MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 2430–2438 doi: 10.1111/j.1365-294X.2010.04664.x

# Epistasis and frequency dependence influence the fitness of an adaptive mutation in a diversifying lineage

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**Abstract**

**The opportunity for a mutation to invade a population can dramatically vary depending on the context in which this mutation occurs. Such context dependence is difficult to document as it requires the ability to measure how a mutation affects phenotypes and fitness and to manipulate the context in which the mutation occurs. We identified a mutation in a gene encoding a global regulator in one of two ecotypes that diverged from a common ancestor during 1200 generations of experimental evolution. We replaced the ancestral allele by the mutant allele, and vice versa, in several clones isolated during the time course of the evolution experiment, and compared the phenotype and fitness of clones isogenic except for the focal mutation. We show that the fitness and phenotype of the mutation are strongly affected by epistatic interactions between genes in the same genome, as well as by frequency dependent selection resulting from biotic interactions between individuals in the same population. We conclude that amongst the replicate population in which it spread, the mutation we identified is only adaptive when occurring in specific genomes and competing with specific individuals. This study thus demonstrates that the opportunity for an adaptive mutation to spread in an evolutionary lineage can only be understood in the light of its genomic and competitive environments.**

*Keywords*: adaptation, adaptive mutation, competitive environment, diversification, experimental evolution, genomic background

*Received 27 January 2010; revision received 1 April 2010; accepted 9 April 2010*

## Introduction

The effect of a mutation is expected to be context dependent. Carefully documenting this context dependence is not an easy task as it requires being able to measure how a mutation affects phenotypes and fitness and to manipulate the context in which the mutation occurs. A few examples have nevertheless been studied in detail (Dean & Thornton 2007; Mitchell-Olds *et al.* 2007). Some clearly illustrate how the fitness of a mutation depends on the environment in which the mutation occurs. For example, in response to predation, a single amino-acid replacement has been shown to induce adaptive changes in the coat colour of the beach mouse living on dark or light coloured soils (Hoekstra *et al.* 2006). The effect of a mutation is also expected to depend on the genomic context in which it occurs and thus on epistatic (i.e. non-additive) interactions with other alleles in the same genome (Bull *et al.* 2000; Phillips *et al.* 2000; Remold & Lenski 2004; Segre *et al.* 2005; Weinreich *et al.* 2005, 2006; Martin *et al.* 2007; Poelwijk *et al.* 2007; Cooper *et al.* 2008; Phillips 2008; Yukilevich *et al.* 2008). The importance of such genetic interactions has been illustrated for five mutations jointly increasing antibiotic resistance in a single β-lactamase allele. Of the 120 possible mutational paths from the ancestral allele to the one conferring strong resistance, only 18 paths allow an increase in antibiotic resistance at each mutational step (Weinreich *et al.* 2006). Finally, the fitness of a mutation is also expected to strongly depend on interactions with competitors, leading to frequency-dependent selection pressures (Ayala & Campbell 1974; Rosenzweig *et al.* 1994; Treves *et al.* 1998; Lunzer *et al.* 2002; Wolf 2003; Fitzpatrick *et al.* 2007). Negative frequency-dependent selection on two alleles has for

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example been shown to be involved in the maintenance of a stable foraging behaviour polymorphism in populations of larval fruitflies (Fitzpatrick *et al.* 2007).

It is, however, virtually unknown how such context dependence might influence the evolutionary trajectory of a potentially adaptive mutation along a single evolutionary lineage.

In the present work, we use an experimental system in which initially isogenic populations of *Escherichia coli* B diversified into populations containing two different ecotypes, i.e. bacterial strains occupying different ecological niches, during 1200 generations of experimental evolution (Le Gac *et al.* 2008; Spencer *et al.* 2008; Tyerman *et al.* 2008). These two ecotypes are coexisting due to frequency-dependent ecological interactions in the evolution environment containing two carbon sources, glucose and acetate. They are named FS (Fast Switcher) and SS (Slow Switcher) in reference to the way they switch from the consumption of one carbon source to the other. SS is a fast glucose consumer and FS an efficient glucose and acetate consumer (Le Gac *et al.* 2008). An analysis of global gene expression (Le Gac *et al.* 2008) revealed that numerous genes controlled by ArcA, a global regulator of gene expression in response to the redox conditions of growth, are differentially expressed in the two ecotypes. Here we report a non-synonymous point mutation in the FS *arcA* gene. We used allelic replacement techniques, associated with phenotypic assays and competition experiments between clones isogenic except for the *arcA* mutation, to quantify the fitness and phenotypic effect of this mutation. By replacing the ancestral allele by the mutant allele and vice versa in several clones isolated at different time points during the experimental evolution, we investigated how the genomic background affects the phenotype and fitness associated with the mutation. By performing the competition experiments in both the presence and absence of the other ecotype, we tested how the population composition affects the fitness of the *arcA* mutation. Results indicate that the phenotype and fitness effects of the mutation are strongly affected by both genomic background and the population composition, illustrating how along a single evolutionary lineage, the fate of an adaptive mutation can be determined by epistatic interactions and frequency-dependent selection.

## Materials and methods

### *Bacterial strains and media*

Bacteria were isolated from a single population that was founded by *E. coli B* REL606 and that evolved during 1200 generations in batch culture. The evolution was conducted by transferring, every 24 ± 2 h, 100 μL of culture in 18 mm-diameter test tubes supplied with 10 mL of sterile Davis Minimal medium supplemented with 0.25 g/L (1.4 mM) glucose and 1.32 g/L (9.7 mM) sodium acetate trihydrate (DMga) as the sole carbon sources and incubating the cultures at 37 °C, 250 rpm (Le Gac *et al.* 2008; Spencer *et al.* 2008; Tyerman *et al.* 2008). FS and SS were distinguished based on their growth rate between 5–10 h after inoculation in fresh medium. During the first 5 h after incubation, SS tend to grow very fast (Fig. 1 and Fig. S1), but almost stop growing after 5 h of incubation (average growth rate between 5–10 h ± SE = 0.039 ± 0.004 $h^{-1}$). FS tend to grow slower than SS during the first 5 h, but continue to grow after the first 5 h (average growth rate between 5 and 10 h ± SE = 0.272 ± 0.014 $h^{-1}$, $t = 17$, DF = 24, $P < 0.0001$).

