Diverse cultures established with an equal density of each of 17 clonal lines of *H. rosa* were left to evolve at two temperatures (25C, 30C, n = 10) for 30–40 generations; 130 d, with 50% water changes every 42 d and the addition of 2 mg of sterile ground fish food flakes (TetraColor) dissolved in sterile deionized water every 6 d.

The 30C replicates were transferred to the 25C growth chamber 6 d (2–3 generations) before the competition experiment to avoid carryover effects of phenotypic plasticity.

At 3 d before the competition experiment, a stock culture of *Tetrahymena* sp. was prepared using a single genotype from a previous experiment. Five milliliters of the source culture were added to a sterile 50 ml conical tube along with 30 ml of media and 200 mg of sterile ground fish food flakes (TetraColor).

On day 0 of the competition experiment, the density of *H. rosa* in each diverse culture was estimated using custom Palmer style counting slides. From each of the 20 cultures, the number of individuals in three 0.1 ml samples was counted using a compound microscope at 40X magnification. To estimate the density of the *Tetrahymena* sp. culture, three 0.1 ml samples were counted by making one lateral pass across the center of the counting slide at 100X magnification. The resulting count was then multiplied by 6.8 to estimate density in each 0.1 ml sample. The mean estimated density of each culture (both *H. rosa* and *Tetrahymena* sp.) was then used to calculate the aliquot needed for each experimental culture, so that the starting density of each replicate remained consistent. This was done by using the equation

(1)

where is the volume of the source culture to be added to the experimental culture and is the volume of source culture that was sampled to obtain , the estimated density of the source culture. Finally, is the target density of the experimental culture. Given = 0.1 ml and = 40 for *H. rosa* and = 80 for *Tetrahymena* sp., the equation can be simplified as Equation 2 for *H. rosa* and Equation 3 for *Tetrahymena* sp.

(2)

(3)

For each culture, was subtracted from targeted total experimental volume of 20 ml

*Back-calculate how much FF is in the TA addition for each rep. and use that to determine how much added at the end (like with media top-off)*