Supplementary Information

Success of a suicidal defense strategy against infection in a structured habitat

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SI Text:

Supplementary Animations:

Supplementary Figures:

Supplementary Tables:

Supplementary References

SI Text 1: Supplementary Figure legends

Figure S1. Changes of the A:S ratio after infection with pathogen Q, which does not induce suicide in either host.

Final A:S ratio as a function of the initial A:S ratio. The averages ± SEM are shown. n = 4 for experiments and n = 100 for simulations. The procedure was as for Fig. 2.

Figure S2. Effect of population size on the A:S ratio change in the absence of spatial structure.

Maximum population size was varied. With P pathogen (a, b) or Q pathogen (c, d). Individual results from simulations are shown for (a) and (c). The procedure was as for Fig. 2.

Figure S3. Detailed time-course analysis.

(a) Time-course of changes in hosts' densities and pathogen's density in an experiment without spatial structure. One out of four similar results. (b(i)) Time-course of changes in hosts' densities and pathogen's density in a simulation without spatial structure.

(b(ii)) Mutant frequencies in the two hosts and A:S ratio in a simulation without spatial structure. (c(i)) Time-course of changes in hosts' densities and pathogen's density in a simulation with spatial structure. (c(ii)) Mutant frequencies in the two hosts and A:S ratio in a simulation with spatial structure. One out of 100 similar simulation results.

Figure S4. Emergence of the pathogen mutants not inducing host suicide.

The measure of the fraction of the mutants is the ratio of the titer on host A to that on host S. It was measured after the infection period. N = 4 for experiments. One out of 100 similar simulation results.

Figure S5. Model for simulation in the presence of spatial structure.

(a) Reproduction of host S (or S^R, A and A^R). (b) Mutation of host S to S^R. Mutation of A to A^R. (c) Infection of host S with pathogen P (or Q). (d) Suicide of host A induced by infection with pathogen P. (e) Mutation of pathogen P to Q. (f) Adsorption of pathogen P (or Q) to S (or I, I^Q and A). (g) Death of host I (or I^Q) accompanied by release of pathogen P (or Q) τ min after infection.

SI Text 2: Detailed description of the model

We modeled our virtual populations in a 10000×10000 dual-lattice (Figure S1). One lattice was for the host, while the other was for the pathogen. We assume that each grid of the lattice has a side length $1\mu m$, and that each grid can harbor only one bacteria cell (whose diameter is about 0.5 μm). The state of the *i*th site ($i = 1,2,\cdots$) in the host lattice at time t is denoted by $s_i^{\mathrm{h}}(t) \in \Sigma^{\mathrm{h}} \equiv \left\{\mathrm{S},\mathrm{S}^{\mathrm{R}},\mathrm{A},\mathrm{A}^{\mathrm{R}},\mathrm{I},\mathrm{I}^{\mathrm{Q}},\mathrm{O}\right\}$ (Table S2). The state S indicates the site occupied by a sensitive non-altruistic (non-suicidal) host, S^{R} does the site occupied by a pathogen-resistant non-altruistic (non-suicidal) host, A does the site occupied by an altruistic (suicidal) host, A^{R} does the site occupied by a pathogen-resistant altruistic (suicidal) host, I does the site occupied by an infected host, I^{Q} does the site occupied by a host infected with a mutant pathogen, while O is an empty site. The state of the *i*th site ($i = 1,2,\cdots$) in the pathogen lattice at time *t* is denoted $s_i^{\mathrm{p}}(t) \in \Sigma^{\mathrm{p}} \equiv \left\{\mathrm{P},\mathrm{Q},\mathrm{O}\right\}$. The state P indicates that the site is occupied by *b* (burst size) individual pathogens, Q indicates that the site is occupied by *b* of individual mutant pathogens, while O is an empty site. The state of the whole lattice population at time *t* is expressed by a vector $\mathrm{W}(t) = ((s_1^{\mathrm{h}}(t), s_1^{\mathrm{p}}(t)),(s_2^{\mathrm{h}}(t), s_2^{\mathrm{p}}(t)),\cdots)$.

Assuming a continuous-time Markov process, we specify the dynamics of W(*t*) by the following transition rates at the *i*th site (*i* = 1, 2, ...):

$(\mathrm{O},{*\mathrm{p}}) \to (\mathrm{S},{*\mathrm{p}}),(\mathrm{A},{*\mathrm{p}})$ at rate $r(1-\mu)n_i(\mathrm{S})/z_r$, and $r(1-\mu)n_i(\mathrm{A})/z_r$, (1)

$(\mathrm{O},{*\mathrm{p}}) \to (\mathrm{S}^{\mathrm{R}},{*\mathrm{p}}),(\mathrm{A}^{\mathrm{R}},{*\mathrm{p}})$ at rate $r\mu n_i(\mathrm{S})/z_r + rn_i(\mathrm{S}^{\mathrm{R}})/z_r$,

and $r\mu n_i(\mathrm{A})/z_r + rn_i(\mathrm{A}^{\mathrm{R}})/z_r$, (2)

$(\mathrm{S},{*\mathrm{p}}) \to (\mathrm{I},{*\mathrm{p}})$ at rate $\beta(1-\mu)m_i^{\mathrm{p}}(\mathrm{P})/Z_d$, (3)

$(\mathrm{A},{*\mathrm{p}}) \to (\mathrm{O},{*\mathrm{p}})$ at rate $\beta(1-\mu)m_i^{\mathrm{p}}(\mathrm{P})/Z_d$, (4)

