Selfish and Altruistic Bacterial Populations Maximize Fitness Under Stress by Local Segregation

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Abstract

Landscapes in ecology have a profound influence on the adaption and evolution of competing populations for resources [1]. We are interested in how altruistic populations survive in the presence of selfish individuals in a non-stirred, closed and complex nutrient landscape. Well-stirred (landscape-free) but closed environments have a depressing future when selfish individuals arise in a population, a fate known as the tragedy of the Commons [2]. Over-exploitation of a well-stirred communal habitat by selfish individuals which do not follow rules of communal self-regulation ends up in the elimination (extinction) of both the original altruistic inhabitants and the selfish population. In the context of bacterial population, the Commons tragedy that occurs is the consumption of limited resources by the individuals, resulting in metabolic stressing of the bacteria and growth advantages to be gained by defection from a "social contract" of altruistic cooperation. There is no avoidance of this tragedy and the collapse of an original altruistic wild-type population by an emergent selfish population in a well-stirred but closed environment is inevitable [3, 4]. However, there is a fundamental difference between resource exploitation in a well-stirred homogenous commons and in a heterogenous landscape of nutrients which is not stirred [6]. We show here using a non-stirred nanofabricated habitat landscape that altruists and selfish bacteria can in fact coexist, that they can maintain phenotype diversity and avoid the tragedy of the Commons. This emergent spatial segregation of competing populations under stress greatly changes, we believe, our perception of the true sophistication of bacterial response to stress and competition, and has broad implications for the adaptive strategies of higher organisms under stress in complex environments.

Within a bacterial context, the entry into stationary phase of bacteria results in restriction of growth and is an altruist strategy. Wild type bacterial cells have evolved sophisticated cell-signalling networks (quorum sensing [7], chemotaxis [8]) to gauge the proper levels of environmental degradation at which this metabolic transition ought to be induced. The expression of proteins is controlled by promoter selective binding on RNA polymerase which, for *E. coli* , is controlled by the known σ factors [9]. This gives rise to the possibility of a global switching of gene expression in bacteria via genetically changing the σ factor genes. In particular, there are a set of σ factor genes, rposD and rposS, which are known to be connected to response to external metabolic stresses [10, 11]. In particular, expression of the *rpoS* gene, which codes for the σ^S factor of RNA polymerase, gives strains more resistance to external stress but also gives rise to poor substrate metabolic competence, that is, expression of *rposS* requires the organism to utilize complex molecules typically present when wild-strains die and lyse under stress. Thus, a *RpoS* mutant might be expected to act as a "cheater" strain if the mutation gives the organism the ability to grow during stationary phase by metabolizing high energy compounds released by the death and lysis of wild-type RpoS bacteria under conditions of stress. The Kolter lab has studied the adaption of *E. coli* to conditions of prolonged metabolic stress [12] and has found that about 4 days into the stationary phase a mutant strain emerges which has a Growth Advantage in Stationary Phase (GASP), that is, it acts as a "cheater", and that the mutation is in the rpoS gene [5]. The rpoS819 mutant that we isolated and sequenced has an interesting sequence: it is simply a 48 bp repeat of the adjoining sequence in

the rpoS gene, indicating that there is a basic, modular gene sequence insertion used to control the basic GASP phenotype.

We constructed fluorescent cheater and cooperator strains of *E. coli* based upon the rpos819 mutant. The selfish strain (rpoS819) was made by P1 phage transduction from the GASP mutant [12] which was initially the strain W3110 before GASP adapation. The selfish rpoS819 mutants were made identifiable by expression of either a green fluorescent protein (GFP) inserted by P1 phage transduction into the lacYZ operon (lacYZ::GFP2, rpoS819 (green) = JEK1032) or by expression of a red fluorescent protein RFP by construction of W3110 lacYZ::mRFP-1, rpoS819 (red)= JEK1033). Cooperator strains (rpoSwt) carrying the wt allele where also made in a similar fashion: (green) JEK1036 = W3110 lacYZ::GFP?2, rpoSwt (green) and (red) JEK1037 = W3110 lacYZ::mRFP-1, rpoSwt. Since both the altruistic strains (JEK1036, green and JEK1037, red) and the selfish strains (JEK 1033, red and JEK 1032, green) where created we verified in control experiments to ensure that the color channels did not bleed between red and green filter sets.

In order to sequence the rpoS locus around the insertion giving rise to the 819 allele, we used a forward primer rpoS-Fwd-Kolter 5-TCACCCGTGAACGTGTTC-3 and reverse primer rpoS-Rev-Kolter 5-GTTAACGACCATTCTCG-3. DNA sequencing of the PCR producs was done using GENEWIZ service to determine the rpoS allele sequence. Sequencing showed that the GASP rpos819 mutant consisted of lateral transfers taken from flanking sequences of the rpoS genome: the first part of the rpoS819 sequence (gcaggggctgaatatcgaa) is actually a lateral transfer of the sequence which follows after the insertion, and the last part of the rpoS819 sequence (gcgccgtttgcgcgaaatcctgcaaac) is a lateral transfer of the rpoS which comes before the insertion. The Supplementary Material shows that the rpoS819 mutation was preserved in JEK1033 and JEK1032, and that the GFP and RFP proteins do not cause differential changes in the growth curves of the bacteria under low stress conditions.

Experiments were carried out in a nanofabricated ecological landscape consisting of a row of Micro Habitat patches (MHPs) which were introduced in a previous publication [13]. The MHP array consists of 85 chambers of dimensions 100 by 100 by 10 μm which are interconnected with 5 microns wide junction channels (JCs). Resources can diffuse into the MHPs through 200 nm deep nanoslits (NS) from feeder channels. While the JCs allow the bacteria to move between the MHPs, the NS only allow nutrients (and waste) to diffuse into and out of the MHPs, since they are too thin for E. coli to pass through. There are many variables at play here in the chip design, thus a huge parameter space exists. However, in the experimement presented here, we focus only on the coupling between the feeder channels and the MHPs: a nutrient landscape was created by modifying the number of nanoslits open in each MHP. We made a simple step function to the nutrient landscape, ie. we created regions of perfectly good food access (where all the nanoslits are open)which spans half the microfluidic chip which we call a "white" region, and a region covering the other half where all the nanoslits are closed off, which we call a "black region". Fig. 1 is a schematic of our rather simple "complex" nutrient landscape device.

A fully computer controlled microscope (Nikon Eclipse 90i) equipped with a motorized stage was used to scan the MHP array. Since the two strains JEK1036 and JEK1033 are not only different alleles but also fluoresce in two different spectral regions (green and red, respectively), the separate populations can be distinguished within the MHP chip. The excitation source was a Hg-Xe arc lamp

