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Rule-based modelling of conjugative plasmid transfer and incompatibility

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Abstract

COSMIC-rules, an individual-based model for bacterial adaptation and evolution, has been used to study virtual transmission of plasmids within bacterial populations, in an environment varying between supportive and inhibitory. The simulations demonstrate spread of antibiotic resistance (R) plasmids, both compatible and incompatible, by the bacterial gene transfer process of conjugation. This paper describes the behaviour of virtual plasmids, their modes of exchange within bacterial populations and the impact of antibiotics, together with the rules governing plasmid transfer. Three case studies are examined: transfer of an R plasmid within an antibiotic–susceptible population, transfer of two incompatible R plasmids and transfer of two compatible R plasmids. R plasmid transfer confers antibiotic resistance on recipients. For incompatible plasmids, one or other plasmid could be maintained in bacterial cells and only that portion of the population acquiring the appropriate plasmid-encoded resistance survives exposure to the antibiotics. By contrast, the compatible plasmids transfer and mix freely within the bacterial population that survives in its entirety in the presence of the antibiotics. These studies are intended to inform models for examining adaptive evolution in bacteria. They provide proof of principle in simple systems as a platform for predicting the behaviour of bacterial populations in more complex situations, for example in response to changing environments or in multi-species bacterial assemblages.

Keywords: Plasmid; Bacteria; Antibiotic; Incompatibility; Horizontal gene transfer; Individual-based model

1. Introduction

Horizontal gene transfer is a crucial driving force in bacterial adaptation and evolution. The three naturally occurring bacterial gene transfer processes, conjugation, transduction and transformation, all contribute to the spread of genes within bacterial populations (Ochman et al., 2000; Frost et al., 2005; Sørensen et al., 2005). Such genetic interplay can lead to the acquisition of new traits, that may in turn confer selection benefits on their hosts for survival in changing environments (Waters, 1999; Barkay and Smets, 2005).

Conjugation has evolved as a process for gene transfer mediated by certain plasmids and transposable elements. Plasmids are extrachromosomal elements that specify mechanisms controlling their own replication and maintenance, and are ubiquitous in bacteria. They can be either conjugative, encoding mechanisms for self-transfer by conjugation, or non-conjugative and hence incapable of initiating conjugation for self-transmission. In addition, plasmids are assigned to incompatibility

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(Inc) groups, depending on their ability to co-exist in the same cell line (Novick, 1987; Actis et al., 1998). Incompatible plasmids belong to the same incompatibility group. They have related replication control mechanisms and fail to co-exist. By contrast, compatible plasmids are from different incompatibility groups. Such plasmids replicate independently of each other, having different control mechanisms and will co-exist in the same cell. Many naturally occurring plasmids are mosaics comprising multiple replicons that have complex incompatability patterns (Osborn et al., 2000), but for the purposes of this study each model plasmid is deemed to have a single incompatability determinant.

Plasmids carry genes for a wide range of functions, including resistance to antimicrobial agents, metabolism of novel carbon sources and virulence. Antibiotic resistance (R) plasmids have a central role in the dissemination of antibiotic resistance and are largely responsible for the rapid emergence of multiple antibiotic resistant bacteria, especially in the hospital environment (Hawkey and Munday, 2004). Generally plasmid-encoded genes are dispensable to their hosts under most conditions; antibiotic resistance being strictly required only when the bacteria are challenged by a specific antibiotic(s). However, the continued presence of antibiotic resistance in the bacterial population would be advantageous against the possibility of future antibiotic exposure. Thus, although maintaining plasmids incurs fitness costs, both genetic and energetic, such genetic elements confer selective advantages on their hosts, particularly in promoting responses to changes in the local environment (Dahlberg and Chao, 2003). Conjugative plasmid transfer thus promotes the spread of beneficial genes within bacterial populations and is a source of variation for adaptive evolution. However, such transfer is limited by various barriers, including incompatibility with resident plasmids. Failure of incompatible plasmids to co-exist can restrict their spread, particularly where the density of a specific plasmid is high within local populations.

The purpose of this study is to develop and validate an individual-based model (IbM), COSMIC-rules (see Gregory et al., 2006, 2007), to simulate plasmid transfer events and incompatibility in bacterial populations. The IbM is used here to develop biologically-realistic simulations of plasmid transfer with predictive potential, for example in examining the spread of antibiotic resistance in clinically-important bacteria. Having validated such simulations using a limited number of parameters, more realistic models reflecting the complexity of natural bacterial populations in their habitats and with predictive value can be addressed. The overall objective is to develop and expand, by using larger-scale Grid technology, the capacity of COSMIC-rules to embrace modelling of complex bacterial genetic systems. In this way the role of horizontal gene transfer in effecting genetic innovations and influencing the behaviour of bacterial populations in changing environments could be explored.

COSMIC-rules models three levels: the genome, the bacterial cell and the environment (for details see Gregory et al., 2007). The genome comprises genes or sets of genes, each represented by a discrete bit string. The environment consists of a multiplicity of substances, including nutrients and antimicrobial agents, into which the bacterial populations are placed. Each bacterial cell is an individual and for individuals to interact there must be compatible pairing of gene types; valid pairings create a successful outcome. In this virtual world each bacterial cell may be subject to mutations and/or genome rearrangements mediated by mobile genetic elements. Such mechanisms contribute to bacterial variation and allow adaptation to a changing environment. COSMIC-rules can create changes in the local environment, e.g. by introducing bacteriocidal antibiotics, in turn generating isolated ecologies where R plasmid-free (antibiotic–susceptible) and R plasmid-containing (antibiotic–resistant) cells can compete for limited resources. The simulations described here use virtual conjugative R plasmids from the same and different incompatibility groups. Spread of the R plasmids is highlighted through displaying infected bacteria as coloured cells and through an environment exposed to antibiotics that provide selection pressures to monitor plasmid dissemination and maintenance.

2. Cell Interactions and R Plasmid Transfer by Conjugation

The genetic makeup (genome) of individual organisms dictates the susceptibility or resistance to substances in the environment. Fig. 1 provides the basic bacterial genome structure and environmental interactions applicable to the conjugative plasmid transfer events described here. Individual bacteria have their own genome that encodes all the functions required for growth and metabolism. The genome comprises the chromosome and any extrachromosomal elements, e.g. plasmids, residing in the cell. The chromosome of donor and recipient cells is isogenic, with two ‘susceptibility’ gene sets that can encode sensitivity to antibiotics A and B, respectively. The donor cell additionally carries a conjugative R plasmid, e.g. α or β, with genes for replication, conjugation and antibiotic resistance. The recipient is plasmid-free or could harbour a com-

