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Adaptation through genetic time travel? Fluctuating selection can drive the evolution of bacterial transformation

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Natural transformation is a process whereby bacteria actively take up DNA from the surrounding environment and incorporate it into their genome. Natural transformation is widespread in bacteria, but its evolutionary significance is still debated. Here, we hypothesize that transformation may confer a fitness advantage in changing environments through a process we term 'genetic time travel': by taking up old genes that were retained in the environment, the bacteria may revert to a past genotypic state that proves advantageous in the present or a future environment. We scrutinize our hypothesis by means of a mathematical model involving two bacterial types (transforming and non-transforming), a single locus under natural selection and a free DNA pool. The two bacterial types were competed in environments with changing selection regimes. We demonstrate that for a wide range of parameter values for the DNA turnover rate, the transformation rate and the frequency of environmental change, the transforming type outcompetes the non-transforming type. We discuss the empirical plausibility of our hypothesis, as well as its relationship to other hypotheses for the evolution of transformation in bacteria and sex more generally, speculating that 'genetic time travel' may also be relevant in eukaryotes that undergo horizontal gene transfer.

1. Introduction

Transformation is the active uptake of naked DNA from the surrounding environment by bacterial cells, followed by the incorporation of this DNA into the bacterial genome. To date, over 60 bacterial species have been reported to undergo transformation [1]. In spite of fairly extensive knowledge about the underlying physiology and molecular biology [2,3], the evolutionary benefits of natural transformation remain elusive [4,5]. One group of hypotheses considers the genetic diversity that results from recombination as the primary evolutionary force responsible for the evolution and maintenance of transformation (reviewed in [6]). Other hypotheses regard recombination as a mere by-product of taking up DNA that serves primarily as a readily available nutrient [5,7,8] or as a protection against DNA damaging stresses [9,10].

One distinctive feature of transformation is that DNA is imported from the surrounding environment instead of being exchanged between living individuals as it usually occurs in eukaryotic recombination. Bacterial cells can supply the DNA pool through either active secretion of DNA or DNA release upon death [5,11–14]. Since DNA molecules can be quite stable in the habitats of transforming bacteria (see Discussion), the DNA pool may have a different genetic composition than the cell population. For example, if deleterious mutations cause the death of bacteria, this may produce an overabundance of such mutations in the medium so that transformation may become detrimental [15,16]. However, genes that are deleterious in one environment may become beneficial in another environment and *vice versa*. Thus, when environmental conditions change constantly, the free DNA in the medium may preserve a record of genes that are beneficial under different environmental conditions. Transformation may then be favoured because it brings about a form of 'genetic time travel', converting the genome of a bacterium back to a past and better-adapted state.

To examine this hypothesis, we studied the dynamics of competitions between transforming and non-transforming bacteria in changing environments, using a mathematical setting with a single locus under selection. The transforming type is capable of importing DNA from a DNA pool, which in turn is supplied by DNA from all bacteria in the population. We studied the competition dynamics in both periodically and stochastically changing environments. Our results demonstrate that transformation is selected for in most of these environments and for a wide range of parameter values. This suggests a novel evolutionary benefit for transformation that can also be scrutinized experimentally.

2. Model

We consider a deterministic continuous time model of an infinitely large population of bacteria. Each bacterium is characterized by (i) its ability or inability to take up DNA and undergo recombination through transformation, and (ii) its allelic state (0 or 1) at a single locus under natural selection.

The population experiences successive environmental changes in the course of adaptation. These changes result in a fluctuating selection regime where selection favours one allele over the other by an amount s. The fitness w_0 of genotype 0 is arbitrarily set to zero, while the fitness $w_1$of genotype 1 fluctuates between the two values s and $-s$. The environment changes either periodically after constant time periods of length T, or stochastically with a uniform distribution of switching time points and a mean time T between subsequent switches.

In addition to the population of bacteria, we also consider a pool of free DNA fragments containing the two alleles 0 and 1. Through death or excretion of DNA, the bacteria of both types supply DNA fragments to this pool. We denote by m the DNA 'turnover rate', that is the net rate at which the genetic composition of the DNA pool is changed through DNA coming from the bacterial population. This rate incorporates both the release rate of DNA by the bacteria (through death or secretion) and the rate at which the DNA in the medium decays or is otherwise removed. (Note that to keep the model simple, we assume that DNA decay removes the DNA molecules from the medium but does not produce damaged DNA molecules that might become deleterious alleles following transformation.) The transforming type is capable of taking up DNA fragments from the pool at a rate u. The imported DNA fragment will then replace the existing allele and may thus cause a genotypic switch from 0 to 1 or *vice versa*. We assume that DNA exists in a sufficient amount in the medium so that DNA uptake does not alter the frequency of fragment types in the DNA pool.

