

Supplementary Material

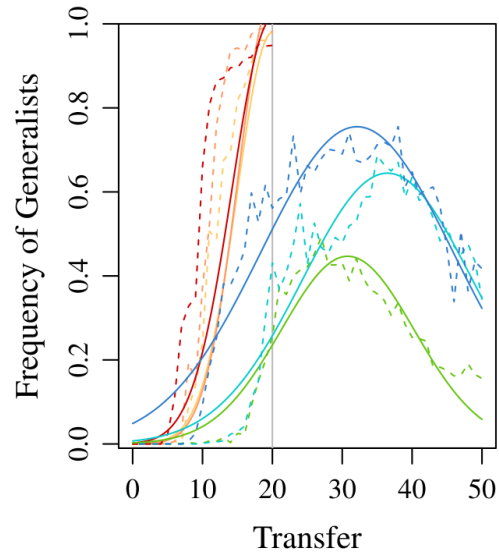


Figure S1. We devised the following statistical test to determine whether the abundance of specialists plummeted and then recovered in the U-shape pattern predicted during evolutionary rescue. We reasoned that the predicted dynamics in the specialists should cause the observed frequency of generalists (dashed lines, identical to solid lines in Figure 2A) to increase to a maximum value well before the end of the experiment and then to decline for a significant number of transfers beyond the maximum. We fit Gaussian curves (solid lines) to the observed data of the form:

$$f_{gen}(t) = ae^{-(t-b)^2/2c^2},$$

where $f_{gen}(t)$ is the frequency of generalists at transfer t , a is the maximum frequency, b is the transfer at which the maximum was obtained, and c is the width of the peak. To fit the curve, we used data only up to a time point where a Gaussian curve gave a reasonable approximation to the

frequency trajectory: transfers 0-20 for the 1:1 host ratio; transfers 0-50 for the 9:1 host ratio.

Our test compared the fit of Gaussian curves in which we either fixed b at t_{\max} the last time point included in the analysis (Model 1) or estimated b from the data (Model 2). Parameters a and c were estimated from the data in both models. For populations evolved in the 9:1 host ratio, the location of the maximum, b , was always estimated to occur significantly earlier than transfer 50 ($N = 10^5$, green, $b = 31$, $F_{1,47} = 6.221$, $p = 0.0081$; $N = 10^6$: light blue, $b = 37$, $F_{1,47} = 3.61$, $p = 0.0355$; $N = 10^7$, blue, $b = 32$, $F_{1,47} = 4.738$, $p = 0.0182$). By contrast, for populations evolved in the 1:1 host ratio, allowing b to deviate from transfer 20 never significantly improved the fit to the data ($N = 10^5$: yellow, $F_{1,17} = 1.713$, $p = 0.1266$; $N = 10^6$, orange, $F_{1,17} = 2.455$, $p = 0.0745$; $N = 10^7$, red, $F_{1,17} = 2.128$, $p = 0.0932$).

