

Defying bacteriophages

Contrasting altruistic with individual-based resistance mechanisms in *Escherichia coli*

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Adaptations by hosts in response to parasitism are generally believed to reduce the susceptibility of the adapted individual. However, recent work on *Escherichia coli* showed that bacteria can fight deadly phage attacks by committing altruistic suicide upon infection, in order to prevent parasite transmission to nearby relatives. Here, we compare the efficiency of suicidal host defense with individual-based resistance. We show that in unstructured environments suicidal host defense is futile since suicide cannot preferentially protect relatives, whereas individual-based resistance is highly efficient in defying phages. In contrast, we found that in structured environments suicidal host defense and individual-based resistance were both efficient in withstanding phages, with the latter type performing slightly better. We propose that the putative lower efficiency of suicidal host defense might be compensated by the fact that suicidal systems usually do not bear pleiotropic costs of resistance, as it is usually the case for individual-based resistance mechanisms.

Organisms are consistently exposed to parasites causing morbidity and mortality. A key interest in parasitology is therefore to understand adaptive responses by hosts to evade or reduce detrimental parasitic effects.¹ While research has traditionally focused on adaptations reducing the susceptibility of individuals,² a valuable alternative seems that natural selection favors the expression of traits in infected individuals that protect others from becoming infected.^{3,4} For instance, it has been observed that infected individuals in social insect and aphid colonies leave the group to die in isolation.^{5–8} Such behavioral patterns suggest that infected individuals do not care for themselves, but remove themselves altruistically from the group (i.e., commit suicide) in order to prevent the spreading of disease among relatives. Although compelling, the idea of altruistic suicide as adaptive host-defense strategy has repeatedly been challenged,^{9,10} and has led to a more general controversy about whether suicide can be adaptive.^{11–13}

This fundamental controversy has now conclusively been solved through a number of studies, which examined abortive infection (Abi) systems in the bacterium *Escherichia coli*. Many bacterial species possess Abi systems, which respond to lethal phage attacks by causing infected cells to die together with the infecting phage.^{14–17} Abi mechanisms usually involve joint actions by a prophage (a phage integrated into the bacterial genome) or a plasmid that encodes the genes for the abortive infection, and the bacterium, which transcribes and synthesizes the abortive

machinery. Although Abi systems have long been described, the key questions of whether they represent altruistic acts and have been selected for that purpose have long remained unaddressed. Recently, Fukuyo et al.,¹⁸ resolved the first question by showing that an engineered Abi system was exclusively selected for in structured environments where abortion preferentially protected clone mates (i.e., relatives) from becoming infected. This finding is compatible with social evolution theory, predicting that altruism can only evolve when its benefit accrues to relatives.^{19,20} The second question was subsequently addressed by Berngruber et al.,²¹ and Refardt et al.,²² who recovered similar evolutionary dynamics for naturally occurring Abi systems, which supports the idea that these systems have been selected for the purpose of committing suicide. Moreover, Refardt et al.,²² showed that altruistic host suicide could spread with ease across a wide range of ecological conditions. This was because suicide in their system incurred negligible costs since infected individuals were moribund, such that the commitment of suicide simply forestalled the inevitable phage-induced death.

While these novel findings have demonstrated that altruistic host suicide is important in combating parasites, they also raise the question of how effective altruistic host-defense is compared with classical individual-based resistance mechanisms. Here, we address this question by comparing the fitness benefits of the two strategies both in structured and unstructured environments.

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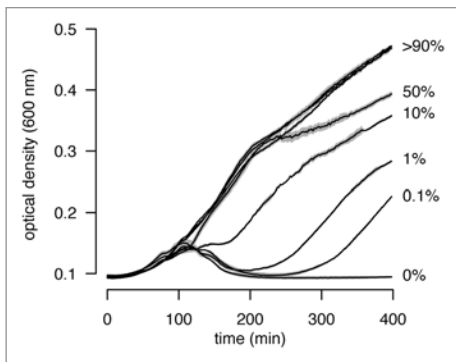


Figure 1. Population growth of mixed cultures containing *E. coli* T4^r (resistant against the obligately lytic phage T4rII) and *E. coli* HK97 (T4rII sensitive) in shaken liquid medium. While phage T4rII eradicated monocultures of *E. coli* HK97, cultures containing *E. coli* T4^r could always grow even when *E. coli* T4^r was initially rare (percentage of *E. coli* T4^r is indicated on the right).