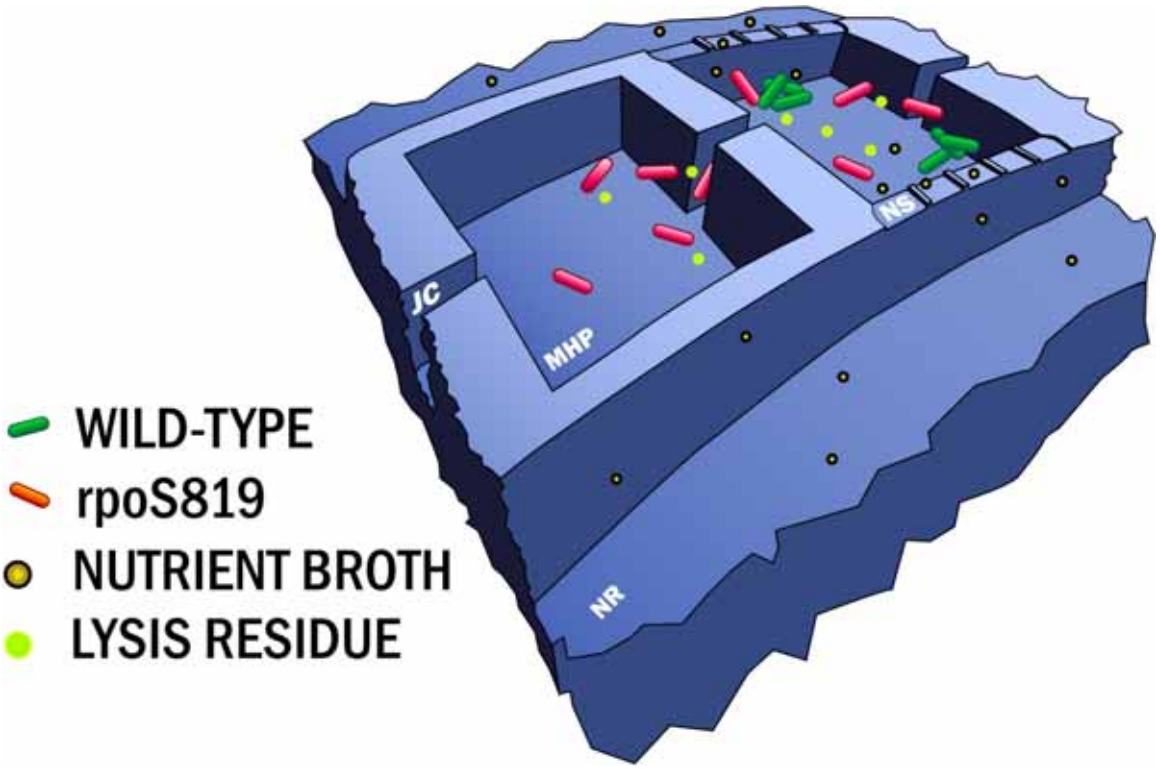

Figure 1: Schematic of a 2-level nutrient landscape device. Wild type bacteria (labelled with green fluorescent proteins) and rpoS819 mutants (labelled with red fluorescent proteins) are free to move within each Micro Habitat patches (MHPs) and may migrate from one region to the other through the Junction Channels (JCs). The Nutrient Reservoir (NR) is coupled to the MHP by Nanoslits (NS). The left-hand side habitat patch is a "black" one with no NS open, the right-hand side one is a "white" patch with full openings. Nutrient and lysis molecules (due to the death of bacteria) are denoted by brown and green spheres respectively and can diffuse in and out of the NS. Each MHP was 100 microns x 100 microns wide x 8 microns deep, the nanoslits were etched 200 nm, the feeding channels were 500 microns wide, 200 microns deep, and 1.5 cm long total. The net feeder channel volume/ MHP volumes was approximately 500/1. volume 500

with customized excitation filters (Chroma Technology) and a computer switched both excitation and emission filters for each MHP to detect separately the JEK1033 (red emitting) cheaters and the JEK1036 (green emitting) cooperators. Our experience with lysed bacteria is that the GFP and RFP proteins are quickly degraded in the lysate in under 0.5 hours, and supported by the fact that we never observed a detectable GFP or RFP signal in the reservoir channels, so we believe that the fluorescence intensity is an accurate and linear measurement of the actual un-lysed bacterial density in the MHP. The cell density in the MHPs is capable of getting to substantially higher levels that occur in a standard bacterial culture tube, but the actual steady-state number is very much a factor of the food supply, that is, on what fraction of the nanoslits are open. We estimate that the highest cell densities that are reached is approximately 0.3 bacteria/micron^3, or approximately 4 OD/cm as measured using light scattering. Our experiments using wild-type and GASP strains of bacteria in a unstirred nutrient landscape with regions of high nutrient stress (all nanochannels blocked off) and regions of relatively low nutrient stress (all nanochannels open) are limited to under 5 days of duration because we want to avoid the genomic changes that lead to the GASP phenotype further evolving in the MHP array.

At a coarse scale we have a very simple nutrient landscape in our MHP array, but within the "black" and "white" regions of high stress and low stress respectively the nutrient landscape is subdivided into relatively small habitat patches which can only hold a limited number of individuals, thus we might expect that under these heterogeneous habitat and nutrient landscapes that quite different phenotype behaviors will be seen as the nutrient landscape evolves due to bacterial cell growth and competition between the selfish and the altruistic strains. Fig. 2 show very different behavior for wild type (JEK1036) and GASP mutant (JEK1033) strains when innoculated into a non-stirred nutrient landscapes. Unlike the well-mixed experiments, our populations of selfish and altruistic bacteria do not obtain local or global dominance (Fig. 2 A) and do not mix even at the local scale (Fig. 2 B) and but instead are spatially anti-correlated within even a single MHP (Fig. 2 C). The segregation of the populations occurs at many different length scales. Not surprisingly, in the "black" region of very high nutrient stress, the selfish strain JEK1033 remains dominant over the entire 72 hours of the experiment, albeit at very low densities. In the region of good resources and at the interface between the good and bad regions of resource availability the situation is far more complex. We have enlarged a set of images of MHPs taken from 50-60 hours after inoculation in Fig. 2 B and it is clear that a very complex segregation of the two populations develops with difference scales for the JEK1033 and JEK1036 strains. This segregation qualitatively changes between the regions of low and high food access, indicating the complex metapopulation dynamics that can occur once stirring is turned off and a complex nutrient landscape is established.

The measure of the aggregate sizes of the two strain can be quantitatively analyzed from the images. This is done by extracting the pixels in the 75^{th} percentile or up and computing the average area of the contiguous domains $\langle \mathrm{A} \rangle$ in the red (selfish) and green (altruistic) color channels, as illustrated in the insets of figure 3. The mean fluorescence intensity $\langle \mathrm{I} \rangle$ of each MHPs, which is proportional to the local population density of altruists or selfish bacteria, is also calculated for both color channels. Although we have a heterogenous nutrient landscape and a dual population of altruistic and selfish bacteria, the multidimensional complexity of the population dynamics is nevertheless captured in fig. 3. We have done a cluster size analysis between hours 45 and 60 into