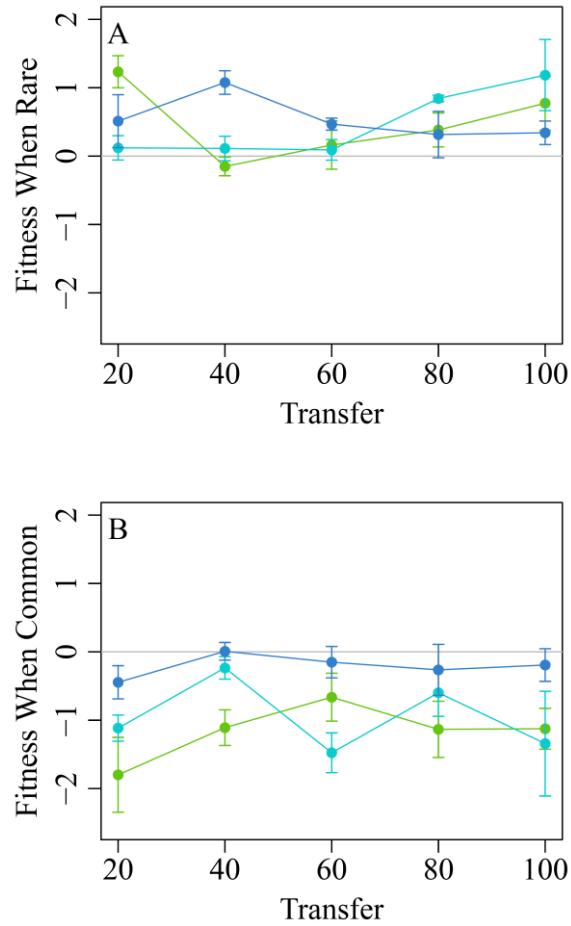


Figure S2. Frequency-dependent selection over time. We conducted frequency-dependent selection assays using generalist and specialist clones isolated from evolving populations every 20 transfers. All assays were performed using the 9:1 ratio of standard: novel hosts in which the populations were evolved. Data are mean $\ln W \pm$ s.e.m. of the generalist relative to the specialist obtained from 5 replicate competition assays initiated at generalist frequencies of $f_{gen} = 0.1$ (A) or $f_{gen} = 0.9$ (B). Colors are as in figure 2 and distinguish replicate lineages propagated using different phage transfer population sizes.

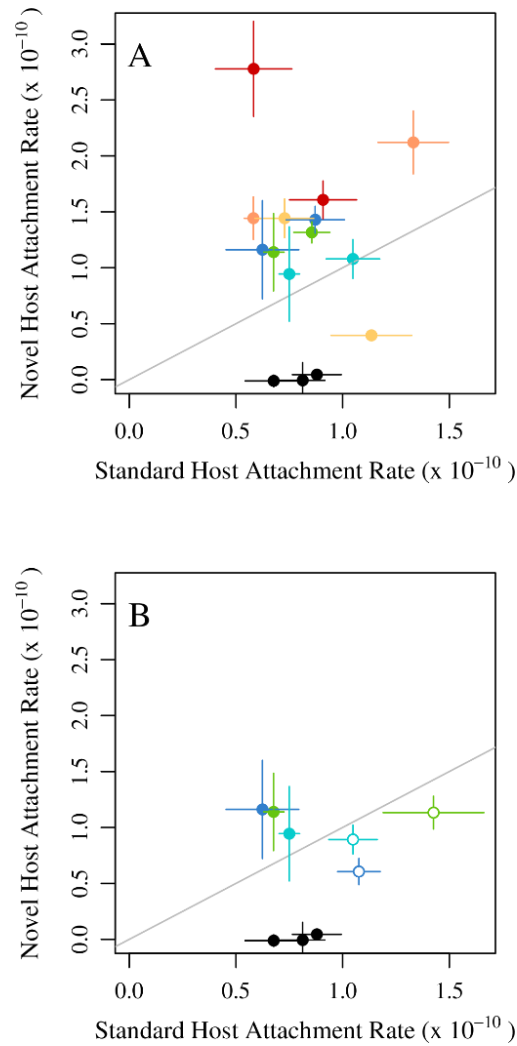


Figure S3. Adsorption rates of evolved generalist phage. Data are means \pm s.e.m. obtained from 6 replicate measures of phage adsorption rates on the standard and novel hosts. Color corresponds to the representation in figure 2. Panel A compares evolved generalist phage isolated at transfer 20 from populations evolved under the 1:1 host ratio (warm colors) versus the 9:1 host ratio (cool colors). Adsorption rates did not differ significantly between host ratio treatments to either the standard host ($F_{1,10} = 0.276$, $p = 0.6108$) or the novel host ($F_{1,10} = 1.864$, $p = 0.2021$). Panel B compares generalist phage isolated after 20 (closed circles) or 100 (open circles) transfers under the 9:1 host ratio. Adsorption rates to the standard host increased significantly

between these time points ($F_{1,7} = 9.726$, $p = 0.0169$). Adsorption rates to the novel host did not ($F_{1,7} = 2.97326$, $p = 0.1283$). Ancestral phage are shown in black for reference. The gray line corresponds to equal adsorption to both hosts.