

RESEARCH ARTICLE – Incubator

Chaperonin overproduction and metabolic erosion caused by mutation accumulation in *Escherichia coli*

José Aguilar-Rodríguez^{1,2,‡}, Mario A. Fares^{3,4,†} and Andreas Wagner^{1,2,5,*}¹Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland,²Swiss Institute of Bioinformatics, Lausanne, Switzerland, ³Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain, ⁴Department of Genetics, Smurfit Institute of Genetics, University of Dublin, Trinity College Dublin, Dublin, Ireland and ⁵The Santa Fe Institute, Santa Fe, New Mexico, USA*Corresponding author: Tel: +41 44 635 6141; E-mail: andreas.wagner@ieu.uzh.ch

‡Present Address: Department of Biology, Stanford University, Stanford, CA, USA; Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA, USA.

One sentence summary: A study of the relationship between chaperone overproduction and genome erosion in bacteria evolved in conditions that mimic the evolution of bacterial endosymbionts.

†Posthumous author.

We dedicate this manuscript to the memory of Mario A. Fares.

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ABSTRACT

Bacterial cells adapting to a constant environment tend to accumulate mutations in portions of their genome that are not maintained by selection. This process has been observed in bacteria evolving under strong genetic drift, and especially in bacterial endosymbionts of insects. Here, we study this process in hypermutable *Escherichia coli* populations evolved through 250 single-cell bottlenecks on solid rich medium in a mutation accumulation experiment that emulates the evolution of bacterial endosymbionts. Using phenotype microarrays monitoring metabolic activity in 95 environments distinguished by their carbon sources, we observe how mutation accumulation has decreased the ability of cells to metabolize most carbon sources. We study if the chaperonin GroEL, which is naturally overproduced in bacterial endosymbionts, can ameliorate the process of metabolic erosion, because of its known ability to buffer destabilizing mutations in metabolic enzymes. Our results indicate that GroEL can slow down the negative phenotypic consequences of genome decay in some environments.

Keywords: metabolism; experimental evolution; molecular chaperones; bacterial endosymbionts

INTRODUCTION

Organisms adapting to a constant environment for many generations tend to lose fitness in other environments, because they do not benefit from maintaining fitness in environments they no longer encounter (Mills, Peterson and Spiegelman 1967; Futuyma 1988; Bennett and Lenski 1993; Fong, Kane and Culver 1995). This evolutionary process is known as ecological specialization, and it can be either driven by natural selection or by genetic drift (Cooper and Lenski 2000; Cooper 2014; Leiby and Marx 2014). Specialization can be driven by natural selection if

an adaptive phenotype shows antagonistic pleiotropy—a phenotype that is selected for being optimal in a given environment is deleterious in other environments due to physiological trade-offs. Alternatively, mutations that are neutral in a particular selective environment can be deleterious in other environments, and such mutations can accumulate in a genome, especially in ‘unused’ genomic regions, by random genetic drift. These two causes of ecological specialization are not mutually exclusive.

Specialization through neutral mutations is especially important in bacterial endosymbionts of some insects such as

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aphids (Moya *et al.* 2008; Toft and Andersson 2010; McCutcheon and Moran 2012). These bacteria live inside host cells that provide a highly constant environment, and have been evolving clonally for millions of generations in this environment. Endosymbionts produce nutrients missing from the host diet, while benefiting from the nutrient-rich and stable intracellular environment of the host. They are maternally inherited by the host, and experience severe bottlenecks during their vertical transmission from generation to generation, which result in small effective population sizes and strong genetic drift. Consequently, natural selection is considerably less efficient in these bacteria than in free-living bacteria. Additionally, a lack of functional DNA repair enzymes (which results in high mutation rates) further accelerate the accumulation of mutations in their genomes, and a lack of recombination prevents the effective purging of deleterious mutations (Moran 1996).