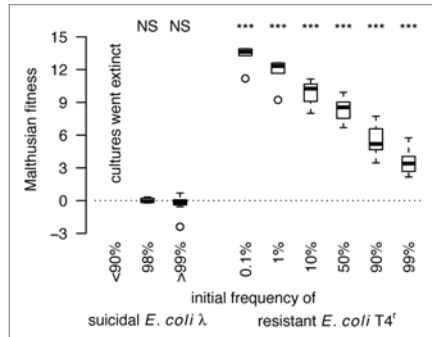


Figure 2. Comparing the performance of suicidal *E. coli* λ and resistant *E. coli* T4^r in competition against *E. coli* HK97 in the presence of phage T4rII in an unstructured environment (shaken liquid medium). While *E. coli* T4^r significantly outcompeted *E. coli* HK97 under all conditions (Malthusian fitness > 0 ; $p < 0.001$), *E. coli* λ either experienced no relative fitness benefit or the entire culture went extinct (these latter results were initially published in Refardt et al.,²² <http://dx.doi.org/10.1098/rspb.2012.3035>). These findings demonstrate that individual-based resistance is the only way of how bacteria can defy bacteriophages in unstructured environments.

Results and Discussion

In all experiments, we exposed bacterial strains to the phage T4rII, which targets lipopolysaccharides embedded in the bacterial outer membrane.²³ We used three different strains of *E. coli* str. K-12 substr. MG1655. As our altruistic suicidal strain, we used *E. coli* carrying the prophage λ (henceforth *E. coli* λ), which encodes the Rex abortive infection system¹⁴ that is triggered by several phages including T4rII.²⁴ As the phage-sensitive strain, we used *E. coli* carrying the Abi-free control prophage HK97 (henceforth *E. coli* HK97). Finally, we used an *E. coli* HK97 strain that has evolved resistance against T4rII (henceforth *E. coli* T4^r). While we compared the performance of phage-sensitive *E. coli* HK97 vs. suicidal *E. coli* λ in our previous study,²² we here compare the competitive abilities of phage-sensitive *E. coli*

HK97 vs. phage-resistant *E. coli* T4^r, and relate it to our previous findings.

We first examined the performance of *E. coli* HK97 and *E. coli* T4^r in an unstructured environment. We did this by growing the two strains together in shaken liquid medium at various frequencies (*E. coli* T4^r varied from 0.1% to 99%) in the presence of T4rII (MOI = 0.001) and followed population growth and the change in relative strain frequency over time. We found that T4rII eradicated monocultures of *E. coli* HK97, whereas *E. coli* T4^r could thrive even when initially rare (Fig. 1). Moreover, *E. coli* T4^r significantly outcompeted *E. coli* HK97 under all conditions (Fig. 2, one-sample t-tests for fitness > 0 : $t_5 > 6.9$, $p < 0.001$). These results demonstrate that individual-based resistance mechanisms can easily spread in a completely unstructured environment, a finding that strongly contrasts with the suicidal host-defense strategy, which either could not prevent population collapse (with *E. coli* λ frequencies $\leq 90\%$) or did not result in fitness benefits (Fig. 2). This comparison reveals that individual-based resistance is the only way of how bacteria can defy bacteriophages in unstructured environments.

We found a different pattern when competitions were performed in a structured environment (Fig. 3). Specifically, when we added 1.5% agar to the medium to limit bacterial dispersal,²⁵ *E. coli* HK97 was significantly outcompeted by both suicidal *E. coli* λ (one-sample t-test for fitness > 0 : $t_{29} = 11.17$, $p < 0.0001$) and *E. coli* T4^r ($t_{11} = 9.52$, $p < 0.0001$). This comparison shows that both altruistic host-defense and individual-based resistance mechanisms are efficient means to defy bacteriophages in a structured environment. However, *E. coli* T4^r seemed to be slightly but significantly more efficient than *E. coli* λ (Fig. 3; two sample t-test: $t_{40} = 4.20$, $p = 0.0001$). A plausible explanation is that suicide always takes its toll, especially at high MOI, which reduces the efficiency of suicidal host defense compared with an individual-based resistance mechanism. Additionally, we also found that the relative fitness of *E. coli* T4^r was greatly reduced in the structured compared with the unstructured environment, an observation that is compatible with the view that medium viscosity also reduces phage dispersal, such that some fraction of *E. coli* HK97 colonies remain unharmed.

To be able to put our findings in an ecological context, we need to become clear about the level of structuring that occurs in natural bacterial populations. While an unifying answer is not possible owing to the fact that bacteria populate an enormous range of different habitats, it seems justified to conclude that some degree of population structure always exists.²⁶ Consequently, we can deduce that altruistic host defense likely represents an efficient strategy to withstand bacteriophages in natural habitats.

