Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic supplementary information

for **Patchiness in a microhabitat chip affects evolutionary dynamics of bacterial cooperation**

Edward W. Tekwa[a], Dao Nguyen[b], David Juncker[c], Michel Loreau[d], Andrew Gonzalez[e]

Fluorescent count calibration

To estimate and correct for fluorescence-related biases in individual counts, 8 independent control experiments are conducted, each of which involves a GFP monoculture, a mCherry monoculture, and a GFP/mCherry mixed culture with cooperators and defectors seeded at half of the monoculture density. First, the Imaris spot detection parameters Threshold (T) and Quality (Q) are varied for each of GFP and mCherry, and the counts for each strain is recorded. Treating the counts as functions of T and Q, we search for the T and Q settings that minimize the differences between GFP and mCherry counts, and between monoculture and mixed culture counts. Finally, the remaining biases are corrected by multiplying experimental counts with correction factors. The final T and Q settings for GFP are 3.83 and 0.5, and for mCherry are 4 and 2. An example of the Imaris spot detection is shown in Fig. S2. The correction factor for mixed culture relative to monoculture counts is 0.64. The correction factor for GFP relative to mCherry counts is 1.11. Using these settings and corrections, the resulting GFP-to-mCherry count ratio is 1 (S.E. 0.074), and the monoculture-to-mixed culture ratio is 1 (S.E. 0.065) across the calibration dataset.

Test tube experiment

As a control with no spatial structure, we grew mixed cultures of wild-type cooperators and mutant defectors in 1mL of media (identical to experiments in MHD) in conventional deep-well plates. After 10 hours in a 30°C shaker incubator, the cultures were diluted and grown on carbenicillin (for cooperators) and tetracycline (for defectors) agar plates for cell count. Defectors outnumbered cooperators (cooperator frequency mean=0.451, SE=0.0097, t_2=-8.80, p=0.013).