Twenty six original clones were isolated on TT plates (Levin *et al.* 1977): three ancestral clones (to check that the plating does not affect phenotypes and fitness; considered as SS based on their growth profiles); three clones (either two FS and one SS, or one FS and two SS) every 200 generations from generation 200 to generation 800; two SS, one FS without the mutation and three FS with the mutation at generation 1000; two FS

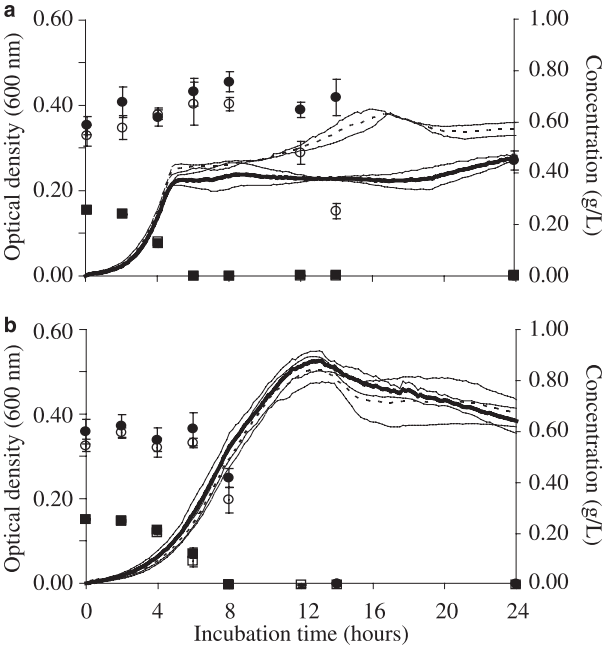


**Fig. 1** Growth profiles and carbon consumption with (dotted line, open symbols) or without (full line, full symbols) the *arcA* mutation of an a.SS and b. FS ecotype isolated after 1000 generations of experimental evolution. The thick lines indicate the growth profile, the circles indicate acetate concentration and the squares indicate glucose concentration. The thin lines and error bars indicate the SE.

with the mutation and three SS at generation 1200 (Fig. S1).

### *Allelic replacement*

To perform the allelic replacement, we amplified the ancestral and mutant *arcA* alleles (arcAF: CAGTAAAGAAGTTACAACGGACGAT and arcAR: GTTATACGCATATTGCCACTCTTCT), cloned the DNA fragments in the pCR2.1-TOPO® (http://www.invitrogen.com) vector, then in the pUC57 vector after digestion with BamHI/XbaI and finally in the pKO3 suicide vector (Link *et al.* 1997) after digestion with NotI/SmaI. The ancestral allele was replaced by the mutant in the 21 clones originally carrying the ancestral alleles and the mutant allele was replaced by the ancestral one in the five clones originally carrying the mutation. The mutation removes one of the Hpy188III restriction sites in the *arcA* gene. This property was used to screen for the actual allelic replacement. After the allelic replacement protocol, for each original clone, two clones were chosen, one with and one without the actual allelic replacement (52 final clones). The *arcA* gene of all final clones was sequenced to ensure the absence of unwanted mutation.

### *Phenotypic assays*

All phenotypic assays were performed in triplicate. For all 52 final clones, growth profiles were generated using a Bioscreen C plate reader (MTX Lab Systems Inc., Vienna, VA USA) by inoculation 200 μL of DMga with 2 μL of overnight culture, incubating at 37 °C and measuring the optical density at 600 nm every 10 min during 24 h (preliminary analysis indicated that the growth profiles are qualitatively similar in test tubes and microplates). Glucose and acetate concentrations were measured in cultures of three original clones (ancestor, one FS and one SS isolated at 1000 generations), with and without the *arcA* mutation, using Megazyme acetic acid (acetate kinase) and D-Glucose HK kits (http://www.megazyme.com).

### *Fitness assays*

All fitness assays were performed in triplicate. Spontaneous ara+ mutants were isolated on MA plates for all the final clones (Lenski 1988). Growth profile analyses and preliminary competition experiments between isogenic clones except for the ara+/− marker did not show any effect of this marker on both the growth profile and the fitness of bacteria. Nevertheless, to minimize any potential effect, the ara+/− markers were assigned randomly for all the competition experiments.

Pairwise competition experiments were initiated between the same original clone with and without the *arcA* mutation, each competitor displaying a different ara+/− marker. Bacteria were grown overnight in 10 mL DMga, then mixed by volume to a proportion mutant:ancestral allele 0.9:0.1 and 0.1:0.9. Competition experiments were initiated by adding 100 μL of this mix in 10 mL DMga and were performed over 6 days (to facilitate the detection of small fitness advantages), following the exact protocol of the evolution experiment (Spencer *et al.* 2008). Initial frequencies of mutant and ancestral alleles, as well as after 2, 4 and 6 days of competition, were measured by plating diluted cultures on TA plates (Levin *et al.* 1977) and counting red (ara−) and white (ara+) colonies after incubation at 37 °C.

To perform the three-way competitions, at each of the 200, 400, 600, 800, 1000 and 1200 generations time points, one FS and one SS clone were chosen randomly (to the exclusion of two original FS clones, one isolated at 200 generations and one at 1200 generations which were discarded due to some peculiarities in terms of growth profile or fitness in the pairwise competitions that seemed specific to these clones). At each time point, four types of competitions were initiated by mixing by volume, 1. 0.9FS*, 0.05FS and 0.05SS; 2. 0.1FS*, 0.45FS, 0.45SS; 3.0.9SS*, 0.05SS, 0.05FS; 4. 0.1SS*, 0.45SS, 0.45FS. Competitions were performed as indicated above for the pairwise competitions. At each time point, FS and SS displayed the same ara+/− marker, while the clone carrying the mutation displayed the opposite marker. We note that with this experimental design we are unable to follow the frequency of FS and SS separately, which is indeed not needed because we are only interested in following the frequency of the *arcA* mutation.

The relative fitness of the mutation was taken to be the slope of the linear regression corresponding to the change in mutant frequency over the 6 days of competition.