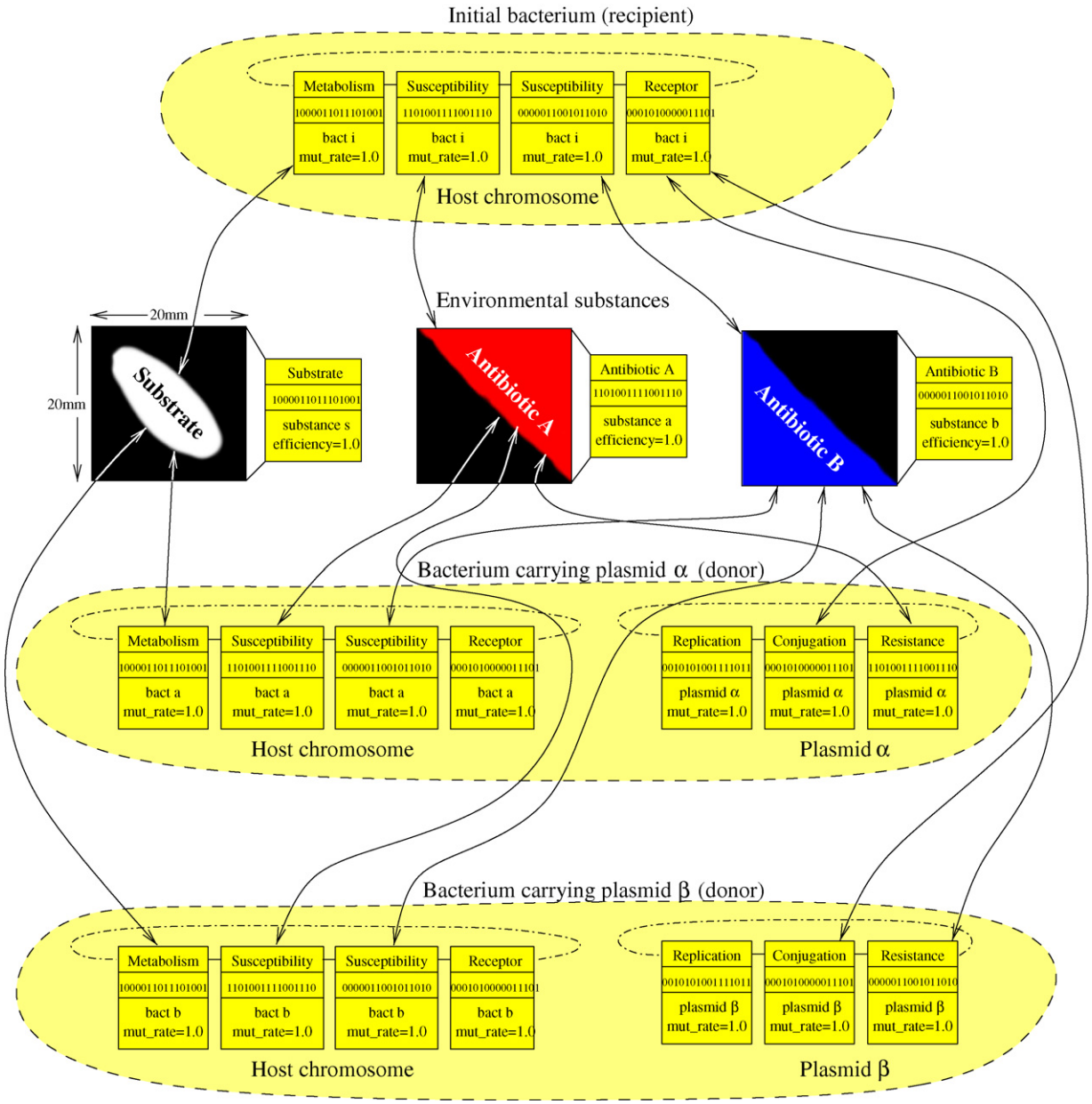

Fig. 1. Genome structure and environmental interactions. The genome of plasmid-free and plasmid-containing bacteria, together with the environmental substances are shown. The connecting arrows show possible interactions. The substrate is shown at the start of a typical simulation (time 0 min). Antibiotic zones for antibiotic A (red) and B (blue) are shown for a typical simulation. Genes/gene sets consist of two main components, a keyword describing the class of interaction, and a bit string that determines specificity. The two susceptibility genes in each bacterium refer to susceptibility to antibiotics A and B, respectively. Additional information such as substance effectiveness (efficiency), or gene-specific mutation rate (mut_rate) can be associated with bit strings (defined as a coefficient of the base rate), creating globally less effective substrates or antibiotics, or creating mutation hot spots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

patible plasmid(s). A collection of tagged bit strings ("tags") (see Gregory et al. (2007)) make up the functional genome. Tags are abstract genes, a tag consists of a bit string and an assigned category or type. The category defines which other tags this tag can interact with. The bit string enforces further specificity by requiring interacting tags to have a similar bit string. We generally consider similar to be up to one bit different to be a match, however

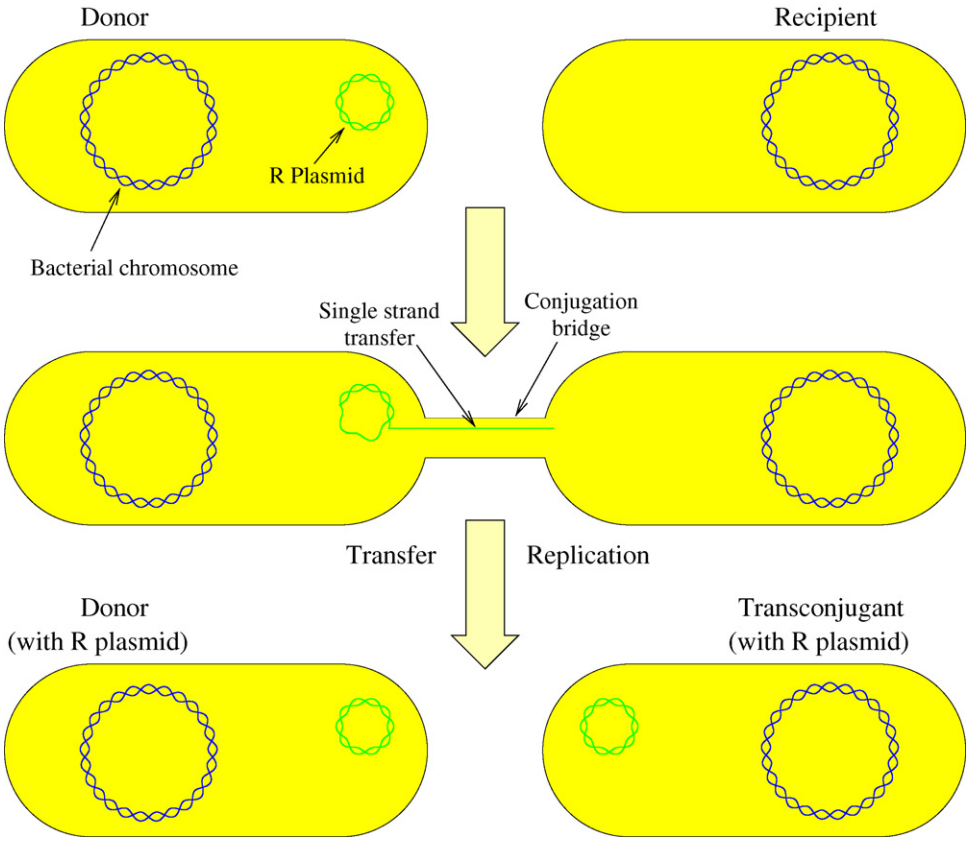

Fig. 2. Diagrammatic representation of the process of bacterial conjugation.

in some circumstances (e.g. incompatibility and superinfection immunity), up to two bits difference is used. Matching is based on counting the number of bits that are different. The two bit strings are exclusive-OR'ed and the number of bits in the result is counted. Each set of tags makes it possible to match particular genes or gene products with other genes or consequences of the expression of individual genes. Only those tags under consideration for R plasmid transfer are shown. Any other necessary functions are assumed, but not displayed. Cell interactions with other cells (e.g. donor–recipient interactions) and with substances (e.g. growth substrates, antibiotics) in the environment involve matching of the bit strings. Possible interactions are shown by the connecting arrows in Fig. 1.