$(\mathrm{S},{*\mathrm{p}}),(\mathrm{A},{*\mathrm{p}}) \to (\mathrm{I}^{\mathrm{Q}},{*\mathrm{p}})$ at rate $\beta\mu m_i^{\mathrm{p}}(\mathrm{P})/Z_d + \beta m_i^{\mathrm{p}}(\mathrm{Q})/Z_d$, (5)

$(\mathrm{I},{*\mathrm{p}}) \to (\mathrm{O},\mathrm{P})$ after lysis time τ, (6)

$({*\mathrm{h}},\mathrm{P}) \to ({*\mathrm{h}},\mathrm{O})$ at rate $a(m_i^{\mathrm{h}}(\mathrm{S}) + m_i^{\mathrm{h}}(\mathrm{I}) + m_i^{\mathrm{h}}(\mathrm{A}))/Z_d$, (7)

where the variable $n_i(\mathrm{s}^{\mathrm{h}})$ is the number of sites with state s^{h} in the nearest-neighbors of the *i*th site of the host lattice, $m_i^{\mathrm{p}}(\mathrm{s}^{\mathrm{p}})$ is the number of sites with state s^{p} in the d step neighbors of the *i*th site of the pathogen lattice (that is, the sites that are no further than d Manhattan distance, the sum of vertical and horizontal moves, from the *i*th site), $m_i^{\mathrm{h}}(\mathrm{s}^{\mathrm{h}})$ is the number of sites with state s^{h} in the *i*th site and the d step neighbors of the *i*th site of the host lattice. The constant $z_r = 4$ is the number of nearest neighbors of a site for host reproduction, and the constant $Z_d = 1 + 2d(d+1)$ is the number of d step neighbor sites for pathogen infection and adsorption. The character $*\mathrm{p}$ is a wildcard state that can be replaced by any state from Σ^{p}, while $*\mathrm{h}$ is a wildcard state that can be replaced by any state from Σ^{h}.

Process (1) represents the reproduction of a sensitive non-altruistic host and an altruistic host to an empty site from a nearest-neighbor site on the host lattice, where μ is a constant parameter representing mutation rate, and r is a constant parameter representing maximum fecundity of a sensitive non-altruistic host and an altruistic host, which is implemented when all nearest-neighbor sites in the host lattice are empty.

Process (2) represents the mutational-reproduction of a sensitive non-altruistic host and an altruistic host, and the reproduction of a resistant non-altruistic host and a resistant altruistic host to an empty site from a nearest-neighbor site in the host lattice.

Process (3) represents the infection of a sensitive non-altruistic host at the *i*th site by a pathogen in its d step neighbors, where β is a constant parameter representing the maximum productivity of pathogens, which is implemented when all the d step neighbor sites are occupied by sensitive non-altruistic hosts.

Process (4) also represents the infection of an altruistic host at the *i*th site by a pathogen in its d step neighbors.

Process (5) represents the mutational-transmission of a pathogen and transmission of a mutant pathogen to a host at the *i*th site from its d step neighbors.

Process (6) represents the lysis process of an infected non-altruistic host, where τ is a constant parameter representing lysis time.

Process (7) represents the adsorption of b individual pathogens at the ith site by a host in its d step neighbors, where $a(=\beta/b)$ is a constant parameter representing the maximum consumption of pathogens, which is implemented when all the d step neighbor sites are occupied by non-resistant hosts in the host lattice.

We refer to the variables $n_i(\mathrm{s^h})/z_r$, $m_i^{\mathrm{p}}(\mathrm{s^p})/Z_d$, and $m_i^{\mathrm{h}}(s^{\mathrm{h}})/Z_d$ as "local density", which is used in the case of local interaction. In the case of global interaction, we refer to "global density" $\rho^{\mathrm{h}}(\mathrm{s^h})$ and $\rho^{\mathrm{p}}(\mathrm{s^p})$ instead.

$(\mathrm{O},*\mathrm{p}) \to (\mathrm{S},*\mathrm{p}),(\mathrm{A},*\mathrm{p})$ at rate $r(1-\mu)\rho^{\mathrm{h}}(\mathrm{S})$, and $r(1-\mu)\rho^{\mathrm{h}}(\mathrm{A})$, (1)'

$(\mathrm{O},*\mathrm{p}) \to (\mathrm{S^R},*\mathrm{p}),(\mathrm{A^R},*\mathrm{p})$ at rate $r\mu\rho^{\mathrm{h}}(\mathrm{S}) + r\rho^{\mathrm{h}}(\mathrm{S^R})$,

and $r\mu\rho^{\mathrm{h}}(\mathrm{A}) + r\rho^{\mathrm{h}}(\mathrm{A^R})$, (2)'

$(\mathrm{S},*\mathrm{p}) \to (\mathrm{I},*\mathrm{p})$ at rate $\beta(1-\mu)\rho^{\mathrm{p}}(\mathrm{P})$, (3)'

$(\mathrm{A},*\mathrm{p}) \to (\mathrm{O},*\mathrm{p})$ at rate $\beta(1-\mu)\rho^{\mathrm{p}}(\mathrm{P})\beta\ (1-\mu)\ \rho^{p}(\mathrm{P})$, (4)'

$(\mathrm{S},*\mathrm{p}),(\mathrm{A},*\mathrm{p}) \to (\mathrm{I^Q},*\mathrm{p})$ at rate $\beta\mu\rho^{\mathrm{p}}(\mathrm{P}) + \beta\rho^{\mathrm{p}}(\mathrm{Q})$, (5)'

$(*\mathrm{h},\mathrm{P}) \to (*\mathrm{h},\mathrm{O})$ at rate $a(\rho^{\mathrm{h}}(\mathrm{S}) + \rho^{\mathrm{h}}(\mathrm{I}) + \rho^{\mathrm{h}}(\mathrm{A}))$, (7)'

where $\rho^{\mathrm{h}}(\mathrm{s^h})$ is the number of sites with state $\mathrm{s^h}$ in the host lattice divided by the lattice size, and $\rho^{\mathrm{p}}(\mathrm{s^p})$ is the number of sites with state $\mathrm{s^p}$ in the pathogen lattice divided by the lattice size.