### *Statistical analysis*

We used three kinds of standard least square statistical models with (i) growth rate between 5 and 10 h in a single batch as the response variable and presence/absence of the mutation (testing whether the presence of the mutation affects the growth rate), generations (testing whether the growth rate is affected by the time points) and the interaction term (testing whether the presence of the mutation affects the growth rate differently at different generations) as the explanatory variables; (ii) fitness of the mutation in the pairwise competitions as the response variable and ecotype (testing whether the fitness of the mutation is different in the two ecotypes), generations (testing whether the

fitness of the mutation is different at different generations) and the interaction term (testing whether the fitness of the mutation in the two ecotypes varies differently at the different time points) as the explanatory variables; (iii) fitness of the mutation as the response variable and ecotype (testing whether the fitness of the mutation is different in the two ecotypes), presence of the other ecotype (testing whether the fitness of the mutation is affected by the presence of the other ecotype) and the interaction term (testing whether the fitness of the mutation is affected differently in the two ecotypes by the presence of the other ecotype) as explanatory variables.

## Results

We focused on an experimental lineage in which an ancestral *E. coli* B diversified into two ecotypes, FS and SS, during 1200 generations of experimental evolution. FS and SS differ in their growth profile in the evolution environment (Fig. 1). SS is a fast glucose consumer, depleting all the glucose available in the environment in less than 6 h (Fig. 1). Once the glucose is depleted, SS eventually switches to acetate consumption, but is unable to consume all the acetate available. FS is an efficient glucose and acetate consumer, depleting the glucose in less than 8 h and the acetate in less than 12 h (Fig. 1). These two ecotypes are detected as early as 200 generations until the end of the evolution experiment. Moreover, the two ecotypes are coexisting by negative frequency dependence selection with a stable equilibrium frequency of about 60% of FS (Fig. S1).

We sequenced the gene *arcA*, a global regulator of gene expression in response to the redox conditions of growth, in FS and SS clones isolated from the beginning until the end of the evolution experiment. A non-synonymous point mutation, responsible for the substitution Thr81Ala at the protein level, was identified in FS clones isolated at 1000 and 1200 generations, but not before that. This mutation, replacing a polar amino-acid by a non-polar one, occurs at a site forming a side chain involved in a hydrogen bond with the phosphate responsible for the activation of ArcA (Toro-Roman *et al.* 2005) and may thus strongly affect the activity or stability of the active protein. We sought to characterize the phenotypic effect, fitness and epistatic interactions of this point mutation in genomic backgrounds isolated from different points in time of the diversifying evolutionary lineage. We replaced the ancestral allele by the mutant allele or vice versa, in a total of 26 FS and SS clones (Fig. S1) isolated every 200 generations from the beginning until the end of the evolution experiment (hereafter, FS and SS refer to the ecotypes without the *arcA* mutation, while FS* and SS* indicate the presence of the mutation). We measured the 24 h growth profile of each clone with and without the *arcA* mutation (Fig. 1 and Fig. S1). When occurring in the SS genome, the mutation considerably improved the growth rate between 5–15 h after inoculation (Fig. 2, Table 1). This improvement was associated, with a faster consumption of acetate (Fig. 1). In contrast, the growth profile of FS* clones did not vary significantly from FS (Figs 1 and 2, Table 1). These results indicate that the mutation interacts very differently with FS and SS genomes. Quite surprisingly, even though it was only observed in the FS ecotype, the mutation has a stronger and potentially beneficial, effect in the SS ecotype.

We also investigated how the mutation acts on the fitness of the 26 FS and SS clones. We performed pairwise competition experiments between the two *arcA* alleles in the genomic background of each of these 26 clones. The competitions were initiated with a mutant frequency of 0.9 and 0.1. The slope of the linear regression over 6 days of competition was considered as a proxy for the relative fitness of the mutation when competing with the ancestral allele, in a common genomic background. As such, a relative fitness of 0 indicates no detectable change in genotype frequency over the

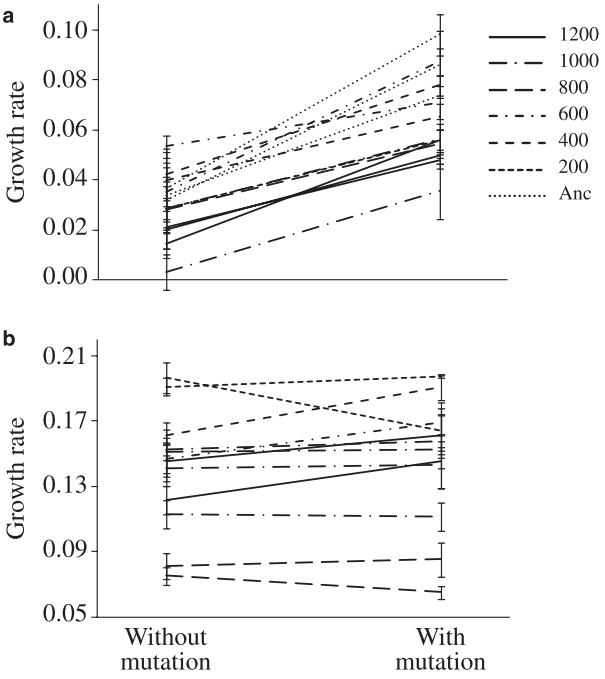


**Fig. 2** Average growth rate (±SEM) between 5–15 h after inoculation of the 26 original (A) SS and (B) FS clones with and without the mutation. The line patterns indicate the generation at which the clones were isolated. Statistical analysis is given in Table 1.

**Table 1** Standard least square model on the effect of the mutation on the growth rate, between 5–10 hours after inoculation, in SS and FS isolated at different generations

| Source | DF | *F* ratio | *P* value |
|---|---|---|---|
| SS, $R^2 = 0.91$ | | | |
| Whole model | 13 | 11.44 | <0.0001 |
| Mutation | 1 | 71.23 | <0.0001 |
| Generations | 6 | 7.76 | 0.0008 |
| Mutation × generations | 6 | 0.99 | 0.47 |
| FS, $R^2 = 0.89$ | | | |
| Whole model | 11 | 9.23 | 0.0003 |
| Mutation | 1 | 1.57 | 0.23 |
| Generations | 5 | 19.42 | <0.0001 |
| Mutation × generations | 5 | 0.75 | 0.60 |

6 days (neutral effect), while a positive fitness indicates an adaptive benefit and negative fitness a deleterious effect. When competitions were initiated with a mutant frequency of 0.9, the fitness of the mutation was always close to 0, indicating that the mutation is neither strongly deleterious nor strongly adaptive when common (Fig. 3, Table 2, Fig. S2). In sharp contrast, the fitness of the mutation was extremely different in FS and SS genomes when competitions were initiated with a mutant frequency of 0.1. The fitness of SS* was strongly positive (mean ± SE = 0.107 ± 0.006), while the fitness of FS* was closer to 0 (mean ± SE = 0.028 ± 0.007) but still slightly positive (Fig. 3, Table 2, Fig. S2). Within the FS ecotype, we can note that there may be some genetic diversity. In the four clones sampled between the generations 400 and 800, the mutation was almost neutral (relative fitness ± SE = 0.004 ± 0.001), while it was slightly adaptive in all of the eight clones sampled at either 200 generations or between 1000 and 1200 generations (relative fitness ± SE = 0.041 ± 0.008).