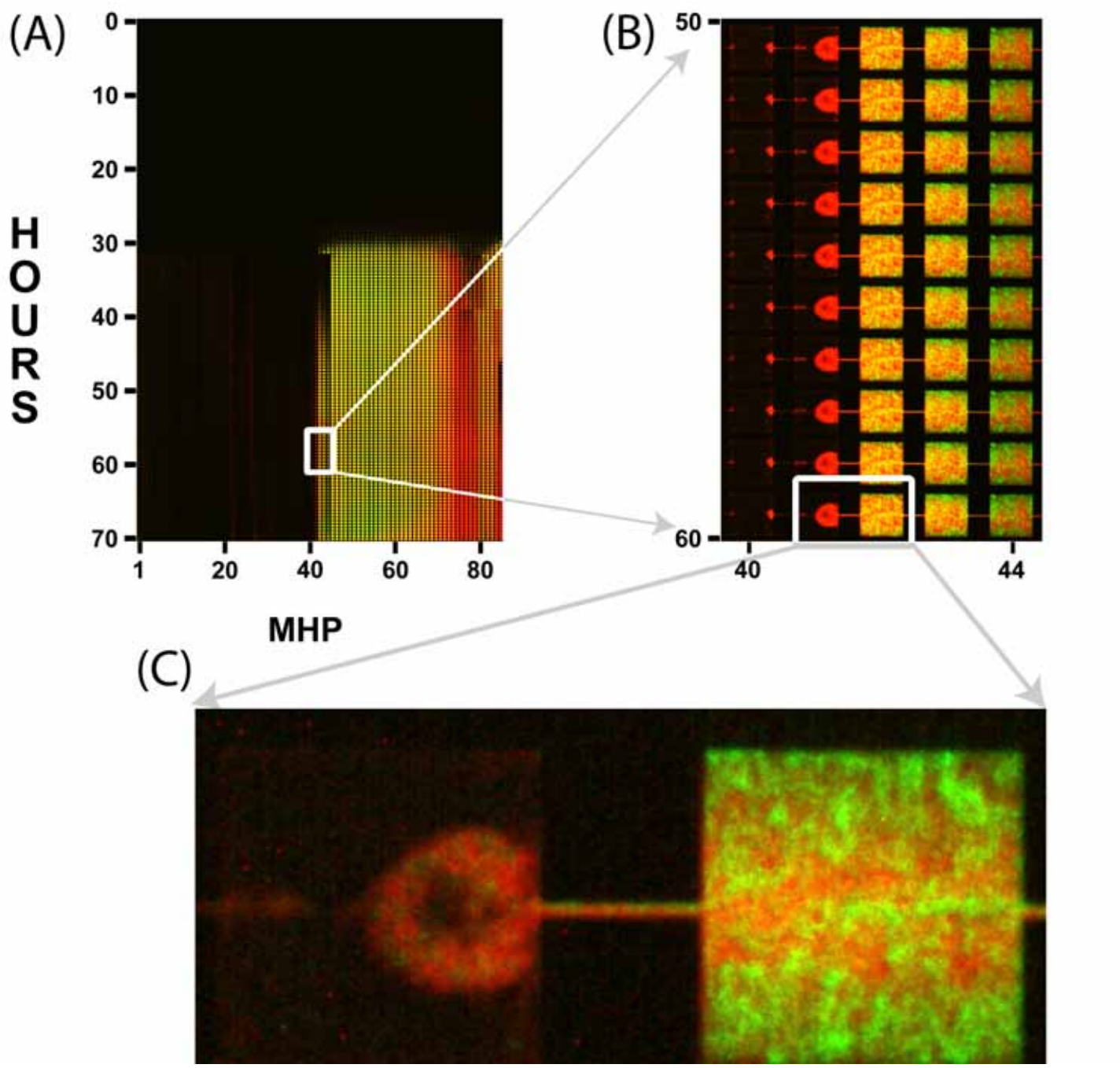

Figure 2: Conflict experiment on a "Black-White" nutrient landscape. The line of 85 patches are scanned approximately every 30 minutes, and the line of images is stacked to create the montage. (A). True color image of the fluorescence of the chain of MHPs stacked vertically for JEK1033 (selfish bacteria, red RFP) and JEK1036 (altruistic bacteria, green GFP) cohabitation. (B). An enlargement of the interface region between the "black" and "white" regions about 50 hours into the run. (C) Two neighboring patches across the interface between zero external nutrient diffusion (left) and full external nutrient diffusion (right).

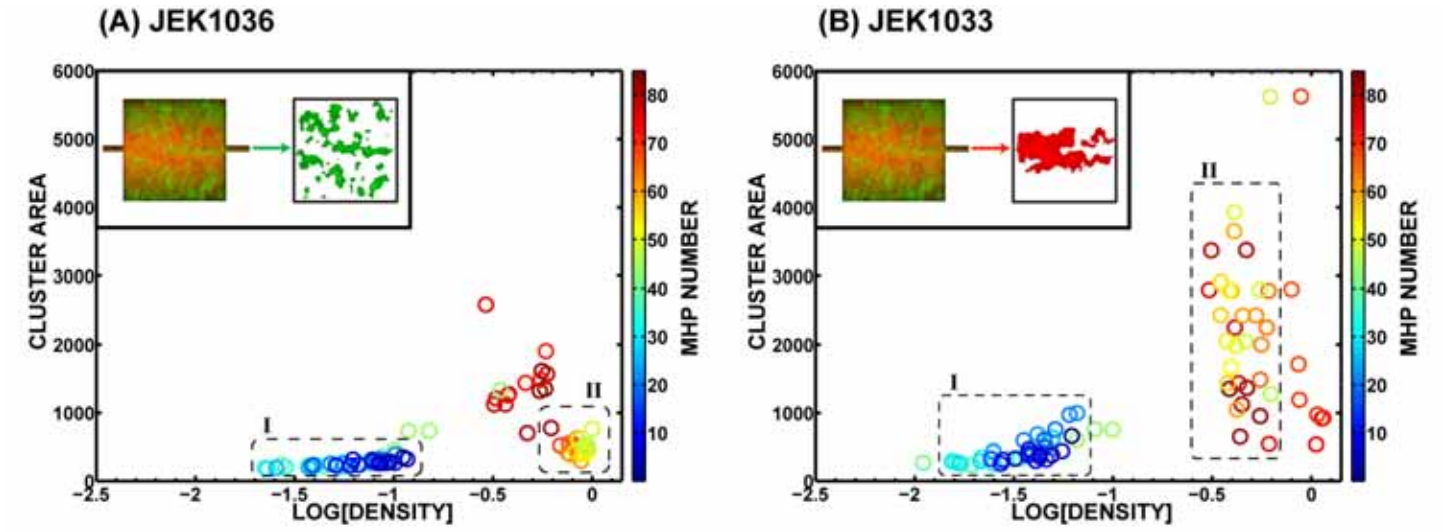

Figure 3: (A) The average continuous altruist patch size in microns^2 versus the average patch cell density (in arbitrary units of fluorescence intensity), with color coding for the nutrient landscape, from "black" (blue) to "white" (red). Note that in region I ("black" part of the device) and region II (at the interface between the "black" and "white" parts), the cluster area remains fairly low. (B) The average continuous selfish patch size versus the average patch cell density, with color coding for the nutrient landscape, from "black" (blue) to "white" (red). The cluster area in region I is slightly increasing with density while in region II, the aggregation size is very large and diffuse. The inserts show a typical MHP in near the "black" and "white" interface and the result of the patch analysis.

the run and used the obtained $\langle A \rangle$ and $\langle I \rangle$ couples, 30 in total, to produce a 2D histogram for each of the 85 MHPs. The main mode of each histograms is recorded and plotted in fig. 3. The datapoints are color-coded by MHP number, the blue end of the spectrum is the "black" region, and the red end of the spectrum is the "white" region. Note that the density axis has been normalized to account for the difference in brightness of the fluorescent proteins. The results of the analysis show that the average local patch size of the selfish JEK1033 strain is approximately 5 times the area of the altruistic JEK1036 strains, while the density of the selfish JEK1033 strain patches is approximately 1/3 of the altruistic JEK1036 strain patches.

We can use this quantitive mapping of the segregation of altruist and selfish bacteria popualtions into a coexisting metapopulation to test our understanding of these dynamics via modeling. Conventional evolutionary game theory is a loop that cycles between games and reproduction. A tournament occurs during which each organism duels other organisms representative of the entire system population. Each organism then receives points. If the points are awarded according to four parameters or "payoff matrix elements" T (temptation), R (reward), P (punishment), and S (sucker's payoff) satisfying the prisoner's dilemma $T > R > P > S$ [14]. Nowak and May in a pioneering study [15] showed that a simple 2-dimensional iterative game played by individuals who always cheat (whom we call selfish individuals in our context) and individuals which always cooperate (whom we call altruists) can give rise to remarkably complex population patterns when

a simple nearest neighbor weighing of the (fixed) strategies of the selfish and altruistic bacteria is iteratively computed to determine the next round of occupants in a lattice. A lattice site becomes occupied by the type of cell, cooperator or defector, that has won the most points in the vicinity in the preceding tournament. For example, the defectors secure more points than the cooperators, as indicated by the size of their letterface. Consequently, the central lattice site becomes occupied by a defector. When prisoner's dilemma matrix elements are used for local games in Nowak's simulation, cooperators and defectors can cluster in fluctuating spatial regions. For some prisoner's dilemmas in Nowak and May's Model, cooperators survive indefinitely and avoid the tragedy of the Commons, as we discuss in the Supplementary material.