Let us denote by x_i and y_i the frequencies of transforming and non-transforming bacteria with allele i, and let f_i be the frequency of allele i in the free DNA pool. Note that $x_0 + x_1 + y_0 + y_1 = f_0 + f_1 = 1$. Based on the above assumptions, the model can then be described by the following system of differential equations:

$$\left.\begin{aligned} \dot{x}_0 &= x_0(w_0 - \bar{w}) - uf_1x_0 + uf_0x_1,\\ \dot{x}_1 &= x_1(w_1 - \bar{w}) - uf_0x_1 + uf_1x_0,\\ \dot{y}_0 &= y_0(w_0 - \bar{w}),\\ \dot{y}_1 &= y_1(w_1 - \bar{w}),\\ \dot{f}_0 &= m(x_0 + y_0 - f_0)\\ \text{and}\quad \dot{f}_1 &= m(x_1 + y_1 - f_1). \end{aligned}\right\} \tag{2.1}$$

Here, $\bar{w}$ is the population mean fitness defined as $(x_0 + y_0)w_0 + (x_1 + y_1)w_1$. Note that as described above, the fitness value w_1 and hence $\bar{w}$ is time-dependent.

We studied the dynamics of the competition between the two bacterial types first analytically and then by numerically solving system (2.1) using MATHEMATICA v. 8.0 (Wolfram Research, Inc.). Throughout, we initiate the population with equal frequencies of the 0 and 1 alleles and of the two types (transforming and non-transforming). The simulations ran for 8000 time units. We obtained the final frequency of the transforming population by integrating the frequency of the transforming type over the last 2000 time points.

3. Results

(a) Constant selection

In order to gain some analytical insight into our model, we first transform our system of differential equations (2.1) as follows. Let $\tilde{x}_1$ and $\tilde{y}_1$ be the frequencies of the 1 allele among all transforming and non-transforming bacteria, respectively (i.e. $\tilde{x}_1 = x_1/(x_0 + x_1)$ and $\tilde{y}_1 = y_1/(y_0 + y_1)$). Moreover, let x be the overall frequency of transforming bacteria ($x = x_0 + x_1$). Applying standard differentiation rules and exploiting $w_0 = 0$, we then obtain from (2.1) the following transformed system of differential equations:

$$\left.\begin{aligned} \dot{\tilde{x}}_1 &= w_1\tilde{x}_1(1 - \tilde{x}_1) + u(f_1(1 - \tilde{x}_1) - (1 - f_1)\tilde{x}_1),\\ \dot{\tilde{y}}_1 &= w_1\tilde{y}_1(1 - \tilde{y}_1),\\ \dot{f}_1 &= m(\tilde{x}_1x + \tilde{y}_1(1 - x) - f_1)\\ \text{and}\quad \dot{x} &= x(1 - x)w_1(\tilde{x}_1 - \tilde{y}_1). \end{aligned}\right\} \tag{3.1}$$

Let us now assume that allele 1 has a constant selective advantage s over allele 0. Comparing the equations for $\tilde{x}_1$ and $\tilde{y}_1$ then reveals that transformation modifies the rate of adaptation within the transforming subpopulation by an amount $u\,(f_1(1 - \tilde{x}_1) - (1 - f_1)\tilde{x}_1)$. This term is positive if and only if $f_1 > \tilde{x}_1$, so that in this case, $\tilde{x}_1$ will increase faster than $\tilde{y}_1$. Hence, assuming equal initial frequencies of allele 1 among the transforming and non-transforming type, the equation for $\dot{x}$ in (3.1) will always be positive, so that the overall frequency of the transforming type will increase. Conversely, when $f_1 < \tilde{x}_1$, the transforming population will exhibit a reduced rate of adaptation, which may in turn produce a decline in the overall frequency of the transforming type.

Let us now consider the case of an adapting population that constantly supplies DNA fragments to the free DNA pool. We assume that initially, the population consists predominantly of individuals carrying allele 0 and the transforming and non-transforming types are equally common. Moreover, the DNA pool initially consists only of allele 0. Figure 1 demonstrates that in this scenario, adaptation is impeded in the transforming subpopulation. This is because although the frequency of allele 1 is also increasing within the DNA pool, it does so only as a response to and at a slower rate than the increase of allele 1 within the bacterial population. As a result of this reduced rate of adaptation of the transforming subpopulation, the transforming type decreases in frequency and eventually becomes extinct. This is the 'bad genes' effect arising through transformation in an adapting population that we have observed before in a different model [16].

