File S1

Cell size did not change throughout the transfers

To ensure that variation in cell size did not bias our estimates of the number of cell divisions between transfers, we measured the cell sizes of 4 MA lines at generations 0 and 1,000. Micrographs were taken of cells from each culture in late log-phase. Length (*L*) and width (*W*) of 100 cells per culture were measured using calibrated scale bars. To calculate the volume of a cell we assume that it is a prolate spheroid (Hellung-Larsen and Anderson 1989): $V = \pi \cdot L \cdot W^2 / 6$. Cell size did not change significantly between the beginning and end of the experiment in the 4 MA lines (Welch's two sample *t*-test on line means: $t = 0.31$, df = 5.7, $P = 0.76$). We conclude that OD_{650} allows us to determine the number of generations elapsed between transfers throughout the course of MA.

Literature Cited

Hellung-Larsen, P., and A. P. Anderson, 1989 Cell volume and dry weight of cultured *Tetrahymena*. J. Cell. Sci. **92:** 319–324.