Figures and figure supplements

Inter-species population dynamics enhance microbial horizontal gene transfer and spread of antibiotic resistance

Robert M Cooper et al
Figure 1. Real-time observation of functional HGT in microfluidic traps. (a–d) Acinetobacter expressing mCherry were mixed with E. coli carrying LacI-repressed pBAV1k-GFP inside a microfluidic device (frames captured from Video 3, see also Supplementary Note 1). (a) t = 1:08, both Acinetobacter (Ab) and E. coli (Ec) were present, and no HGT had occurred. (b–d) t = 3:18, all E. coli in the field had been lysed, and several independent lineages of GFP-expressing Acinetobacter deriving from HGT were visible (circled). (e–k) HGT renders Acinetobacter resistant to antibiotic treatment (frames captured from Video 7). Kanamycin was added 11:42 after seeding the device. Still images were captured at indicated times after kanamycin addition, after several independent HGT events had already occurred (arrows in e–g). (h–k) Newly kanamycin-resistant Acinetobacter began outcompeting both E. coli and the sensitive parent (h), and by 13 hr after kanamyin addition (i–k), they dominated the environment.

DOI: https://doi.org/10.7554/eLife.25950.003
Figure 1—figure supplement 1. Microfluidic chip design. Cells were loaded from the port on the right, which was also used to remove waste media. Fresh media was flowed in from the ports on the left. For media switching, the top left port was used to flow in LB with kanamycin by raising the attached syringe. There are four rows of traps (green), each with a slightly different design. The top two rows have feeding channels (blue) on both the top and the bottom of the traps, while the third row of traps is only open to feeding channels on one side. The fourth row was designed to have narrow feeding channels at the backs of the traps (yellow), but these proved too challenging to fabricate and were omitted from the physical devices, making this row functionally equivalent to the third.

DOI: https://doi.org/10.7554/eLife.25950.004
Figure 2. Enhancement of HGT efficiency by neighbor killing. Communities were seeded with 2 ul droplets containing indicated strains of Acinetobacter with genomic spect resistance, mixed with genomically cm-resistant E. coli carrying the kan-resistant donor plasmid pBAV1k. Spots were incubated at 30°C overnight (a) or at 37°C for 150 min (b–d) or for the indicated time (e–i). Lower limits on y-axes are the limits of detection. (a) E. coli (cm+kan, green bars), Acinetobacter (spect, orange bars), and newly double-resistant Acinetobacter (spect+kan, blue bars) present after growth either alone or in co-culture. (b) Survival of E. coli after growth alone (-,-) or with wild type (+,-), non-killing Δhcp (-,+), or both (+,+ strains of Acinetobacter. Spots were seeded with Acinetobacter at optical density (OD) five and E. coli at OD 1. (c) HGT during the experiment shown in b, measured as the proportion of wild type (WT - spect, left bar group) or Δhcp (tetracycline, right bar group) CFUs that were also resistant to kan. Two-strain cultures (dark bars) correspond to the center two bars in b, while the three-strain cultures (light bars) corresponds to the rightmost bar in b. (d) Transformation efficiency of Acinetobacter as in c, but mixed with purified pBAV1k DNA just before spotting. (e–g) Time course showing CFUs of E. coli (dotted green), total Acinetobacter (solid orange) and double-resistant Acinetobacter (dashed blue) in two-strain cultures (wild-type Acinetobacter in e and Δhcp Acinetobacter in f). (g) The proportion of wild type (solid line) and Δhcp (dashed line) Acinetobacter cells that have acquired kan resistance during the experiment in e-f. (h,i) Time course showing HGT complementation by killing in trans. The proportion of double-resistant cells is shown for wild type (solid lines) and Δhcp (dashed lines) Acinetobacter during the experiment in e-f. (j) A conceptual model for killing-enhanced HGT to Acinetobacter. Statistical significance levels are: * = p<0.05 with significance calculated using raw data, and ** = p<0.001, with significance calculated on log10-transformed data (see Materials and methods for sample sizes and statistical analysis, and see main text for exact p-values).

 DOI: https://doi.org/10.7554/eLife.25950.013
Figure 2—figure supplement 1. Effect of genomic context on killing-enhanced HGT. (a) Proportion of kan-resistant *Acinetobacter* after overnight growth with *E. coli* carrying different bait plasmids. (b) Dependence of HGT on genetic location of the resistance gene and genomic homology. Error bars indicate standard errors from three culture replicates, and lower limits on y-axes are the limits of detection for each experiment.