To initialize the simulation, every host lattice site was randomly and independently

assigned one of the state in $\Sigma^{h} \equiv \left\{S, S^{R}, A, A^{R}, I, I^{Q}, O\right\}$, and every pathogen lattice site was assigned as O. In the case of global interaction, each host is on a host lattice site, but it can interact with a pathogen wherever it is on the pathogen lattice. In the case of local interaction, each host can interact only with a pathogen in the nearest-neighbor site and its own site. Simulations were repeated 100 times for each condition.

The diffusion coefficient for phage, *D*, is described using the Stokes-Einstein relation:

$$D = \frac{k_{B} T}{6\pi\eta R}, \tag{8}$$

where *R* is approximately the radius of the phage head, and η is the viscosity of the medium, *T* is absolute temperature, and k_{B} is the Boltzmann constant. The maximum adsorption rate *k* corresponds to the case of total cell absorption, where the surface of the cell is entirely covered by receptors:

$$k = 4\pi c D, \tag{9}$$

where *c* is the cell radius. From equation (8) and (9), we calculated $k = 47.5\ \mu m^{3}\ s^{-1}$ when $R = 30$ nm, $T = 37$ °C = 310 K, $\eta = 0.01$ P = 0.01 g cm^{-1} s^{-1} [33], $c = 0.5$ μm, and $k_{B} = 1.38 \times 10^{-23}$ m^{2} kg s^{-2} K^{-1}. We approximated *k* as the infection rate of the pathogen.

After b phage particles are released at time $t = 0$ at the origin, the concentration of the phage, *C*, would obey the diffusion equation:

$$\frac{\partial C(x,y,t)}{\partial t} = D\frac{\partial^2 C(x,y,t)}{\partial x^2} + D\frac{\partial^2 C(x,y,t)}{\partial y^2}, \tag{10}$$

where *x, y* is the position coordinate. The solution to (10) with the point initial concentration b/l^3 (with $l = 1\ \mu\text{m} = 10^{-6}\,\text{m}$) at the point (x,y), is

$$C(x,y,t) = \frac{b/l^3}{4\pi Dt} e^{-d^2/4Dt}, \tag{11}$$

where $d = \sqrt{x^2 + y^2}$. The maximum concentration of bacteria when all the grids of the lattice with side length $l = 10^{-6}$ m are filled is $B = l^{-3} = 10^{18}$ m^{-3}. Thus the maximum absorption (infection) rate is $\beta = Bk = 47.5$ s^{-1}, yielding the mean time to absorption $t_a = (\log b)/\beta = 0.0715$ s. The infection radius d^* then satisfies the equation

$$\frac{1}{l^3} = \frac{b}{l^3}\frac{1}{4\pi Dt_a}\exp\left[-\frac{d^{*2}}{4Dt_a}\right], \tag{12}$$

or $d^* = 7.94\ \mu\text{m}$. The infection radius on the lattice is given by the integer part of d^* in units of μm: $d = 7$.

The growth rate of the host, *r*, satisfies the equation:

$$1 + r = 2^{1/t_B}, \tag{13}$$

where t_B is the doubling time of the host. From equation (13), we calculated $r = 0.023$ min^{-1} given that $z_r = 4$, $t_B = 30$ min.

