



Individual- and Population-based Diversity in Restriction-modification Systems

LUDO PAGIE[†] AND PAULIEN HOGEWEG

Utrecht University,
Bioinformatics group,
Padualaan 8,
3584 CH, Utrecht,
The Netherlands

E-mail: L.Pagie@bio.uu.nl

Restriction-modification (RM) systems are cognate gene complexes that code for an endonuclease and a methylase. They are often thought to have developed in bacteria as protection against invading genetic material, e.g., phage DNA. The high diversity of RM systems, as observed in nature, is often ascribed to the coevolution of RM systems (which ‘invent’ novel types) and phages. However, the extent to which phages are insensitive to RM systems casts doubts on the effectiveness of RM systems as protection against infection and thereby on the reason for the diversity of RM systems. We present an eco-evolutionary model in order to study the evolution of the diversity of RM systems. The model predicts that in general diversity of RM systems is high. More importantly, the diversity of the RM systems is expressed either at the individual level or at the population level. In the first case all individuals carry RM systems of all sequence specificities, whereas in the second case they carry only one RM system or no RM systems at all. Nevertheless, in the second case the same number of sequence specificities are present in the population.

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1. INTRODUCTION

Restriction-modification (RM) systems are cognate gene complexes that code for an endonuclease and a methylase. The endonuclease of RM systems cuts DNA molecules at or near specific short nucleotide sequences unless it is methylated at these sequences. The DNA of the host bacterium is protected against the endonuclease activity by the methylase which recognizes (and methylates) the same sequence as the endonuclease. In natural bacterium populations a large number of different ‘RM types’ are found, i.e., RM systems that recognize different nucleotide sequences [for reviews see: Bickle and Kruger (1993); Barcus and Murray (1995); Redaschi and Bickle (1996)].

[†]Current address: Santa Fe Institute, 1399 Hyde Park Road, Santa Fe NM 87501, U.S.A.

It is generally believed that the main function of RM systems is to protect bacterium cells against foreign DNA, i.e., phage infections. If a bacterium is infected by a phage the DNA of the phage is cut by the endonuclease and the infection is aborted. However, a probability exists that the endonuclease fails to cut an invading DNA molecule. For typical RM systems this probability ranges from 10^{-2} to 10^{-6} (Wilson and Murray, 1991; Korona *et al.*, 1993). As a result of this failure the invading phage DNA becomes inadvertently methylated and thereby protected against the endonuclease activity just like the DNA of the host bacterium. When such methylated phages infect bacteria that carry the corresponding set of RM types, or a subset thereof, the phages are not hindered by the presence of the RM systems. Only when the methylated phage infects a bacterium that lacks (some of) the RM types does the phage lose the methylation patterns and become sensitive again.

Previous theoretical models have shown that under well-mixed conditions novel RM systems can invade existing bacterium–phage communities as a result of frequency-dependent selection (Levin, 1988). The *common* RM types are assumed always to be accompanied and limited by correspondingly modified phages, whereas *novel* RM types protect their host against infections. Once RM systems are established they will remain in the population due to frequency-dependent *infection*. The (high) diversity of the RM-systems is then a consequence of subsequent invasions by RM-systems and theoretically is only limited by the bacterium population size.

Data from natural and laboratory bacterium strains show that many bacteria are resistant to many phages in ways other than through restriction. Also, many phages appear to be insensitive to many RM types (Korona *et al.*, 1993). Moreover, in experiments with sensitive laboratory bacterium strains RM systems seem to provide the bacteria with only transient protection from the phage. Quickly, bacteria arise that are immune to the phage, due to evolution of receptor-based resistance (Korona and Levin, 1993). They suggest that RM systems only favour bacteria in colonization events in spatially explicit environments in which phages are already present.

Frank (1994), however, showed that by increasing the number of RM systems in the bacterium population a transition occurs from a bacterium population dominated by resistant bacteria to a bacterium population dominated by sensitive bacteria that carry RM systems. A further increase of the diversity of RM systems can lead to a situation in which a phage population can no longer be sustained. This is caused by a decrease in the number of *effective* phage infections, i.e., infections by modified phage of bacteria that carry the corresponding RM type. With the disappearance of the phage population the selection for novel RM systems is lost as well.