As a consequence of their high mutational load, such endosymbionts have evolved a mechanism to buffer deleterious mutations. Specifically, they overproduce proteins that help fold other proteins, molecular chaperones such as GroEL and DnaK, which can reduce the fitness cost of destabilizing mutations in proteins (Baumann, Baumann and Clark 1996; Fares *et al.* 2002; Wilcox *et al.* 2003; McCutcheon, McDonald and Moran 2009; Stoll, Feldhaar and Gross 2009; McCutcheon and Moran 2012; Bennett and Moran 2013; Fan *et al.* 2013; Oakeson *et al.* 2014). In the rich chemical environment that their host cells provide, selection on many metabolic genes is relaxed in these endosymbionts (Moya *et al.* 2008; Moran, McLaughlin and Sorek 2009; Toft and Andersson 2010; Latorre and Manzano-Marín 2017). Therefore, these genes tend to accumulate loss-of-function mutations that cause metabolic erosion, a process by which an organism loses metabolic abilities, such as the ability to metabolize nutrients.

In the Long-Term Evolution Experiment (LTEE), 12 populations of *Escherichia coli*—the closest free-living relative to the well-studied endosymbiont of aphids *Buchnera aphidicola* (Moran and Wernegreen 2000)—have evolved for more than 60 000 generations (and counting) in a minimal medium containing glucose as the only source of carbon and energy with an effective population size of $\sim 3.3 \times 10^7$ (Lenski *et al.* 1991), which is larger than in bacterial endosymbionts. Adaptation to this single environment has resulted in decreased performance in other environments (Cooper and Lenski 2000; Leiby and Marx 2014). Some populations have evolved hypermutable (mutator) phenotypes (Sniegowski, Gerrish and Lenski 1997). In these populations the higher mutation rate caused substantial metabolic erosion, measured as the number of different chemical environments where fitness has declined. This suggests that rather than physiological trade-offs underlying antagonistic pleiotropy, the neutral process of mutation accumulation suffices to cause metabolic specialization in the LTEE (Leiby and Marx 2014). Additionally, the fitness reduction of mutator populations is ameliorated by growth at a lower temperature. Most amino acid substitutions are slightly deleterious because of their destabilizing effects, and destabilizing mutations tend to increase a protein's heat sensitivity (DePristo, Weinreich and Hartl 2005). Therefore, temperature-dependent fitness reduction suggests that in these populations metabolic erosion is caused by destabilizing mutations affecting metabolic enzymes (Leiby and Marx 2014). If this is the case, the overproduction of chaperones that can buffer mutations affecting protein stability could have a similar effect to low temperature in ameliorating metabolic erosion caused by mutation accumulation.

GroEL is a member of the family of chaperones known as chaperonins, which are large double-ring complexes that

enclose target proteins for folding within a cylindrical folding chamber (Hartl, Bracher and Hayer-Hartl 2011). GroEL can buffer destabilizing mutations in its target proteins (Fares *et al.* 2002; Maisnier-Patin *et al.* 2005; Tokuriki and Tawfik 2009; Bogumil and Dagan 2010; Williams and Fares 2010; Bershtein *et al.* 2013; Wyganowski, Kaltenbach and Tokuriki 2013; Pechmann and Frydman 2014; Sabater-Muñoz *et al.* 2015). Many of these proteins are metabolic enzymes (Takemoto, Niwa and Taguchi 2011). Enzymes evolved under GroEL overproduction accumulate twice as many mutations, and these mutations have higher destabilizing effects than in the absence of GroEL overproduction (Tokuriki and Tawfik 2009). These observations make it plausible that chaperones such as GroEL could slow down the process of metabolic erosion in endosymbionts.

One of the first demonstrations that GroEL can buffer deleterious mutations came from *E. coli* populations with high mutational loads (Fares *et al.* 2002). These populations had evolved for more than 3000 generations via single-cell bottlenecks on solid glucose-limiting minimal medium. Half of the populations evolved at a high mutation rate. The evolution of these populations was dominated by genetic drift, due to the extreme bottlenecks they experienced during their evolution, which are similar to bottlenecks experienced by endosymbionts during their vertical transmission between hosts. At the end of the experiment, the evolved populations had considerably reduced their fitness (growth rate) in the experimental environment. Moreover, overproducing GroEL in the evolved populations restored fitness to almost ancestral levels. This restoration was observed after supplementing the minimal growth medium with amino acids, otherwise, the energetic cost of overproducing GroEL was so great that no fitness recovery was observed. Similar results were later obtained in mutator populations of *Salmonella typhimurium* (Maisnier-Patin *et al.* 2005). Both studies focused on a single environment. In this study, we investigate whether GroEL overproduction can ameliorate the metabolic erosion experienced by bacteria evolved under conditions that favor the accumulation of mutations in a large number of environments. To address this question, we study mutator *E. coli* populations evolved for thousands of generations under conditions that emulate those of bacterial endosymbionts evolution and we assay their ability to metabolize different carbon substrates using phenotype microarrays.