Supplementary References

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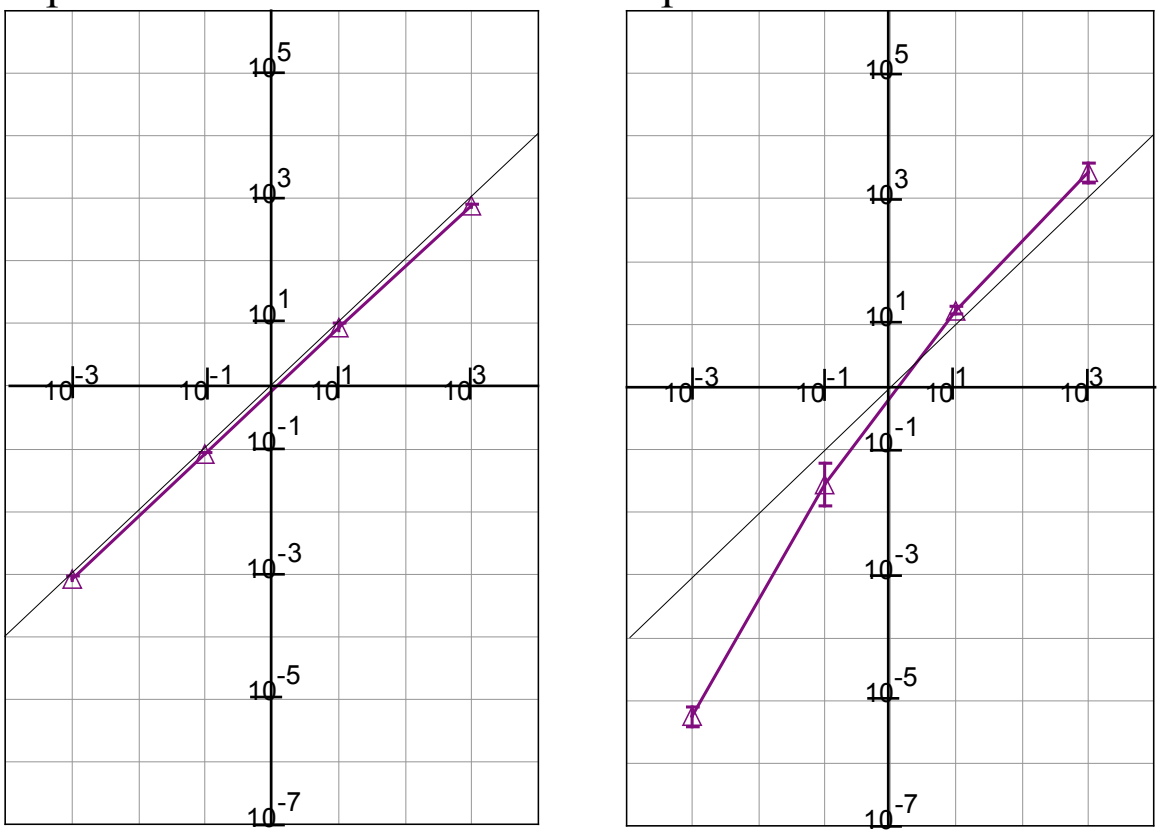
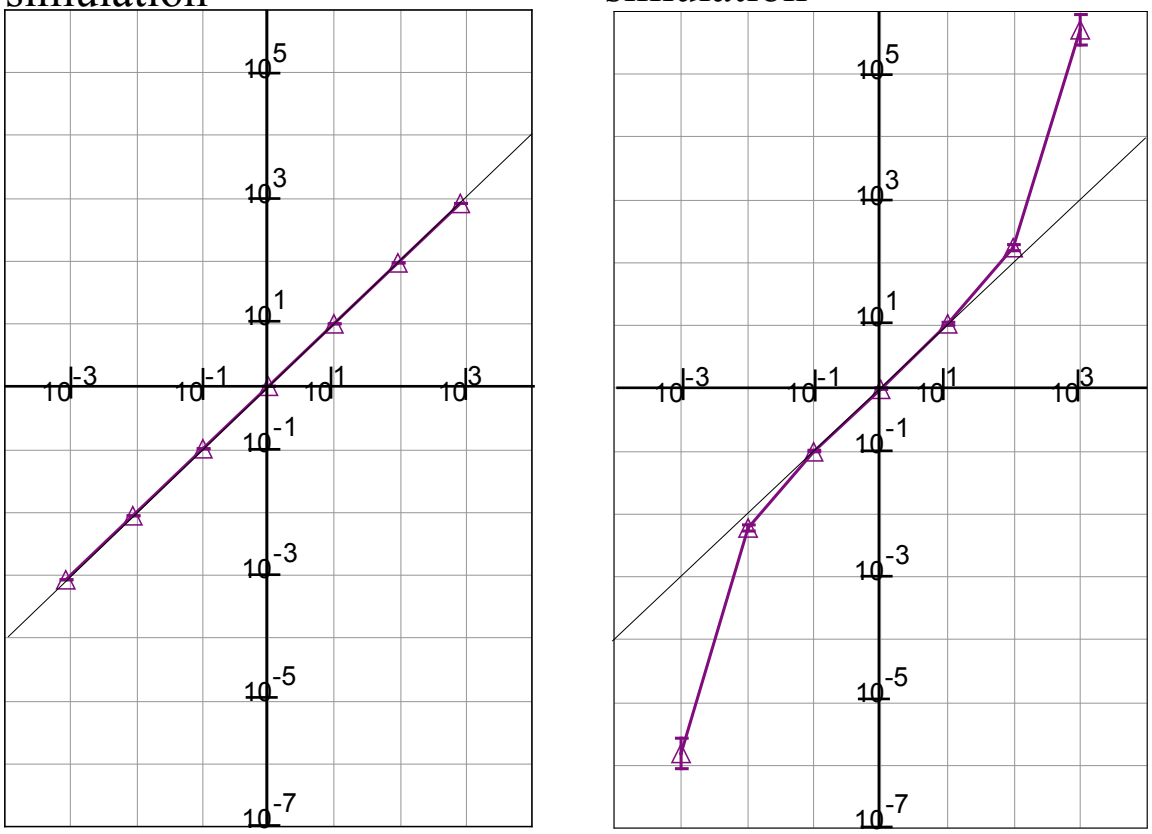
(a) with spatial structure, experiment