Plasmid transfer by conjugation requires cell interactions of a donor and recipient (Thomas and Nielsen, 2005). For Gram-negative bacteria the sex pilus, encoded by the conjugative plasmid carried by the donor, contacts an appropriate recipient. Retraction of the pilus brings the cells closer together to create a conjugation bridge for transfer of the plasmid from donor to recipient. Conjugative DNA synthesis generates a copy of the R plasmid in both donor and recipient (Fig. 2). The recipient acquiring the plasmid is termed a transconjugant. Donor and transconjugant can now donate plasmids to new recipients in a further round of mating and both are resistant to the antibiotic(s) specified by the R plasmid (Kohiyama et al., 2003). In the simulations the donor–recipient interactions for plasmid transfer involve bit string matching between a conjugation ligand, encoded by the plasmid in the donor, and a cognate receptor on the recipient (see Fig. 3). Bit string matching can be used to create specificity for the interaction, allowing productive interaction on the one hand (successful contact for conjugation) or preventing interaction on the other (mimicking the phenomenon of surface exclusion). Conjugation and plasmid transmission will not occur if the receptor bit string of the recipient is mutated to be sufficiently different to the ligand bit string of the donor so as not to match (more than one bit different). Matching between substance (antibiotic) and susceptibility bit strings determines antibiotic action and matching between substance (antibiotic) and resistance bit strings determines resistance, in what would otherwise be a sensitive population. Where the recipient in the mating already carries an

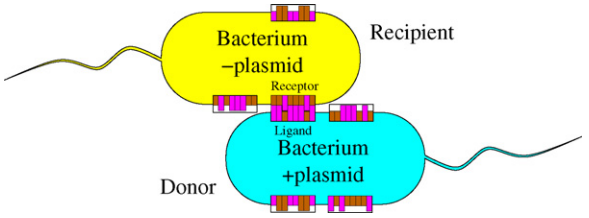

Fig. 3. Conceptual view of ligand–receptor interaction in plasmid transmission. The donor (blue cell) contains a conjugative plasmid, which encodes a conjugation ligand (sex pilus) specific to the receptor carried by the recipient bacterium (yellow cell). Contact between donor and recipient involves interaction of the ligand and receptor. The key like boxes are analogous to the bit strings in our tags, except that this analogy matches its inverse. For successful conjugation to occur, the donor must have a ligand key that fits the receptor key of the recipient. In reality, the donor would also pull the recipient sufficiently near to achieve direct contact and permit conjugative transfer. However this detail is not modelled. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

incompatible plasmid, the incoming plasmid will be eliminated from the host, due to failure to replicate. For compatible replicons both plasmids will be maintained in the recipient. Each plasmid carries a specific replication tag to denote the plasmid species. A plasmid will not be maintained in a recipient if it already carries a plasmid with the same replication tag, i.e. an incompatible plasmid. However, both plasmids will be maintained where the replication tags are different, i.e. for compatible plasmids (see Section 3.1, rule 5).

The process of conjugation thus effects the transmission of R plasmids and the passing on of antibiotic resistance, and potentially the ability to metabolise other substrates, within bacterial populations.

3. Rules for Plasmid Transfer

Rules are a critical component of COSMIC-rules and define conditions and outcomes when bacteria or their genomes interact with the environment or other bacteria (Gregory et al., 2007). Rules are used to provide the likelihood of a quantitative outcome for interactions between individual bacteria and other bacteria, plasmids, phages and/or their environment. The rules define the possible interactions between particular phenotypes and environmental conditions and define quantitative responses or probabilities of potential outcomes. Rules are applied to and not part of the genome, the bacteria or the environment. They are informed by the principles of bacterial genetics and simulation parameters are biologically realistic values determined experimentally in laboratory and microcosm studies of conjugative R plasmid transfer (Shaw and Cabelli, 1980; Freter et al., 1983; Licht et al., 1999; Hayes, 2003; Sørensen et al., 2005). Thus all the time values described here represent biological time. The implemented rules use these same parameters.

For plasmid transfer the following rules apply.

3.1. Horizontal Transmission of Plasmids

(1) Cell movement uses a weighted random walk (Berg, 2000). Cell position consists of two floating point numbers, x and y. For each time step, the position is changed every second by up to 2.5 μm. This is much slower than *E. coli* typically swims in a liquid medium, but has been implemented here in order to enhance and stabilise the patterns of plasmid spread. The direction of travel is random and Gaussian weighted to favour the existing direction. Cell position in the environment is thus non-discrete, but cell movement occurs once every second in instantaneous jumps from old to new position. A two-dimensional array of floating point numbers records substance concentration for each defined substance, consequently, each non-discrete cell location maps to a discrete local substance concentration (see Table 1).

(2) Conjugal contact and transfer of plasmids between donors and recipients only occurs when (i) the participating cells come within 12 μm (10 × the cell dimension) of each other; (ii) the recipient receptor tag is no more than one bit different to the plasmid

Table 1
Simulation parameters

Parameter description	Parameter	Unit/domain
Default world size	0.02	m
Initial bacterial population	4000	Individuals
Peak number of bacteria	670,000	Individuals
Environment space	3D	Topology
Substance concentration	2D array	Floating point
Floating point numbers per dimension of array	512	4 byte floats
Mutation rate	10^{-8}	Per bit per tag per second
Simulation step size (iteration)	1	s
Generation time (shortest)	20	min
	1200	Iterations
Visualisation step size	100	s
Maximum cell movement per time step	25	μm
Maximum distance for plasmid transfer	12	μm
Default allowed bit difference	1	Bit
Incompatibility allowed bit difference	2	Bits

conjugation ligand tag; (iii) the donor has a replication tag more than two bits different to any existing replication tags in the recipient. The maximum distance condition is to mimic the proximity needed for sex pilus contact and mediation of cell-to-cell contact following pilus retraction. Transfer depends on random encounters related to the density of donors (or transconjugants) and recipients. To account for the stepwise movement of cells, the proximity is the smallest distance between cells when moving from their old to current positions. That is, for every pair of potentially interacting individuals, it is assumed that cells travel from their previous to their current positions at a constant speed. With this assumption, it is possible to calculate the closest distance that cells ever come to each other. This distance must be within 12 μm. The second condition ensures the recipient is a compatible species for the donor, the third condition stops multiple copies of the same plasmid entering the cell. Increasing the similarity threshold to two bits (rather than the one bit used for most other tag comparisons) ensures that new potential plasmid "species" are created at biologically realistic rates (see also rule 5).

(3) Under normal steady state circumstances (for exception see rule 4) transfer occurs at a frequency of 1×10^{-3} per cell (i.e. on average only 1 in 1000 cells will conjugate).

(4) In newly infected recipients, the transfer frequency goes up to 1 (100% of newly infected cells can transfer) for the period of one or two generation times after infection; the generation time (time between cell divisions) being around 20 min for the bacterial population, under optimal nutritional conditions (see Table 1 and Gregory et al., 2007). The higher frequency applies to a single recipient cell and to both daughter cells, where the bacterium acquires the plasmid in the middle of its division cycle, until the middle of the cycle of the two daughters derived from it. This mimics the transient epidemic spread phenomenon experienced with conjugative plasmids (Ghigo, 2001). After about a generation the system returns to the steady-state, with a frequency of 1×10^{-3} per cell. Generation time is generally used to describe an average over the whole population. In reality and for COSMIC-rules, generation time varies according to local substrate concentration and the resulting localised growth rate.

(5) All plasmid-carrying cells are unable to act as recipients for a second copy of that plasmid from the same or another donor, due to mechanisms of surface exclusion and incompatibility. In order to avoid the problem of undue sensitivity that would be caused by simple point mutations effectively creating new plasmid species, the threshold for bit string matching has been modified for the simulation of incompatibility. Thus plasmids are incompatible if their Replication bit strings match exactly or are up to two bits different (as opposed to the normal condition of up to only one bit different). In the model, any plasmid, whether a mutant of the original or an unrelated plasmid introduced into the cell, will be compatible if the bit strings differ by more than two bits.

(6) The conjugative transfer of the plasmid is instantaneous.

(7) Having donated the plasmid, a donor cell requires a "recovery period" of about 10 min before it can re-donate the plasmid to a second recipient.