That the mutation provides the most adaptive benefit to an SS genotype, yet was sampled solely among FS ecotypes in the evolutionary experiment, is intriguing. However, the fitness effects of the mutation may not only be influenced by the intra-genomic background, but also by the inter-genomic environment, i.e. by the presence of other ecotypes. FS and SS ecotypes coexisted for more than 1000 generations of experimental evolution. To understand how the fitness of FS* and SS* is affected by the presence of the other ecotype, from the 26 initial clones we chose a pair of FS and SS clones every 200 generations, starting at generation 200 until the end of the evolution experiment. For each time point, three-way competition experiments were initiated in the evolution environment between SS*, SS, FS and between FS*, SS, FS. Competitions were initiated with

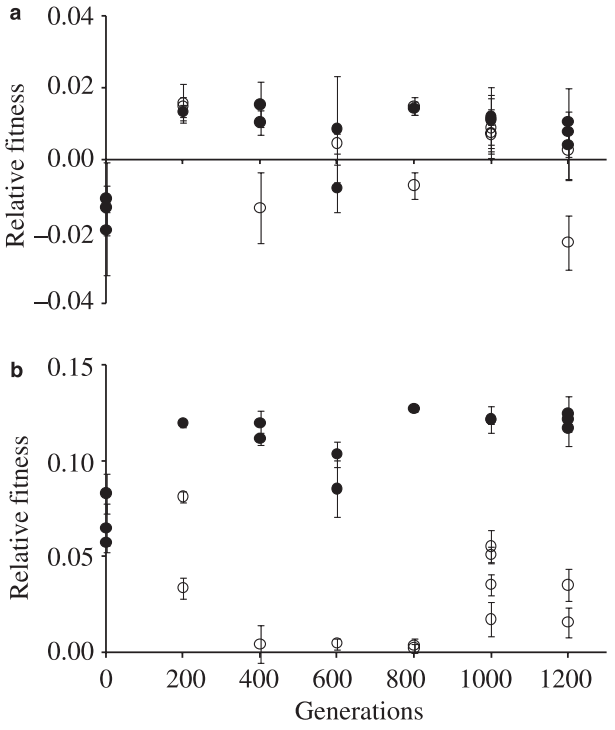


**Fig. 3** Relative fitness (±SEM) of the *arcA* mutation when competing only with the ancestral allele in FS (open circles) and SS (full circles) at different generations, starting from initial frequencies of (a) 0.9 and (b) 0.1. Statistical analysis is given in Table 2.

**Table 2** Standard least square model testing how the mutation affects the fitness of the ecotypes at different generations. The ancestral clones are excluded for this analysis

| Source | DF | *F* ratio | *P* value |
|---|---|---|---|
| Initial mutant frequency: 0.9, $R^2 = 0.66$ | | | |
| Whole model | 11 | 1.93 | 0.15 |
| Ecotype | 1 | 4.98 | NA |
| Generations | 5 | 2.02 | NA |
| Ecotype × generations | 5 | 1.57 | NA |
| Initial mutant frequency: 0.1, $R^2 = 0.95$ | | | |
| Whole model | 11 | 20.31 | <0.0001 |
| Ecotype | 1 | 189.04 | <0.0001 |
| Generations | 5 | 2.68 | 0.08 |
| Ecotype × generations | 5 | 1.52 | 0.26 |

90% or 10% of bacteria carrying the mutation (the remaining proportion being a mix of 50% FS, 50% SS), the frequency of the mutation was measured every other day during 6 days and fitness was measured as above. When competitions were initiated with 90% of bacteria carrying the mutation, the fitness of FS* and SS* changed from almost null in the pairwise

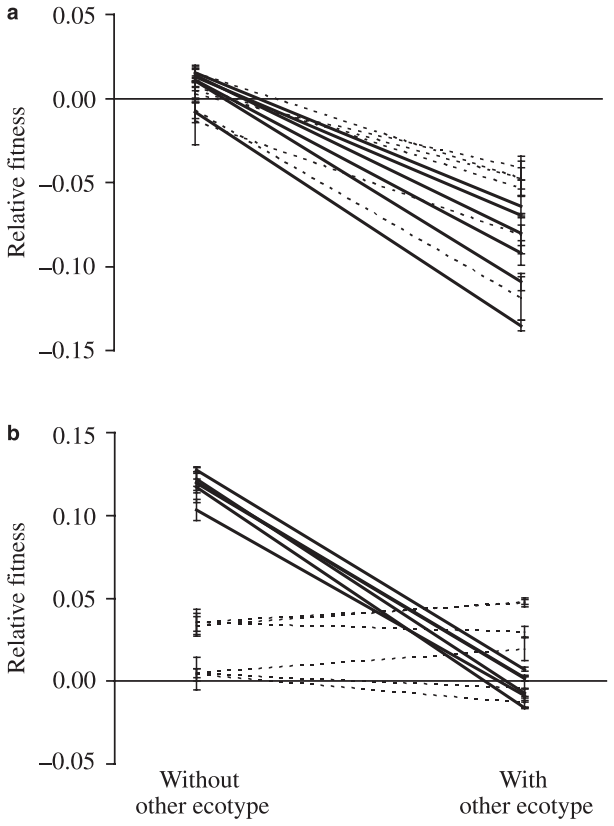


**Fig. 4** Relative fitness (±SEM) of the *arcA* mutation when competing with the ancestral allele in SS (full line) and FS (dotted line) at initial frequencies of (A) 0.9 and (B) 0.1 in the absence and presence of the other ecotype. Statistical analysis is given in Table 3.