(b) without spatial structure, experiment



(c) with spatial structure, simulation

(d) without spatial structure, simulation



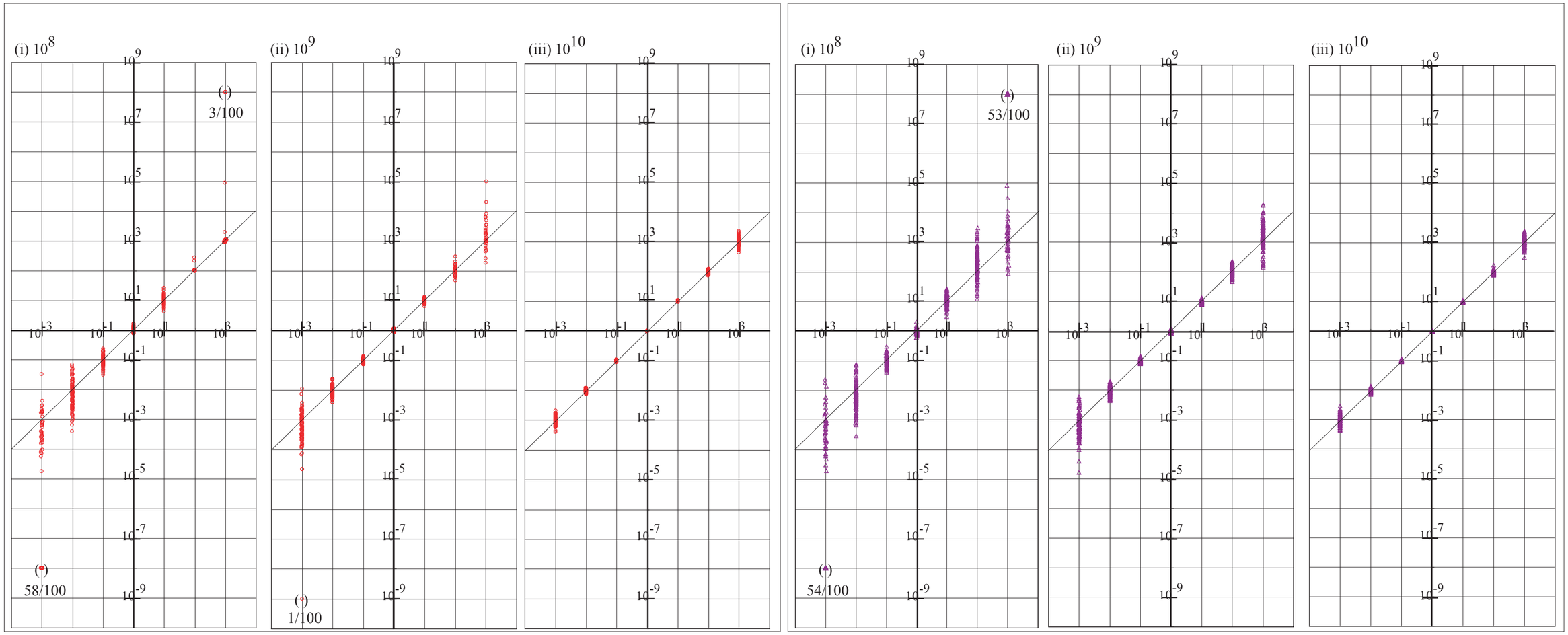
after incubation [A = altruistic host] / [S = non-altruistic host]

initial [A = altruistic host] / [S = non-altruistic host]