Most work on the evolution of the diversity of RM systems has been concerned with the invasion of bacterium populations by bacteria that carried only single RM systems (Levin, 1988; Korona and Levin, 1993; Frank, 1994). We study the eco-

Parameter	Value	
field size S	300×300	
growth penalty p	0.05	
plasmids per bacterium n	0...9	
growth rate g	1.0	if (cell=empty)
infected growth rate g	0.0	ColonizeFromNeighbours()
colonization rate C	$\sum_1^8 \frac{(g-np)}{8}$	else {
death rate d_w	0.1	if (Uniform_Probability() < death_rate)
infected death rate d_i	0.45	Death()
phage influx i	10^{-6}	else {
inadvertent methylation m	10^{-4}	RM-system_Dynamics()
RM decay mutation μ_d	10^{-4}	Phage_Dynamics()
RM acquisition rate μ_g	10^{-8}	}
horizontal transfer rate μ_t	10^{-6}	}

Table 1. Parameters and their default values.

Figure 1. Pseudo-code that determines the local transitions and is applied for each grid cell in a random order.

evolutionary dynamics of RM systems in bacterium–phage communities on longer time-scales in which we explicitly allow for the competition or cooperation between RM systems in individual bacteria, as well as at the level of the bacterium population. We find that, as a result of the frequency-dependent selection of novel RM systems, the diversity of RM systems increases if the bacterium population is infected by phages. More importantly, we find that the diversity is expressed in two modes. Both modes occur as stable attractors if the system is viable, i.e., if phages, bacteria and RM systems are present. Although each mode may contain large numbers of RM types the ecological dynamics of the two modes differ greatly. The selection pressure of novel RM systems also differs as a result of the different ecological dynamics.

2. THE MODEL

We modelled the interactions of the bacteria in a spatially explicit, discrete event and discrete variable model with probabilistic updating. The bacterium population is a partially open system (e.g., an intestine or a sewer) which can be infected by bacteria, phage and RM systems from external sources. The bacteria in the model can be interpreted as individual cells or as monomorphic bacterium strains [see also Pagie and Hogeweg (1999)]. Phages are not modelled explicitly but as infected bacteria. RM systems are modelled as independent genetic elements that can be carried by plasmids or on the host genome. We assume that the RM systems do not code for any functionality influencing the behaviour of the bacterium host other than the endonuclease and methylase activity. They impose a penalty on the growth rate of their host due to the forced maintenance of endonuclease and

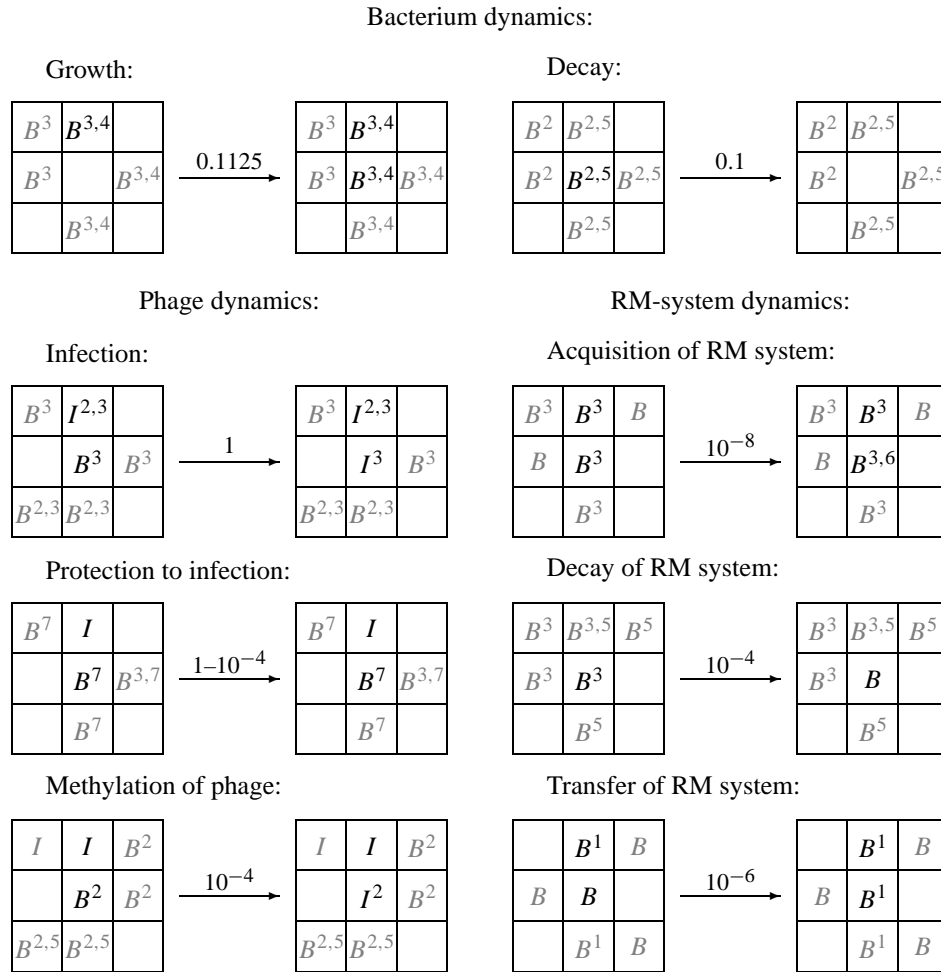


Figure 2. An overview of the possible transitions in the model. The probabilities at the arrows apply to the transitions of the cells with the black content.

