizes using two-factor ANOVA, with Evolution Conditions, Assay Conditions, and their interaction as factors. For each evolution treatment – constant salinity versus increasing salinity – we used a two-factor ANOVA with the Assay Conditions, Cell Lysate treatment, and their interaction as factors. we determined differences in transformation capacity using a general linearized model with a negative binomial

distribution to account for positive skew. To determine statistical differences in the number of non-transforming populations (zeros) we used a two-part hurdle model from the hurdle package in R, as it specifies one process for zero counts and one process for positive counts, and is commonly used for positively skewed data with lots of zeros (Gonzales-Barron et al. 2010; Hofstetter et al. 2016).

Results

Growth Rate

P. stutzeri adapted to changes in salinity after ~330 generations of serial transfer. Both evolution treatments (constant vs. increasing salinity) on average grew faster than the ancestor in the high salt environment. However, populations evolved in the increasing salinity environment grew faster in both the low and high salt environment (Fig. 2A; Salinity $p < 0.001$; Treatment*Salinity $p = 0.052$). In addition, populations exposed to the gradual increase in salt, exhibited higher growth rates than those adapted to the constant salt concentration but only when tested at the lower salinity (Fig. 2A**;** $p= 0.0468$). Interestingly, both of the evolved populations had larger population sizes in the high salt environment – relative to the low salt environment and to the ancestor (Fig. 2B; $p <0.001$). This was surprising given the evolved populations grew significantly slower in that environment compared to the low salinity one.

Loss of Transformability

Evolved populations exhibited a significant loss of transformation capacity relative to the ancestor (Fig. 3; constant low salinity, $p=0.005$; increasing salinity, $p=0.02$). At the end of the experiment between 39% and 64% of evolved populations – depending on the treatment and test conditions still underwent transformation. In the remaining populations there were no transformants at a detectable level. In addition, there was a striking similarity between the two evolution treatments in terms of how many populations underwent transformation (Fig. 3). In both evolution treatments, there were significantly more populations undergoing transformation when tested at the higher salinity, despite no known difference in genotype (Fig. 3; $p <0.001$ for both treatments).

Transformants and total cells

There was no relationship between the number of transformants and the number of recipient cells in independently evolving populations (Fig. 4). This trend was true across the treatments, as well as agreeing with preliminary work showing that transformation is not limited by population size in larger populations (Fig. S1A**).** Therefore, we report the number of transformants standardized by the amount of eDNA (transformation efficiency). However, we also report (in Fig. 5B) the number of transformants standardized by the number of recipient cells (transformation frequency). This is done to account for the fact that, on average, there were significantly more transformants and recipient cells at higher salinities, suggesting the increase in the average number of transformants could be correlated with the increase in the average number of recipient cells – despite there being no evidence of such a correlation within the individual populations.

High variation in transformation efficiency

At the end of the experiment the transformation efficiency (transformants/µg DNA) was significantly lower in the low salt environment, regardless of the evolution conditions (Fig. 5A; $p < 0.0001$). However, the populations

that transformed eDNA and evolved at constant salinity, did so at a higher efficiency than the ancestor – but only when tested in the high salt environment (Fig. S2; $p = 0.0216$). When the number of transformants was standardized by the number of recipient cells, there were no statistically significant differences in transformation frequency (Fig. 5B). Although numerically the transformation frequency was highest for populations evolved in constant low salinities and moved to the high salinity environment for the transformation assay.

Evolving with cells lysates or populations of dead cells did not affect transformation efficiency in a uniform manner (Figure 6; Figure S3; see Table S3 for expanded results). In general, the transformation efficiency was higher in the high salt environment. However, standardizing by the population size indicated there was no difference in transformation frequency between the low and high salt environment – as the number of transformants and the number of total cells was larger in the high salt environment (Fig. S4). Moreover, there were no consistent changes in growth rate or population size with the addition of different cell lysates (Fig. S5; see Table S4 and S5 for expanded results). There was a high level of congruency between the two evolution treatments in terms of which populations underwent transformation (Fig. 6; comparing the top and bottom panels). These effects may have appeared early in the experiment, since the two treatments diverged from a single set of evolving populations on day 50 of the experiment.

Trade-off between growth rate and transformation

There was no trade-off between the transformation efficiency and the growth rate (Fig. 7; the same being true for transformation frequency – data not shown). While there may have been tradeoff between growth rate and transformation within individual strains, we were unable to detect such a tradeoff in the population-level measurements conducted here.

Discussion

Understanding the evolutionary origins and fitness consequences of transformation can shed light on the larger question of why organisms undergo genetic recombination, and to what extent the traits governing selection are themselves selected upon. Here, I conclude that evolving *P. stutzeri* with different sources of eDNA or evolving them in constant versus increasing salinities did not have a large effect on growth rate or transformation efficiency. However, I did find that the transformation capacity – the ability for evolved populations to transform eDNA – changed dramatically over just ~330 generations or 100 days of serial transfer.

By the end of the experiment, around 50% of the evolved populations did not transform any of the provided extracellular antibiotic resistance genes, although the exact percentage varied from 36-61% depending on the treatment. This was true, regardless of whether the number of transformants was reported as the transformation efficiency (transformants/µg eDNA) or the transformation frequency (transformants/total cells). We report both metrics here to account for differences in average population size in the low versus high salinity environment, even though the total population size only limits transformation in very small populations of *P. stutzeri* (smaller than those reported here; Fig. S1B). Overall, we focus the discussion on the variation in transformation capacity across the

evolved lineages, as this is true irrespective of how the data is analyzed (transformation efficiency vs. transformation frequency).

Several other bacterial species, in addition to *P. stutzeri*, undergo transformation irrespective of population density. For instance, *Vibrio parahaemolyticus* and *V. campbellii* both undergo transformation in the absence of quorum sensing which is the ability to regulate gene expression with population size. Meanwhile, their close relative *V. cholerae,* and *Streptococcus* species both require quorum sensing for successful transformation (Shanker and Federle 2017; Simpson et al. 2019). Interestingly, the genetic features that underpin the differences in quorum sensing across *Vibrio* species have yet to be identified. Similarly, different isolates of the same bacterial species often exhibit large differences in their transformation capacity, with the genetic variation underpinning these differences often impossible to discern. For instance, isolates of *P. stutzeri* collected from different soil environments had highly variable transformation frequencies, with about one-third of isolates considered non-transformable (Sikorski et al. 2002). Similar observations have been made in *Vibrio* species that inhabit different environments, and spurred the recent suggestion that transformation may be lost in environments where it is no longer beneficial (Jaskolska et al. 2018; Simpson et al. 2019).