3.2. Vertical Transmission of Plasmids

(1) The normal complement of copies of the R plasmid is 2 at cell division. For vertical transmission there is faithful partition, in which each daughter receives one copy at division and this replicates inside the cell to produce two copies at the end of the cycle and so on. There is stable plasmid inheritance, with no loss of plasmids at division.

3.3. Cost Rules for Plasmid Maintenance

(1) Plasmid-carrying cells suffer a 1% reduction in growth rate at wild-type copy number, i.e. generation time is increased by 1%.

3.4. Benefit Rules for Plasmid Maintenance

(1) The individual bacterial host is rendered resistant to the antibiotic(s) or other toxic substance encoded by the plasmid carried.

3.5. Experimental Set-up

The simulation runs on a cluster of 16 dual Xeon 2.4 GHz machines with gigabit networking. It has been largely written in C++, using the Standard Template Library and Gnu Scientific Library. Most interprocess communication is achieved using the Parallel Virtual Machine library, bash and perl scripts provide visualisation generation, state saving and runtime optimisation.

The simulation partitions the world into 256 equally sized areas called demes. Each deme is a UNIX process and so deme to machine mapping can be varied

to ensure efficient load balance across the cluster. Each deme contains individual bacteria that move, and can interact with other bacteria. For the duration of a time step, the deme is isolated from the other demes. At the end of a time step (one simulated second), individuals move and that movement could place them in another deme. A master process ensures that cross deme migration is fair, repeatable and efficient. The master process only provides synchronisation and book keeping, deme migration occurs directly between demes.

Considering demes in isolation makes the simulation computable as it removes the need for cell to cell synchronisation between demes. Exact reproducibility depends on having the same number of demes. As with changing the seed (see Section 4.4), changing the number of demes changes the deme boundaries, subtly changing the course of events but reproducing the same overall events.

Typical simulation parameters are shown in Table 1.

4. Simulations of Conjugative Plasmid Transfer

In the following simulations no allowance is made for the release of nutrients from any dead or dying bacteria.

4.1. Plasmid Transfer and Antibiotic Resistance

This simulation demonstrates the spread of a conjugative R plasmid (designated β) through a plasmid-free, antibiotic-sensitive bacterial population. The environment (Fig. 4) contains a growth substrate and a bacteriocidal antibiotic, to which the plasmid specifies resistance.

Fig. 5 (a) provides snapshots of events during the simulation. The substrate is spread along the diagonal of the environment, shown as a white area in panel 1 (Fig. 5(a)), with antibiotic B added as a circular source from the lower right-hand side to the centre (as shown in Fig. 4). Diffusion of the antibiotic is not represented in the simulation. The environment is initially inoculated with the plasmid-free, antibiotic-sensitive bacteria and, as the population grows, the white area fades to black, due to the bacteria (in yellow) consuming the substrate. The bacteria are initially placed randomly throughout the whole environment, but as the simulation progresses they tend to occupy the substrate rich area. No bacteria survive in the region containing the antibiotic, since they are susceptible to its action (Fig. 5(a), panel 2). In this area the concentration of substrate is preserved at its initial level. At time 333:20 min, an inoculum of resistant bacteria carrying the conjugative R plasmid β (encoding

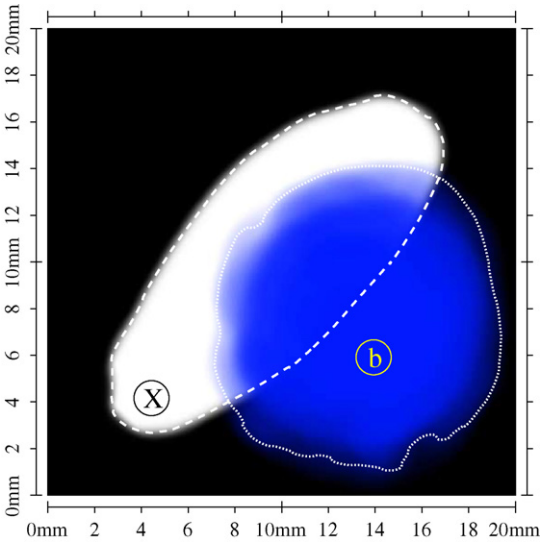

Fig. 4. Substrate and antibiotic zones in the environment for simulation in Fig. 5. An antibiotic B (in blue, with dotted white outline) covers a circular area (b) of the environment, this intersects with an area of substrate (in white, with dashed white outline), stopping antibiotic-sensitive bacteria from growing in this area of overlap. Antibiotic-sensitive bacteria are introduced randomly over the whole environment. X denotes the area where R plasmid carrying bacteria resistant to antibiotic B are introduced.

resistance to antibiotic B), is added to the bottom left of the population (Fig. 5(a), panel 3) at X (as in Fig. 4). Plasmid transfer occurs by conjugation, with the blue coloured bacteria carrying plasmid β. Such bacteria are resistant to antibiotic B and begin to colonise the region containing the antibiotic (Fig. 5(a), panel 4). With growth of plasmid-containing cells and further conjugal plasmid transfer the resistant bacteria with plasmid β gradually spread, consuming the substrate and colonising the entire area (panels 5–8, Fig. 5(a)). Fig. 5(b) summarises Fig. 5(a) showing a rise in the plasmid-free bacterial population from time 0 to around 333 min to a population density of 20,000 cells. At time 333:20 min, 200 R plasmid (β)-containing bacteria are added to the bottom left of the plasmid-free population. Plasmid transfer and cell growth result in an increase in the plasmid-containing cells that can survive in the presence of antibiotic B and by 600 min there are only 80 plasmid-free cells remaining. These could include variants unable to participate in conjugation due e.g. to a defective receptor on the recipient caused by mutation events.

4.2. Transfer of Incompatible Plasmids

This simulation demonstrates the spread of two conjugative R plasmids (α and β), from the same