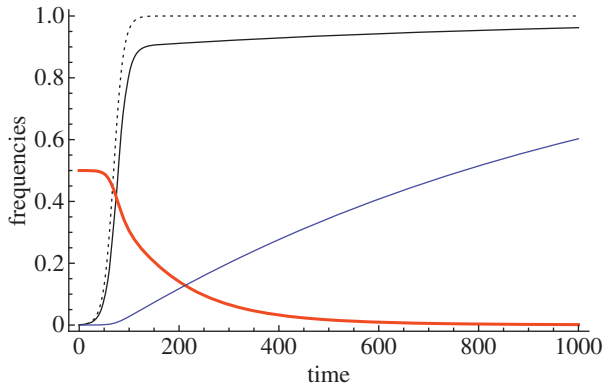

Figure 1. Example dynamics of the competition between transforming and non-transforming bacteria in a constant environment where allele 1 has a selective advantage over allele 0. The four lines denote the frequency $\boldsymbol{x}$ of the transforming bacteria (red), the frequency $\boldsymbol{f}_1$ of allele 1 in the DNA fragment pool (blue), the frequency $\tilde{\boldsymbol{x}}_1$ of allele 1 within the transforming population (solid black) and the frequency $\tilde{\boldsymbol{y}}_1$ of allele 1 within the non-transforming population (dashed black). The population initially consists of equal numbers of transforming and non-transforming bacteria and the initial frequency of the beneficial allele 1 within both types is 0.001. Parameters take the values $s = 0.1$, $u = 0.01$, $m = 0.001$.

(b) Periodically changing selection

We next examined the dynamics assuming that the selection regime changes periodically at regular time intervals so that an allele that was deleterious in one time period, becomes beneficial in the next period. Figure 2 shows two example dynamics. In figure 2*a*, as in figure 1, the frequency of allele 1 is initially lower in the DNA fragment pool than in the cell populations, and this causes the transforming type to decline slightly in frequency. However, upon the first switch in the environment when allele 0 becomes the beneficial allele, the frequency of the transforming type increases strongly. This is due to two related reasons. First, because transformation impedes adaptation in the first phase, there will be a higher initial frequency of the beneficial allele within the transforming bacteria and this initial 'head start' means that the transforming type adapts faster. Second, when the environment changes, the frequency of the beneficial allele is (until time point approx. 130) higher in the DNA pool than within the population of transforming bacteria, so that transformation further drives the frequency of the transforming type upwards. In the subsequent periods, the frequency of the transforming bacteria exhibits damped oscillations, but eventually the transforming type becomes fixed.

However, fluctuating selection can also produce selection against the transforming type when the transformation rate is high (see figure 2*b* for an example). Here, adaptation is generally strongly impeded in the transforming population, because this population is bound to follow closely the pool of free DNA in its allelic composition. This can be seen especially in the second phase (from time point 100) where allele 1 (the deleterious one in that phase) increases in frequency within the transforming bacteria (figure 2*b*). Thus, transformation can completely overpower natural selection and in this example, the reduction in the frequency of the transforming type through the 'bad genes' effect is so strong that it cannot be offset by the subsequent increase in frequency caused by maintaining the beneficial allele 0 in the transforming subpopulation.

In order to obtain a more complete understanding of the conditions favouring transformation in a changing environment, we performed a screen of the parameter space for the three parameters u, m and T. (Since the strength of selection s is important only relative to the other parameters, this parameter can be kept fixed without loss of generality.) For different combinations of these parameters, we numerically solved our system of differential equations and recorded the final frequencies of the transforming type as described in the Model section.

This screening revealed three different regions in the parameter space (figure 3). When the transformation rate is very low and/or the DNA turnover rate is very high, transformation is neutral. In the case of a low transformation rate, this effect is obvious because the difference between the transforming and non-transforming bacteria diminishes. On the other hand, with increasing DNA turnover rate, the allelic composition of the free DNA pool and the bacterial populations will become increasingly similar, so that with very high DNA turnover rates transformation will not affect the allele frequency within the transforming bacteria. In a second region transformation is selected against. This occurs with high transformation rates, fairly high DNA turnover rates and slow changes in the environment—conditions that ensure that the detrimental effects of transformation described above prevail. Finally, in the majority of the parameter space that we screened, the transforming type reaches fixation in the population. This indicates that the beneficial effect of transformation shown in figure 2*a* predominates for a wide range of parameters.