**Table 3** Standard least square model testing how the mutation affects the fitness of the ecotypes in presence and absence of the other ecotype

| Source | DF | *F* ratio | *P* value |
|---|---|---|---|
| Initial mutant frequency: 0.9, $R^2 = 0.83$ | | | |
| Whole model | 3 | 33.06 | <0.0001 |
| Ecotype | 1 | 1.19 | 0.29 |
| Other ecotype | 1 | 94.00 | <0.0001 |
| Ecotype × other | 1 | 3.99 | 0.06 |
| Initial mutant frequency: 0.1, $R^2 = 0.97$ | | | |
| Whole model | 3 | 197.48 | <0.0001 |
| Ecotype | 1 | 161.62 | <0.0001 |
| Other ecotype | 1 | 267.08 | <0.0001 |
| Ecotype × other | 1 | 163.75 | <0.0001 |

competition to negative in the presence of the other ecotype (Fig. 4, Table 3, Fig. S3). This result could indicate two things: the mutation might be deleterious in the competitive environments of the three-way competition experiments and/or the mutation might be able to coexist via negative frequency-dependence with some of the competitors. When competitions were initiated with 10% of FS*, the fitness of the mutation remained almost neutral or slightly adaptive in the presence of the SS ecotype (Fig. 4, Table 3, Fig. S3). When slightly adaptive, this indicates that the fitness of the mutation is frequency-dependent (i.e. the frequency declines from high frequency and increases from low frequency) and can coexist with some of the competitors. Due to the experimental design of the three-way competitions, we do not know whether FS* coexist with FS, SS or both. However, as in the pairwise competitions, some of the FS* are able to invade FS, it seems reasonable to think that these same FS* can coexist with SS in the three-way competitions. When initiated with 10% of SS*, the fitness of the mutation changed from strongly adaptive to almost neutral in presence of the FS ecotype. Thus, in a population already containing both the FS and the SS ecotypes, the mutation is no longer adaptive in SS, probably explaining why the mutation did not occur in the SS lineage (Fig. 4, Table 3, Fig. S3).

## Discussion

We identified a mutation in a global regulator gene in one of two ecotypes that diversified from a common ancestor during an evolution experiment (Le Gac *et al.* 2008; Spencer *et al.* 2008; Tyerman *et al.* 2008). By replacing the ancestral allele with the mutant allele and vice versa in clones isolated at various points in the frozen record of the evolutionary process and by performing phenotypic and fitness assays, we showed that the effects of the mutation are very different in the two ecotypes. When occurring in a pure population of bacteria specialized in fast glucose consumption (SS), the mutation dramatically improves the consumption of the non-preferred carbon source (acetate) without having any visible antagonistic pleiotropic effects on glucose consumption. As a result, in such a population, the mutation is strongly adaptive and would probably quickly replace the ancestral allele. When occurring in a pure population of efficient glucose and acetate consumers (FS), the mutation does not lead to profound phenotypic modifications. Even though we were unable to detect the phenotypic modifications associated with this mutation in the FS genomes, the mutation is slightly adaptive in some genomes and almost neutral in others. There are indications that the mutation is adaptive in the FS genomes isolated at 200 and then 1000 and 1200 generations (see Fig. 3, and Figs S2 and S3), suggesting two potential invasion windows in this ecotype, an early one and a late one, during the time course of the evolution experiment.

Apparently, invasion occurred in the second of those windows in our experiment. These results show that the epistatic interactions between the mutation and the genomes of FS and SS are very different. In particular, it illustrates that the phenotypic and fitness effects of a mutation strongly depend on the genomic background in which the mutation occurs. As the *arcA* mutation was identified in FS, it is intriguing to see that it is more adaptive when occurring in SS than FS genomes. In principle, one could imagine that the *arcA* mutation actually occurred in an SS genome and then led to the formation of FS, but this seems unlikely. First, when occurring in a SS genome, the *arcA* mutation does not change a SS growth curve into a FS one and changing the mutant allele by the ancestral one in FS does not restore an SS growth profile. Second, the *arcA* mutation was first identified at 1000 generations, while FS were present since generation 200. The occurrence of the mutation in a SS genome would thus require the *arcA* mutation, at least another unidentified mutation that would change the SS* growth profile into a FS one (which would be necessary and sufficient to explain the growth profile difference between FS and SS) and the competitive exclusion of the FS lineage preexisting since generation 200 by the newly formed FS ecotype. This scenario appears to be much less likely than the simple occurrence of the *arcA* mutation in the pre-existing FS lineage, especially since the mutation seems to have occurred in the right time window in the FS lineage.

We also demonstrated that the fitness of the *arcA* mutation is not only affected by epistasic interactions, but also by frequency dependent selection pressures, by showing that the fitness of the *arcA* mutation strongly depends on the genomic composition of the population. In the presence of the FS ecotype, the mutation is no longer adaptive when occurring in the SS genome, which is another argument against the occurrence of the *arcA* mutation in the SS lineage, as it may curtail the establishment of the mutation in the SS ecotype. This is probably because by the time SS* switches to acetate consumption after glucose depletion, FS already consumed most of the available acetate. However, the fitness of FS*, when rare, is not affected by the presence of SS. This is probably because the *arcA* mutation affects the competitiveness for acetate consumption and SS probably consumes virtually no acetate in the presence of FS.

To summarize the results, in one of the ecotypes (SS), the mutation broadens the ecological niche (i.e. leads to a more generalist strategy) by improving the consumption of a non-preferred resource (the acetate). However, the mutation is only adaptive in SS when the FS strain is not present (i.e. when the FS niche is not already occupied). In the FS ecotype, the mutation probably does not affect the width of the ecological niche, but rather the competitiveness within the niche, generating some fine-tuned adaptation not affected by the presence or absence of the SS ecotype. Thus, according to our results, the *arcA* mutation could have spread in the ancestral (SS-like) lineage prior to diversification, but not in SS after diversification, as the mutation is no longer adaptive in SS once FS is present. Because of epistatic interactions, the mutation only seems adaptive in some FS ecotypes, namely the ones we isolated before 200 or after 1000 generations. Thus, in FS the mutation could have spread either very early after the appearance of the first FS-like strains or late in the evolution experiment (in actual fact, the mutation spread in FS during this second invasion window).

