Electronic supplementary material (M. Barfield and R.D. Holt)

Origin of Mutations in Host Shift Experiments

Using data published in Dennehy et al. (2006), the source phage population in the host shift experiment is expected to be greater than 10^{10} at the end of each growth period; assume it is $10^{10.5}$. 10^4 virions go to the sink, while the rest are diluted by 10^6, leaving $10^{4.5}$ to go to the next passage in the source. These phage reproduce to > 10^{10} and this cycle likely repeats for the duration of the experiment.

The population levels in the sink are not as clear. Again using Dennehy et al. (2006) as a reference, the population should increase to about $10^{9.5}$ following the initial inocula with 10^4 phage. Subsequently this population is reduced to about $10^{3.5}$ during serial passage transfer. If no adaptation occurs and 10^4 new phage are added each day, the population levels should approach $10^{4.2}$ after source phage are added, and to $10^{9.7}$ after growth. However, the absolute fitness of the static source is about $10^{-1.4}$ in Figure 4 of the current manuscript, and at the end of the experiment the host shift sink has an absolute fitness only a little greater than 1 in spite of a $10^{1.6}$ increase in relative fitness. If the change in absolute fitness is similar to that in relative fitness, this implies an absolute initial fitness of about $10^{-1.5}$, in reasonable agreement with the static source value. The Dennehy et al. (2006) results, used above, show that absolute fitness on ERA is approximately $10^{-0.5}$. If the fitness is an order of magnitude lower, then the sink population should fluctuate between about 10^4 and $10^{8.5}$. Call the final sink population N.

A new mutant can appear in the sink on any day because of a mutation in the sink that day, or due to a mutant in the source that is transferred to the sink. If the mutation rate is u per virion, then the expected number of sink mutations in the last replication is Nu. If each phage replication increases the population by a factor of F, then the phage population produced in the last-but-one replication is N/F, so the number of mutations would be Nu/F. A mutant produced in this replication would produce on average wF phage, where w is the fitness of the mutant relative to the non-mutants (per replication cycle), for a total number of mutants in the final population due to this replication's mutations equal to Nuw. With 5 replications/day, the total number of mutants expected is $Nu(1 + w + w^2 + w^3 + w^4)$, with 10^{-6} of these passaged to the next sink, or $Nu(1 + w + w^2 + w^3 + w^4)/10^6$.

To these are added 10^4 virions from the source. Using a similar calculation for the number of source mutants per day gives $Mu(1 + v + v^2 + v^3 + v^4)$, where M is the peak source population and v is the relative fitness of the mutant in the source. The fraction of these that go to the sink is $10^4/M$, so the number of mutants occurring in the source on one day that go to the sink on the next passage is $10^4u(1 + v + v^2 + v^3 + v^4)$. If $v = w$, then the number of mutants expected from the source is more than that from the sink if $N < 10^{10}$. Both estimates of N above are below this value (basically because the sink is a sink).

However, presumably we are interested in mutations that are beneficial in the sink. Figure 2 implies that such mutations are probably detrimental in the source. Therefore, v is probably less than 1 and w greater than 1. This would increase the sink's contribution – for a single day, if $N < 10^{10}(1 + v + v^2 + v^3 + v^4)/(1 + w + w^2 + w^3 + w^4)$, the source's contribution is larger.

This only considers mutants arising in one day for the source. Another possibility is for a mutant to go from source to sink that arose in the source on a previous day. The number of mutants in the source on one day passaged to the next source is $Mu(1 + v + v^2 + v^3 + v^4)/10^6$. After 5 replications, the number is $Mu(1 + v + v^2 + v^3 + v^4)v^5$, of which $10^4u(1 + v + v^2 + v^3 + v^4) v^5$ go to the sink. This is v^5 times the source contribution above, and for even earlier days the contributions would be v^{10}, v^{15}, etc. So after many days, the number of mutants coming from the source would be $10^4 u(1 + v + v^2 + v^3 + v^4)/(1 - v^5)$, and the condition for this to be greater than the new sink mutations is $N < 10^{10}(1 + v + v^2 + v^3 + v^4)/[(1 + w + w^2 + w^3 + w^4)(1 - v^5)]$. Thus we conclude it is highly likely that beneficial mutations entered the sink via the source rather than arising *de novo* in the sink.