Hence, it is possible that transformation was not maintained in several of the evolved lineages because it did not provide a fitness benefit during experimental evolution. Weak selection for transformation could have been due to osmotic stress, the application of only a mild stress, or due to infrequent fluctuations in the environment. For instance, previous studies found transformation was beneficial when the environment shifted every 2-3 transfers (Perron et al. 2012; Engelmoer et al. 2013), as opposed to every 20-30 transfers as was done in this study. Therefore, an interesting follow-up study would be to compare the distribution of transformation phenotypes after evolving *P. stutzeri* in a constant optimal environment, a constant but very stressful environment, and a rapidly fluctuating environment – to better understand how stress, or fluctuations in stress, shape the evolution of transformation.

Another possibility is that transformation only provides a fitness benefit in response to very specific stressors. For instance, several studies have found that transformation was beneficial when evolving populations where exposed to periodic inputs of sub-inhibitory concentrations of antibiotics (Perron et al. 2012; Engelmoer et al. 2013). Because transformation allows the reversible integration of resistance genes, and antibiotics are usually transiently present in the environment, transformation could be a mechanism well-suited to handling antibiotic stress. In contrast, transformation may be less beneficial in response to stressors like changes in osmotic pressure which are encoded by large and connected gene networks. In general, more work needs to be done on the specific stressors that transformation confers a benefit to, as prokaryotic genes appear to adapt to either vertical or horizontal transmission, meaning that not all processes may be well-adapted to evolve via horizontal gene transfer (Novick and Doolittle 2020).

A final consideration is the role of osmotic pressure in altering the efficiency of eDNA uptake. Populations adapting to

high osmolarity environments generally have a large fraction of mutations in genes associated with cell wall synthesis (Cesar et al. 2020). Therefore, it could be that changes in the cell wall altered the pilus structure which captures eDNA from the environment (Graupner et al. 2000). *P. stutzeri* has two pili that interact to regulate transformation. The type IV pilus acquires eDNA from the environment, while the second pilus is believed to translocate eDNA into the cytoplasm and when knocked out decreases transformation ~90% (Graupner et al. 2001). Therefore, changes in the cell wall in response to salt stress could have altered the interaction between these two pili, creating the gradient of transformation capacity evident in the evolved lineages. Follow-up investigations which involve whole genome sequencing, will hopefully elucidate if mutations in osmoregulatory genes could have altered transformation capacity.

Despite evidence of high variation in transformation capacity in many bacterial lineages, very few studies have quantified changes in transformation after experimental evolution. To date, six experimental evolution studies have focused on the fitness effects of transformation but only one study has quantified transformation before and after experimental evolution. In that one study, transformation did not provide a fitness benefit and the evolved lineages repeatedly lost the capacity to undergo transformation (Bacher et al. 2006). Several other studies have identified transformation-mediated fitness benefits (primarily in stressful environments), but none of them quantified the prevalence of transformation at the end of the experiment (Table 1).

Moving forward, quantifying transformation after adaptation could help disentangle the benefits of transformation from the overall benefits of competence – which is the physiological state a bacterium must enter to undergo transformation and is often part of a larger stress response. For instance, Pseudomonads also use the Type IV pilus for flagellum-independent movement or twitching motility (Graupner et al. 2001). While *Bacillus subtilis*, a well-studied soil-dweller, upregulates transformation as part of a general stress response prompted by DNA damage or antibiotics (Claverys et al. 2006). Therefore, future studies that quantify changes in transformation efficiency during and after experimental evolution, will be critical in disentangling the specific benefits of transformation within the larger regulatory network of competence.

Overall, this study provides novel experimental evidence that the ability to undergo transformation can change over relatively short timescales and may be more plastic across space and time than is generally accepted. The substantial decrease in transformation efficiency in the low salt environment where *P. stutzeri* evolved, suggests that transformation did not provide a fitness benefit during salt adaptation. Taken together, this work suggests that transformation is an adaptive, selectable trait, that may increase or decrease rapidly in response to selection.

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Table 1 Review of transformation-mediated fitness effects**.** Experimental evolution studies that have quantified the fitness effects of transformation.

Citation	Bacterial Taxa	Adaptation Conditions	Transformation increased adaptation	Exogenous DNA provided	Changes in transformation
Bacher et al. 2006 [55]	*Acinetobacter baylyi*	High Salinity & Temperature	No	No	Decreased
Baltrus et al. 2007 [50]	*Helicobacter pylori*	Novel Laboratory Conditions	Yes	No	Not reported
Perron et al. 2012 [126]	*Acinetobacter baylyi*	Periodic Antibiotics (3-4x/wk)	Yes, when provided resistance genes	Yes	Not reported
Engelmoer et al. 2013 [51]	*Streptococcus pneumoniae*	Periodic Antibiotic (kanamycin 2x/wk)	Yes	No	Not reported
Utnes et al. 2015 [127]	*Acinetobacter baylyi*	Novel Laboratory Conditions	Yes, but only during early stationary phase	Yes	Not reported
Mcleman et al. 2016 [128]	*Acinetobacter baylyi*	Parasitic Phage	Yes, from phage-sensitive or resistant DNA	Yes	Not reported