Figure S1

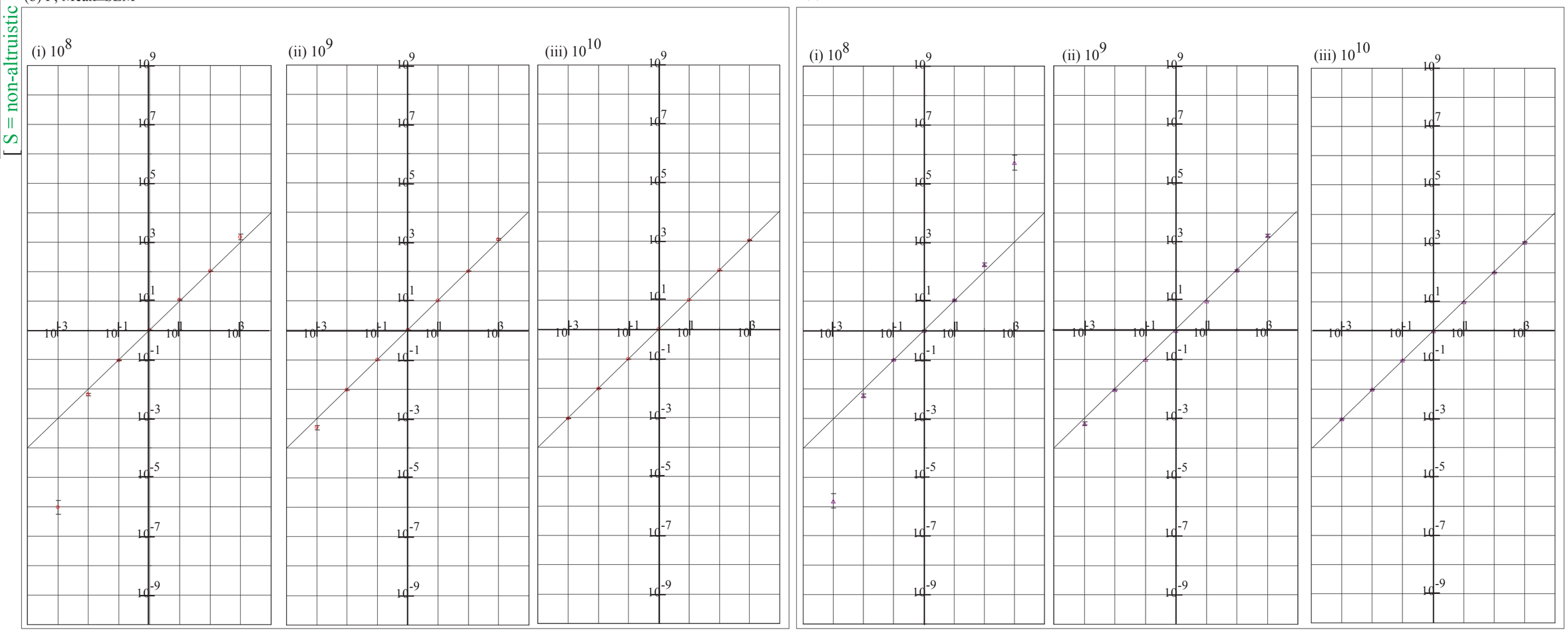
(a) P; raw data

(c) Q; raw data



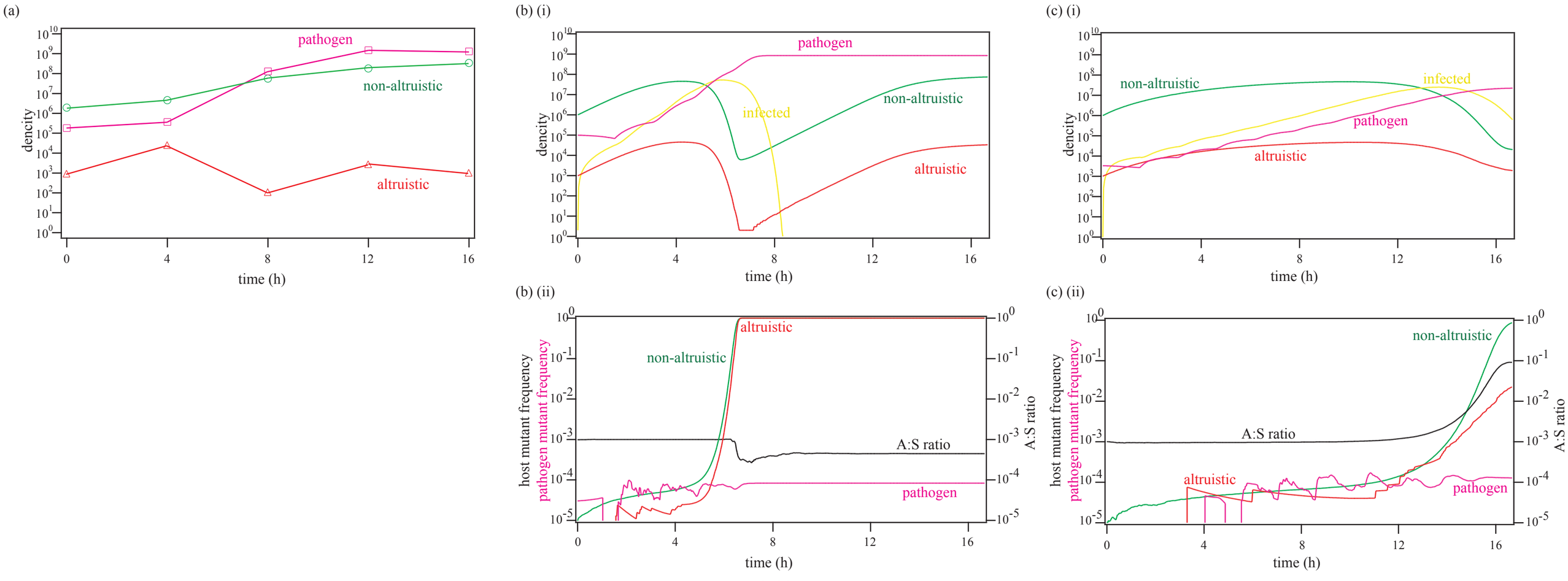
(b) P; Mean±SEM

(d) Q; Mean±SEM



$$\text{initial } \frac{[\text{ A = altruistichost }]}{[\text{ S = non-altruistic host }]} \text{ after incubation}$$

FigS2


(a)
pathogen
non-altruistic
altruistic
dencity
time (h)
(b) (i)
pathogen
infected
non-altruistic
altruistic
dencity
time (h)
(b) (ii)
altruistic
non-altruistic
A:S ratio
pathogen
host mutant frequency
pathogen mutant frequency
A:S ratio
time (h)
(c) (i)
non-altruistic
infected
pathogen
altruistic
dencity
time (h)
(c) (ii)
non-altruistic
A:S ratio
altruistic
pathogen
host mutant frequency
pathogen mutant frequency
A:S ratio
time (h)