MATERIALS AND METHODS

Strains and plasmids

Strains E1, E2 and E3 derive from *E. coli* K12 substr. MG1655 Δ mutS. They evolved in parallel in a long-term mutation accumulation experiment on solid LB medium at 37°C (Alvarez-Ponce *et al.* 2016; Sabater-Muñoz *et al.* 2017), where each population was passaged for 250 days through a single-cell (clonal) bottleneck after 24 hours of growth. Passaged colonies were chosen randomly.

We derived the control plasmid pGro7c from the plasmid pGro7 (Takara, Cat. #3340) via PCR amplification using primers TGATAACTCTCCTTTGAGAAAGTCGG and TTGCCCTG CACCTCGCAGAAATAA, and Phusion® High-Fidelity DNA polymerase (NEB), after having obtained permission from Takara to modify pGro7. We digested the PCR products with Dpn1 to remove pGro7. We separated the digested PCR products on a 0.8% agarose gel, excised the band corresponding in size to the desired amplification product, purified the product using the QIAquick Gel extraction kit (Qiagen, #50928704), and quantified

it using a Nanodrop spectrophotometer. We generated pGro7c by ligating the amplification product using T4 DNA ligase (NEB). We validated the sequence of pGro7c using Sanger sequencing. We transformed the ancestor (A) strain, and the evolved lines (E1, E2 and E3) with pGro7 and pGro7c to generate eight different strains: A/pGro7, E1/pGro7, E2/pGro7, E3/pGro7, A/pGro7c, E1/pGro7c, E2/pGro7c and E3/pGro7c.

Phenotype microarrays

We used Biolog phenotype microarray PM1 (Biolog, Inc., Hayward, California, USA) to assay the carbon utilization phenotypes of each of the eight strains on 95 different carbon sources. Both cell growth and respiration contribute to these metabolic phenotypes, because respiration can be independent of growth (Leiby and Marx 2014). We performed four replicate Biolog assays for each strain, for a total of $8 \times 4 = 32$ microarrays. To do so, we streaked each strain from glycerol stocks onto LB agar plates supplemented with 25 $\mu\text{g}/\text{mL}$ of chloramphenicol (Sigma-Aldrich #C0378). We incubated the plates at 37°C for 24 hours, and re-streaked the colonies onto fresh plates of the same type, which we incubated at the same temperature for the same period of time. We resuspended the colonies from the latter plates in IF-0 solution (Biolog, Inc., Hayward, California, USA) using sterile cotton swabs, and then centrifuged the suspension at 3000 g for 3 min. We resuspended the cell pellet in fresh IF-0 to an optical density at 600 nm (OD_{600}) of approximately 0.05 (as measured in a 200 μL suspension volume). We diluted (1:5) this suspension in IF-0 + dye (Biolog, Inc., Hayward, California, USA) supplemented with 25 $\mu\text{g}/\text{mL}$ of chloramphenicol, L-methionine (5 μM), cyanocobalamin (0.125 μM) and 0.2% (w/v) L-arabinose (Sigma-Aldrich #3256). We added 100 μL of the resulting solution to each well of a PM1 microarray, which we incubated without shaking at 37°C for 24 hours. We measured OD_{600} and optical density at 750 nm (OD_{750}) at 0 min and 24 hours using a microplate reader (Tecan Spark 10 M). We performed nine reads per well in a 3-by-3 square grid, and computed the average.