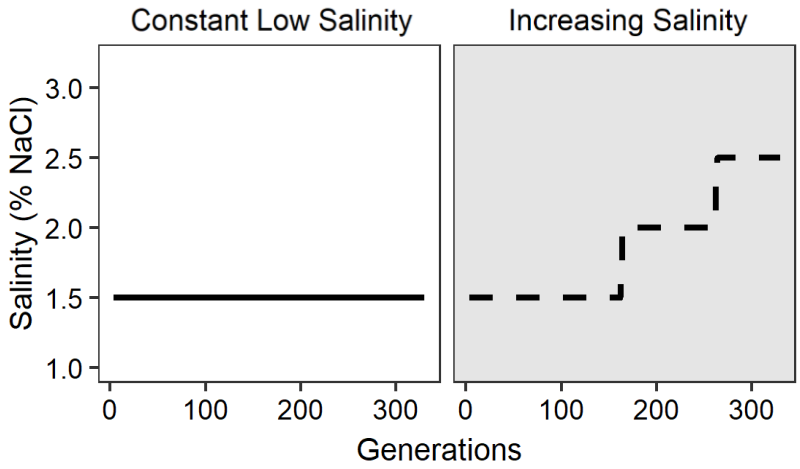

Fig. 1 The serial transfer conditions for the two evolution treatments. The left panel shows the conditions for populations adapted to a constant low salinity environment (1.5% salinity) and the right panel shows the conditions for populations adapted to increasing salinities (1.5% to 2.5% salinity).

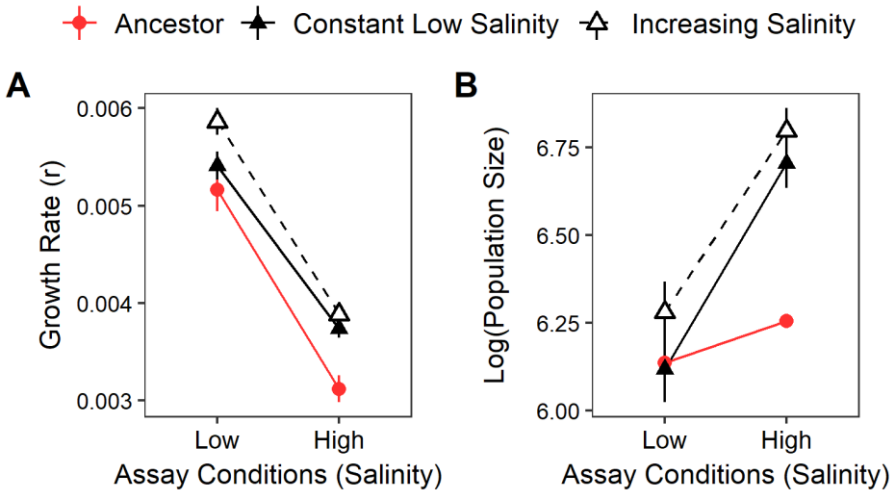

Fig. 2 Effects of adaptive evolution. (A) Growth rate and (B) log transformed population size for the ancestor (red circles), constant low salinity (black closed triangles), and increasing salinity evolution treatments (black open circles) in low (1.5%) and high (3%) salinity. The points show the average across the ancestral (n=8) and evolved populations, and the error bars indicate the standard error (constant low, n = 86; increasing n = 94)**.**

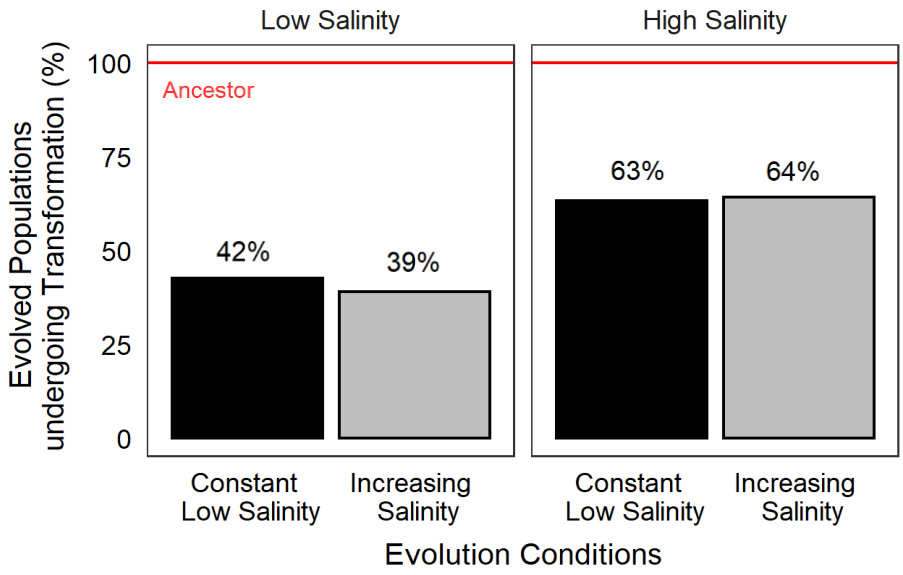

Fig. 3 The number of evolved populations with a detectable number of transformants in the low and high salinity test environment for populations evolved in constant or increasing salinities (count data). The red line corresponds to the beginning of the experiment when transformants could be detected in 100% of the ancestral populations.