We extended our model with periodic environmental changes in several ways (see the electronic supplementary material for details). First, we found that transformation is still favoured for many parameters even when it comes at a small-to-intermediate constitutive fitness cost (see electronic supplementary material, figure S1). Second, we performed an invasion analysis of bacterial types with a certain transformation rate into resident populations with another transformation rate. This analysis revealed the existence of evolutionarily stable transformation rates: for given parameters T, m and s, there exists a transformation rate that is stable against invasion of bacteria with a different transformation rate (see electronic supplementary material, figure S2). Finally, we also incorporated mutation into our model, which usually has the effect of diminishing selection for transformation (see electronic supplementary material, figure S3). The reason for this is that mutation maintains genetic variation in the non-transforming subpopulation towards the end of the adaptive phase. When the environment changes, this genetic variation then increases the efficacy of natural selection so that the transforming type is less strongly favoured over the non-transforming type.

(c) Stochastically changing selection

After studying the effect of periodically changing environments, we next asked whether our results also hold for stochastically changing environments. To this end, we constructed sets of environmental scenarios by randomly drawing time points for environmental switches from a uniform distribution. We then simulated the evolutionary dynamics in each of these scenarios and measured the mean frequency of the transforming population at the end of the simulation time (see Model section).

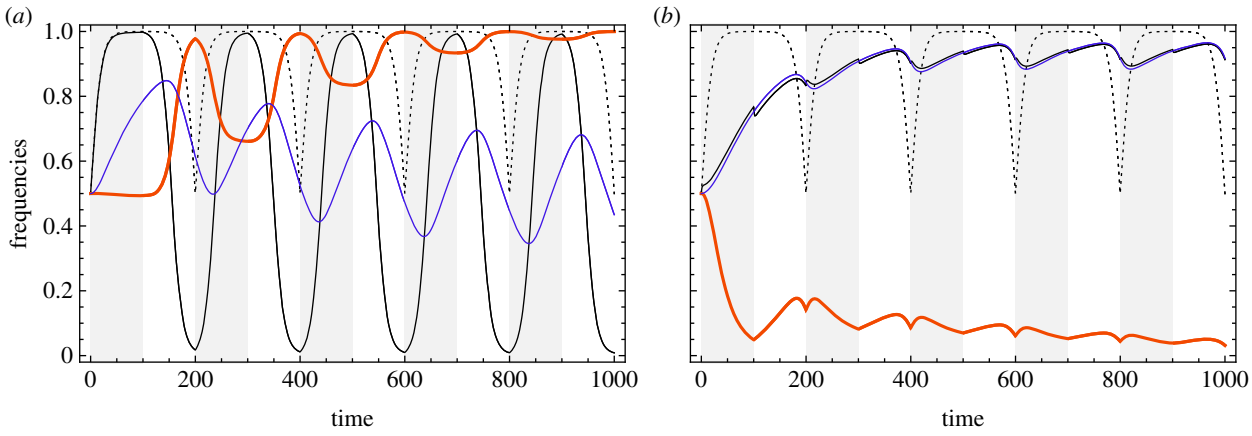

Figure 2. Example dynamics of the competition between transforming and non-transforming bacteria in a periodically changing environment. Red, blue, solid black and dashed black lines indicate the same frequencies as in figure 1. The selection pressure for and against allele 1 is shown as grey and white time periods, respectively. The population initially consists of equal numbers of transforming and non-transforming bacteria and the initial frequency of allele 1 in both sub-populations and the fragment pool is 0.5. Parameters take the values $\boldsymbol{s} = 0.1$, $\boldsymbol{m} = 0.01$, and (*a*) $\boldsymbol{u} = 10^{-3}$ and (*b*) $\boldsymbol{u} = 1$.