DOI: https://doi.org/10.7554/eLife.25950.014
Figure 2—figure supplement 2. Confirmation of HGT from replicating and homology plasmids. (a) plasmid DNA isolated with a standard miniprep kit and digested using SspI. Lanes 1–3: Kanamycin-resistant *Acinetobacter* clones resulting from HGT of pBAV1k. Lanes 4–6: Kanamycin-resistant *Acinetobacter* clones resulting from HGT of pRC03H-homology. Lane 7: *E. coli* carrying pBAV1k. (b) inverse PCR with primers located in the kan gene using genomic DNA isolated from kanamycin-resistant *Acinetobacter* clones resulting from HGT of pRC03H.

DOI: https://doi.org/10.7554/eLife.25950.015
Figure 3. Determining quantitative parameters for natural transformation and contact-dependent neighbor killing. Error bars indicate measurement standard deviations of experimental data for a single spot harvested from an agar plate, and lines represent simulations using the shared best fit parameters (Table 1). Note all y-axes are log scale except for l. Orange lines indicate Acinetobacter (spect-, or in k, tet-resistant), blue lines indicate transformed Acinetobacter (additionally kan-resistant), green lines indicate E. coli (cm-resistant), and black or grey lines indicate the fraction of

Figure 3 continued on next page
Figure 3 continued

_Acinetobacter_ that have been transformed. (a–c) Transformation of _Acinetobacter_ via homologous recombination of exogenous DNA (pRC03H-2S) added just before spotting the cells. (a,b) Time courses of transformation of _Acinetobacter_ mixed with limiting (a) or saturating (b) plasmid DNA. (c) Fraction of _Acinetobacter_ transformed by a DNA dilution series, harvested after either 30 min or 2 hr. (d–j) Measuring killing of _E. coli_ by _Acinetobacter_. (d–i) A selection of independent time courses seeded with varying densities of _E. coli_ and _Acinetobacter_ (see also Figure 3—figure supplement 3). (j) A contact-dependent killing assay using a dilution series of _Acinetobacter_ mixed with _E. coli_ at the same ratio, but varying total concentration. (k,l) Time series of the number of CFUs (k) and fraction of transformed killing-deficient _AcinetobacterΔhcp_ (l) after growth with _E. coli_ on agar plates, used to fit DNA ‘leakage’.

DOI: https://doi.org/10.7554/eLife.25950.017
Figure 3—figure supplement 1. Logistic growth fit for Acinetobacter spots on agar starting with various initial CFUs.
DOI: https://doi.org/10.7554/eLife.25950.018
Figure 3—figure supplement 2. Logistic growth fit for *E. coli* spots on agar starting with various initial CFUs.

DOI: https://doi.org/10.7554/eLife.25950.019
Figure 3—figure supplement 3. Fitting contact-dependent killing. The full set of time courses used to fit the killing parameters. A subset of time courses are shown in Figure 3d–i. Each row represents experiments done on a different day. Each time course was seeded with different densities of *Acinetobacter* and *E. coli*. As in Figure 3d–i, solid orange: total *Acinetobacter*, dotted green: *E. coli*, error bars indicate measurement standard deviation for a single harvested spot.

DOI: https://doi.org/10.7554/eLife.25950.020
Figure 3—figure supplement 4. Simulations of contact-dependent killing with and without restriction of killing to perimeter cells. This is the same experimental data shown in Figure 3—figure supplement 3 and simulated using the same, best-fit parameters. Here, solid lines indicate simulations using $N^*$ to restrict killing to the perimeters of micro-colonies, and dashed lines indicate the same simulation without that restriction (i.e. replacing $A^*$ with $A$ and $E^*$ with $E$). Green indicates *E. coli*, and orange indicates *Acinetobacter*. 

DOI: https://doi.org/10.7554/eLife.25950.021
Figure 3—figure supplement 5. Effective perimeter cells. The effective number of micro-colony perimeter cells (Equation 5, solid blue) is shown in comparison to the total number of cells (Equation 4, dashed orange, small micro-colony limit), and the large micro-colony limit (Equation 3, dotted green). The two panels are the same, but panel b is zoomed out by a factor of 10. The initial number of CFUs at seeding; i.e., \( N_0 \) in Equations 3, 5, is 1000 (black asterisk).