Both the presence of oxidized tetrazolium and that of cells cause absorbance at 600 nm. The absorbance at 600 nm due to the presence of cells can be removed by subtracting the OD_{750} from the OD_{600} , because tetrazolium has almost no absorbance at 750 nm. We therefore used these cell-density corrected values in our study ($\text{OD}_{600-750}$), and computed the metabolic phenotype M_S for substrate S as $\text{OD}_{600-750,24\text{ h}} - \text{OD}_{600-750,0\text{ h}}$, where $\text{OD}_{600-750,24\text{ h}}$ is the corrected optical density after 24 hours, and $\text{OD}_{600-750,0\text{ h}}$ is the corrected optical density at the start of the experiment. We used a minimum threshold to detect respiration in a given substrate S (Sprouffs et al. 2018). To obtain this threshold we computed the absolute differences between all pairs of 3072 $\text{OD}_{600-750,0\text{ h}}$ values (8 strains \times 4 replicates \times 96 wells). Because at time 0, cells have not started to metabolize yet, these differences must be caused by experimental noise. We only consider differences in M_S as significant if they are greater than 0.039, which is the 98-th percentile of all the differences between wells with no growth.

For each evolved strain we compared the four experimental measurements of M_S with the corresponding measurements for the ancestor using a two-tailed t-test. In particular, we compared the three evolved populations with pGro7 (E1/pGro7, E2/pGro7 and E3/pGro7) against A/pGro7, and the three evolved populations with pGro7c (E1/pGro7c, E2/pGro7c and E3/pGro7c) against A/pGro7c. We adjusted the P values for multiple testing using the false discovery rate method. We consider that a metabolic phenotype has not declined significantly if $P > 0.05$, and both

the evolved and the ancestral average M_S values are greater than 0.039 (the noise threshold).

We quantified metabolic erosion in a given evolved population as

$$\frac{P - C}{P} \quad (1)$$

P refers to the number of carbon sources where M_S is greater than the noise threshold in A/pGro7 for evolved strains overexpressing GroEL, and in A/pGro7c for evolved strains that do not overexpress GroEL. C refers to the number of carbon sources where the metabolic phenotypes has not declined significantly in the evolved population.

RESULTS

Our experiment begins with three independent clonal populations from a mutation accumulation experiment that had been initiated from the same mutator clone (*E. coli* K12 MG1655 ΔmutS). The populations had been evolved by daily passaging through single-cell bottlenecks on solid rich growth (LB) medium at 37°C (Alvarez-Ponce et al. 2016; Sabater-Muñoz et al. 2017) for 250 days or approximately 5500 generations (~22 generations between bottlenecks). In such an evolution experiment, the efficiency of natural selection is severely reduced because of the extreme bottlenecks to which the evolving lineages are exposed. In consequence, non-lethal mutations can accumulate freely under the influence of genetic drift (Barrick and Lenski 2013).

We transformed the ancestor and a clone from each of the three evolved populations with the plasmid pGro7 (Nishihara et al. 1998). This plasmid contains the operon *groE*, which encodes GroEL, and its co-chaperone GroES, under the regulation of a single promoter inducible by L-arabinose. We also transformed the ancestor and each of the three evolved clones with a control plasmid pGro7-DEL(*groE*) (pGro7c) that lacks the operon *groE* but is otherwise identical to pGro7 (Materials and Methods). In the presence of the expression inducer L-arabinose, only cells harboring the plasmid pGro7 overproduce GroEL. In total, we thus generated eight strains from the three evolved clones (E1, E2 and E3) and their ancestor (A). Four of these strains contain the plasmid pGro7 (A/pGro7, E1/pGro7, E2/pGro7 and E3/pGro7), and the other four contain the control plasmid pGro7c (A/pGro7c, E1/pGro7c, E2/pGro7c and E3/pGro7c). L-arabinose cannot be used as a carbon source by any of these strains.

To study metabolic erosion in the evolved populations, we used Biolog phenotype microarrays, which allow the high-throughput measurement of growth and cellular respiration in multiple environments contained in different wells of a 96-well microtiter plate (Bochner, Gadzinski and Panomitros 2001; Bochner 2009). These arrays measure metabolic activity using tetrazolium, a redox dye that absorbs the electrons from the electron transport chain, and changes color when being reduced by respiring cells. Biolog assays are widely used, and have been useful to map the phenotypes of various genotypes (e.g. gene knock-out mutants) (Bochner, Gadzinski and Panomitros 2001; Zhou et al. 2003; Pommerenke et al. 2010), to study metabolic innovation (Toll-Riera et al. 2016), to study macroevolutionary patterns of phenotypic evolution in bacteria (Plata, Henry and Vitkup 2015), or to characterize the phenotypes of experimentally evolved lineages (Le Gac et al. 2012; Hug and Gaut 2015). Specifically, we used PM1 plates, which measure cellular respiration and growth in 95 chemical environments distinguished by their carbon source, to determine the metabolic phenotypes

Table 1. Metabolic erosion in the presence and absence of GroEL overproduction for each of three populations evolved in a mutation accumulation experiment for more than 5000 generations.

