The Evolution of a 'Tragedy of the Commons' in a Host-Pathogen Metapopulation

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1 Abstract

Recent studies have suggested that the manner in which hosts of disease-causing organisms move and contact one another can have profound consequences on the ecological and evolutionary dynamics of the disease itself. We are using a model host-pathogen system to address the role of such "population structure" in the ecology and evolution of disease. The host is the bacterium *Escherichia coli* and the pathogen is the virus T4 coliphage that infects and kills the bacterium. From knowledge of how isolated populations of the bacterium and its virus interact, we construct a theoretical framework for exploring how metapopulations (collections of many populations linked by occasional migration) behave. Theoretical predictions are then tested experimentally with metapopulations of bacteria and bacteriophage using a high throughput robot to perform migrations between subpopulations. We find that the way in which migration takes place between subpopulations affects the ecological dynamics of the host-pathogen system. Furthermore, the topology of the metapopulation influences the evolution of the pathogen. A "tragedy of the commons" evolves in our simple system, where "rapacious" phage that overexploit their bacterial host can outcompete "prudent" phage that use their host in a more restrained manner. This "tragedy" is resolved differently depending on how the metapopulation is structured—specifically, spatially restricted migration favors prudent pathogens. We believe there are potential connections of this work to the evolution of avirulence in disease systems, prudent predation in predator-prey systems, and the evolution of cooperation more generally.

2 Introduction

Biological communities are commonly fragmented into subpopulations that are loosely linked through migration. The dynamics of such metapopulations have been investigated using theoretical models, statistical tools, field studies and laboratory experiments. While extremely valuable, experiments on multi-species metapopulations have focused on ecological dynamics (e.g., abundance data) in relatively small networks. Experimental evolution of interacting species in large metapopulations remains comparatively unexplored.

Here we use a model host-pathogen system to investigate how the pattern of migration within large metapopulations affects eco-evolutionary dynamics. The bacterial host is *Escherichia coli* and its viral pathogen is T4 coliphage (Fig 1). The experiments (described below) embed this host-pathogen system inside 96-well microtiter plates, yielding a metapopulation structure for the host-pathogen community.

We find that the bacteria and virus cannot coexist within a single well and therefore prolonged ecological coexistence at the metapopulation scale must rely on asynchrony in local extinctions and continual recolonization through migration.

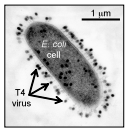

Figure 1: A micrograph of an *E. coli* cell being infected by several T4 phage particles. The virus binds to the surface of the bacterium and injects its DNA. After production of new progeny phage (the dark particles within the cell), the bacterium is lysed and the viral progeny are released. (TEM plate courtesy of John Wertz).

3 Metapopulation Modeling

We can characterize the state of a microtiter well as belonging to a set of discrete states (bacteria-filled, phage-filled, and empty). By performing dilutions and migrations, we empirically determine the transition matrix for our well-states (Table 1). We use this matrix to run lattice-based simulations of metapopulation dynamics.

Our simulations predict coexistence in the metapopulation (Figs 2a,b). Wells exhibit a kind of 'rock-paper-scissors' dynamic: Migrating phage 'beat' bacteria (through infection and decimation), migrating bacteria 'beat' empty wells (through colonization), and empty wells 'overtake' phage (without host cells, the phage are diluted to extinction). Spatially restricted migration leads to clumping of well types (Fig 2c) and a dampening of inherent oscillatory dynamics (compare Figs 2a & 2b). With spatially restricted migration, only phage at the boundaries between phage clumps and bacterial clumps can access fresh host. Relative to unrestricted migration, this limitation on host access is predicted to reduce the average phage density and increase the average bacterial density (compare Figs 2a and 2b).

future well state

current well state	B	P1	P2	P3	E
B	B, E, ∅	P1, P2, P3			
P1		B	P1, P2, P3, E, ∅		
P2		B	P1	P2, P3, E, ∅	
P3		B	P1	P2	P3, E, ∅
E	B		P1	P2	P3, E, ∅

Table 1: The transition matrix. All entries in this table give the identity of the state of the source well of migration that allows a focal well to transition from the relevant row state to the relevant column state (**B**--bacteria; **P1-P3** – phage of decreasing titer; **E**--empty well). The symbol ∅ refers to transitions in which there is no migration event (i.e.,straight dilution of a focal well followed by incubation). The filled entries correspond to transitions that cannot occur.