By investigating the fate of a mutation in an important gene regulating the central carbohydrate metabolism in *E. coli*, our study demonstrates experimentally that epistatic interactions and frequency dependent selection pressures can profoundly affect evolutionary trajectories in recently diverged evolutionary lineages. Along an evolutionary lineage, the opportunity for a potentially adaptive mutation to increase in frequency in a population may strongly depend on the genome in which it occurs (Bull *et al.* 2000; Phillips *et al.* 2000; 2008; Remold & Lenski 2004; Segre *et al.* 2005; Weinreich *et al.* 2005, 2006; Yukilevich *et al.* 2008; Martin *et al.* 2007; Poelwijk *et al.* 2007; Cooper *et al.* 2008) and, crucially for the evolution of diversity, may be strongly affected by the genetic composition of a population (Ayala & Campbell 1974; Rosenzweig *et al.* 1994; Treves *et al.* 1998; Lunzer *et al.* 2002; Wolf 2003; Fitzpatrick *et al.* 2007).

## Acknowledgements

We thank Melanie Bertrand, Sarita Balabhadra, Erik Hanschen for technical assistance, the Church lab for the pKO3 vector and two anonymous reviewers for helpful comments. This project is supported by NSERC (Canada) and by the Human Frontier Science Program (EU).

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Mickael Le Gac is an evolutionary biologist using experimental evolution approaches to investigate diversification and adaption processes. Michael Doebeli is an evolutionary biologist interested in the mathematical theories of the evolution diversity and the evolution of cooperation, as well as in evolutionary experiments testing these theories.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** (a) Growth profiles of the 26 original clones without (in black), and with (in red) the *arcA* mutation. The numbers indicate the generations. SS growth profiles are indicated in the upper part of the figure, FS profiles in the lower part. For each growth profile, thick lines indicate the average growth profiles, thin lines indicate the standard error of the mean, the x axis indicates time since inoculation and the y axis indicates the optical density (600 nm). A * indicates the presence of the mutation in the original clone. (b) Frequency dependence between FS and SS. Each graph indicates the result of competition experiments, initiated at two different frequencies, between one FS and one SS clone isoalted at the same time point during the evolution experiment. The *x*-axis indicates the number of days of competition, the *y*-axis the frequency of FS clones. SE are indicated.

**Fig. S2** Results of the competition experiments, initiated with two different frequencies, between strains isogenic except for the *arcA* mutation (a) in SS clones and (b) in FS clones. Each graph corresponds to one clone isolated during the evolution experiment. The *x*-axis indicates the days of competition (each mark corresponds to 2 days). The *y*-axis indicates the frequency of the mutation (each mark corresponds to 0.2). SE are indicated.

**Fig. S3** Results of competition experiments, initiated with two different frequencies, between strains isogenic except for the *arcA* mutation (left column) and between these same strains plus a clone from the other ecotype isolated at the same time

point during the experimental evolution (right column) (a) in SS clones and (b) in FS clones. The *x*-axis indicates the days of competition (each mark corresponds to 2 days). The *y*-axis indicates the frequency of the mutation (each mark corresponds to 0.2). SE are indicated.

Supporting information

Figure S1.
a. Growth profiles of the 26 original clones without (in black), and with (in red) the *arcA* mutation. The numbers indicate the generations. SS growth profiles are indicated in the upper part of the figure, FS profiles in the lower part. For each growth profile, thick lines indicate the average growth profiles, thin lines indicate the standard error of the mean, the x axis indicates time since inoculation, and the y axis indicates the optical density (600nm). A * indicates the presence of the mutation in the original clone.
b. Frequency dependence between FS and SS. Each graph indicates the result of competition experiments, initiated at two different frequencies, between one FS, and one SS clone isoalted at the same time point during the evolution experiment. The x axis indicates the number of days of competition, the y axis the frequency of FS clones. sem are indicated.