In our case we have a more complex form of Nowak and May's evolutionary game: the bacteria (over the time scale of these experiments) have fixed strategies, but the environment itself is allowed to change due to the bacterial selfishness and altruism, and nutrients can diffuse in from the external world: this is a substantially more complex but realistic landscape of nutrients and populations we have created then a simple prisoner's dilemma. Surprisingly, it is possible to model such a complex system using a fairly simple nearest-neighbor lattice algorithm and achieve informative and intuitively appealing results. We can include a Commons tragedy aspect to the problem: the altruists are not allowed to consume to the lysis results of the death of bacteria, while the selfish bacteria are allowed to consume lysis results and the nutrients. On the other hand, the selfish bacteria need the conditioning of the medium by the altruists in order to grow rapidly: in that sense the selfish bacteria are dependent on the altruists for survival. The selfish bacteria can be said to not truly seek a tragedy of the Commons, for if they did they would be destroyed as well as their host.

Our basic algorithm is sketched out in Fig. 4, it follows the lattice iteration model of Nowak and May but we now have two new variables: the nutrient broth concentration $\mathcal{F}$ and the lysis residue concentration $\mathcal{G}$, which is due to the death (lysis) of cooperator bacteria. Only the selfish bacteria can consume the lysis residue, both altruist and selfish bacteria can consume $\mathcal{F}$, but the altruists have large consumption quanta per iteration, $\Delta\mathcal{F}_{\dashv}$. The selfish bacteria can consume lysis molecules in quanta of $\Delta\mathcal{G}$, but consume less nutrient media per iteration $\mathcal{F}_{\lceil}$. We further allow the nutrient to be consumed by the bacteria and to diffuse into a region dependent on the availability of the nutrient as determined by openings of the nanoslits, which are put on the boundaries of the lattice simulation. The altruist and selfish bacteria reproduce themselves by evaluating the average food in the nearest neighbor vicinity of lattice site i, the probability of reproduction is proportional to the average food in the area, $<\mathcal{F}>$ for the altruists and $\mathcal{F}+\mathcal{G}$ for the selfish bacteria. If the bacteria have quantal needs of food which are greater than the amount food present they can only get the remaining food and exhaust the site. Food is allowed to diffuse in the nanoslits through the edges, where it propagates using a discretized form of the diffusion equation. Thus, our iterative lattice game rules at lattice position i for the nutrients available to the altruists $\mathcal{F}_{\rangle}^{\dashv}$ and selfish bacteria $\mathcal{F}_{\rangle}^{\int}$ are:

$$\mathcal{F}_i^a = min(\mathcal{F}_i, f^a) \tag{1}$$

$$\mathcal{F}_i^s = min(\mathcal{F}_i, f_s) \tag{2}$$

$$\mathcal{G}_i^s = min(\mathcal{G}_{\rangle}, g_s) \tag{3}$$

The birth rates $\mathcal{B}$ of the altruists and selfish bacteria are computed by doing an average of the nearest neighbor food source $< F^a >$ and $< F_s + G_s >$, the probability of a new birth is weighed by the amount of food available divided by the cost per reproduction δb, newly formed bacteria occupy sites and replace the previous inhabitants of the lattice site. In the process of replacement of a previous occupant we also create lysis material. Birthing costs nutrient amount b_a and b_s for altruists and selfish bacteria respectively:

$$\mathcal{B}_i^a = \frac{< \mathcal{F}_a >}{\delta b^a} \tag{4}$$

$$\mathcal{B}_i^s = \frac{< \mathcal{F}_s > + < \mathcal{G}^s >}{\delta b^s} \tag{5}$$

We cannot possibly model at present the full dynamics of a full 85 patch experiment, but we can capture the segregation dynamics of 2 microhabitat patches at the interface: the one on the left has no external diffusion input of nutrient (the "black region") and the right side has ample access (the "white region"). The lattice simulation starts with no lysis products and a random occupation of the 3-D lattice sites with two coupled habitat patches. In spite of our simplifications, the simulation reproduces many of the complex features found in a nutrient landscape with altruists and selfish bacteria as can be seen in Fig. 4. For example, if you compare the simulation of Fig. 4B with the data from Fig. 2D you can see that the basic (stable) segregation of the altruists and the selfish bacteria is reproduced, particularly the clustering of the selfish bacteria in the "black" region around the entry channel to the "white" region, and the dispersion of the selfish bacteria in the "white" region and the clustering of the altruists in the "white" region. The clustering of the selfish bacteria in the "black" region around the entry channel to the "white" region occurs because the selfish bacteria actually depend on the altruists for lysis food nutrient sources and so are driven near the "white" region to consume lysis products diffusing from the white region where the altruists can grow. In the "white" region, altruists are driven away from selfish bacteria because of their Commons tragedy phenotype and cluster, yet the selfish bacteria cannot assume dominance since they need the altruists to condition the medium: a complex pattern of segregation results. The analysis of the patch sizes of the segregated strains shown in Fig. 4 shows that as in our experiments patch sizes scale with the phenotype of the strain, and the segregation is stable in time, as long as the genome is stable.

What do we learn from these experiments? First, we think we should move away from the prisoners dilemma terminology of "defectors" and "cooperators" with the pejorative implications for the defectors, although our terminology of "selfish" and "altruists" is equally pejorative. In reality, probably both strains of altruists and selfish bacteria are necessary for the stable existence of the species in the presence of the complex and ever-changing nutrient landscape that is presented outside the confines of the microbiology laboratory. The facile change of the genome from the wild-type altruist genome JEK1036 to the rpoS mutant JEK1033 in a few days under stress with a 48 bp repeat of the adjoining sequence in the rpoS gene surely indicates that the GASP phenotype is programmed into the survival strategy of the collective organisms and is not a random event [16]. We have shown here that the GASP phenotype does not necessarily result in a tragedy of the Commons ecological disaster as long as the nutrient landscape is allowed to develop and is complex in topology, although admittedly ours is a very simple form of complexity. There are other variables

at play here that we have not net explored. While the lack of stirring may be a major reason that selfish bacteria do not overrun the populations in our experiments, the small population sizes in our system compared to chemostats or even the mL culture tubes used in microbiology surely plays a role [17, 18]. The real test of the general importance of this result will be the extrapolation of these experiments to true 2 and 3-D nutrient landscapes and the use of eucaryotic cells in addition to bacteria. Ultimately, we believe that by learning the rules of engagement and observing the dynamics and steady-state solutions that competing communities develop as they cope with a rough fitness landscape that we will gain insight into a fundamentally analog systems problem which cannot be coded on conventional digital computers: how do agents improve fitness while competing for scarce resources? Do metapopulations follow the logic of Game Theory or do they find solutions that are illogical from a game theory perspective yet maximize strain fitness?

References

[1] Fragile Dominion: Complexity and the Commons, Simon Levin, Perseus Publishing, Cambridge, MA, 2000.