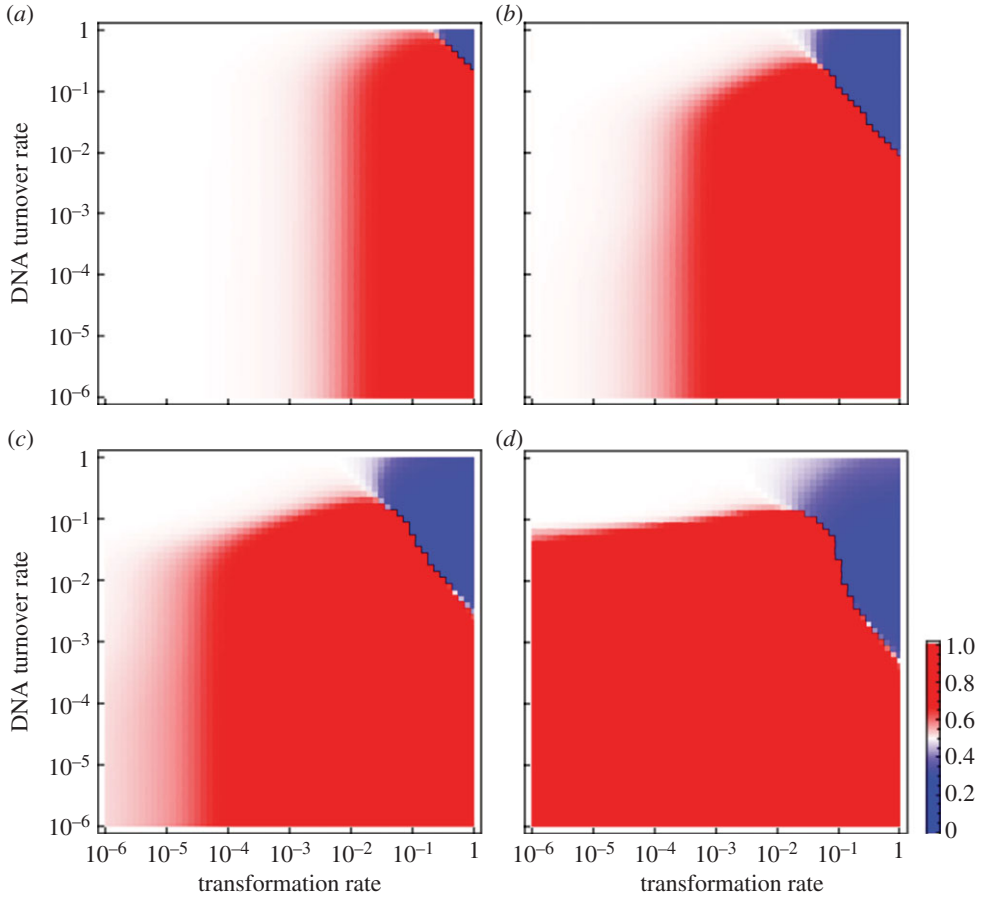

Figure 3. The mean of frequencies of the transforming type in the last 2000 time points of simulation in periodically changing environments at different values of the transformation rate and the DNA turnover rates at different time intervals (*a*) $\boldsymbol{T} = 10$, (*b*) $\boldsymbol{T} = 50$, (*c*) $\boldsymbol{T} = 100$ and (*d*) $\boldsymbol{T} = 320$. Each plot shows the average final frequencies of the transforming type for 60 parameter values of transformation and DNA turnover rates.

Figure 4 reveals marked differences between the competition dynamics in the stochastically and periodically changing environments under the same (average) time periods of environmental change. These differences are pronounced when the DNA turnover rate and the transformation rate are either both low or high and when the environment changes frequently. For low DNA turnover and transformation rates (figure 4*a*), transformation is neutral in periodically changing environments but favoured in stochastically changing environments. For high DNA turnover and transformation rates (figure 4*d*), the transforming type becomes fixed in periodically changing environments but declines in frequency in the stochastically changing environments.

This discrepancy can be explained intuitively as follows. In the stochastically changing environments, there will be variation in the length of time intervals with a particular selection regime, with occasional time intervals that are much longer than the average time period. In these long time intervals, when transformation and DNA turnover rates are both high, the bad gene effect becomes pronounced and consequently