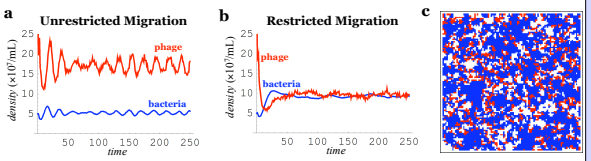

Figure 2: Stochastic cellular automata predictions. Global densities of bacteria and phage on a lattice of 100×100 wells with (a) an "Unrestricted" migratory neighbourhood and (b) a "Restricted" neighbourhood (only the north/south/east/west wells around a focal well can serve as sources of migration). Migration probability is m=0.45 in both cases. (c) A snapshot of Restricted neighbourhood lattice from time step 100, showing clumping of bacterial wells (blue), phage wells (red) and empty wells (white).

4 Hypotheses

1) ***Despite the lack of coexistence within a subpopulation, phage and bacteria can coexist in a metapopulation.***

2) ***The dynamics with spatially restricted migration are more stable than with unrestricted migration.***

3) ***The mean phage density is lower with spatially restricted migration (and the mean bacterial density is higher).***

5 Experimental metapopulations

We tested these theoretical predictions using experimental metapopulations of 192 subpopulations (two 96-well plates) propagated by serial transfer on a 12-hour cycle. Immediately after transfer, each metapopulation was exposed to one of three migration treatments (Fig 3). In the 'Restricted' treatment, migration into a focal well occurred from one of the north, south, east, or west neighbours with probability m=0.45 (we employed 'wrap-around' boundaries so that every well had four neighbours). In the 'Unrestricted' treatment, migration into a focal well occurred from one of any other well in the metapopulation with the same probability m=0.45. Though they varied with regard to the pattern of migration, both Restricted and Unrestricted treatments imposed some degree of population structure. In the 'Well-Mixed' treatment, all population structure was destroyed every 12-hour cycle by thoroughly mixing the entire metapopulation in a large reservoir and then redistributing the mixture into a fresh set of wells. All dilutions, migrations and mixing were done with a high-throughput robot (Fig 4). Each treatment was run for 20 cycles and replicated four times.

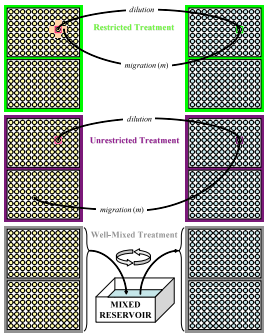

Figure 3: A transfer event is shown for the three treatments. Like the 'example focal well' (boxed in bright pink), each well in the Restricted treatment is diluted from spent media (yellow) into fresh media (blue). Then, with probability m=0.45, migration occurs from one of the four nearest wells (highlighted in pink) into the focal well. The Unrestricted treatment is identical except that any well in the metapopulation can serve as the migration source. In the Well-Mixed treatment, all wells are diluted into a common reservoir, thoroughly mixed, and redistributed into a fresh set of wells.

Consistent with theoretical predictions, we observed that population structure is critical to coexistence. The Well-Mixed treatment lost both bacteria and phage, whereas both types persisted in the other treatments (Figs 5a & 5b).

Population instability was gauged by the coefficient of variation (CV) in phage or bacterial abundance over all time points. By this measure, bacteria in the Restricted treatment were more stable than in the Unrestricted treatment, although not significantly so ($CV_{Restricted}$=0.951, $CV_{Unrestricted}$=1.276, p= 0.2). Phage in the Restricted treatment were significantly more stable than in the Unrestricted treatment ($CV_{Restricted}$=0.572, $CV_{Unrestricted}$=0.884, p= 0.02857). Thus, restricted migration tends to stabilize community dynamics as predicted by our simulations.

In contrast to our predictions, we found no significant differences across treatments in average bacterial density ($N_{Restricted}$=4.18×10^7, $N_{Unrestricted}$=4.03×10^7, p=0.89) or in average phage density ($N_{Restricted}$=2.5×10^8, $N_{Unrestricted}$=2.76×10^8, p=0.34).

Thus, we have experimental support for hypotheses 1 and 2, but not hypothesis 3. The appearance and maintenance of a polymorphism in phage plaque morphology led us to consider the role of pathogen evolution in the failure of hypothesis 3.

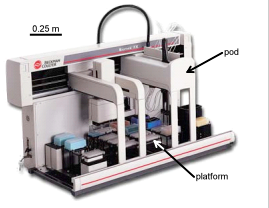

Figure 4: The high-throughput liquid handling robot used in the experiments (the *Biomek FX, Beckman Coulter*). The microtiter plates are placed on the platforms and one of the pods sends down pipettes to transfer small portions of one well to another well within a collection of subpopulations (that is, the robot executes migrations between microbial subpopulations).

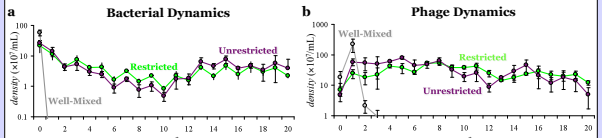

Figure 5: Ecological results for experimental metapopulations. (a) Average bacterial density (±SEM) and (b) average phage density (±SEM) through time for each of the three treatments.