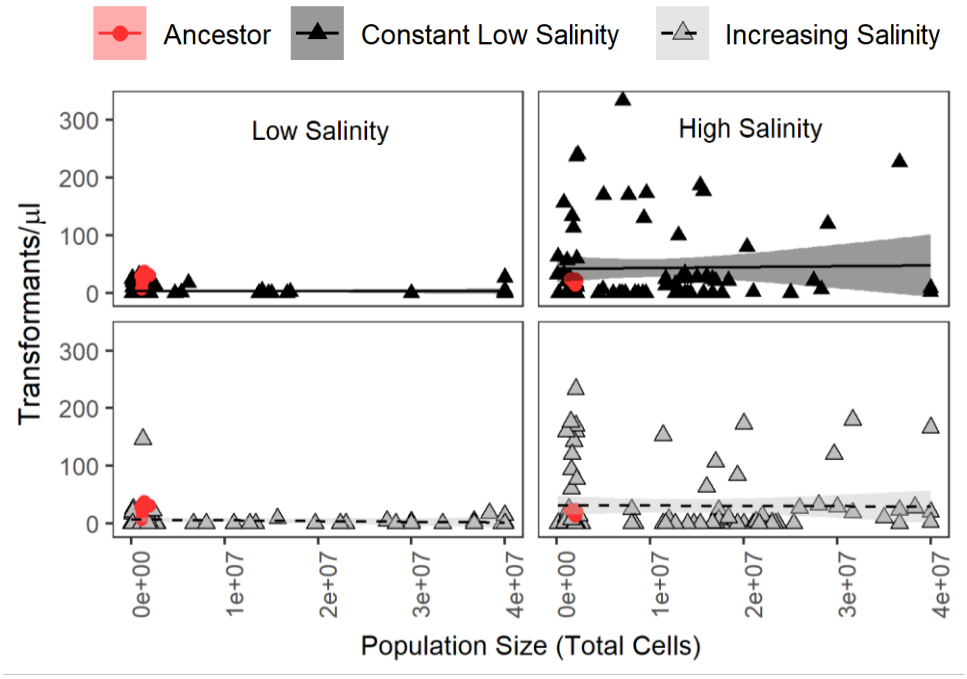

Fig. 4 The relationship between transformants and total cells**.** In the ancestral and evolved populations, the number of transformants does not increase as the population size or total number of cells increases. Each point represents an individual population-level measurement. The linear relationship between transformants and total cells is indicated by the lines with the shaded areas showing the 95% confidence interval (n=8 ancestor, n=86 constant low and n=94 in the increasing salinity treatment).

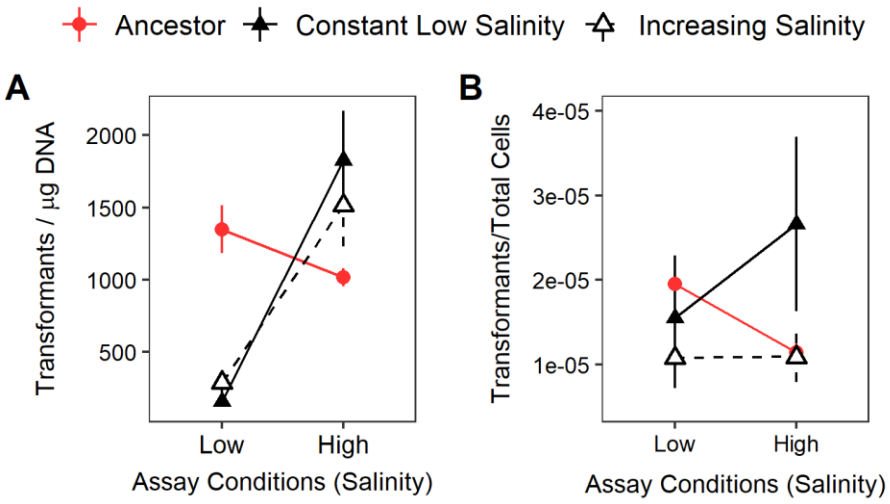

Fig. 5 Changes in transformation in response to experimental evolution. (A) Transformation efficiency (transformants/µg DNA) and (B) transformation frequency (transformants/total Cells) for the ancestor (red circles) and evolved populations (constant low salinity = solid black lines; increasing salinity = dashed lines). The points show the average across the ancestral (n=8) and evolved (constant low = 86, increasing =94) populations, and the error bars indicate the standard error (averages include transforming and non-transforming populations).

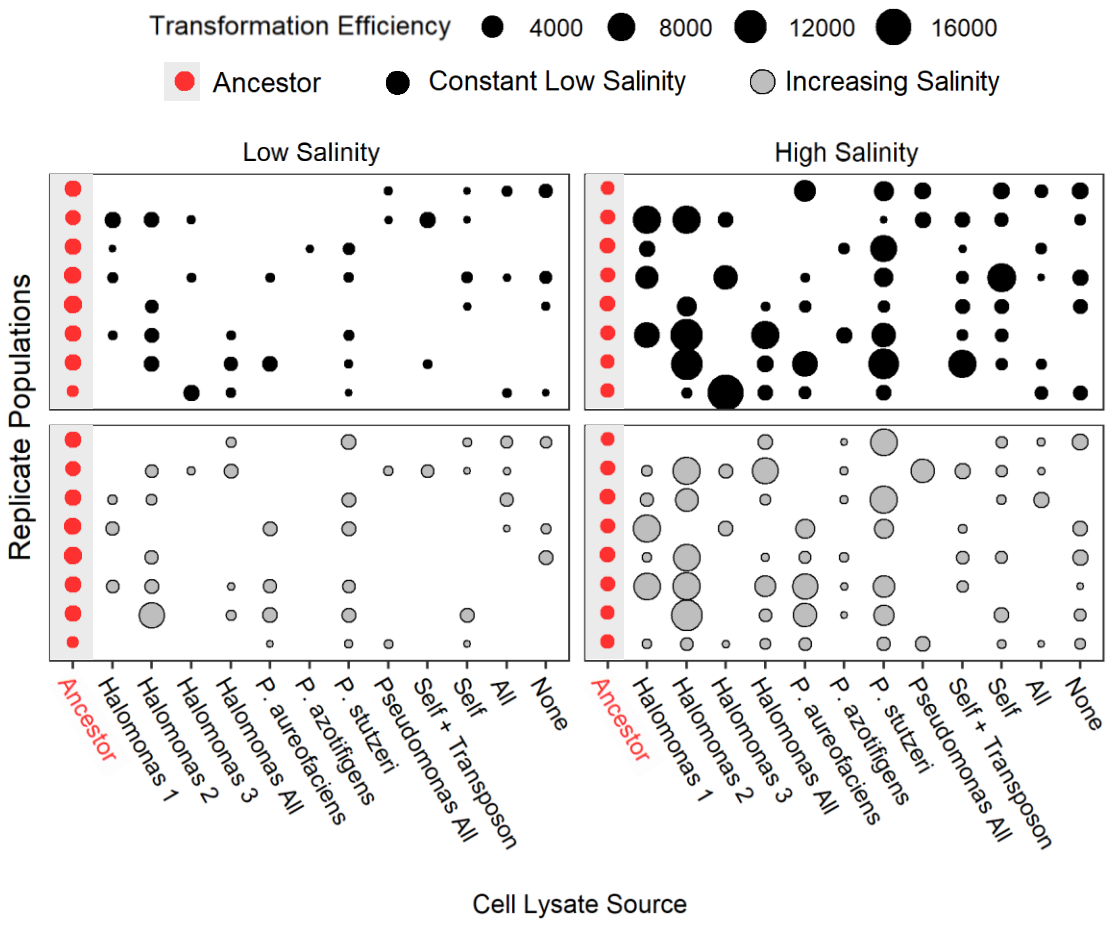

Fig. 6 Variation in transformation efficiency. Transformation efficiency for the ancestral and evolved populations in low and high salinities. Higher transformation efficiencies are denoted by larger circles with the populations evolved at a constant low salinity in the top panel and those evolved in increasing salinities in the bottom panel. The cell lysates are shown on the x-axis and the replicate populations on the y-axis corresponding to a 96-well plate layout (n=8 replicates per cell lysate source).