FigS3

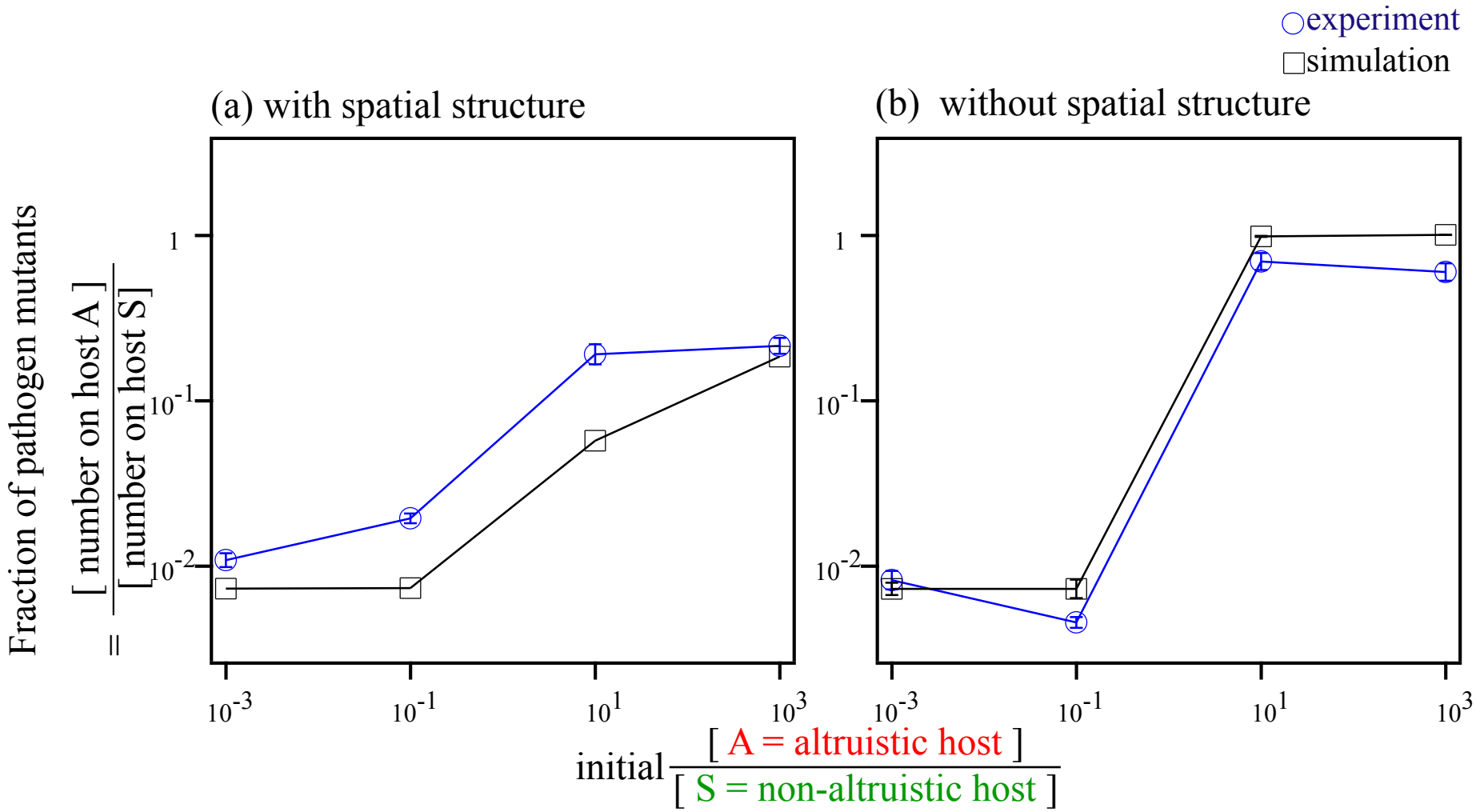

experiment
simulation
(a) with spatial structure
(b) without spatial structure
Fraction of pathogen mutants
= [number on host A] / [number on host S]
1
10^-1
10^-2
10^-3
10^-1
10^1
10^3
initial [A = altruistic host] / [S = non-altruistic host]