DOI: https://doi.org/10.7554/eLife.25950.022
Figure 4. Comparison between predicted and actual killing-enhanced HGT in microbial communities. Plotted is the fraction of Acinetobacter that have become double antibiotic-resistant due to HGT of kan resistance from E. coli. Data are from the same experiments as Figure 3d–i and Figure 3—figure supplement 3, which shows the total CFUs. Solid lines are model predictions, and error bars are standard deviations of experimental results. Each row of plots is from a different day, and plots within a row are for varying seeding densities of the two species (shown in the Figure Supplement at time 0).

DOI: https://doi.org/10.7554/eLife.25950.024
Figure 5. Simulations showing the effects of initial species density and interaction time on HGT, and the degree to which contact-dependent killing enhances HGT. The axes in a–c,f,g indicate the initial cell count ($A_0$ and $E_0$) relative to the carrying capacity ($K_A$ and $K_E$) for *Acinetobacter* and *E. coli*, respectively. Contour levels indicate 10-fold changes in a,b, two-fold changes in c,g, and 30 min changes in f. (a) Simulated HGT frequency to wild-type *Acinetobacter* grown with *E. coli* at varying seeding densities for 2 hr. (b) Simulated HGT frequency as in a, but for killing-deficient *Acinetobacter* Δhcp. (c) The HGT enhancement factor provided by killing depending on seeding density; that is, the ratio of HGT to the wild type (a) divided by HGT to the killing mutant (b). (d) HGT frequency over time for agar surfaces seeded with both species at $10^{-3}$ of their respective carrying capacity. Solid line: wild type *Acinetobacter*, dotted gray line: killing mutant Δhcp *Acinetobacter*. (e) HGT enhancement factor provided by killing over time for the simulation in d. (f) Incubation time at which killing provides the greatest HGT enhancement, for varying seeding densities. (g) HGT enhancement as in c, but after 10 hr, by which time even sparsely seeded communities had approached simulated growth saturation.

DOI: https://doi.org/10.7554/eLife.25950.025
Figure 6. Simulated inhibition of HGT by DNase (a–d) or competing DNA (e–h) in simulated microbial communities seeded with both Acinetobacter and E. coli at $10^{-3}$ of their respective carrying capacities and grown for 2 hr. All contour levels indicate two-fold differences, and all axis and colorbar scales are log10, except DNA half life in a. (a) Fraction of wild type (solid line) and non-killing (dotted grey line) Acinetobacter that have undergone HGT as a function of DNA half life in the extracellular space. (b,c) Fraction of wild type (b) and killing mutant (c) Acinetobacter that have undergone HGT with the half life of extracellular DNA set to 1 min, for varying initial cell counts. The axes indicate the initial cell count $A_0$ and $E_0$ for Acinetobacter and E. coli, respectively. (d) The degree to which killing increases HGT as a function of seeding density, with DNA half life set to 1 min; i.e., the ratio of HGT to the wild type (b) divided by HGT to the killing mutant (c). (e) HGT efficiency as in a, but with the addition of varying amounts of competing DNA rather than a finite DNA lifetime. (f,g) Efficiency of HGT as in b,c, but with the addition of $10^{11}$ kb of competing DNA at time 0 rather than DNA decay. (h) Enhancement of HGT provided by killing, as in d, but with $10^{11}$ kb of competing DNA at time 0 and no DNA degradation.

DOI: https://doi.org/10.7554/eLife.25950.026
**Figure 7.** Experimental tests of model predictions. (a–d) Dependence of HGT on cell seeding density. a HGT frequency to wild-type *Acinetobacter* spotted together with *E. coli* at the indicated cell counts ($A_0$ and $E_0$, respectively) and grown for 2 hr at 37°C. b HGT frequency as in a, but for *Acinetobacter Δhcp*. The missing bar at the bottom indicates data below detection. (c) HGT enhancement provided by killing, i.e., the ratio of data in a to that in (b). (d) Same as in c but after overnight growth. (e) Reduction of HGT to wild type (blue dot) or Δhcp (green X) *Acinetobacter* seeded with *E. coli* at equal high density (approximately $3 \times 10^6$ CFUs each), or to wild type with both species seeded at low density ($3 \times 10^4$ CFUs each, orange diamond), all mixed with the indicated amount of DNase before spotting and grown for 2 hr. Missing data points indicate HGT was below detection ($5 \times 10^4 \text{CFUs}$), and HGT to *Acinetobacter Δhcp* was below detection ($5 \text{CFUs}$) for all tested levels of DNase. Reduction is relative to the same experiment with no DNase added. (f) HGT reduction as in e, but adding $10^{11}$ kb of competing DNA before spotting. Error bars indicate the propagated standard error.

DOI: https://doi.org/10.7554/eLife.25950.027