Evolved population	Plasmid ^a	Metabolic erosion ^b
E1	pGro7c	98.7% (76/77)
E1	pGro7	88.6% (62/70)
E2	pGro7c	90.9% (70/77)
E2	pGro7	75.7% (53/70)
E3	pGro7c	100% (77/77)
E3	pGro7	95.7% (67/70)

^aCells harboring the plasmid pGro7 overproduce GroEL in the presence of the expression inducer L-arabinose, while cells harboring the control plasmid pGro7c do not.

^bThe fraction of those carbon sources on which evolution caused significant metabolic erosion, based on carbon sources which the ancestor metabolizes above the noise threshold of 0.039 out of 95 tested carbon sources (Materials and Methods).

for each of the eight strains used in this study (Materials and Methods, Table S1, Supporting Information).

We define metabolic erosion as the fraction of carbon sources that an evolved population metabolizes more slowly than the ancestor strain from which the mutation accumulation experiment started. To measure metabolic erosion, we only consider carbon sources that the ancestor was able to metabolize above a minimum threshold that is defined by measurement noise (Materials and Methods). We consider that a metabolic phenotype has not declined if it is above this noise threshold, and if it is not significantly lower than the ancestral phenotype (*t*-test, false discovery rate < 0.05, Materials and Methods). To evaluate the extent of this erosion in the evolved populations, we first focused on the strains that harbor the control plasmid pGro7c, that is, they do not overproduce GroEL. After having experienced 250 single-cell bottlenecks, that is, ~5500 generations of mutation accumulation, the evolved populations (E1/pGro7c, E2/pGro7c and E3/pGro7c) fare considerably worse than their ancestor (A/pGro7c) in a majority of the carbon sources tested (between 100% to 90.9%; Table 1).

The evolved lineages E1 and E2 had been sequenced in a previous study (Alvarez-Ponce *et al.* 2016). Analyzing the sequence data, we find that metabolic erosion and the number of accumulated nonsynonymous mutations are not associated in a straightforward pattern. For example, population E1 accumulated 303 mutations and metabolizes 98.7% of carbon sources more poorly than the ancestor, while population E2 accumulated more (731) mutations and shows metabolic erosion on fewer (90.9%) carbon sources. Of the 304 and 731 nonsynonymous mutations accumulated in lineages E1 and E2, 139 and 277 mutations (45.9% and 37.9%, respectively) affected metabolic enzymes (Keseler *et al.* 2013). Lineages E1 and E2 had 182 and 224 metabolic pathways (43% and 53%, respectively) affected by nonsynonymous mutations (Keseler *et al.* 2013). The observation that E2 cells express GroEL and DnaK more highly than E1 cells may help explain the poor correlation between metabolic erosion and the number of accumulated mutations. Similarly to GroEL, DnaK is a chaperone that can help buffer the effects of mutation, and that is highly expressed in bacterial endosymbionts (Aguilar-Rodríguez *et al.* 2016; Kadibalban *et al.* 2016).

To find out whether chaperones can indeed mitigate metabolic erosion, we turned to the mutation accumulation populations that overproduce GroEL. To analyze data from these populations, however, we needed to take into account that chaperone overproduction carries energetic costs (Fares *et al.* 2002;