[2] G. Hardin, Science 162 (3859), 1243 (1968).

[3] M. Vulic and R. Kolter, Genetics 158 (2), 519 (2001).

[4] E. R. Zinser and R. Kolter, Res Microbiol 155 (5), 328 (2004).

[5] E. R. Zinser and R. Kolter, J Bacteriol 181 (18), 5800 (1999).

[6] S. P. Diggle, A. S. Griffin, G. S. Campbell et al., Nature 450 (7168), 411 (2007).

[7] B. L. Bassler, Cell 109 (4), 421 (2002).

[8] E. F. Keller and L. A. Segel, J Theor Biol 30 (2), 225 (1971).

[9] A. Ishihama, Annu Rev Microbiol 54, 499 (2000).

[10] K. Tanaka, Y. Takayanagi, N. Fujita et al., P Natl Acad Sci USA 90 (8), 3511 (1993).

[11] R. Hengge-Aronis, J Mol Microb Biotech 4 (3), 341 (2002).

[12] S. E. Finkel and R. Kolter, P Natl Acad Sci USA 96 (7), 4023 (1999).

[13] J. E. Keymer, P. Galajda, C. Muldoon et al., P Natl Acad Sci USA 103 (46), 17290 (2006).

[14] Kaminski, M.M. Games Prisoners Play Princeton University Press, Princeton (2004)

[15] M. A. Nowak and R. M. May, Nature 359 (6398), 826 (1992).

[16] D. J. Earl and M. W. Deem, P Natl Acad Sci USA 101 (32), 11531 (2004).

[17] M. M. Desai, D. S. Fisher, and A. W. Murray, Curr Biol 17 (5), 385 (2007).

[18] G. Hartvigsen and S. Levin, P Roy Soc Lond B Bio 264 (1388), 1677 (1997).

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The authors declare that they have no competing financial interests. Correspondence and requests for materials should be addressed to Juan E. Keymer. `J.E.KeymerVergara@tudelft.nl`.

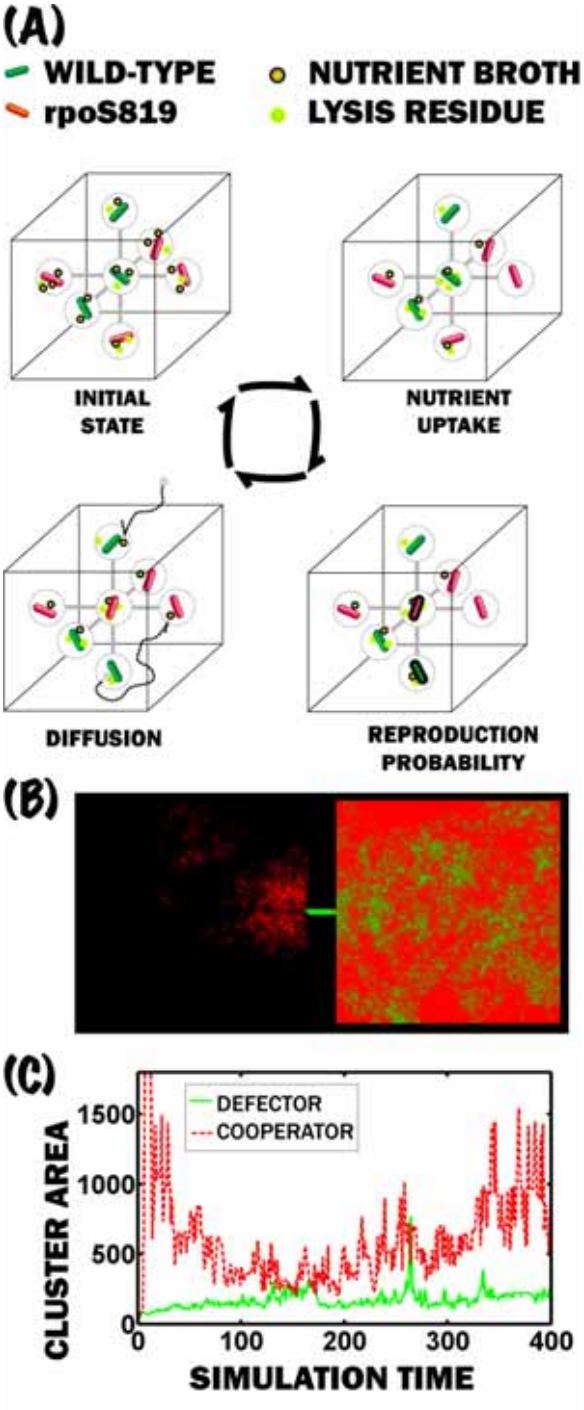


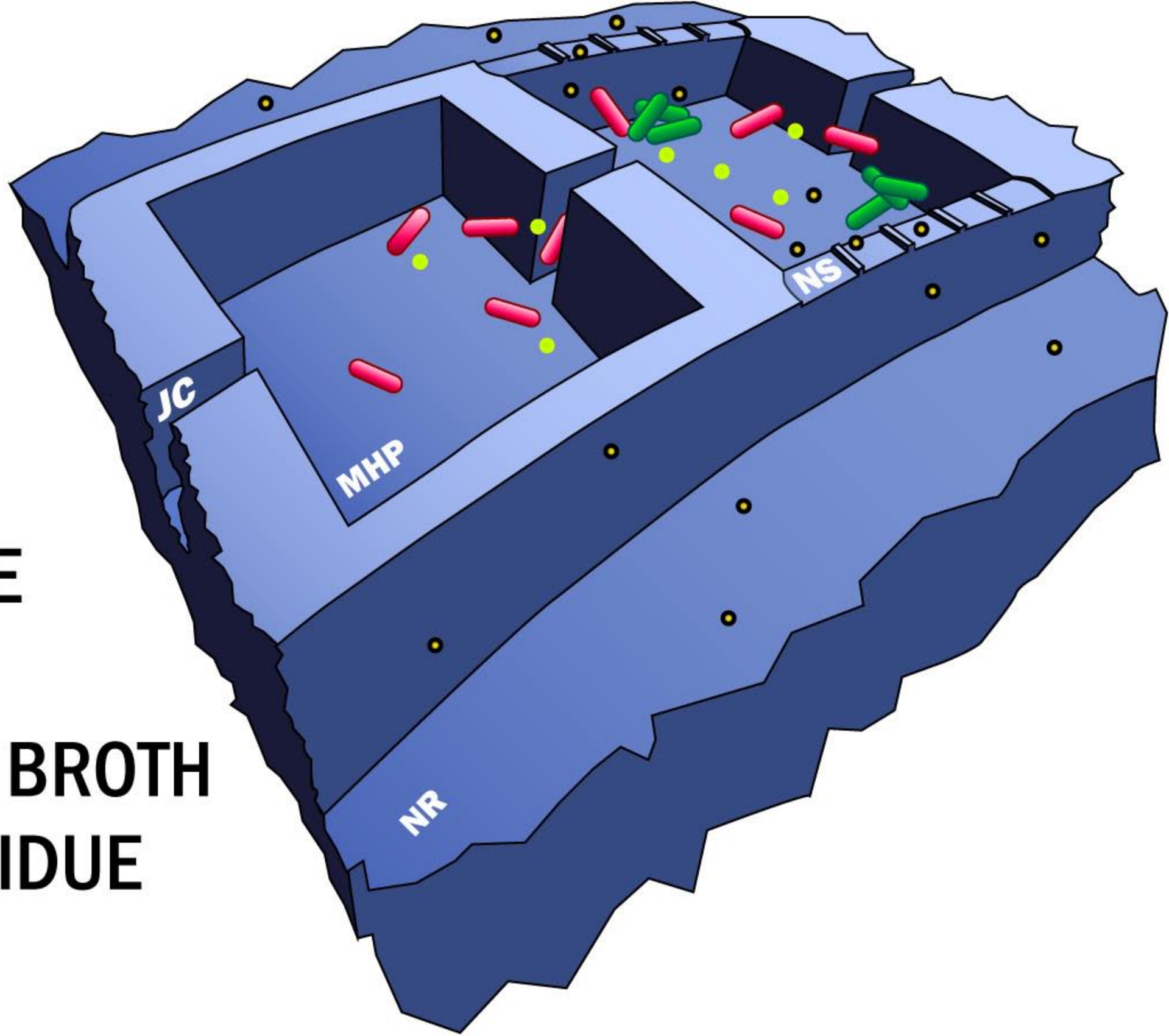
Figure 4: (A) Lattice sites now include concentrations of nutrient (brown circles) and lysis products (green circles. Altruists cannot consume lysis products, and consume nutrients at a lower rate than selfish bacteria can. (B) Simulation image representative of the steady state that stabilizes after 400 loops for a simple 2-habitat patch, the left side has no nutrient diffusional entry from the top and bottom sides of the patch, the right side has full nutrient entry. (C) Development of segregated cluster sizes vs. iterations in the game for altruist and selfish bacteria.

