Supplemental Information for

Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity

Mariann Landsberger[1], Sylvain Gandon[2], Sean Meaden[1], Hélène Chabas[1§], Angus Buckling[1], Edze R. Westra[1*] and Stineke van Houte[1*]

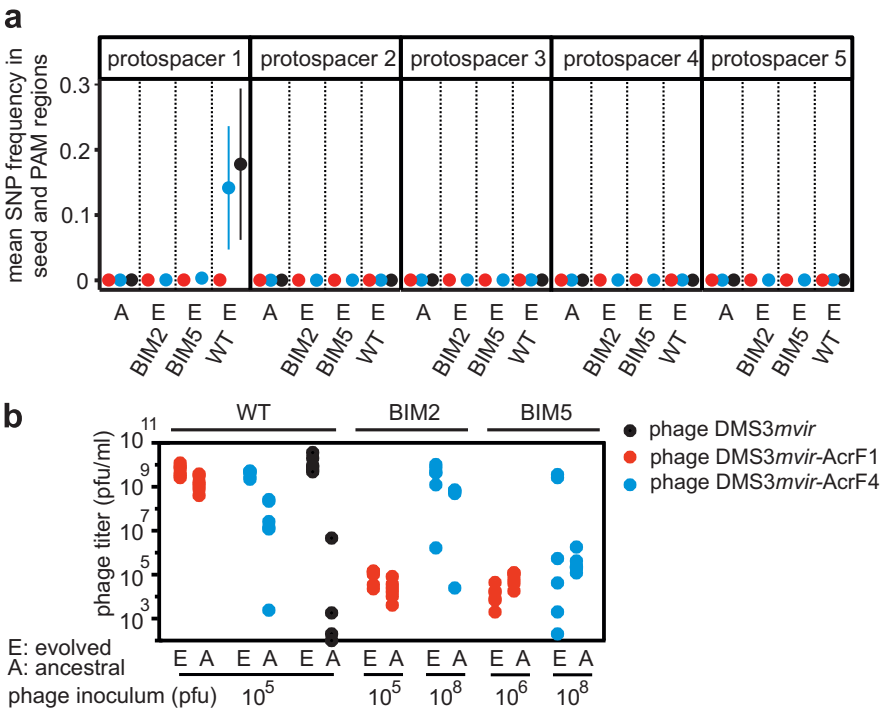
* Correspondence: westra.edze@gmail.com, vanhoute.stineke@gmail.com.

This PDF file includes:

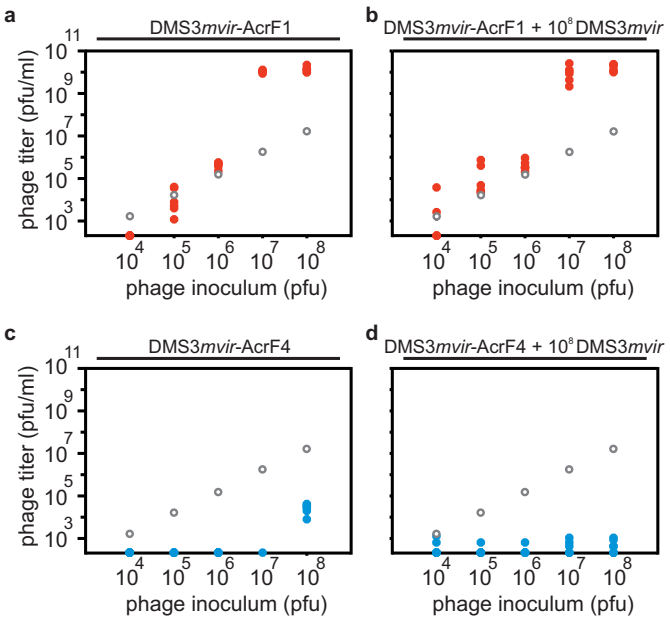
Supplementary Figures 1 – 4

Supplementary information on mathematical modelling

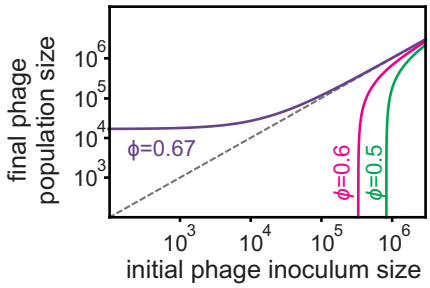
Supplementary figures



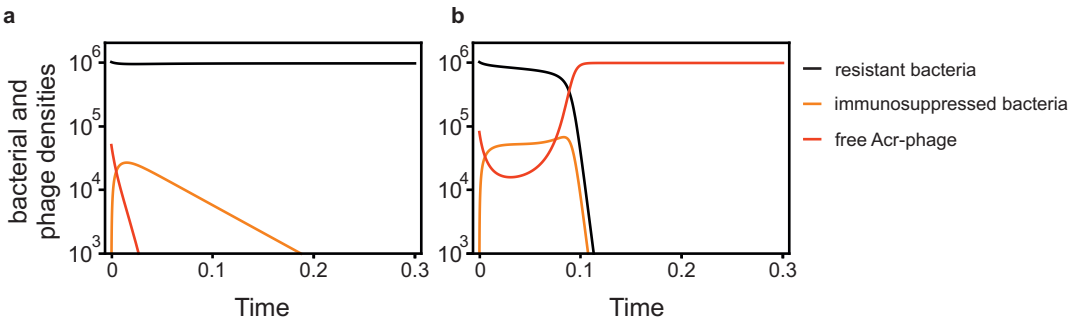
Supplementary Figure 1. Epidemiological tipping points cannot be explained by phage evolution (related to Figure 2). (**a**) Deep sequencing of protospacer sequences of phages DMS3*mvir* (black data points), DMS3*mvir*-AcrF1 (red data points) or DMS3*mvir*-AcrF4 (blue data points), either ancestral (A) or evolved on WT, BIM2 or BIM5 hosts (DMS3*mvir* only on WT). Protospacer 1 is targeted by WT, BIM2 and BIM5, protospacer 2 is targeted by BIM2 and BIM5, and protospacers 3, 4 and 5 are targeted by BIM5. Mean SNP frequency across the seed and PAM region (in total 10 nucleotides) of each protospacer is shown, error bars indicate the 95% c.i. (**b**) Density-dependent epidemiological tipping points are not due to phage evolution. Viral titers at 24 hpi of phage DMS3*mvir* (black data points), DMS3*mvir*-AcrF1 (red data points) or DMS3*mvir*-AcrF4 (blue data points) on bacteria PA14 WT, BIM2 or BIM5. Below each diagram is indicated which phage amounts (pfus) were added in the experiment. A indicates ancestral phage; E indicates evolved phage (isolated from the experiments depicted in Fig. 2). Each dot represents an independent biological replicate (n=6). The limit of detection is 200 pfu/ml.



Supplementary Figure 2. Epidemiological tipping points are not due to Csy-complex sequestration by WT phage (related to Figure 3). (**a**) Viral titers at 24 hpi with 10^4, 10^5, 10^6, 10^7 or 10^8 pfus DMS3*mvir*-AcrF1. (**b**) Viral titers at 24 hpi with 10^4, 10^5, 10^6, 10^7 or 10^8 pfus DMS3*mvir*-AcrF1 in the presence of 10^8 pfus DMS3*mvir*. (**c**) Viral titers at 24 hpi with 10^4, 10^5, 10^6, 10^7 or 10^8 pfus DMS3*mvir*-AcrF4 (**d**) Viral titers at 24 hpi with 10^4, 10^5, 10^6, 10^7 or 10^8 pfus DMS3*mvir*-AcrF4 in the presence of 10^8 pfus DMS3*mvir*. Grey circles indicate the phage titers (pfu/ml) at the start of the experiment (corresponding to the addition of 10^4, 10^5, 10^6, 10^7 or 10^8 pfus). Colored points represent phage titers at 24 hpi; each data point represents an independent biological replicate (*n*=6). The limit of detection is 200 pfu/ml.