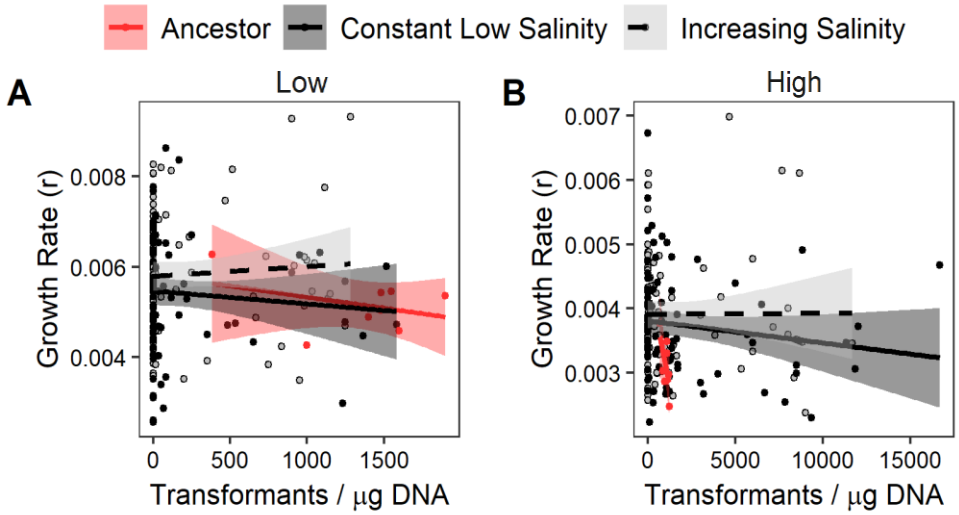

Fig. 7 Trade-off between growth rate and transformation efficiency. Average population-level growth rate regressed against the transformation efficiency in the (A) low salinity and (B) high salinity environments. Each point represents an individual population-level measurement. The linear relationship between transformants and total cells is indicated by the line, with the shaded area showing the 95% confidence interval (n=8 ancestor, n=86 constant low and n=94 in the increasing salinity treatment).

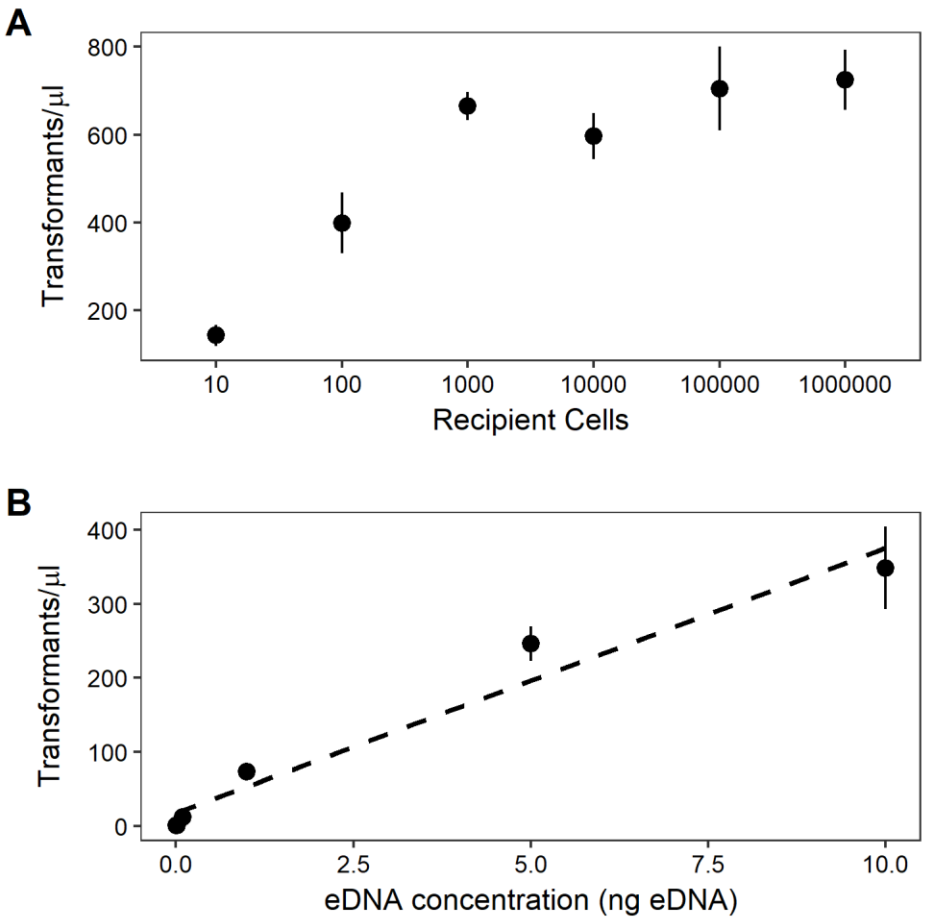

Fig. S1 Preliminary transformation assays. (A) The effect of total population size on the number of transformants when the eDNA concentration is held constant. (B) The relationship between the concentration of eDNA and the number of transformants under laboratory conditions (0.001, 0.01, 0.1, 1, 5, 10 ng/µl eDNA) – in large populations (~1,000,000 recipient cells).