Fig. S4

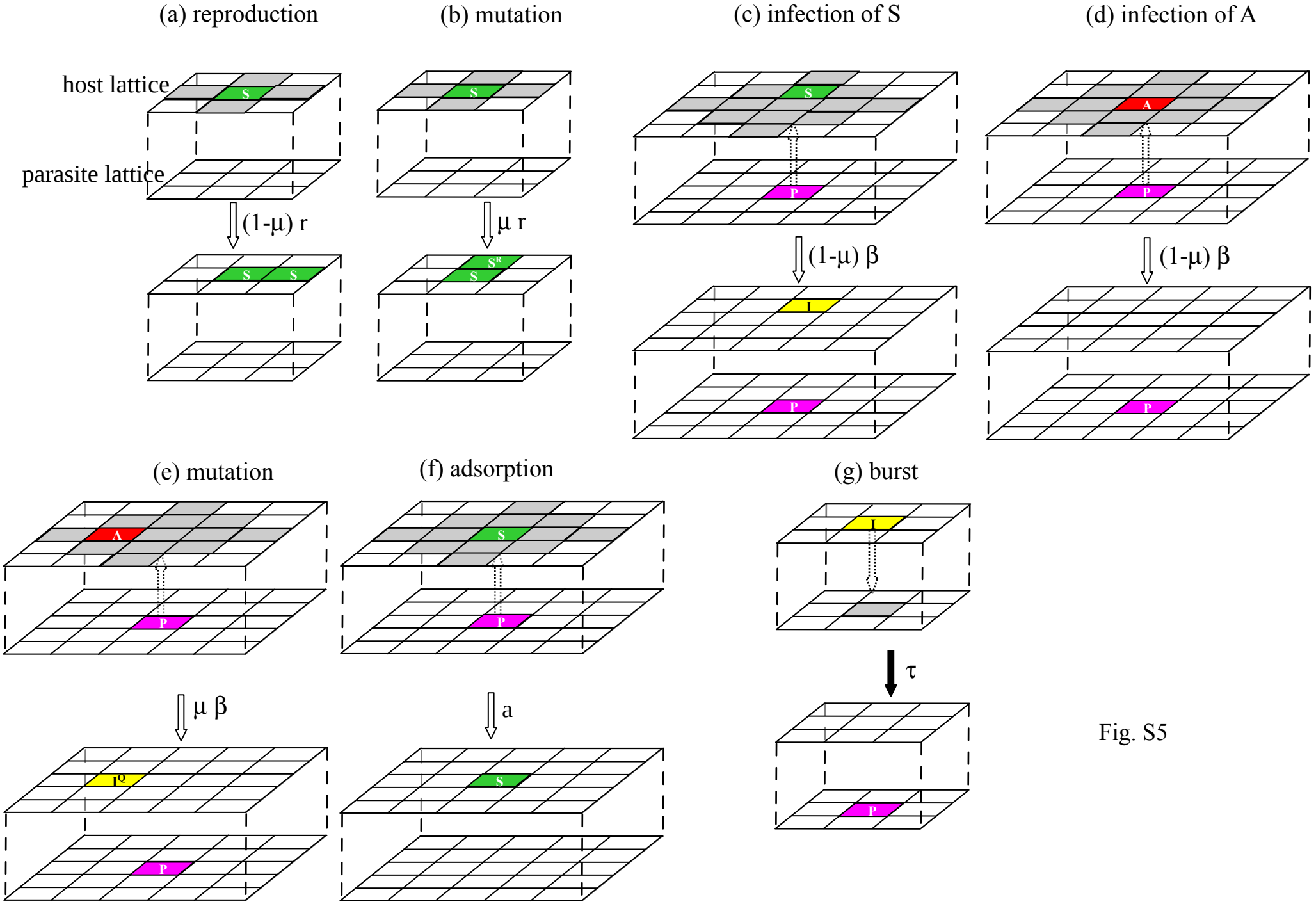

(a) reproduction
(b) mutation
(c) infection of S
(d) infection of A
host lattice
parasite lattice
S
A
P
I
(1-μ) r
μ r
(1-μ) β
(e) mutation
(f) adsorption
(g) burst
μ β
a
τ
I^Q
S^R

Fig. S5

Table S1. Pathogen mutants.

Tube number	Isolate number	Nucleotide position	Codon change	Amino acid change
1	3	186	AAA -> AAAa	K62frameshift
	4	371	GGT -> GtT	G124V
	5	186	AAA -> AAAa	K62frameshift
	6	371	GGT -> GtT	G124V
2	7	923	TTA -> T-A	L308frameshift
	8	923	TTA -> T-A	L308frameshift
	9	425	CCT -> CaT	P142H
	9	858	GAA -> GA-	E286frameshift
	10	425	CCT -> CaT	P142H
3	11	434	TGG -> TtG	W145L
	12	118	GAA -> tAA	E40stop
	13	823	GGC -> tGC	G275C
	14	118	GAA -> tAA	E40stop
4	15	187	GAA -> aGAA	E63frameshift
	15	923	TTA -> TT-	L308frameshift
	16	923	TTA -> TTtA	L308frameshift
	17	187	GAA -> aGAA	E63frameshift
	18	187	GAA -> aGAA	E63frameshift

Table S2. Bacteria, phages and plasmids

Symbol	Bacterial strain	Genotype	Comments	Source and reference
	JC8679	F$^-$ *thr-1 leuB-6 phi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44 recB21 recC22 sbcA23 his-328* λ$^-$		A. J. Clark, (22)
	JW5871	BW25113 *mcrB::kan*		National Institute of Genetics, (23)
	MG1655	F$^-$ lambda$^-$ *ilvG*$^-$ *rfb* -50 *rph* -1		D. Biek, (24)
	BIK2146	JC8679 *lacZ::cml*		N. Handa, (25)
	BMF1	JC8679 *mcrB::kan*	P1 from JW5871 to JC8679	this work
A	BMF3	MG1655 *lacZ::cml*	P1 from BIK2146 to MG1655	this work
S	BMF5	MG1655 *mcrB::kan*	P1 from JW5871 to MG1655	this work
	BMF22	BMF3 carrying pMF8		this work
	BMF24	BMF5 carrying pMF8		this work
	BMF28	BMF5 carrying pMF9		this work
	BMF30	BMF3 carrying pMF9		this work
	BMF35	BMF5 carrying pYNEC323		this work
	BMF37	BMF5 carrying pYNEC324		this work

Symbol	Phage strain	Genotype	Source and reference
P	LEF1	Bam1^0 ΔB *int*$^-$ *pvuIIM* Δ(*red-gam*) *imm21 nin5 shn6*^{0}	E. Fukuda, (7)
Q	LIK891	Bam1^0 ΔB *int*+ Δ(*red-gam*) *imm21 nin5 shn6*^{0}	N. Handa, (26)
	λvir		A.Miura, (21)
	P1 vir		H. Ikeda, (27)

Plasmids	Relevant characteristics	Prototype plasmid	Drug resistance	Source and reference
pYNEC323	*pvuIIRMC cml*	pIK153	Cml	Y. Nakayama, (28)
pYNEC324	*pvuIIR*$^-$*MC cml*	pIK153	Cml	Y. Nakayama, (28)
pMF8	*mrfp*	pUC19	Amp	this work
pMF9	*gfpuv*	pUC19	Amp	this work

kan, kanamycin-resistance gene; *cml*, chloramphenicol-resistance gene.

Table S3. Symbols.

Symbol	Definition	
Host		
S	Susceptible; Non-altruistic type	
A	Altruistic type	
I	Infected form of S by P	
S^R	Mutant form of S resistant to pathogen	
A^R	Mutant form of A resistant to pathogen	
I^Q	Infected form of S by Q	
Pathogen		
P	Induces suicide in S host	
Q	Mutant defective in induction of suicide	
Host lattice		
O-site	Empty	
S-site	Occupied by non-altruistic host	
S^R-site	Occupied by resistant non-altruistic host	
I-site	Occupied by infected host	
I^Q-site	Occupied by mutant infected host	
A-site	Occupied by altruistic host	
A^R-site	Occupied by resistant altruistic host	
Pathogen lattice		
O-site	Empty	
P-site	Occupied by pathogen	
Q-site	Occupied by mutant pathoger	
invariable		Value
z_r	Number of nearest-neighbors for reproduction	4
Z_d	Number of d (=7) step neighbor sites for infection and adsorption	113
r	Growth rate of host	0.023 min^{-1}
β	Infection rate of pathogen	47.5 s^{-1}
τ	Lysis time of infected hos	60 min (30)
μ	Mutaion rate of host and pathogen	1.00E-5
a	Adsorption rate of lambda phage	1.58 s^{-1}
d	Infection radius	7
b	Burst size of pathogen	30 (29)