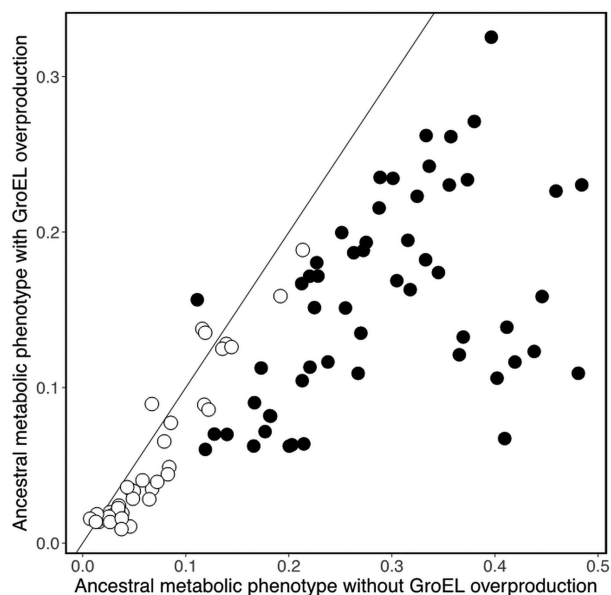


Figure 1. GroEL overproduction incurs metabolic costs. Metabolic phenotypes of the ancestor on 95 different carbon sources. Each circle represents the rate at which the ancestor can metabolize one of the carbon sources averaged over four technical replicates of the ancestor without GroEL overproduction (horizontal axis) and with GroEL overproduction (vertical axes). The diagonal line indicates equal metabolic rates with and without GroEL overproduction. Circles below the diagonal line represent carbon sources where GroEL overproduction causes a reduction in metabolic rate. Filled circles indicate environments where the difference in metabolic rate with and without chaperonin overproduction is statistically significant, i.e. greater than experimental measurement noise (Materials and Methods).

Sabater-Muñoz *et al.* 2015). The ATP consumed in the reaction cycle of the chaperonin contributes less to this cost than the synthesis of large amounts of GroEL and GroES proteins (Fares *et al.* 2002; Bogumil and Dagan 2012). The cost is evident by comparing the ancestral strains A/pGro7 with A/pGro7c, where GroEL overproduction causes a decline in metabolic rates in most environments (Fig. 1). To take this cost into account, we compared the metabolic phenotypes of evolved strains overexpressing GroEL (E1/pGro7, E2/pGro7 and E3/pGro7) with the corresponding ancestor (A/pGro7). We find that populations overexpressing GroEL show less metabolic erosion than populations with no GroEL overproduction (Table 1). However, this reduction in metabolic erosion is small (it occurs in only 9.9% of environments on average; e.g. D-Galactose, D-Gluconic Acid and L-Glutamic Acid for both E1 and E2) because the number of environments where there is no decline of the metabolic phenotype with GroEL overproduction is small (average = 9.3). We observe that populations with a higher level of metabolic erosion show lower amelioration of this erosion after GroEL overproduction (Table 1). However, this correlation is not significant (Pearson's correlation coefficient = -0.910, *P* = 0.273), probably because it is based on little data (*n* = 3).

DISCUSSION

Bacteria evolving for many generations in the same chemical environment experience a process of metabolic specialization by which they lose or reduce their ability to metabolize many different metabolic substrates. Arguably, bacterial endosymbionts provide the best well-known examples of this process (Latorre and Manzano-Marín 2017). In some cases,

the decline of metabolic phenotypes in these bacterial species can cause substantial fitness reductions in the host (Latorre and Manzano-Marín 2017). Mutation accumulation experiments mimic some of the conditions present during the evolution of intracellular bacteria, and bacteria evolved in this type of experiment acquire some characteristics common to endosymbionts (Sabater-Muñoz et al. 2017), such as high mutational loads (Lee et al. 2012; Sabater-Muñoz et al. 2015; Aguilar-Rodríguez et al. 2016), genome reduction (Nilsson et al. 2005) or higher expression of molecular chaperones such as GroEL and DnaK (Maisnier-Patin et al. 2005; Sabater-Muñoz et al. 2017). In this study, we analyzed mutator populations subject to daily single-cell bottlenecks for more than 5000 generations in a nutrient-rich environment. At the end of this mutation accumulation experiment, these bacterial populations showed similar patterns of genome reduction, mutational biases and gene expression (Sabater-Muñoz et al. 2017).