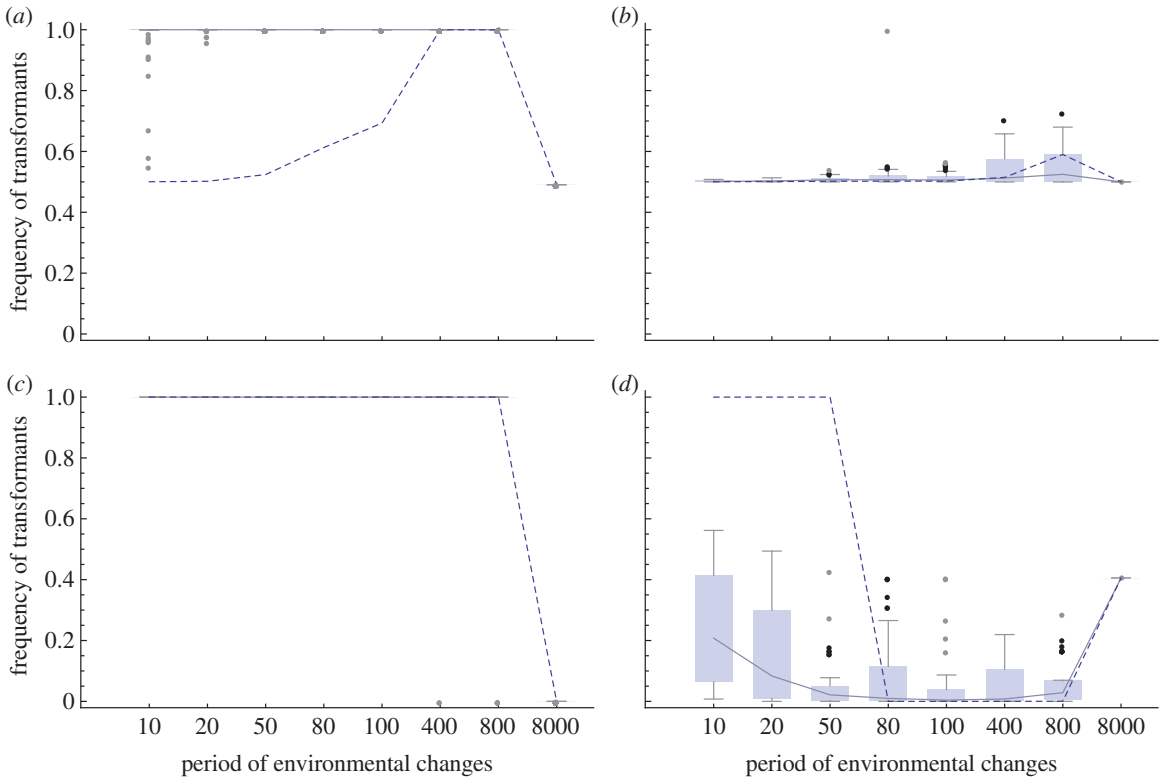

Figure 4. The final frequencies of the transforming type at the end of the simulation in stochastically changing environments. For each time period ***T***, 50 fluctuating environments were generated and the dynamics simulated for each of these scenarios. Transformation and DNA turnover rates take the values (*a*) $\boldsymbol{u} = 0.00001$, $\boldsymbol{m} = 0.00001$, (*b*) $\boldsymbol{u} = 0.00001$, $\boldsymbol{m} = 0.1$, (*c*) $\boldsymbol{u} = 0.1$, $\boldsymbol{m} = 0.00001$ and (*d*) $\boldsymbol{u} = 0.1$, $\boldsymbol{m} = 0.1$. The *x*-axis gives the mean period time. The *y*-axis gives the mean frequency of the transforming subpopulation during the last 2000 time points. Solid lines show medians and boxes indicate the interquartile range of the 50 simulations. Outliers, shown as points, are defined as exceeding 1.5 times the interquartile range. The whiskers are extended from the ends of boxes to the farthest points that are not outliers. The dashed lines show the corresponding values in the periodically changing environment with the same time period. (Online version in colour.)

the frequency of the transforming type decreases. This is owing to the fact that selection has more time to act on the population in these intervals and thereby reduce genetic variation. Consequently, the efficacy of selection in the subsequent phases decreases and the frequency of the transforming type remains almost constant. Conversely, when both DNA turnover rate and uptake rates are low, the transforming type can benefit from the DNA pool with a different allelic composition and increases in frequency in one of the relatively long time intervals. In most cases, the transforming type finally becomes fixed and fluctuating selection has no further impact.

We observed the same effect for the two above-mentioned cases in periodically changing environments where only the second time interval is prolonged, and also in stochastically changing environments with switches that happen with different random deviations from the mean T (results not shown).

4. Discussion

In eukaryotes, recombination through meiosis results in a shuffling of parental genes originating from the same gene pool. Therefore, the only effect of recombination at the population level is to break up statistical associations between alleles at different loci (linkage disequilibrium, LD). Hypotheses for an evolutionary benefit of meiotic recombination must therefore explain how LD builds up in the first place and why breaking up LD is beneficial [17]. By contrast, recombination through transformation in bacteria can, in addition to reducing LD [16], also directly alter allele frequencies within the population because the DNA source (the environment) may be different in its allele frequencies from the recipient population of transforming bacteria. Thus, recombination through transformation may be governed by selective pressures different from those acting on sex and recombination in eukaryotes. This observation is at the core of our 'genetic time travel' hypothesis for the evolutionary benefit of transformation in changing environments, according to which transformation is favoured because it allows the incorporation of genes from a reservoir preserving ancestral genetic information.

Owing to this fundamental difference, previous models for the evolution of sex and recombination in changing environments are quite distinct from our model (e.g. [18–20]; reviewed in [21]). These models require at least two loci under selection as well as either fluctuating epistasis or stochastic effects. A related class of models are models where fluctuating selection is mediated by coevolving parasites (Red Queen models; [22,23]); these models also usually produce fluctuating epistasis. By contrast, our model is deterministic and involves only a single locus under selection.

Our model is also distinct from recently proposed models incorporating 'episodic selection' [24,25]. It is known that many bacteria must enter a state of competence before they can undergo natural transformation. This state is often induced by environmental stress (e.g. starvation) and is characterized by reduced birth and/or death rates that allow the bacteria to survive harsh conditions. Because of this functional link between a persister-like state and competence for transformation, episodic stressful conditions may indirectly select for transformation [24,25]. (See also [26] for an analysis of

how—in the absence of DNA uptake—the optimal switching rates between a persister and non-persister state are determined by the period of environmental change.) Here, we have not incorporated a specific state of competence affecting birth and death rates into our model, thus enabling us to study our hypothesis in isolation from other factors influencing selection for transformation. However, it would be interesting to also explore the relative importance of direct and indirect benefits of DNA import in changing environments in future studies.