While our comparison revealed that both host-defense strategies can be efficient in laboratory settings, it remains unclear, which of the two strategies is more prevalent in nature. Individual-based resistance might be more common because it is easier to evolve, since a single point mutation can lead to resistance.²⁷ In contrast, suicidal defense systems are often based on complex machineries¹⁷ that eventually have first evolved for other purposes, and have only later been coopted into suicidal

systems.²² Although more complex, we propose that suicidal host-defense systems might be cheaper to maintain in the long run because they are often decoupled from other functions, and might therefore entail little pleiotropic cost. Individual-based resistance, on the other hand, is typically achieved by altering a molecule on the bacterial cell surface that is recognized by a phage and to which the phage adsorbs.¹⁷ Such an adaptation may impair the original function of the molecule and consequently reduce bacterial fitness (as observed in T4 phages²⁸), even more so if further modifications are necessary to resist different phages, or to respond to phage counter-resistance.²⁹⁻³¹ These potential cost differences might also affect the co-evolutionary dynamics between phages and bacteria. Moreover, it would be interesting to see whether both resistance mechanisms can occur in the same individual, or whether they are mutually exclusive, with one resistance mechanisms eventually replacing the other one over evolutionary time scales. While speculative at this stage, these considerations will hopefully stimulate future work.

Finally, another unresolved issue about Abi systems is that they often involve the action of prophages. A plausible explanation for this is that prophages already possess mechanisms that can kill bacteria during induction (i.e., when a prophage enters the lytic phase), which can eventually be coopted into suicide machineries. More sinister is the possibility that bacteria are not always free to choose their doom.¹¹ For instance, it has been observed that competition between two prophages in the same bacterial cell leads some prophages to induce premature cell death, possibly to prevent the other prophage from replicating.^{32,33}

Methods

To obtain *E. coli* T4^r, we diluted overnight cultures of *E. coli* HK97 100-fold, and incubated 100 μ l together with 10⁴ pfu of phage T4rII overnight in a plate reader. Cultures whose growth pattern indicated the evolution of resistance were selected and serially passaged (three times) in 3 ml medium. Before every passage, we diluted cultures 1,000-fold and added 10⁸ pfu of phage T4. This was done to select for full resistance, and to reduce possible resistance costs through compensatory mutations. We then streaked out cultures, and picked a single colony to establish a stock culture, stored at -80°C. We verified resistance by cross-streaking against lysates of both phages T4 and T4rII. We chose this procedure because we were interested in obtaining a strain that exhibits a different defense mechanism against phage T4rII, but otherwise grows comparably to *E. coli* λ and *E. coli* HK97 (which do not differ in their growth rate in the absence of phages²²). Indeed, when grown in mixed cultures, we found that neither *E. coli* T4^r nor *E. coli* HK97 experienced a selective advantage in the absence of phages ($t_4 = 0.48$, $p = 0.66$, difference in Malthusian fitness = 0.02). The downside of this procedure is, however, that we cannot infer the initial cost of phage resistance.

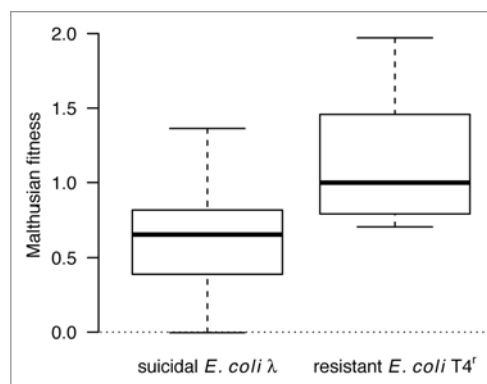


Figure 3. Comparing the performance of suicidal *E. coli* λ and resistant *E. coli* T4^r in competition against *E. coli* HK97 in the presence of phage T4rII in a structured environment (1.5% agar plate). Both strains significantly outcompeted *E. coli* HK97, indicating that suicidal host defense as well as individual-based resistance were efficient in withstanding phages. A direct comparison between the two strategies reveals that individual-based resistance is slightly but significantly better under these conditions. But note that although the experiments were performed following the exact same protocol, they were performed at different dates, which might explain some of the differences.

We performed all experiments following the same protocol as described previously.²² In short, experiments shown in **Figures 1 and 2** were performed in 96-well microtiter plates in 100 μ l of medium. Initial concentration of both competing strains together was 5×10^7 cfu/ml, initial concentration of phage T4rII was 10⁵ pfu/ml. Experiments for **Figure 3** were performed in 24-well plates with wells filled with 1.5 ml LB 1.5% agar each. Between 10 and 10⁶ cfu of *E. coli* λ and 10⁴ cfu of *E. coli* T4^r were added together with 10⁴ pfu of phage T4rII.

To be able to distinguish bacterial strains in mixed competition, we marked all strains with plasmids pGFP and pDsRed carrying genes for fluorescent proteins under an inducible promoter as described previously.²² Competition assays in unstructured and structured environments followed protocols previously described,²² except that experiments in structured environments were performed on agar instead of agarose.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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