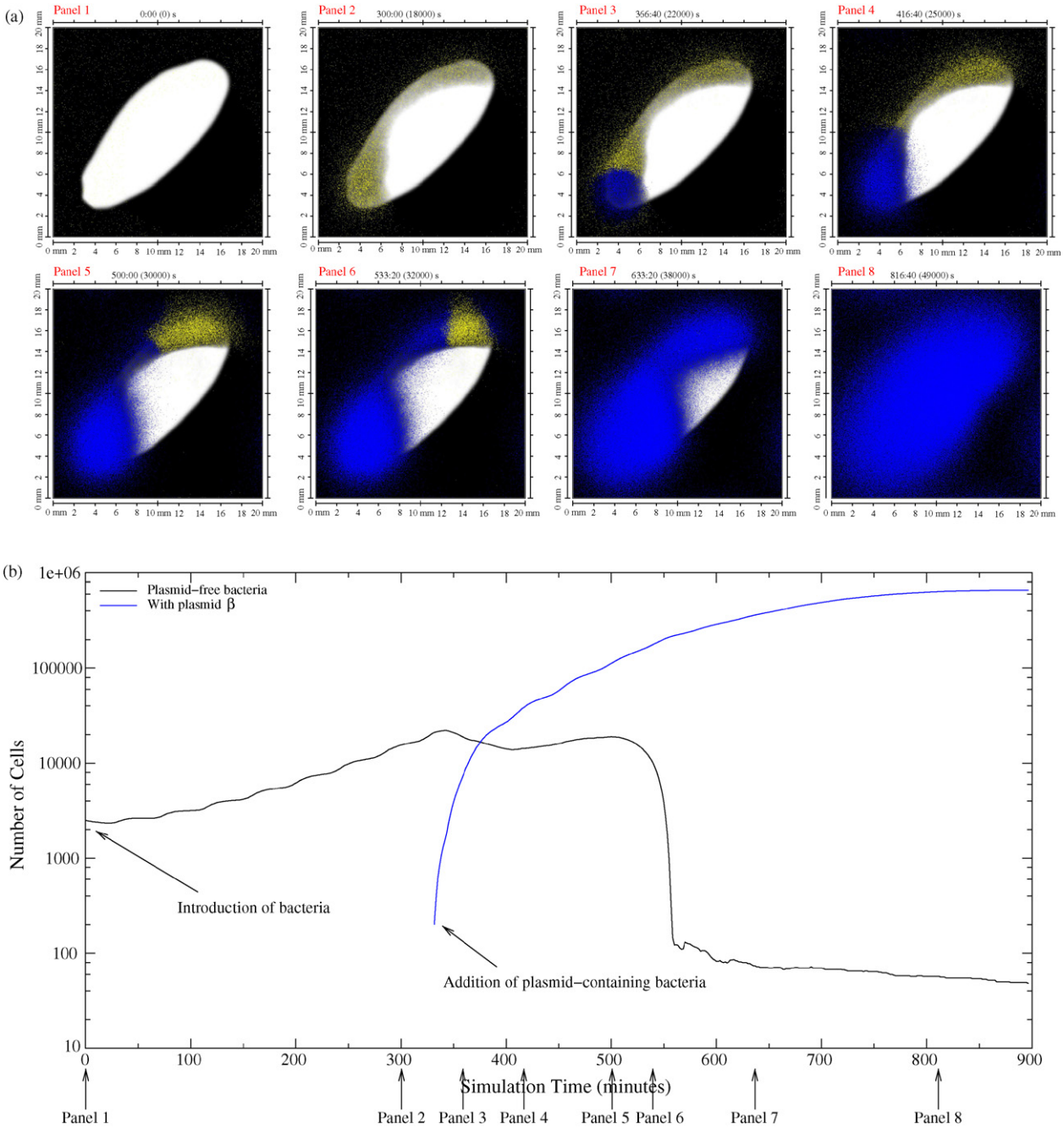

Fig. 5. Conjugative plasmid transfer. (a) Panels 1–8 show key moments in the simulation of plasmid spread: the growth of the initial population around the antibiotic (panels 1 and 2), and the addition of bacteria with antibiotic resistance conferred by plasmid β and plasmid transfer (panels 3–8). (b) Graphical summary of panels showing an exponential rise of the bacterial population, followed by the rapid spread of plasmid infection throughout the bacterial population.

incompatibility group, within a plasmid-free, antibiotic-sensitive bacterial population. The plasmids encode resistance to different antibiotics and have mutually incompatible modes of infection. Plasmid transfer and maintenance is detected and confirmed by exposure to the appropriate antibiotics.

Fig. 6 is a colourised version of the substrate and antibiotic zones present in the environment during the

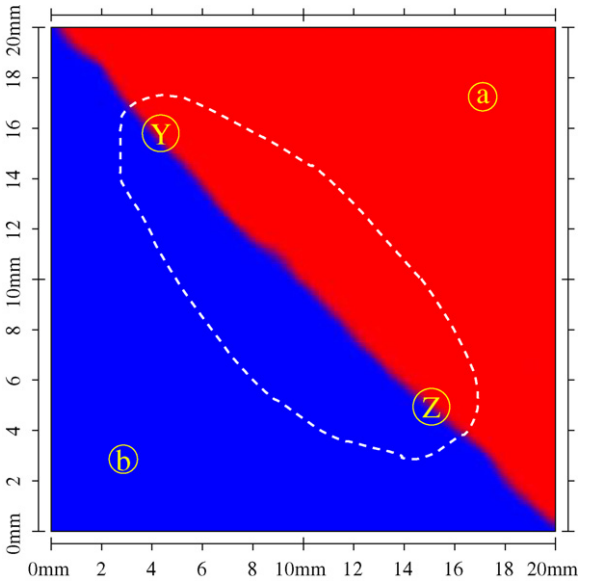

Fig. 6. Substrate and antibiotic zones in the environment for simulations in Figs. 7 and 8. From time 583:20 min, two antibiotics (A (in red) and B (in blue)) cover the whole environment with no overlap. a and b represent regions where antibiotics A and B are introduced, respectively; Y and Z, areas where R plasmid carrying bacteria are introduced. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

simulations depicted in Fig. 7(a) and Fig. 8(a). The growth substrate (outlined with a white dashed line) is spread along a diagonal of the environment. 583:20 min into the simulation, two types of bacteriocidal antibiotic, A and B, are added at 'a' and 'b', respectively, each covering half the environment, with no overlap between them. (This simplifies the system by avoiding the simulation burden of diffusion.) Antibiotic A is shown in red, antibiotic B in blue. For bacteria to continue living in these regions they must be resistant to the appropriate antibiotic. Fig. 7(a) provides snapshots of the events that occur in the simulation when the plasmid-free and antibiotic–susceptible bacterial population is mixed with two inocula of resistant bacteria carrying distinct incompatible, conjugative R plasmids (α and β) encoding resistance to antibiotics A and B, respectively, and then exposed to the antibiotics. Panels 1–3 (Fig. 7(a)) show the plasmid-free bacterial population growing on substrate alone, which is depicted as a dashed white outline in Fig. 6. As the population grows the white area fades to black due to the bacteria (in yellow) consuming the substrate. At time 333:20 min, the two inocula of resistant bacteria, each carrying one of the incompatible R plasmids, are added (200 of each) to the opposite ends of the growing population (Fig. 7(a), panel 4). Bacteria with plasmid α at Y and with plasmid β at Z (as in Fig. 6). Plasmid transfer occurs by conjugation: the red coloured bacteria receive plasmid α encoding resistance to antibiotic A, the blue coloured bacteria plasmid β encoding resistance to antibiotic B. Over the next two panels (Fig. 7(a), panels 5 and 6) the plasmids spread and are maintained throughout the population, until encountering a recipient carrying an incompatible plasmid, as shown in panel 7 (Fig. 7(a)). At time 583:20 min, antibiotics A and B are added: A to zone 'a' and B to zone 'b' (see Fig. 6). Introduction of the antibiotics kills susceptible bacteria, whilst those acquiring and maintaining the appropriate R plasmid survive in their, respective halves (Fig. 7(a), panel 8). Few, if any bacteria survive across the border where the two antibiotics meet and where cells from either zone can migrate. Such cells need to be resistant to both antibiotics and this would normally necessitate acquisition and maintenance of plasmids α and β, but both R plasmids cannot co-exist in the same cell line due to incompatibility. Fig. 7(b) summarises Fig. 7(a), showing the rise of the plasmid-free bacterial population from time 0 to around 375:00 min to a population density of 275,000 cells. At time 333:20 min the two inocula of R plasmid-containing bacteria are added (200 of each) to opposite ends of the plasmid-free population. Plasmid transfer occurs rapidly and by 450 min there are very few plasmid-free cells remaining. Note the similarity between the rate of spread of the two plasmids in the population (blue and red) and that there are no bacteria carrying both plasmids, due to incompatibility. At time 583:20 antibiotics A and B are added, each covering half the surface of the environment, with the border along the diagonal (see Fig. 6). The introduction of these antibiotics kills susceptible cells (i.e. those without the appropriate R plasmid: α in the presence of antibiotic A and β in the presence of antibiotic B), resulting in a rapid drop in the bacterial population. However, acquisition and maintenance of the relevant R plasmid ensures survival of plasmid-containing bacteria in their respective halves of the environment. Also present are a few cells resistant to one or other antibiotic through mutation.

4.3. Transfer of Compatible Plasmids

This simulation demonstrates the spread of two compatible conjugative R plasmids (γ and β), from different incompatibility groups, through a plasmid-free, antibiotic-sensitive bacterial population. The plasmids encode resistance to different antibiotics. Plasmid transfer and maintenance is detected by exposure to the appropriate antibiotics. The substrate and antibiotic zones during the simulation are as described for transfer of incompatible plasmids (see Section 4.2 and Fig. 6).