6 Pathogen Evolution

We sampled four random phage isolates from the last transfer of each replicate. Regardless of the initial multiplicity of infection (ratio of phage to bacteria), phage from the Unrestricted treatment were significantly less productive than phage from Restricted treatment (Fig 6a). Hence, evolution in the phage accounts for the failure of hypothesis 3. At each MOI, phage from the Unrestricted treatment were significantly more competitive than phage from the Restricted treatment (against a common "marked" phage strain, Fig 6b). Moreover, a negative correlation between phage productivity and competitive ability was found at all three MOI levels and was significant in two cases (Kendall's test: low MOI τ=-0.25, p=0.04545; intermediate MOI τ=-0.14, p=0.2655; high MOI τ=-0.44, p=0.00033).

These findings are consistent with a 'tragedy of the commons', as the 'rapacious' phage evolving in the Unrestricted treatment can outcompete the 'prudent' phage found in the Restricted treatment, but the prudent phage is more productive when alone.

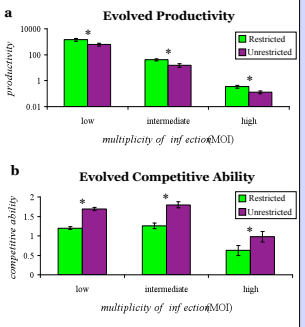

Figure 6: Evolution. (a) Productivity and (b) competitive ability of evolved phage at three multiplicities of infection. We plot mean±SEM and asterisks denote significance at the 0.05 level.

7 Evolutionary Model

We extended our earlier simulation to include two phage types, prudent and rapacious, with a trade-off between productivity and competitive ability. If sufficiently productive, the rapacious type can displace the prudent type in the Unrestricted treatment (Fig 7a), but not in the Restricted treatment (Fig 7b), a result that is robust to the shape of the trade-off (Fig 7c). Rapacious phage fare better in the Unrestricted treatment because: (1) the increased probability of reaching fresh hosts reduces the likelihood of extinction and (2) the increased probability of mixing phage types favors the rapacious competitor. In the Restricted treatment, wells of the less productive rapacious type 'burn out' before spreading very far and prudent types persist by default.

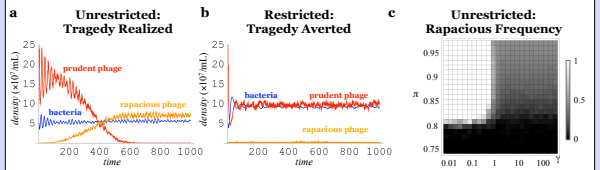

Figure 7: Evolutionary lattice-based simulations. In mixed subpopulations, rapacious phage out-compete prudent cohabitants with probability β>0, but enter sub-critical levels one dilution earlier with probability $(1-\pi)^\gamma$>0 (π is a measure of productivity/persistence). Let β=$(1-\pi)^\gamma$ specify the trade-off between competitive ability and persistence (γ specifies the shape of the trade-off). The fate of rapacious phage (introduced by mutation into a 100×100 lattice with bacteria and prudent phage with m=0.45, π=0.87 and γ=0.1) is shown given (a) unrestricted and (b) restricted migration. (c) The frequency of phage that is rapacious after 3000 cycles is shown on a greyscale for a number of different parameter combinations (each square is the average rapacious frequency over five simulation runs). With unrestricted migration, rapacious phage that are sufficiently persistent (large π) completely displace prudent types if the trade-off is sufficiently concave (small γ). None of the parameter combinations shown allow rapacious phage to persist at high frequencies given restricted migration.

8 Potential Mechanism

We next investigated the latent period (the time from phage adsorption to host cell lysis) of our evolved phage isolates. We placed each isolate in a microtiter well with abundant host at low MOI. The optical density (OD) of these wells was compared with control wells with only bacteria. The time of OD deviation between phage and control wells gave us the period for two rounds of host cell lysis (twice the latent period). Evolved phage from the Restricted treatment had a longer latent period. Thus, phage from the Unrestricted treatment had evolved a more virulent life history.

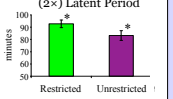

Fig 8: The average of ~ twice the latent period from both treatments

9 Conclusions

We demonstrate that the topology of migration within a host-pathogen metapopulation affects eco-evolutionary dynamics. Our results likely apply to other victim-exploiter interactions embedded within metapopulations (e.g., Fig 9). Specifically, restricted migration between subpopulations may promote the evolution of prudent predation or restrained consumption. In general, restraint in the use of a common resource is a form of cooperation, and we have shown that spatial restrictions in migration can favour relatively cooperative use of the common resource, thus averting the tragedy of the commons.

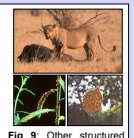

Fig 9: Other structured victim-exploiter systems

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