Supplementary Figure 3. Partial immunity alone cannot explain the observed epidemiological tipping points (related to Figure 4). Model predictions of the effect of initial Acr-phage inoculum density on the phage density after 24h of incubation for different values of Acr strength when no immunosuppressive state *S* is assumed in the model (ϕ = 0.67, 0.6 and 0.5; purple, magenta, green respectively); other parameter values: B = 5, α = 0.001, γ = 20, ρ = 0.7. Grey line corresponds to the initial amount of phage and values below this line indicate a lack of phage amplification.



Supplementary Figure 4. Model predictions of the temporal population dynamics of Acr-phage and resistant and immunosuppressed hosts (related to Figure 5). Model predictions for the densities of resistant bacteria (black), immunosuppressed bacteria (orange) and phages (red) across time for two initial inoculum sizes: **(a)** V(0)=5*10^4, **(b)** V(0)= 8*10^4. Other parameter values: B = 5, α = 0.001, γ = 20, ρ = 0.7.

Supplemental information

Mathematical modelling

We develop an epidemiological model to understand the dynamics of bacteriophages that carry an anti-CRISPR mechanism (Acr) in a resistant host population. Resistant bacteria may be in a normal state (the density of these bacteria is noted $R(t)$) or in a immunosuppressed state (the density of these bacteria is noted $S(t)$).

Initially the host population is homogeneous and the density of resistant bacteria is $R(0)$. Then an inoculum of free phage particles with density $V(0)$ is introduced in the host population. Free phage particles adsorb to the bacteria at a rate α. When a free phage adsorbs to a resistant bacteria three outcomes are possible: (i) The phage is destroyed and there is no change in bacterial resistance with probability ρ. In other words ρ is a measure of bacteria resistance (ρ increases with the number of spacers targeting the phage), (ii) The phage is not destroyed because of its Acr activity with probability $(1-\rho)\phi$ which leads to cell lysis and the release of B new phage particles. The efficacy of Acr activity is thus measured with ϕ. (iii) Finally, the phage fails to complete its lytic cycle but blocks bacterial immunity and the bacteria becomes immunosuppressed with probability $(1-\rho)(1-\phi)$. This state is reversible and immunosuppressed bacteria become resistant again at rate γ. If an immunosuppressed bacteria is exposed to a phage, the absence of immunity allows the phage to complete its lytic life cycle. This yields the following set of ordinary differential equations (see **Fig. 4a**):

$$\begin{aligned}
\dot{R}(t) &= -\big(a\,(1-\rho)\,V(t)\big)R(t) + \gamma\,S(t) \\
\dot{S}(t) &= a(1-\rho)(1-\phi)V(t)R(t) - \big(\gamma + a\,V(t)\big)S(t) \\
\dot{V}(t) &= a(1-\rho)\phi B\,V(t)R(t) + aB\,V(t)S(t) - a\,\big(S(t)+R(t)\big)V(t)
\end{aligned} \tag{1}$$

Initially there are no immunosuppressed bacteria around (i.e. $S(0)=0$) and if a few virus particles are introduced the phage population will increase if the Acr activity is higher than a threshold value:

$$\phi > \phi_0 = \frac{1}{(1-\rho)B} \tag{2}$$

In other words, when ϕ is low and ρ is high the phage population cannot take off when the inoculum of the phage is small (**Fig. 4b,c**).

Yet, if one introduces a sufficiently high density of Acr-phages they will shut down the immunity of the bacterial population and will increase the density of immunosuppressed bacteria. At time T the following condition may be verified

$$\phi > \phi_0 - \frac{S(T)(B-1)}{R(T)(1-\rho)B} \quad (3)$$

and the phage population may thus take off for lower values of Acr activity. Note that conditions (2) and (3) indicate a range of values of ϕ where the epidemiological outcome depends on the initial density of the virus (epidemiological bistability). Note that the threshold value of the density of virus leading to an epidemic depends also on the duration of the immunosuppression (see **Fig. 4d**).

In **Fig. S4** we illustrate the epidemiological dynamics taking place when the initial density of the phage is either below or above the threshold value of the phage inoculum. Below the threshold (**Fig. S4a)**, the density of immunosuppressed bacteria does not reach a high enough level to satisfy condition (3) and the phage population goes extinct. Above the threshold (**Fig. S4b)**, the immunosuppressed bacteria reaches a high enough density, condition (3) is verified and the phage population can exploit the whole bacteria population.