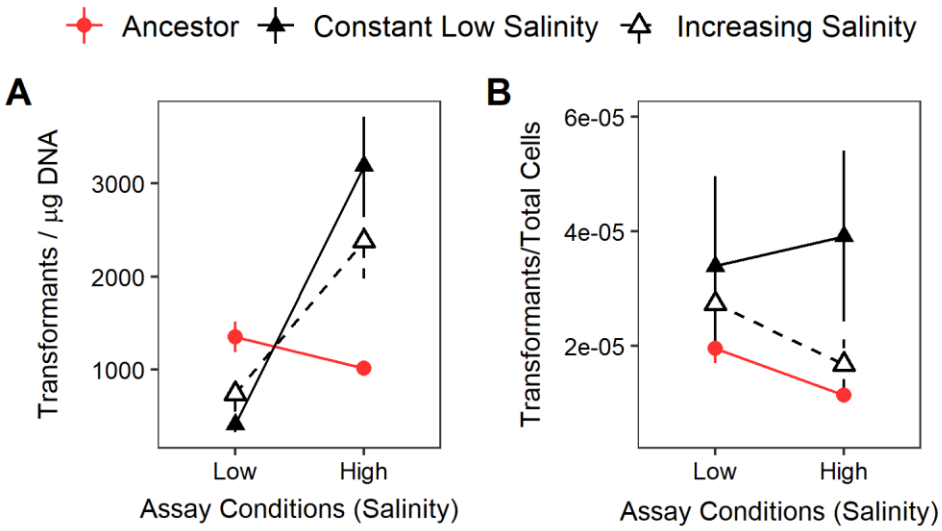

Fig. S2 Transformability in the populations that transformed eDNA. (A) Transformation efficiency (transformants/µg DNA) and (B) transformation frequency (transformants/total Cells) for the ancestor (red circles) and evolved populations (constant low salinity = solid black lines; increasing salinity = dashed lines). The points show the average across the ancestral (n=8) and evolved (constant low = 86, increasing =94) populations, and the error bars indicate the standard error (averages include only populations that underwent transformation).

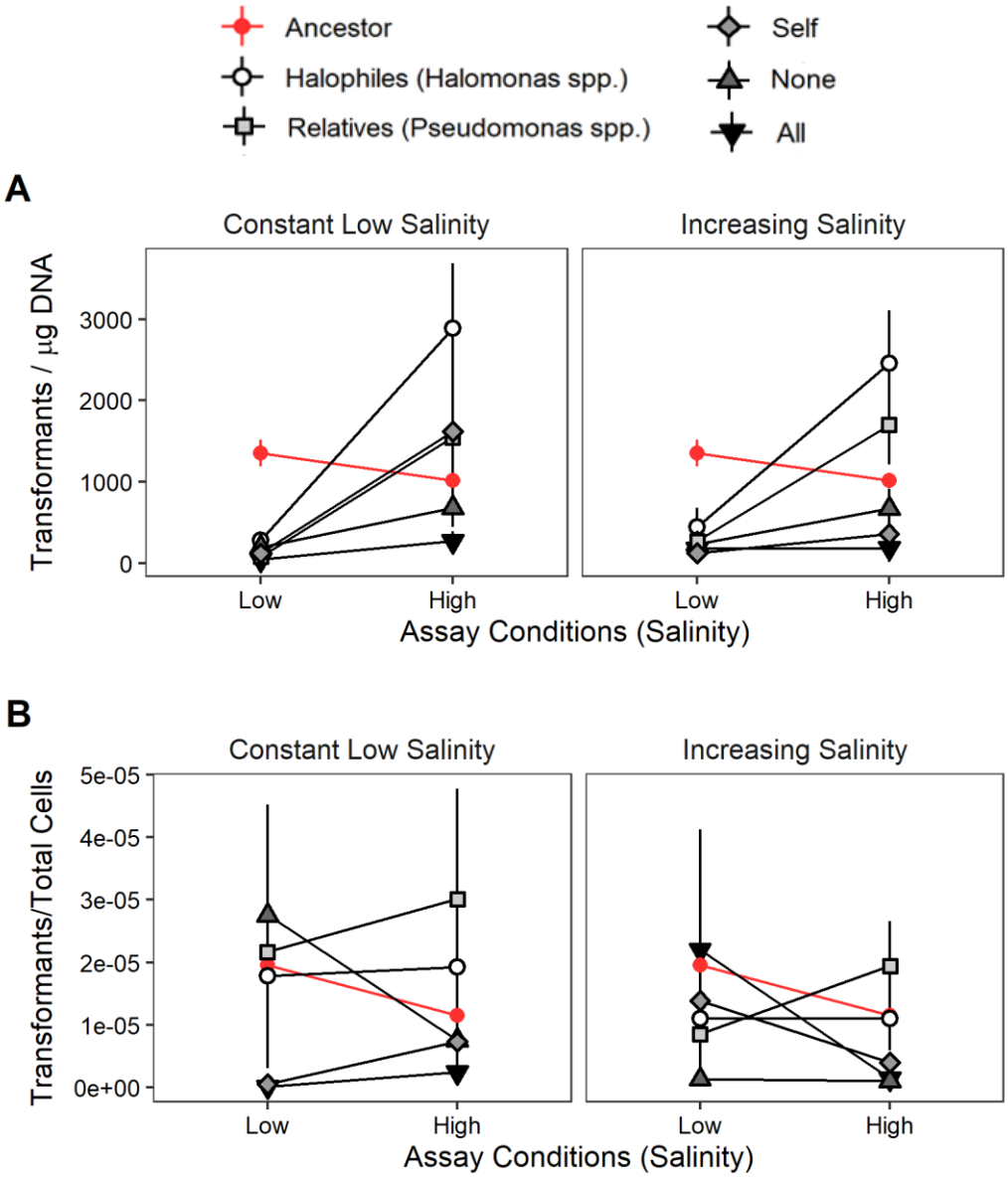

Fig. S3 Effect of cell lysates on transformation. (A) Transformation efficiency, and (B) transformation frequency for each cell lysate treatment. The ancestor (red circles), the constant low salinity (left panel) and the increasing salinity treatments (right panel, dashed lines) are shown at low and high salinity (n=8 replicate populations across treatments, excluding treatments that went extinct, see table S4.2).