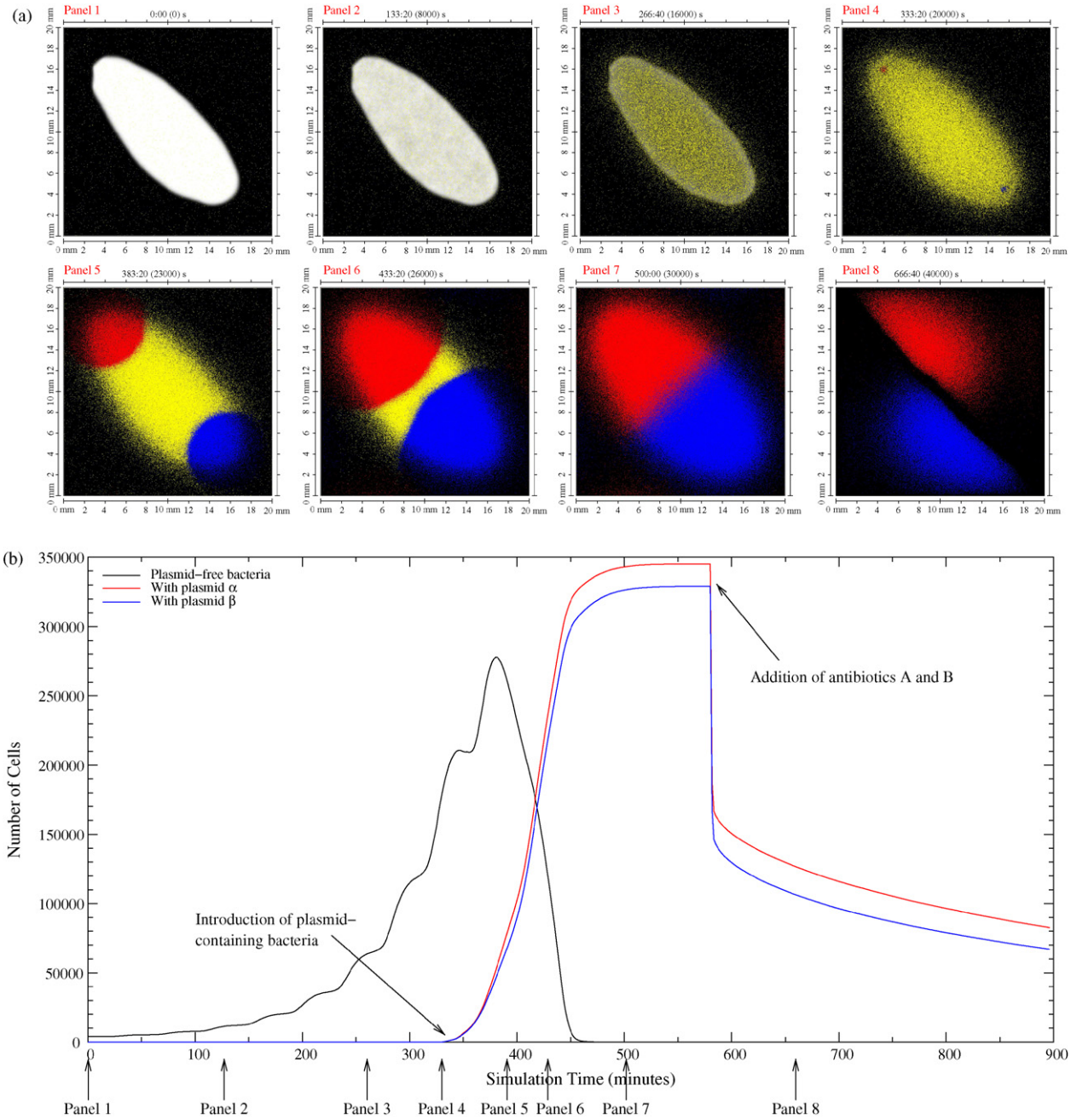

Fig. 7. Incompatible plasmid transfer and maintenance. (a) Panels 1–8 show snapshots of the environment at key moments in the simulation. Panels 1–4 demonstrate the initial increase in antibiotic-sensitive bacteria. Panels 5–7 show the spread of two incompatible plasmids (α and β), each determining resistance to a single antibiotic, and added to opposite ends of the bacterial population. Only one or other plasmid can be maintained in recipients due to incompatibility. Panel 8 demonstrates selection of resistant bacteria that maintain the appropriate plasmid-encoded resistance upon exposure to the antibiotic, added as in Fig. 6. (b) Graphical summary of panels showing the rise in the plasmid-free population, followed by the rise in two plasmid-carrying populations (with α and β). Upon addition of antibiotics A and B, both populations rapidly fall, to around half their initial size.