WILD-TYPE

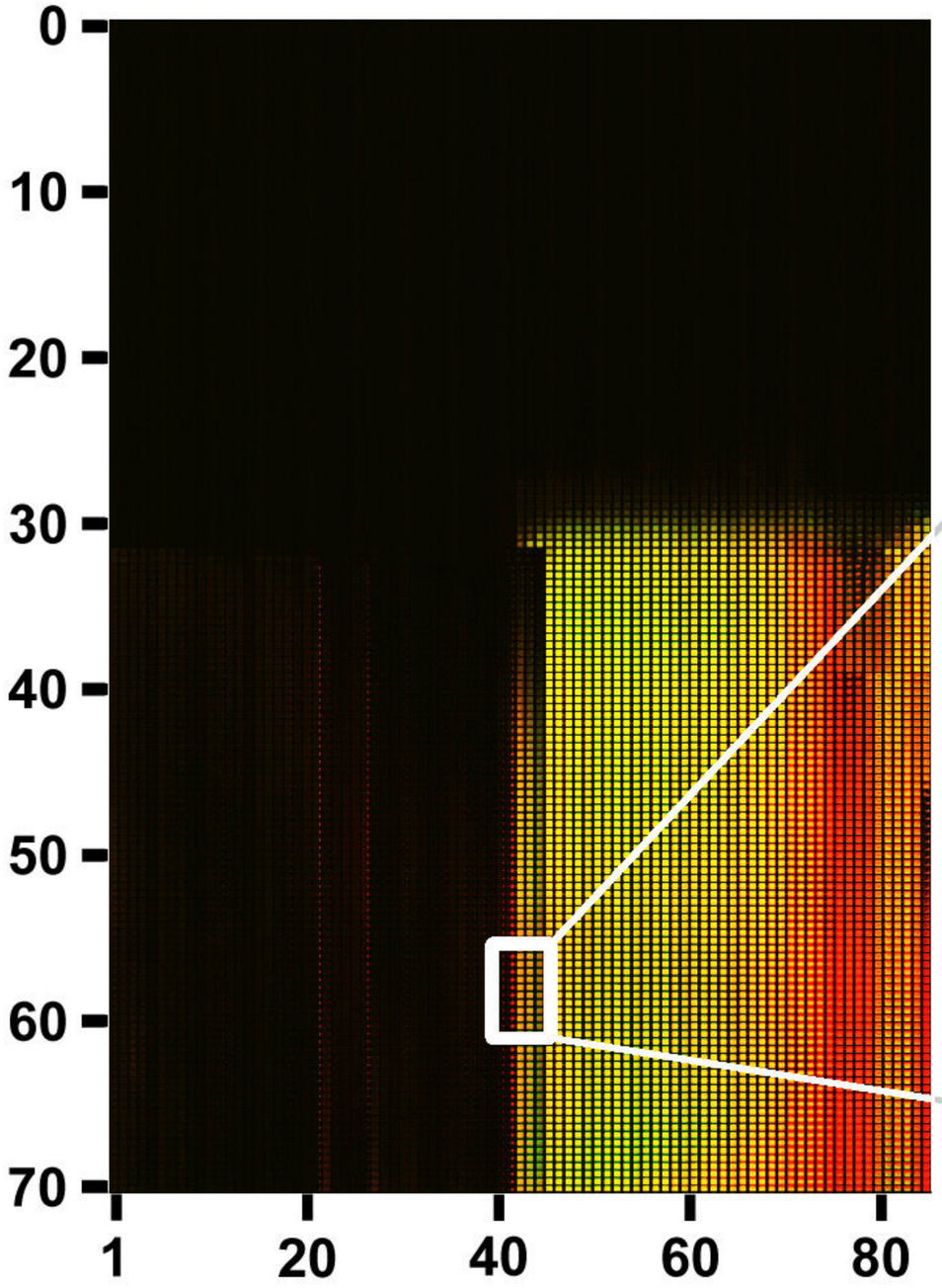
rpoS819

NUTRIENT BROTH

LYSIS RESIDUE



(A)