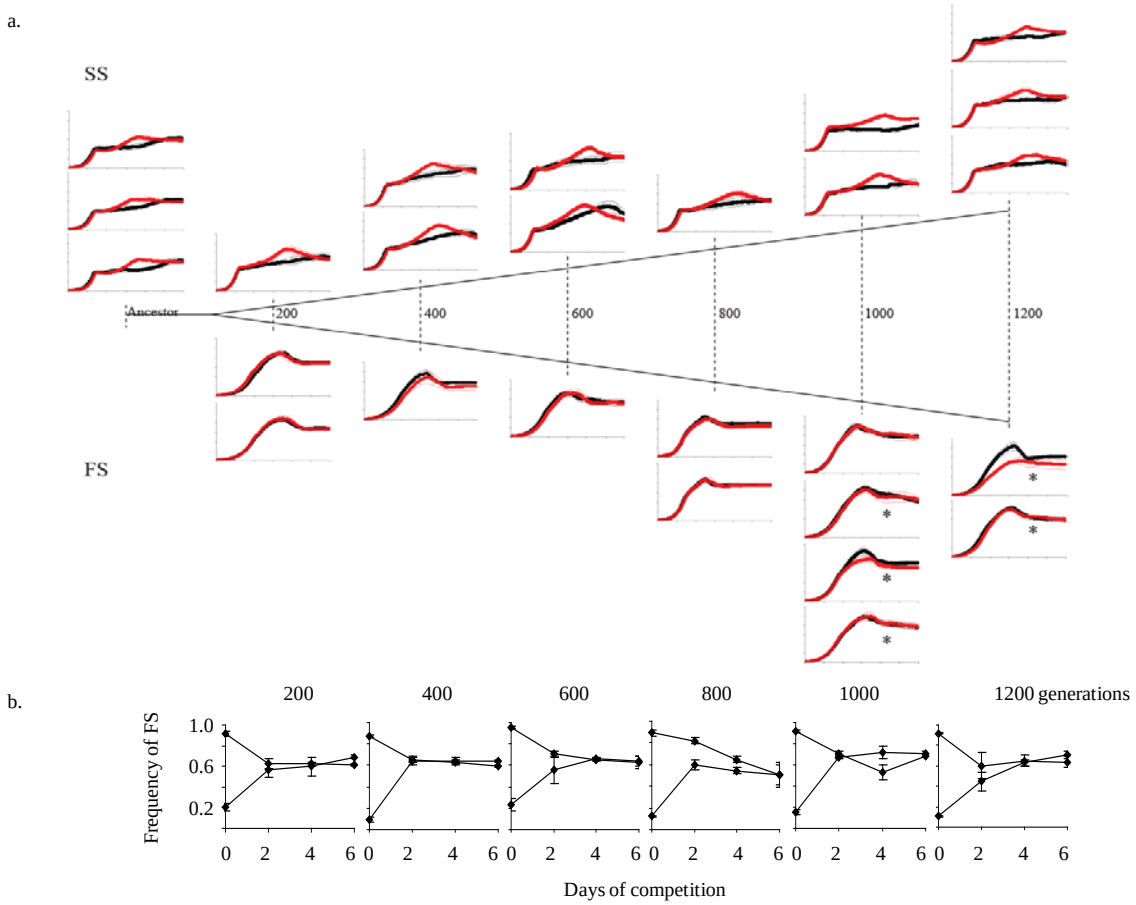
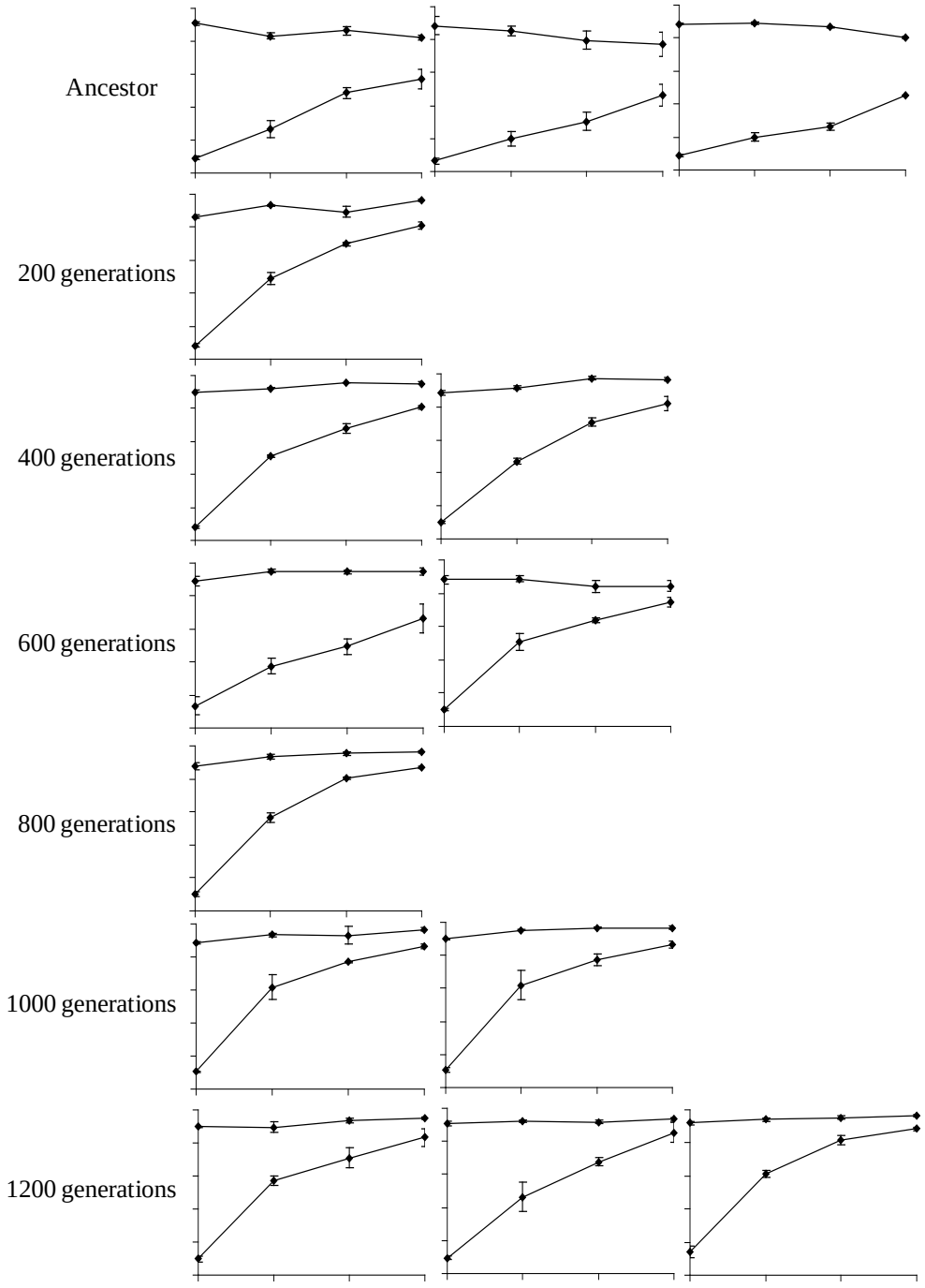


Figure S2
Results of the competition experiments, initiated with two different frequencies, between strains isogenic except for the *arcA* mutation, a. in SS clones, b. in FS clones. Each graph corresponds to one clone isolated during the evolution experiment. The x axis indicates the days of competition (each mark corresponds to two days). The y axis indicates the frequency of the mutation (each mark corresponds to 0.2). sem are indicated.