methylase levels in the cytoplasm plus the maintenance of the RM system itself. RM systems can be of different, discrete types, i.e., recognize different nucleotide sequences. In all other respects, such as growth penalty or endonuclease efficiency, RM systems of different types are identical.

Table 2 gives an overview of all parameters used in the model, together with the default values. Figure 1 shows a program in pseudo-code that is applied to every cell in the grid in a random order. In Fig. 2 we have given an overview of the possible state changes of the bacteria, the phages, and the RM systems. At the transition arrows we indicate the probabilities that this particular transition occurs per time step. Note that the indicated transition probabilities only apply to the cells with black contents. For instance, under growth, the probability that the bacterium in the northern cell colonizes the centre cell is 0.1125, as indicated.

2.1. RM systems. RM systems are acquired and lost independently of the rest of the genome of the host bacterium. The processes by which RM systems are transferred within the bacterium population and by which RM systems are acquired from external sources are not modelled at a detailed level. In natural bacterium populations genetic material is readily exchanged within and between bacteria populations through a variety of processes, e.g., conjugation, transduction, and transformation. We generalized with respect to these processes by assuming a single process to account for the exchange of existing RM systems between bacteria within the population, with rate μ_t , and a single process to account for the acquisition of ‘novel’ RM systems that are not yet present in the population, with rate μ_g . Novel RM systems are acquired in a bacterium in addition to any RM systems that the bacterium may already carry. A scenario can be that phages from external sources carry a novel RM type (e.g., by transduction) and are cut up during the infection process by the endonuclease of the RM systems which are present in the bacterium cell. The novel RM system is incorporated in the bacterium genome by homologous recombination. RM systems are lost, e.g., by mutation or by segregation, with rate μ_d .

We do not take into account the number of RM systems per type that are carried by a bacterium. Also, we assume that all RM systems are compatible, i.e., a single bacterium can carry all RM types (provided the total growth penalty does not get too large).

2.2. Spatial embedding and local interactions. The space in the model is a 2D, square, regular grid of 300×300 sites with a neighbourhood consisting of the eight nearest sites (i.e., the ‘Moore-neighbourhood’). Each site is either empty or occupied by a bacterium. Empty sites can be colonized by bacteria from the eight neighbouring sites, each with a probability based on their growth rate. A wild-type bacterium, i.e., an uninfected bacterium that does not carry any RM systems, has a growth rate $g = 1.0$. Infected bacteria do not grow; they have a growth rate $g = 0.0$. The growth rate of bacteria that carry RM systems is lowered linearly

according to the number of RM systems they carry; $g = 1.0 - n \times p$, where n is the number of RM systems and p is the growth penalty per RM system. The linearity between growth rate and number of RM systems is chosen in order to avoid the occurrence of a priori nonlinearities in the interaction between bacteria and the number of RM systems that they carry. The death rate d_w of bacteria is 0.1 and the death rate d_i of infected bacteria is 0.45.

2.3. Phages. Phages are modelled as infected bacteria. A susceptible bacterium is infected if it has an infected bacterium in its neighbourhood. A bacterium is susceptible if it is not protected by an ‘effective’ RM system (see below). Phages can escape the endonuclease activity of an effective RM system during infection with probability m and subsequently acquire the same methylation patterns as the host bacterium. Such phages are no longer sensitive to the corresponding RM types. The latter are no longer ‘effective’ against these modified phages. The probability that a bacterium that carries N effective RM types will be infected by a phage is m^N . If a phage that carries methylation patterns infects a bacterium that lacks (some of) the corresponding RM types the phage loses these methylation patterns and once more becomes sensitive to these RM systems. We used an influx of phages at rate i per bacterium in order to maintain a bacterium–phage–RM system interaction under all circumstances (see Section 3.1).

3. RESULTS

We studied the evolutionary consequences of the interaction of multiple RM systems in a bacterium population in a large number of simulations, for a wide range of parameter values. The simulations were started with a wild-type bacterium population, i.e., bacteria without RM systems, infected by phages. The acquisition of RM types by the bacteria occurred on a long time-scale.