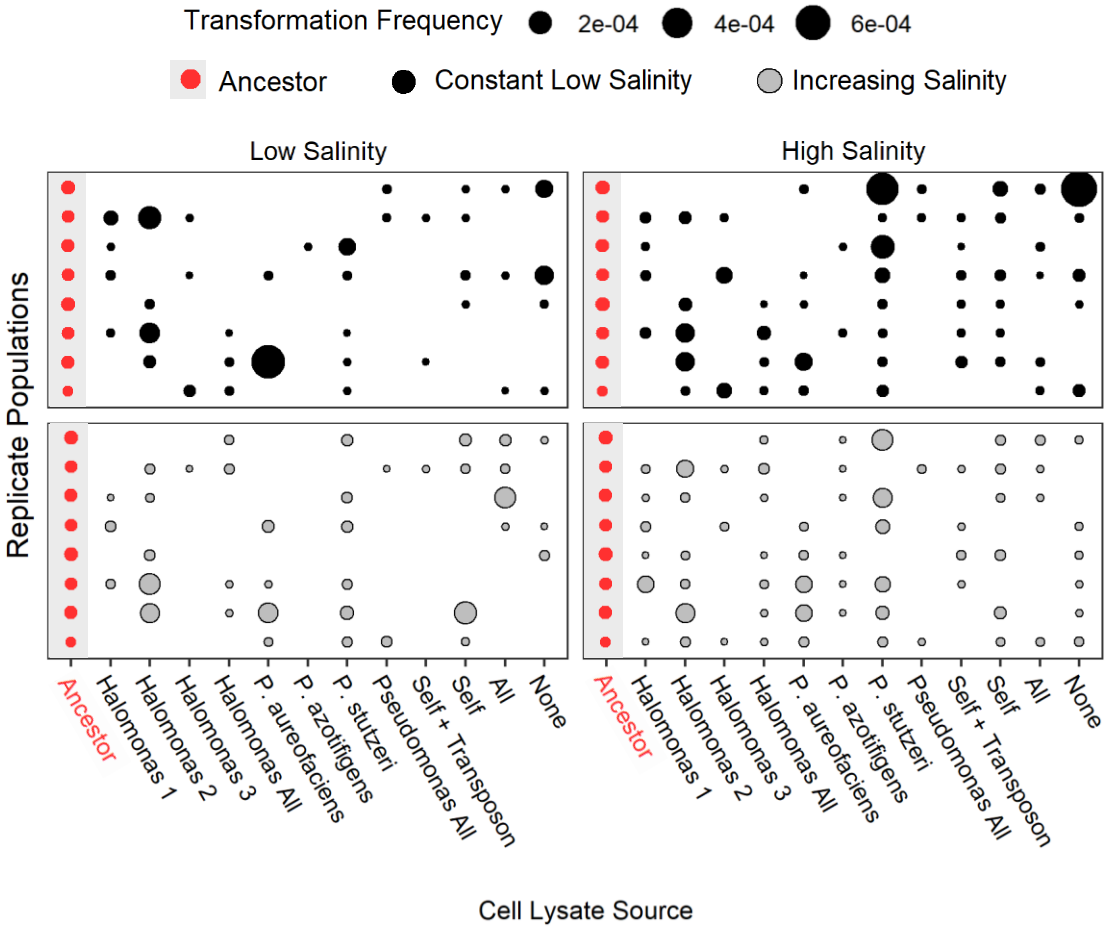

Fig. S4 Variation in transformation frequency. Transformation frequency for the ancestral and evolved populations in low and high salinities. Higher transformation frequencies are denoted by larger circles with the populations evolved at a constant low salinity in the top panel and those evolved in increasing salinities in the bottom two panels. The cell lysates are shown on the x-axis and the replicate populations on the y-axis corresponding to a 96-well plate (n=8 replicates per cell lysate).

