Supplemental Data

Host Mixing and Disease Emergence

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Supplemental Experimental Procedures

Growth of bacteria and phage under selective conditions

We measured the densities after 48 hours (one experimental transfer) of the ancestral susceptible and resistant bacteria and phage. To distinguish resistant and sensitive bacteria, we used an isogenic mutant of SBW25 as the susceptible strain: SBW25-*LacZ* [1]. This strain has a promoter-less *lacZ* insertion, which results in the formation of blue colonies when grown on LB agar plates supplemented with X-gal (40μg/ml). Crucially, the strain does not have a pleiotropic fitness cost relative to its ancestor [1], allowing a direct comparison to be made between the *lacZ* marked strain and the ancestral strain used in the selection experiments. Approximately 10^7 bacteria (from overnight cultures shaken at 0.9 g at 28C) and 10^5 phage particles were inoculated into 30 ml glass tubes containing 6 ml King's Media B. Three replicate populations were set up for each of the susceptible: resistant bacteria ratios as used in the selection experiment. We also set up 3 replicates of the 1:1 ratio in the absence of the phages.

Densities of bacteria (based on colony forming units, cfus) were determined by plating onto LB agar supplemented with X-gal, and phage densities (based on plaque forming units, pfus) by plating onto soft KB agar containing exponentially growing ancestral

bacteria at the start of the experiment and after 48 hours growth under the selective conditions.

There was a near-perfect positive correlation between the frequencies of susceptible bacteria at the start and end of the growth cycle (Supplementary Figure; $\log_{10}$-transfromed ratios: $r = 0.997$, $n = 12$, $P < 0.001$), demonstrating that the expected qualitative differences in selection pressures between treatments were maintained throughout a 48 hour growth period. The slope of this relationship was however significantly less than 1 (slope of log10-transformed ratios = 0.86; $t = 44.31$, $P < 0.001$), indicating that the relative fitness of the susceptible strain decreased with increasing starting frequency. This is presumably because larger susceptible host densities supported larger phage densities (Supplementary Figure). In the absence of phages, there was a significant cost associated with phage resistance (ratio of susceptible to resistant hosts increased by 25%; $t = 7$, $n = 3$, $P = 0.02$). Note that data from the 0.1 and 0.01% susceptible host treatments were not included, because counts of the susceptible bacteria using dilutions that allowed individual colonies to be distinguished were too low to obtain accurate estimates of the relative frequencies. Accurate calculation is impossible in the absence of selective markers, such as antibiotic resistance. These markers invariably have pleiotropic effects, making the results not comparable with the ancestral bacteria.

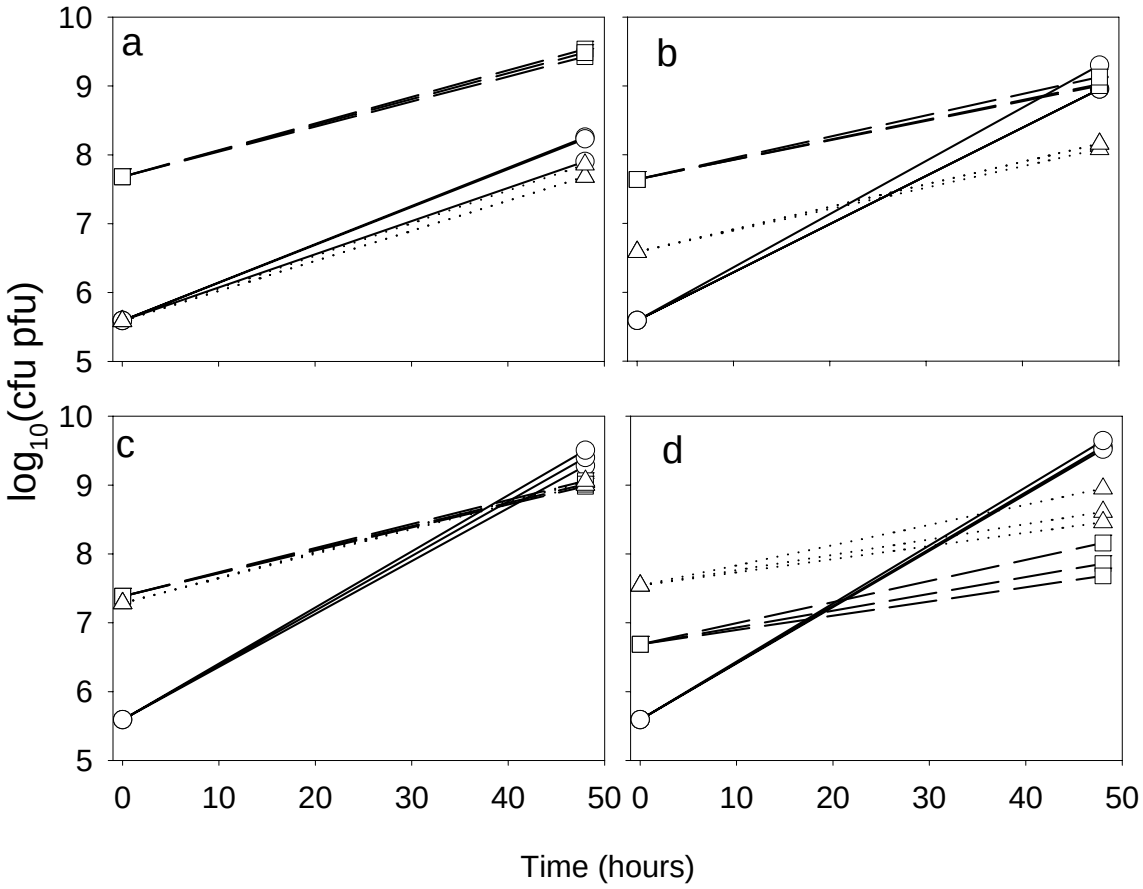

Figure S1. Bacteria (colony forming units; cfus) and phage (plaque forming units; pfus) densities through time at different susceptible host frequencies: a) 1%; b) 10%; c) 50%; d) 90%. Resistant bacteria are indicated by dashed lines and squares; susceptible bacteria by dotted lines and triangles & phages by solid lines and circles. Three replicates were carried out for each susceptible host frequency.

Supplemental Reference

1. Zhang, X.X. & Rainey, P. B. (2007). Construction and validation of a neutrally-marked strain of *Pseudomonas fluorescens* SBW25. J. Microbiol. Methods *71*, 78-81