Supplementary figures

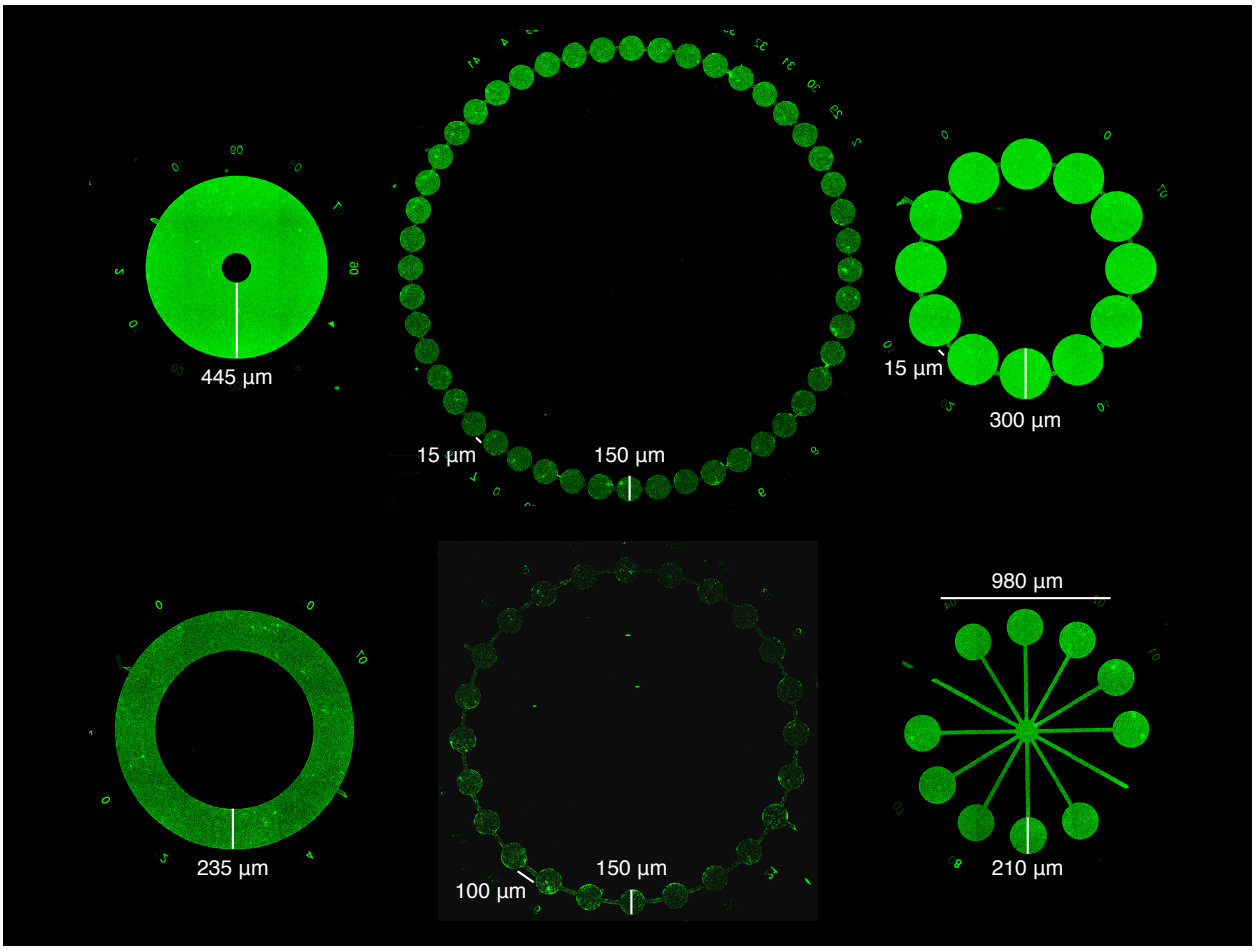

Figure S1. Additional habitat variations. The habitats were inoculated with green cooperators and are imaged at T=8. Clockwise from the top left corner, the diameters and depths (µm) are: 1050x20, 2670x20, 1500x20, 1405x10, 2060x10, 1380x20. All corridors are 24 µm wide. The habitat areas are: 0.85, 0.87, 0.85, 0.64, 0.48, 0.85 mm^2. The edge-to-area ratios, or patchiness measures, are: 0.0045, 0.028, 0.014, 0.047, 0.032, 0.0084 μm^{-1}. We kept habitat areas close to either 0.85 or 0.42 and patch sizes similar when possible, but some topologies, such as the star (4^{th} habitat in this figure), necessitated some departures.

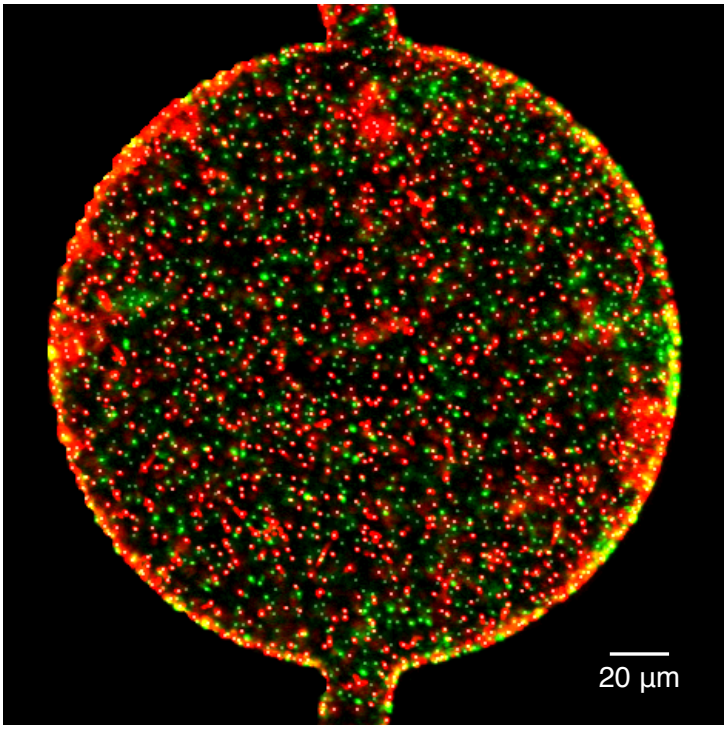

Figure S2. An example of the bacteria spot detection at T10. The detected green cooperators and red defectors were labelled with grey rectangular squares.