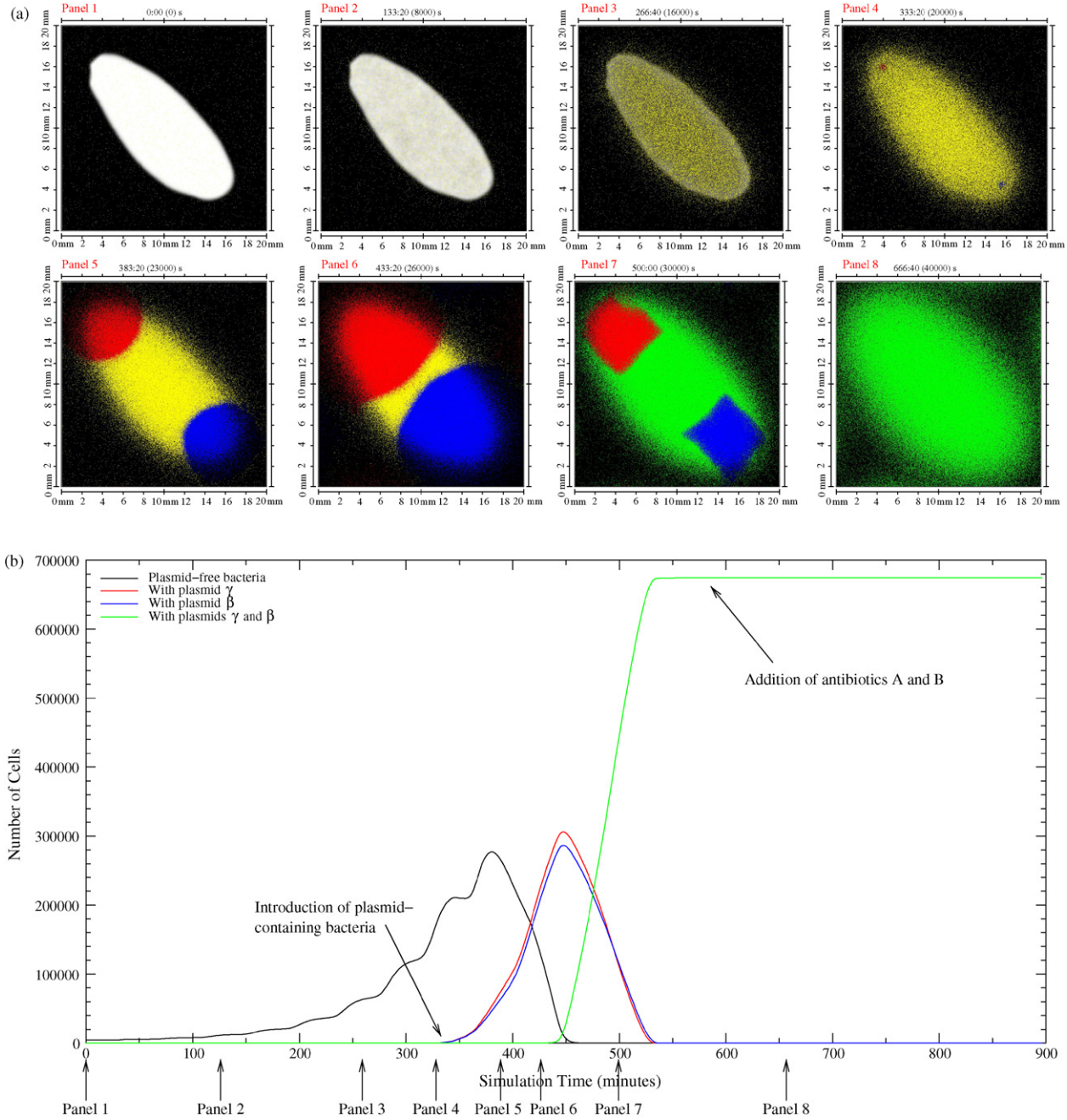

Fig. 8. Compatible plasmid transfer and maintenance. (a) Panels 1–8 show snapshots of the environment at key moments in the simulation. Panels 1–6 are identical to those of Fig. 7(a) except that compatible rather than incompatible plasmids are introduced in panel 4, the difference is initially seen in panel 7 where bacteria containing one plasmid encounter bacteria containing the other. Panels 1–4 demonstrate the initial increase in antibiotic-sensitive bacteria. Panels 5–7 show the spread of two compatible plasmids, each determining resistance to a single antibiotic and added to opposite ends of the bacterial population. Unlike Fig. 7(a), in panel 7 the plasmids freely exchange, green denoting bacteria maintaining both plasmid γ and β. Panel 8 demonstrates the extent of plasmid spread, by selecting for bacteria resistant to antibiotic A or B after addition of the antibiotics as in Fig. 6. There is no discernible reduction in the population, as all visible cells are green, due to carriage of both plasmids γ and β, and hence resistant to both antibiotics. (b) Graphical summary of the panels, showing the rise in the plasmid-free population, followed by the rise in two plasmid-carrying populations (with γ and β). The addition of antibiotics has no effect on the population overall, since, by this time all the cells have acquired resistance to both antibiotics. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

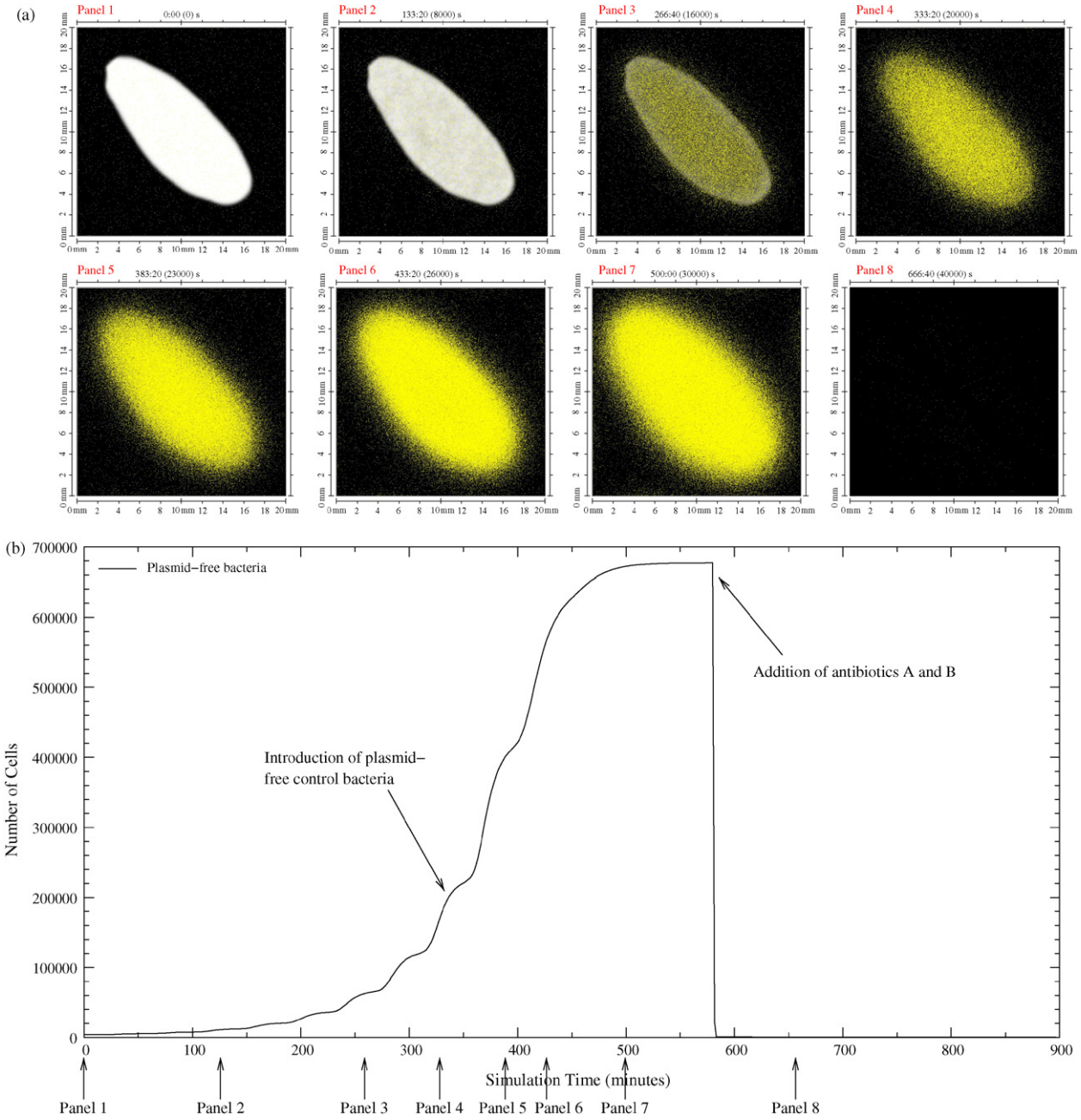

Fig. 9. Plasmid-free control simulation. (a) Panels 1–8 show snapshots of the environment at key moments in the simulation as for Figs. 7 and 8. Panels 1–4 are identical to those of Fig. 7(a) and 8(a), except that plasmid-free rather than plasmid-containing cells have been added to the existing plasmid-free population in panel 4. Panels 1–7 demonstrate the initial increase in antibiotic-sensitive bacteria, consuming the vast majority of the substrate. Antibiotics A and B are introduced at time 583:20 min (as in Fig. 6) and kill nearly all the cells, only a few resistant mutants remain by time 666:40 min (panel 8). (b) Graphical summary of the panels, showing the long rise in the plasmid-free population, followed by the rapid reduction when antibiotics are introduced.