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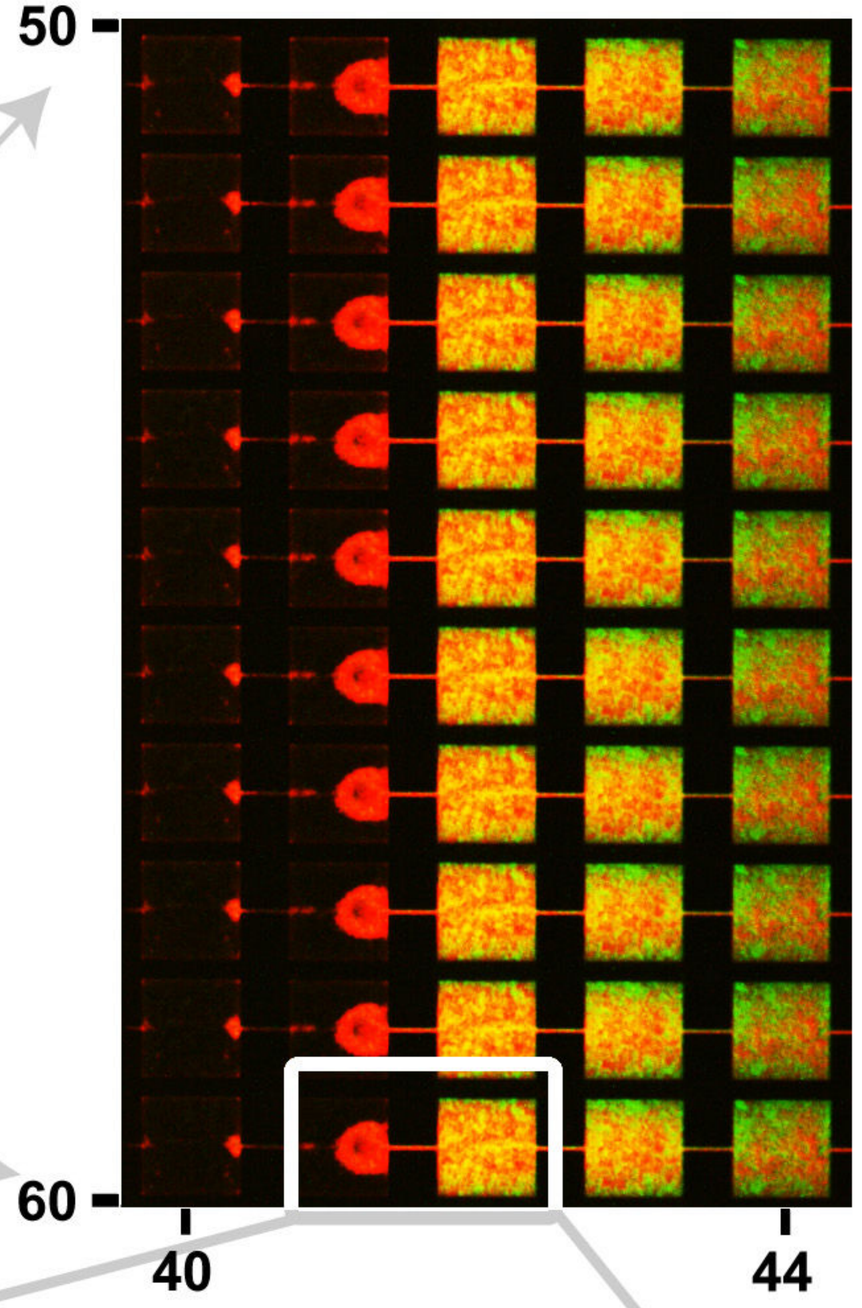
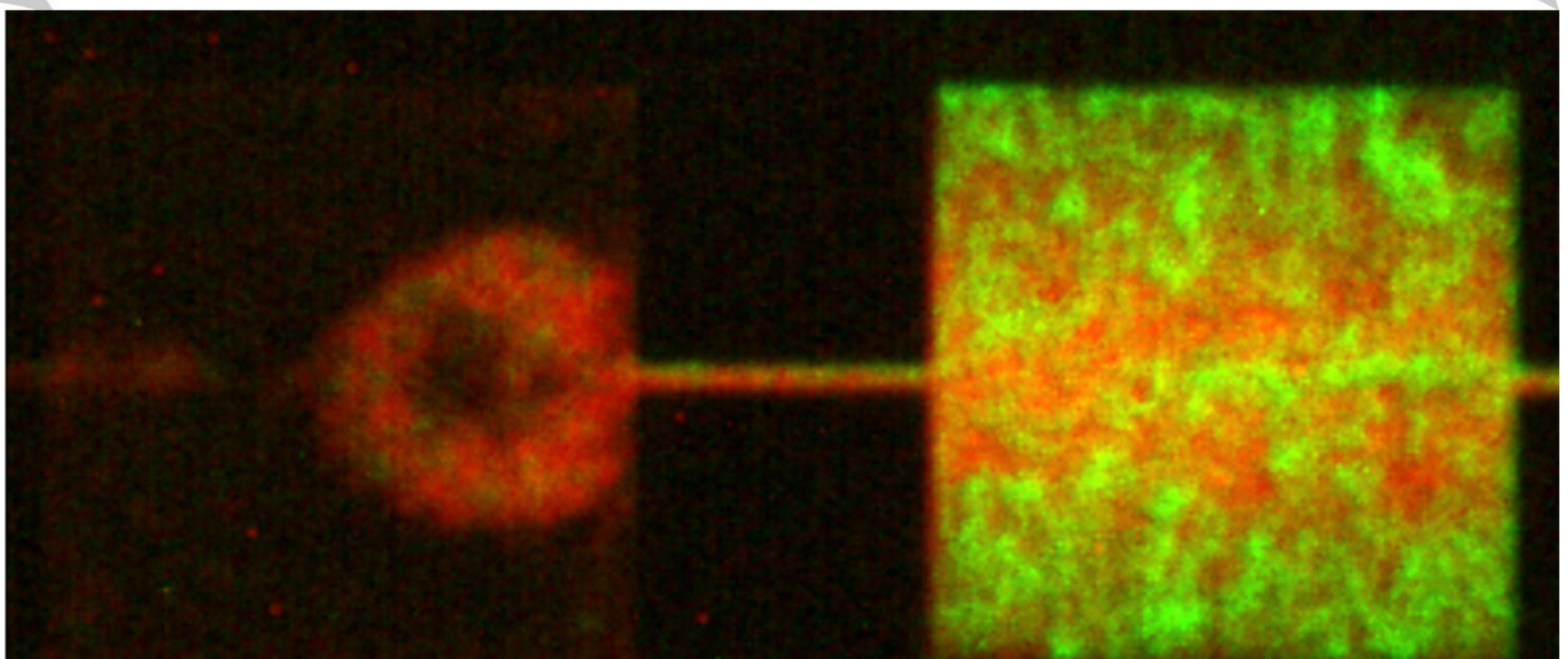
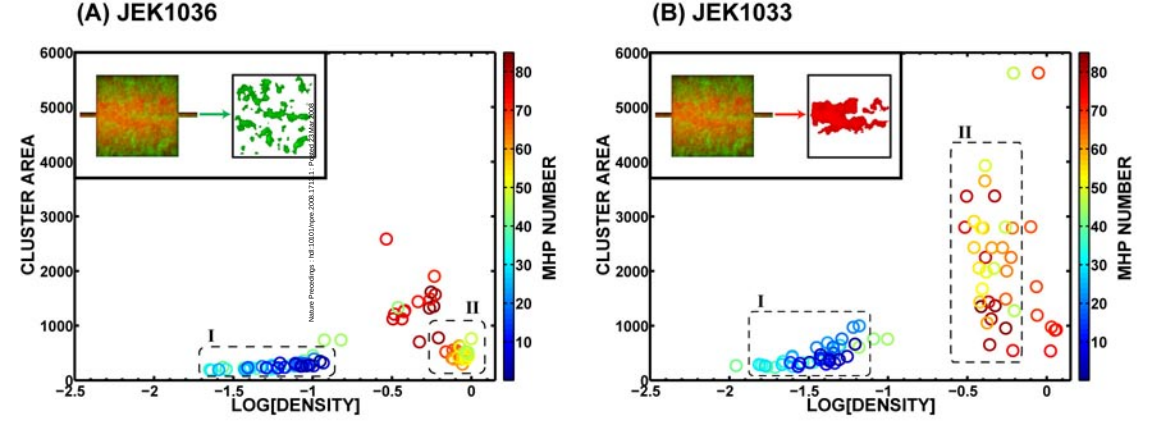


(C)




(A) JEK1036
(B) JEK1033
CLUSTER AREA
LOG[DENSITY]
MHP NUMBER
I
II
Nature Precedings : hdl:10101/npre.2008.1731.1 : Posted 25 Mar 2008

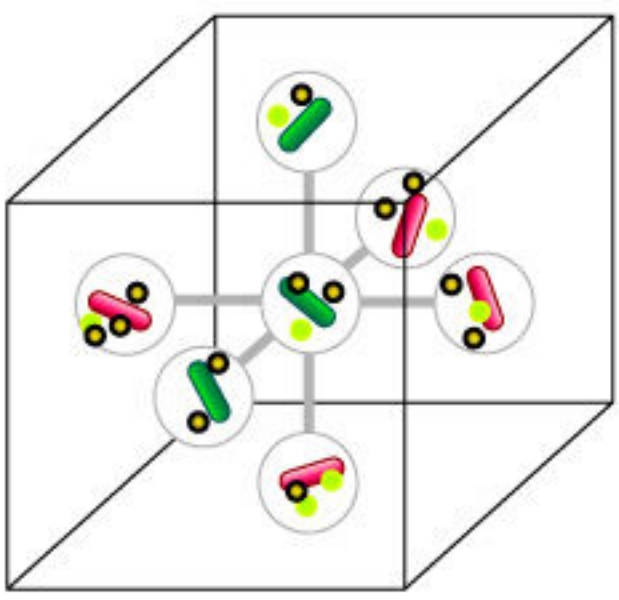
(A)