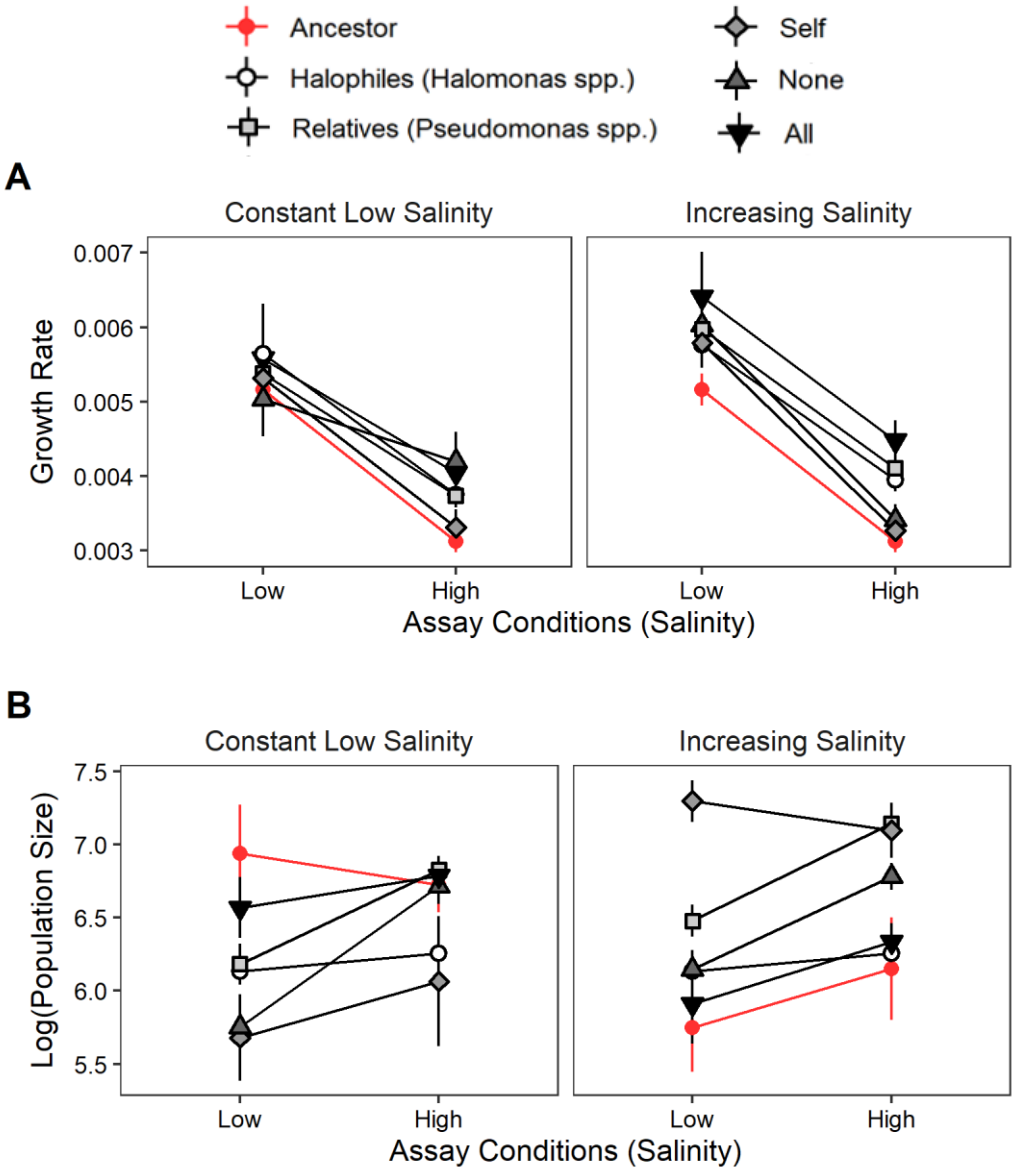

Fig. S5: Effect of cell lysates on growth rate and population size. (A) Growth rate, and (B) log transformed population size for each cell lysate treatment. The ancestor (red circles), the constant low salinity (left panel) and the increasing salinity treatments (right panel, dashed lines) are shown at low and high salinity (n=8 replicate population across treatments).

Table S1 Summary of Cell lysate sources. The halophiles were collected from salt springs in the Namib dessert. The conductivity of the spring at the time of collection is listed for *Halomonas spp*. The *Pseudomonas* relatives were purchased from the German culture collection (accessions number listed).

Strain	Cell Lysate Category	Strain Source	Conductivity (mS/cm)
Halomonas spp. 1 (*Halomonas taeanensis*)	Halophile	Namib Springs Aub Canyon	7.3
Halomonas spp. 2 (*Halomonas*)	Halophile	Namib Springs Kai-As	1
Halomonas spp. 3 (*Halomonas*)	Halophile	Namib Springs Swartmodder	190
Halomonas spp. All (1+2+3)	Halophile		
Pseudomonas chlororaphis subsp. *aureofaciens*	Relative	DSM6698	
Pseudomonas azotifigens	Relative	DSM17556	
Pseudomonas stutzeri JM300	Relative	DSM10701	
Pseudomonas spp. All	Relative		
Self-Lysate + Transposon (*P. stutzeri* + miniTn7)	Self	Baltrus lab	
Self-Lysate (*P. stutzeri*)	Self	Baltrus lab	
None	None		
All	All	A combination of all strains listed above	

Table S2 Populations that did not revive. List of populations that did not revive at the end of the experiment. One population was contaminated (3c).

96- Well Plate	Evolution Treatment	Cell Lysate Treatment
1a	Constant Low Salinity	Halomonas spp. 1 (*Halomonas taeanensis*)
2a	Constant Low Salinity	Halomonas spp. 2 (*Halomonas*)
3a	Constant Low Salinity	Halomonas spp. 3 (*Halomonas*)
4a	Constant Low Salinity	Halomonas spp. All (1+2+3)
4b	Constant Low Salinity	Halomonas spp. All (1+2+3)
5c	Constant Low Salinity	*Pseudomonas chlororaphis* subsp. *aureofaciens*
6g	Constant Low Salinity	Pseudomonas azotifigens
6h	Constant Low Salinity	Pseudomonas azotifigens
10c	Constant Low Salinity	Self Lysate (*P. stutzeri*)
11b	Constant Low Salinity	None
1a	Increasing Salinity	Halomonas spp. 1 (*Halomonas taeanensis*)
3c	Increasing Salinity	Halomonas spp. 3 (*Halomonas*)

Table S3 Expanded transformation efficiency results. Letters for pairwise comparisons across the cell lysate treatments with p-values listed at the bottom of the column.

	Transformation Efficiency			
	Constant Salinity		Increasing Salinity	
	1.50%	3.00%	1.50%	3.00%
Ancestor	a	a	b	a
Self Lysate	b	a	a	a
No Lysate	b	a	ab	a
Halophile Lysate	b	a	ab	a
Relatives Lysate	b	a	a	a
All spp.	b	a	ab	a
	p<0.0001	p=0.055	p=0.0207	p=0.0506

Table S4 Expanded growth rate results. Letters for pairwise comparisons across the cell lysate treatments with p-values listed at the bottom of the column.

	Growth Rate (r)			
	Constant Salinity		Increasing Salinity	
	1.50%	3.00%	1.50%	3.00%
Ancestor	a	a	a	a
Self Lysate	a	ab	ab	ab
No Lysate	a	b	a	abc
Halophile Lysate	a	b	ab	bc
Relatives Lysate	a	b	b	c
All spp.	a	b	ab	c
	p=0.697	p<0.001	p=0.08	p<0.001

Table S5 Expanded population size results. Letters for pairwise comparisons across the cell lysate treatments with p-values listed at the bottom of the column.

	Log_{10} (Populations size)			
	Constant Salinity		Increasing Salinity	
	1.50%	3.00%	1.50%	3.00%
Ancestor	abc	ab	a	ab
Self Lysate	bc	ab	a	ab
No Lysate	ab	a	b	c
Halophile Lysate	abc	b	ab	c
Relatives Lysate	a	ab	a	bc
All spp	c	ab	a	a
	p=0.008	p=0.02	p<0.001	p<0.001