

Prolonged culture and long lasting infections select
for poorly transmitted bacterial variants —
Supplementary materials S4

July 25, 2019

In vitro kinetics of variants emergence

This experiment aimed distinguishing the emergence of group 2 from that of group 3 variants in prolonged static cultures of group 1 variants, using bacterial motility as a diagnostic character. For this purpose, we performed 25 independent cultures of G1#23, and we streaked these cultures every 24 hours onto NBTA plates. Each time red colonies were observed on the NBTA plate, a maximum of five red colonies and an identical number of blue colonies were sampled and their hemolytic capacity and their motility were measured. Petri dishes where swarming was observed were excluded from analysis, as they did not allow reliable motility measurement.

In this experiment, NBTA plates being incubated for 48 hours, we knew that red variants were present in a culture only two days after they have been sampled. Because of this delay, we stopped sampling bacteria from a culture only two days after red variants have appeared. In the results we present here, we include measurement

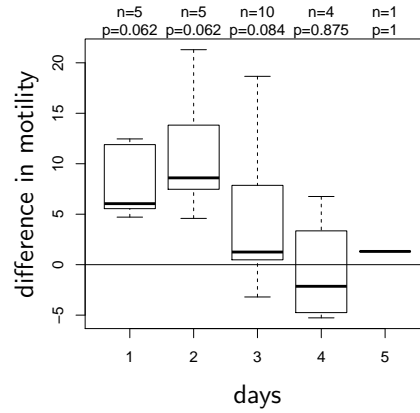


Figure S4-1: Difference in motility between red and blue isolates (i.e. mobility of red cells minus mobility of blue cells), as a function of the day they were sampled in a LB culture.

15 performed on bacteria sampled one day after red variants have been first detected
 16 in a culture. This increases sample size and provides us with better estimates of
 17 bacterial traits. But, as we want to focus on variants that just appeared, we will not
 18 consider bacteria sampled more than one day after red variants are first detected in
 19 the *X. nematophila* culture.

20 All cultures did contain red variants after five days of incubation. Seventy-five
 21 out of the 146 red colonies we sampled were non or weakly haemolytic, while all of
 22 the 143 blue colonies we sampled were fully haemolytic. In addition, we found that
 23 the difference in motility between red and blue colonies was significantly positive
 24 over the first two days of the experiment (Wilcoxon signed rank test: $p = 0.001953$)
 25 but did not significantly depart from zero afterwards (Wilcoxon signed rank test:
 26 $p = 0.625$, see figure above. As a result, we found that the difference in motility
 27 between red and blue colonies significantly decreased over time (Spearman rank
 28 correlation: $\rho = -0.614$, $p = 0.001095$).