The extracellular DNA pool is supplied by microorganisms through active secretion [11–14] and cell lysis [11,12]. Our hypothesis rests on the assumption that this DNA can persist for sufficiently long time spans in the medium relative to the evolutionary dynamics of the bacterial population. Stability of free extracellular DNA in the environment depends on several factors, including the concentration of bacterial DNases, UV radiation and temperature [11,27,28]. However, several lines of evidence indicate that free DNA molecules can be very stable and retain their transformability up to several days in water [28,29] and up to 10 weeks in soil [11,27,30]. These numbers are well within realistic time scales for bacterial evolution.

We made two important assumptions regarding transformation and the pool of free DNA in our model. First, we only consider frequencies of DNA fragments in the DNA pool, but not overall abundances (but see [16]). This has the advantage that the model becomes more tractable and that DNA release and decay can be captured with a single parameter m (the DNA turnover rate) that determines how closely the genetic composition of the free DNA pool follows that of the living bacteria. Neglecting total abundance of DNA molecules in the medium is justified as long as variation in this abundance does not affect transformation rates. The second assumption is that DNA decay does not lead to damaged DNA that can then function as strongly deleterious alleles (independent of the environment) when taken up and incorporated into the genome. Clearly, including this effect into the model would diminish the advantage of transformation.

In order to keep our model as simple as possible and to study the 'genetic time travel' effect in isolation from other effects of recombination we have considered only a single locus in our model. In reality, selection for or against transformation will depend on how selection acts on many loci simultaneously. For example, selection for transformation caused by a single locus under fluctuating selection may readily be overpowered by selection against transformation caused by many loci that are under constant selection pressure. With several loci under fluctuating selection caused by different environmental factors (with different time periods), selection on transformation rates may be hard to predict based on expectations for each locus individually. These and other scenarios involving several loci as well as the effects of epistasis and random genetic drift in models of transformation with fluctuating selection remain to be explored in future studies.

In conclusion, our model suggests a novel evolutionary role for transformation: it may enable bacteria to adapt faster to changing environments by taking advantage of the genes stored in the free DNA pool. This role may complement other evolutionary functions of transformation, for example, as a means of acquiring nutrition [5,7] or increasing genetic diversity through recombination [3,6]. The viability of our hypothesis should be readily testable in evolution experiments with naturally competent bacteria. Finally, we would like to note that 'genetic time travel' through incorporation of free DNA may not only be beneficial in prokaryotes: bdelloid rotifers, for example, undergo rampant horizontal gene transfer [31] and at the same time are potentially under strong fluctuating selection through frequent desiccation of their habitat.