a. Fitness of the mutation in SS



b. Fitness of the mutation in FS

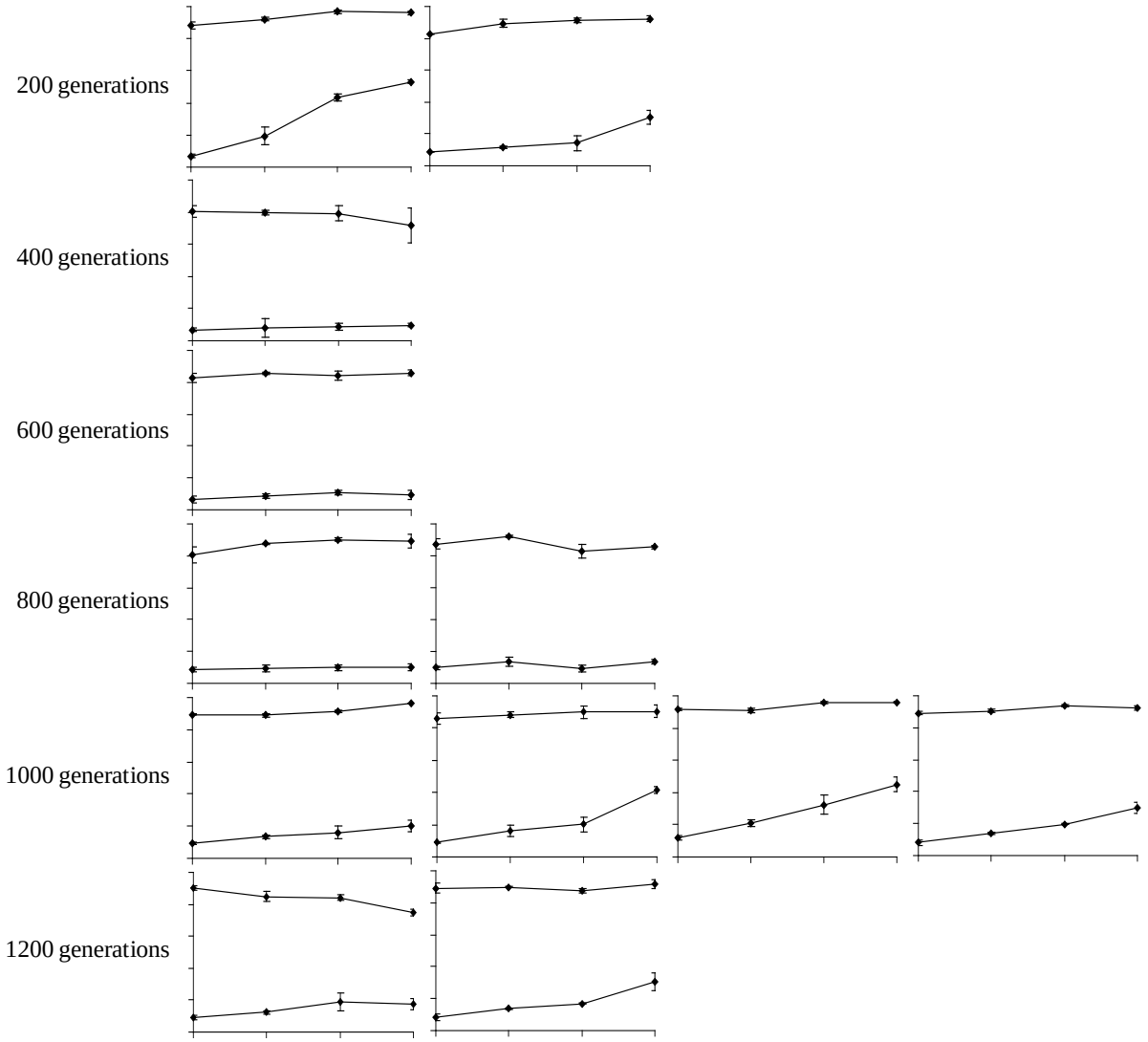
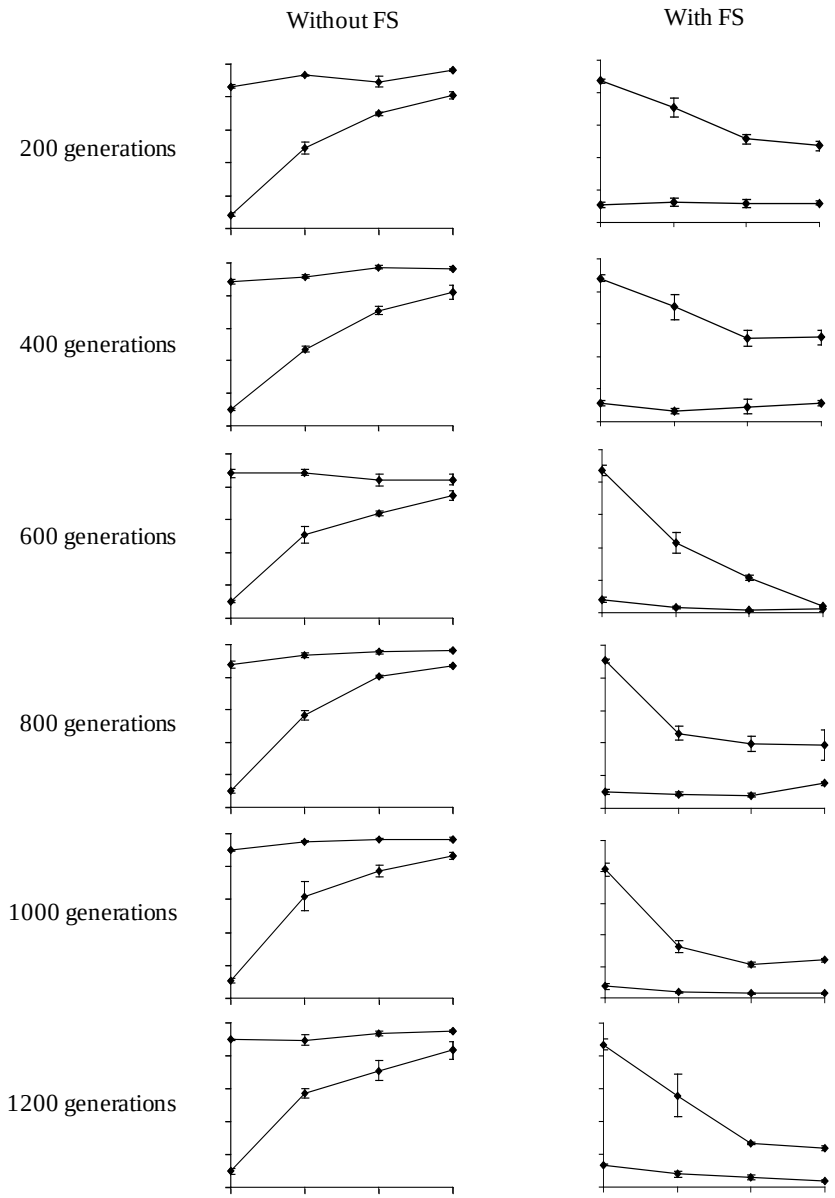


Figure S3
Results of competition experiments, initiated with two different frequencies, between strains isogenic except for the *arcA* mutation (left column), and between these same strains plus a clone from the other ecotype isolated at the same time point during the experimental evolution (right column). a. in SS clones, b. in FS clones. The x axis indicates the days of competition (each mark corresponds to two days). The y axis indicates the frequency of the mutation (each mark corresponds to 0.2). sem are indicated.

a. Fitness of the mutation in SS with and without FS



b. Fitness of the mutation in FS with and without SS