WILD-TYPE

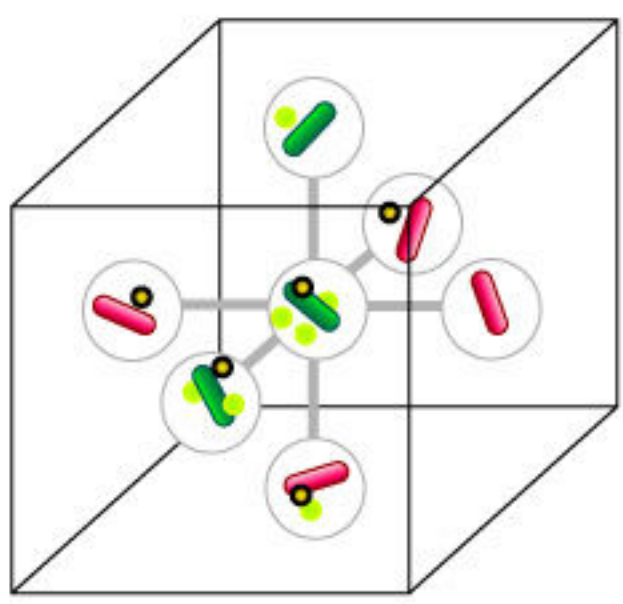
rpoS819

NUTRIENT BROTH

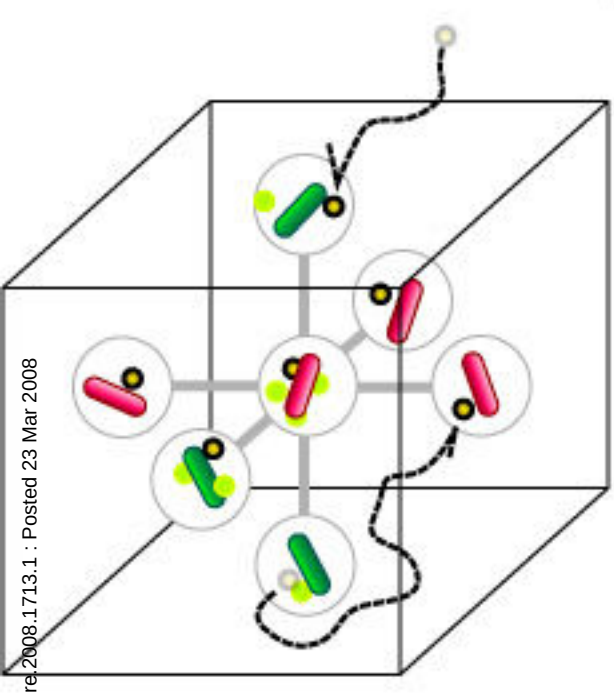
LYSIS RESIDUE



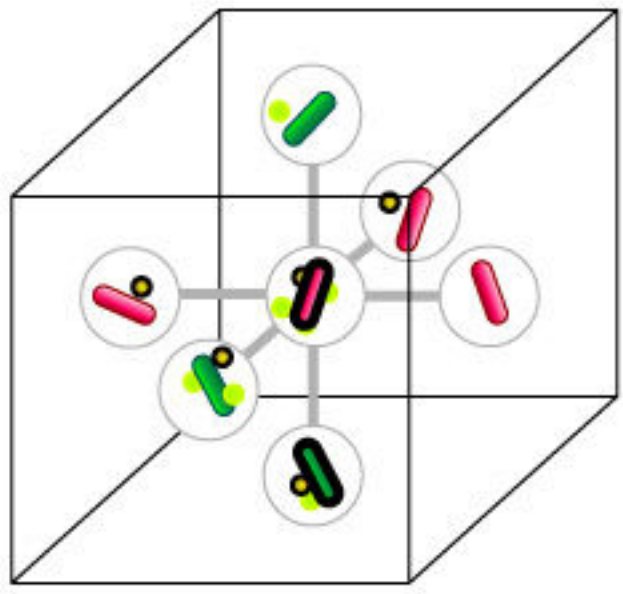
INITIAL STATE



NUTRIENT UPTAKE

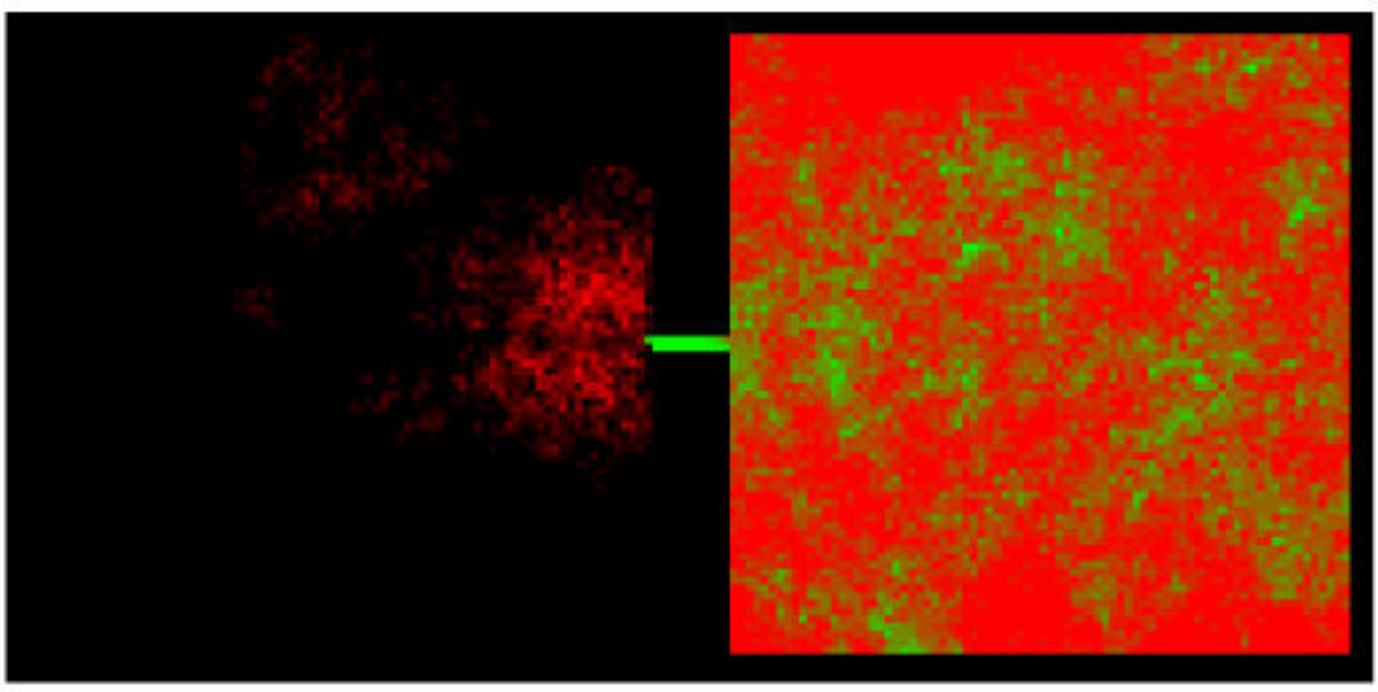


DIFFUSION



REPRODUCTION PROBABILITY

(B)



(C)