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References

1. Johnsborg O, Eldholm V, Havarstein LS. 2007 Natural genetic transformation: prevalence, mechanisms and function. *Res. Microbiol.* **158**, 767–778. (doi:10.1016/j.resmic.2007.09.004)
2. Chen I, Dubnau D. 2004 DNA uptake during bacterial transformation. *Nat. Rev. Microbiol.* **2**, 241–249. (doi:10.1038/Nrmicro844)
3. Dubnau D. 1999 DNA uptake in bacteria. *Annu. Rev. Microbiol.* **53**, 217–244. (doi:10.1146/annurev.micro.53.1.217)
4. Narra HP, Ochman H. 2006 Of what use is sex to bacteria? *Curr. Biol.* **16**, R705–R710. (doi:10.1016/J.Cub.2006.08.024)
5. Redfield RJ. 2001 Do bacteria have sex? *Nat.* Rev. Genet. **2**, 634–639. (doi:10.1038/35084593)
6. Vos M. 2009 Why do bacteria engage in homologous recombination? *Trends Microbiol.* **17**, 226–232. (doi:10.1016/j.tim.2009.03.001)
7. Redfield RJ. 1993 Genes for breakfast: the have-your-cake-and-eat-it-too of bacterial transformation. *J. Hered.* **84**, 400–404.
8. Finkel SE, Kolter R. 2001 DNA as a nutrient novel role for bacterial competence gene homologs. *J. Bacteriol.* **183**, 6288–6293. (doi:10.1128/JB.183.21.6288-6293.2001)
9. Michod RE, Bernstein H, Nedelcu AM. 2008 Adaptive value of sex in microbial pathogens. *Infect. Genet. Evol.* **8**, 267–285. (doi:10.1016/J.Meegid.2008.01.002)
10. Engelmoer DJP, Rozen DE. 2011 Competence increases survival during stress in *Streptococcus pneumoniae*. *Evolution* **65**, 3475–3485. (doi:10.1111/J.1558-5646.2011.01402.X)
11. Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D. 2007 Release and persistence of extracellular DNA in the environment. *Environ. Biosafety Res.* **6**, 37–53. (doi:10.1051/ebr:2007031)
12. Lorenz MG, Wackernagel W. 1991 High-frequency of natural genetic-transformation of *Pseudomonas stutzeri* in soil extract supplemented with a carbon energy and phosphorus source. *Appl. Environ. Microbiol.* **57**, 1246–1251.
13. Hamilton HL, Dominguez NM, Schwartz KJ, Hackett KT, Dillard JP. 2005 *Neisseria gonorrhoeae* secretes chromosomal DNA via a novel type IV secretion system. *Mol. Microbiol.* **55**, 1704–1721. (doi:10.1111/J.1365-2958.2005.04521.X)
14. Backert S, Meyer TF. 2006 Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr. Opin. Microbiol.* **9**, 207–217. (doi:10.1016/J.Mib.2006.02.008)
15. Redfield RJ. 1988 Evolution of bacterial transformation—is sex with dead cells ever better than no sex at all. *Genetics* **119**, 213–221.
16. Moradigaravand D, Engelstädter J. 2013 The evolution of natural competence: disentangling costs and benefits of sex in bacteria. *Am. Nat.* **182**, E112–E126. (doi:10.1086/671909)
17. Otto SP. 2009 The evolutionary enigma of sex. *Am. Nat.* **174**, S1–S14. (doi:10.1086/599084)
18. Charlesworth B. 1976 Recombination modification in a fluctuating environment. *Genetics* **83**, 181–195.

19. Sasaki A, Iwasa Y. 1987 Optimal recombination rate in fluctuating environments. *Genetics* **115**, 377–388.
20. Kondrashov AS, Yampolsky LY. 1996 Evolution of amphimixis and recombination under fluctuating selection in one and many traits. *Genet. Res.* **68**, 165–173. (doi:10.1017/S0016672300034054)
21. Otto SP, Michalakis Y. 1998 The evolution of recombination in changing environments. *Trends Ecol. Evol.* **13**, 145–151. (doi:10.1016/S0169-5347(97)01260-3)
22. Hamilton WD, Axelrod R, Tanese R. 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl Acad. Sci. USA* **87**, 3566–3573. (doi:10.1073/Pnas.87.9.3566)
23. Salathe M, Kouyos RD, Bonhoeffer S. 2008 The state of affairs in the kingdom of the Red Queen. *Trends Ecol. Evol.* **23**, 439–445. (doi:10.1016/J.Tree.2008.04.010)
24. Johnsen PJ, Dubnau D, Levin BR. 2009 Episodic selection and the maintenance of competence and natural transformation in *Bacillus subtilis*. *Genetics* **181**, 1521–1533. (doi:10.1534/Genetics.108.099523)
25. Wylie CS, Trout AD, Kessler DA, Levine H. 2010 Optimal strategy for competence differentiation in bacteria. *PLoS Genet.* **6**, e1001108. (doi:10.1371/journal.pgen.1001108)
26. Kussell E, Kishony R, Balaban NQ, Leibler S. 2005 Bacterial persistence: a model of survival in changing environments. *Genetics* **169**, 1807–1814. (doi:10.1534/Genetics.104.035352)
27. Levy-Booth DJ *et al*. 2007 Cycling of extracellular DNA in the soil environment. *Soil. Biol. Biochem.* **39**, 2977–2991. (doi:10.1016/J.Soilbio.2007.06.020)
28. Alvarez AJ, Yumet GM, Santiago CL, Toranzos GA. 1996 Stability of manipulated plasmid DNA in aquatic environments. *Environ. Toxicol. Water Qual.* **11**, 129–135. (doi:10.1002/(Sici)1098-2256(1996)11:2<129::Aid-Tox8>3.0.Co;2-B)
29. Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011 Persistence of environmental DNA in freshwater ecosystems. *PLoS ONE* **6**, e23398. (doi:10.1371/journal.pone.0023398)
30. Romanowski G, Lorenz MG, Sayler G, Wackernagel W. 1992 Persistence of free plasmid DNA in soil monitored by various methods, including a transformation assay. *Appl. Environ. Microbiol.* **58**, 3012–3019.
31. Gladyshev EA, Meselson M, Arkhipova IR. 2008 Massive horizontal gene transfer in bdelloid rotifers. *Science* **320**, 1210–1213. (doi:10.1126/science.1156407)