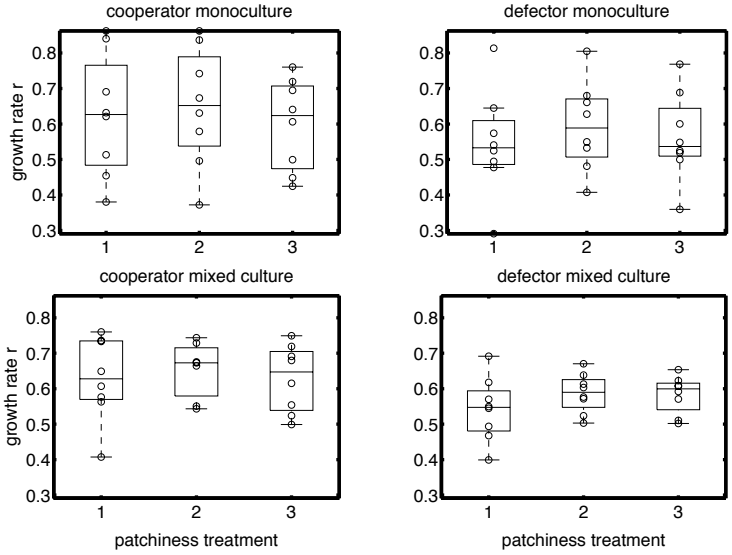

Figure S3. Maximum growth rate *r* estimates for cooperators and defectors in monocultures and mixed cultures as functions of habitat connectivity treatments. According to ANOVA, *r* is not significantly different in terms of strain and culture type (p=0.096), patchiness treatment (p=0.77), or their interaction (p=0.88).

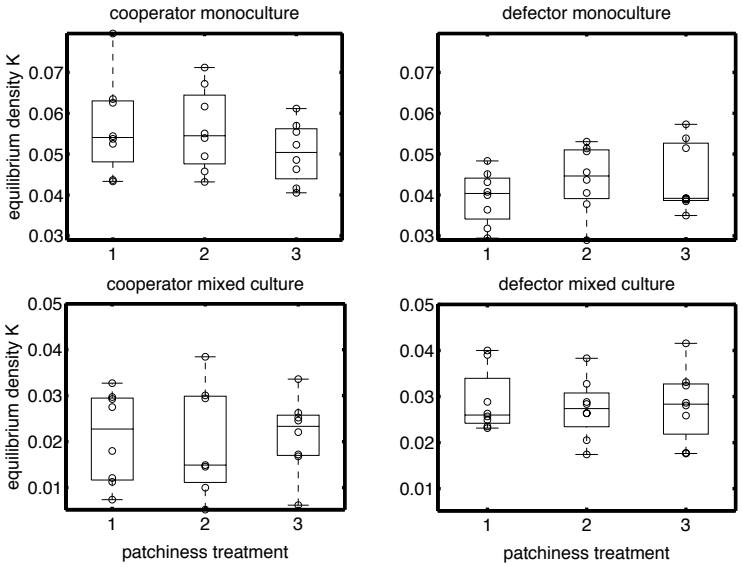

Figure S4. Equilibrium density *K* estimates for cooperators and defectors in monocultures and mixed cultures as functions of habitat connectivity treatments. According to ANOVA, in monocultures, *K* is significantly higher for cooperators than for defectors (p=2.9e-05), but is not significantly different in terms of patchiness treatments (p=0.81) and the interaction between strain and patchiness (p=0.081). In mixed cultures, *K* is significantly lower for cooperators than for defectors (p=0.0063), but is not significantly different in terms of patchiness treatments (p=0.96) and the interaction between strain and patchiness (p=0.83).