Previous studies of bacteria evolved in mutation accumulation experiments have shown that they can experience great fitness reductions, which can be partially compensated by the overproduction of chaperones (Fares et al. 2002; Maisnier-Patin et al. 2005; Sabater-Muñoz et al. 2015; Aguilar-Rodríguez et al. 2016). However, these studies did not explore fitness reduction in environments different from the environment in which evolution took place. Here, using phenotype microarrays, we assayed evolved populations in 95 different environments distinguished by their carbon sources. We find that bacterial populations evolved under strong genetic drift can lose or reduce their ability to metabolize many distinct carbon sources. Because these populations have evolved under conditions that considerably reduce the power of natural selection to drive the fixation of beneficial mutations, the most likely cause for metabolic erosion is the accumulation of mutations driven by genetic drift, rather than the fixation of advantageous mutations that show antagonistic pleiotropy (Leiby and Marx 2014).

However, we note that there is no correlation between the number of accumulated nonsynonymous mutations and metabolic erosion in two of the evolved populations for which we have whole-genome sequences from a previous study (Alvarez-Ponce et al. 2016). There is also no correlation between the number of nonsynonymous mutations in metabolic enzymes, and metabolic erosion. There are at least two possible explanations for why the relationship between mutational load and erosion may not be straightforward. First, neutral or slightly deleterious mutations in metabolic enzymes can be highly pleiotropic because of the organization of metabolism as a reaction network. A loss-of-function mutation in a highly connected enzyme can simultaneously and negatively affect several distinct metabolic phenotypes. The second non-exclusive possibility is that the relationship between mutational load and erosion may be influenced by chaperone expression. We find that the population with a higher chaperone expression shows less metabolic erosion (Sabater-Muñoz et al. 2017). The effect of chaperone expression on metabolic erosion may be mediated through the buffering of destabilizing mutations in metabolic enzymes (Tokuriki and Tawfik 2009). Such mutations may have also caused the erosion observed in populations from the LTEE (Leiby and Marx 2014).

GroEL and the Hsp70 chaperone DnaK are the most abundant proteins in the cytosol of endosymbiotic bacteria (Baumann, Baumann and Clark 1996; Wilcox et al. 2003; McCutcheon, McDonald and Moran 2009; Stoll, Feldhaar and Gross 2009; McCutcheon and Moran 2012; Bennett and Moran 2013; Fan et al. 2013; Oakeson et al. 2014). For instance, GroEL expression is 7.5

times higher in *B. aphidicola* than in its close free-living relative *E. coli* (Baumann, Baumann and Clark 1996). These observations suggest that GroEL and DnaK help these cells cope with their high mutational loads by buffering the negative effects of destabilizing mutations in proteins, and this hypothesis has experimental support (Fares et al. 2002; Maisnier-Patin et al. 2005; Sabater-Muñoz et al. 2015; Aguilar-Rodríguez et al. 2016). Here, we find some further evidence that chaperones could ameliorate some of the negative effects of metabolic erosion. In particular, we observe that evolved populations overproducing the chaperonin GroEL show less metabolic erosion than in the absence of chaperonin overproduction. However, we only observe this mitigating effect of GroEL overproduction in a few environments. This is not remarkable if we consider that GroEL is not an all-powerful molecular machine, capable of buffering any possible deleterious mutation. The buffering ability of GroEL is most certainly restricted to certain proteins and types of mutations (Wang et al. 2002; Kerner et al. 2005; Fujiwara et al. 2010; Tartaglia et al. 2010; Williams and Fares 2010). However, it would be worth exploring if GroEL overproduction could show greater amelioration of metabolic erosion in populations evolved for fewer generations, and therefore with smaller mutational loads. Another interesting avenue for further work would be to study metabolic erosion with different expression levels of GroEL to better map the trade-off between expression cost and buffering benefit (Sabater-Muñoz et al. 2015).

In summary, we show that bacterial populations evolving in conditions emulating the evolution of bacterial endosymbionts experience a severe reduction in their ability to metabolize many distinct carbon substrates. We find that the relationship between this metabolic erosion and mutational load is not straightforward, and could be influenced by both pleiotropy and chaperone expression. Finally, we show that GroEL overproduction can mitigate metabolic erosion in some environments. In so doing, we provide evidence that molecular chaperones can reduce in more than one environment the negative impact of genome decay on cell metabolism.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSLE](https://academic.oup.com/femsle/article-abstract/36/6/10/fnz121/5509575) online.

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