Fig. 8(a) presents snapshots of the simulation, with panels 1–6 showing events as described for Fig. 7(a) (panels 1–6), except that the two inocula carry compatible, rather than incompatible, conjugative R plasmids, γ and β, encoding resistance to antibiotics A and B, respectively. Both these plasmids spread by conjugation and are maintained throughout the test population (as indicated by the green coloured bacteria (panel 7, Fig. 8(a)),

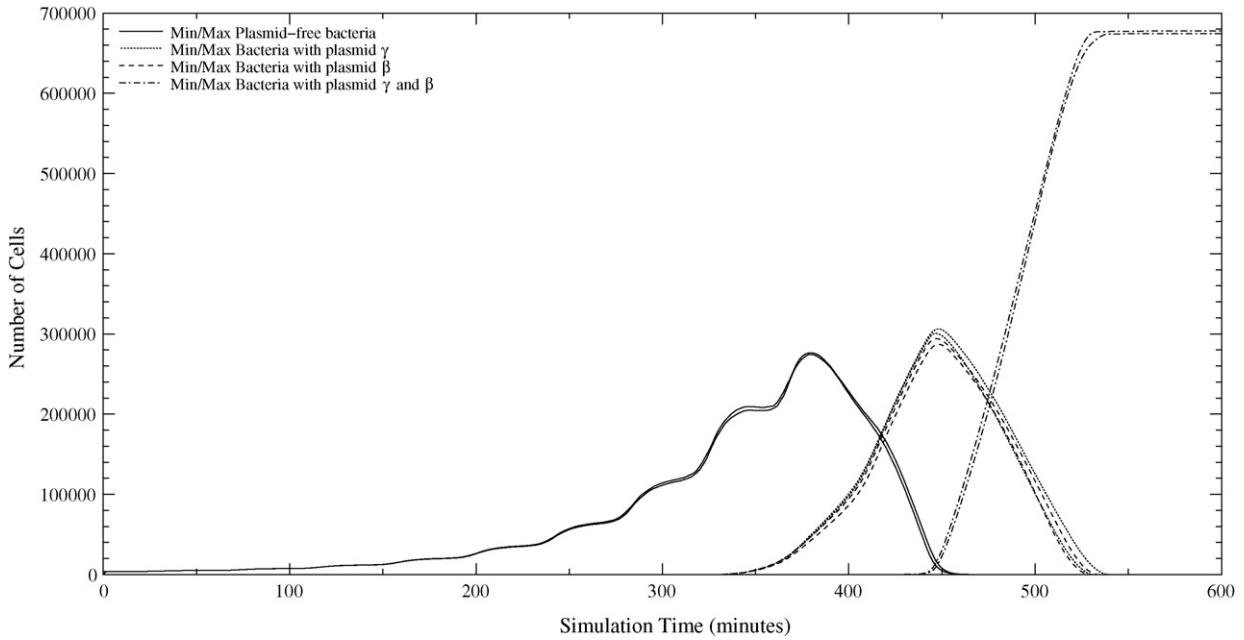

Fig. 10. Simulation repeatability when modelling plasmid transfer. This is based on three identical runs of the plasmid compatibility simulation of Fig. 8. The graph shows the minimum and maximum total population densities for each group of bacteria, while changing only the initial random number seed.

instead of being confined to plasmid-free recipients, as is the case with the incompatible plasmids. The population thereby acquires and maintains resistance to both antibiotics A and B. Accordingly, by the end of the simulation all the cells (with the exception of a few variants) carry plasmids γ and β, indicating that these elements can co-exist. Thus upon exposure to the antibiotics, which are added in the same way and at the same time as for the simulation with incompatible plasmids, there is no reduction in the number of green coloured bacteria. This contrasts with the reduction in numbers of blue and red coloured bacteria in the simulation with the incompatible plasmids (see panel 8 (Fig. 8(a)) and compare panel 8 (Fig. 7(a))).

Results are displayed graphically in Fig. 8(b) and show an identical rise in the plasmid-free bacterial population from time 0 to around 375:00 min, as for the incompatibility simulation (compare Fig. 7(b)). At time 333:20 min the two inocula of R plasmid-containing bacteria are added (200 of each) to opposite ends of the plasmid-free population. Bacteria with plasmid γ at Y and with plasmid β at Z (as in Fig. 6). Plasmid transfer occurs rapidly and by 583:20 min, when antibiotics A and B are added, both plasmids γ and β have spread and are maintained throughout the entire population, indicating their compatibility. There is no fall in the population upon exposure to either of the antibiotics, due to carriage of both plasmids, encoding the resistance determinants, in the cells. The only cause of death would be starvation. However, the low maintenance rate of the cells ensures that such deaths are far beyond the time-frame of these simulations.

4.4. Robustness of Simulations

A control simulation for the case studies is presented in Fig. 9, in which plasmid-free, rather than plasmid-containing, inocula are added at time 333:20 min to the test plasmid-free population (at Y and Z, see Fig. 6), following growth on substrate, as described in Figs. 7 and 8. At time 583:20 min, antibiotics A and B are added, each covering half the surface of the environment, as shown in Fig. 6. With the exception of a few variants, all the cells, being susceptible to antibiotics, are killed. This demonstrates a requirement for acquisition and maintenance of the appropriate R plasmid in order for bacteria to survive when challenged by the antibiotics, as indicated in Figs. 7 and 8.

All the simulations are repeatable. With the same random number seed, the simulation follows exactly the same path. The parallel algorithm takes into account small (and sometimes large) differences in timing by keeping the nodes synchronised to a common time frame. With different seeds, the simulation follows a slightly different path.

Fig. 10 demonstrates the variation for three identically initialised simulations, modelling plasmid spread as found in Fig. 8. The minimum and maximum population sizes across all simulations are shown and demonstrate that they are similar, but not exactly the same. The small variation is to be expected; a change in seed will lead to a random event occurring when it previously had not, or not occurring when it previously had occurred. In a uniformly distributed dense population this would have no effect, since an opportunity missed by one individual would be taken up by the next. However, here the heterogeneously distributed population amplifies the effect of random decisions.

5. Conclusion

This paper reports the application of COSMIC-rules to simulations of conjugative plasmid transfer between bacteria. Transfer of conjugative R plasmids has been demonstrated to occur in these simulations in a biologically meaningful and realistic manner. The results support our original concept (Gregory et al., 2007) that compressing the representation of the genome within COSMIC-rules is valid and retains biological relevance. Acquisition of an R plasmid confers an antibiotic resistance phenotype on the host, allowing survival in the presence of the particular antibiotic. As in real living situations, for transfer of two incompatible R plasmids into the same cell, one or other fails to co-exist in the cell line. In turn, a portion of the population maintains one of the R plasmids, whilst the remainder harbours the other plasmid. This has been detected following "exposure" to the antibiotics, such that bacterial cells survive in the presence of the antibiotic to which the acquired and stably maintained plasmid encodes resistance. Incompatibility is therefore predicted from this model to be a significant limiting factor for plasmid spread in localised bacterial populations. For two compatible plasmids, both are capable of transferring to and being maintained in the entire population, as demonstrated by survival of all the plasmid-containing cells in the presence of the antibiotics. In contrast, incompatible plasmids fail to survive in the same cells. Our model thus performs to expectation with the limited set of parameters applied. Plasmid transfer between individual donors and recipients that form mating pairs has been effectively modelled based on an IbM. A number of other IbM approaches to bacterial simulations, for example those of Kreft et al. (1998), Ginovart et al. (2002) and Prats et al. (2006), have been reported, but they have focussed on metabolic and growth issues rather than the genetic events studied here. Our findings thus demonstrate the validity of the IbM approach to studies on gene transfer by conjugation, as was also proposed recently by Sørensen et al. (2005), after our studies had been initiated.

The ability to simulate gene transfer events has applications in examining, for example, many aspects of adaptive evolution and phenomena such as the spread of antibiotic–resistant bacteria (DeNap et al., 2004) and the ability of bacterial populations to degrade xenobiotic pollutants (Basta et al., 2004). Our current framework provides building blocks for generating more complex simulations. We plan to produce future simulations of multiple genetic events, including modelling the behaviour of transposable elements and lysogenic bacteriophages in bacteria exposed to varying environmental conditions. Modelling complex scenarios that combine different horizontal gene transfer processes and varying gene expression with changing conditions will permit predictive modelling of evolution in natural environments. This will be particularly relevant to analysing the effects of a dynamic gene pool on bacterial evolution in response to natural and man-made environmental change. More complex models will be essential for predictive modelling of adaptive and evolutionary responses in assemblages of different bacterial species as exemplified by biofilms (Davey and O'Toole, 2000; O'Toole et al., 2000).

Movies of all these simulations and source code are available at: http://www.csc.liv.ac.uk/~greg/biosys/plasmid/

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