In a typical simulation we find that at first novel RM types are integrated into all bacteria in the population, notwithstanding the (additional) growth penalty which they impose on the bacteria. Thus, a homogeneous bacterium population is maintained with increasing numbers of RM systems per bacterium. However, in this period the phages also accumulate all methylation patterns that correspond to the RM types, as the latter are established in the bacterium population. Thus, the phages maintain insensitivity to all RM types that are present in the bacterium population; the bacteria are consequently not protected against phage infections by the RM systems that they carry, except shortly after the first introduction of a novel RM type.

In this period, the population dynamics and the spatio-temporal patterns that we find in the model are typical for spatially embedded host–parasite systems with a high infection rate: here, a large phage population size, a relatively small host population size, and turbulent wave patterns (Johansen, 1996; Savill *et al.*, 1997).

Of course, RM systems cannot continue to accumulate indefinitely in bacteria

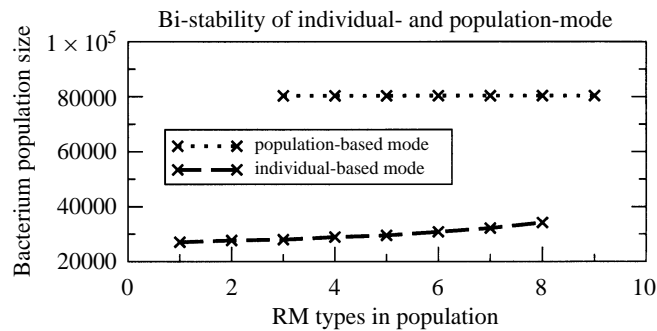


Figure 3. Bacterium population sizes of individual-based mode and population-based mode versus the number of RM systems in the bacterium population.

because their growth rate is finite. At a certain point, following the introduction of additional RM types into the population, the accumulated diversity of RM systems in each individual bacterium breaks down. This leads to a situation in which bacteria carry only one RM system or even no RM system. The total number of RM types in the bacterium population, however, is maintained at the same level during and after this breakdown. The different RM types are now distributed over the bacterium population. During the breakdown event the phage population is greatly reduced and loses all modifications, or it even dies out completely, whereas the bacterium population size increases very much. The population dynamics and spatio-temporal patterns after this transition differ very much from those before the transition.

Simulations like the ones described above showed the transition at arbitrary numbers of RM systems present in the population suggesting bi-stability between two dynamical modes. We analysed the bi-stability of the system in simulations in which we initialized the model with different numbers of RM types in the population, and with a homogeneous bacterium population or a heterogeneous bacterium population. In Fig. 3 we plot the average bacterium population-size against the number of RM types in the population. The dotted line indicates simulations in the population-based mode, the dashed line indicates simulations in the individual-based mode. Both modes are stable for a large range of RM types in the population.

Thus, the model can best be described in terms of the two dynamical modes which we denote as *individual-based diversity* and *population-based diversity*. In the first mode the bacterium population is almost homogeneous with respect to the composition of RM types, i.e., all bacteria carry all RM types that are present in the population. In the second mode the bacterium population is heterogeneous, i.e., bacteria carry none or only one of the RM types present in the population.

In order to understand the transition between the two modes we discuss simulations in which novel RM types are introduced explicitly at regular time intervals. First, however, we will show the characteristics of the individual- and population-based modes in 'ecological' simulations, i.e., simulations which have already been

initialized in either of the two modes and which do not include the introduction of novel RM types, i.e., $\mu_g = 0.0$.

3.1. Individual- and population-based diversity. In Fig. 4 we show snapshots and space–time plots of two simulations which have equal parameter values but which were initialized differently. In both simulations five different RM types are present in the bacterium population. The first simulation [Fig. 4(a)] is initialized with wild-type bacteria and with bacteria that carry all five RM systems: the individual-based mode. The second simulation [Fig. 4(b)] is initialized with wild-type bacteria and bacteria that carry only one of the five RM types: the population-based mode. Colours other than white (empty space), red (wild-type bacteria) and black (infected bacteria) denote uninfected bacteria that carry different combinations of RM systems.

In the individual-based mode we see that almost all bacteria either carry all five RM systems that were present in the initial population (i.e., pink) or they are infected with phage (i.e., black). A few bacteria (e.g., blue, orange, purple) have lost one RM system due to decay mutations; the set of RM types that they carry is a *subset* of the set of RM types that the predominant bacteria carry. The space–time plot shows that these ‘mutant’ bacteria, despite their higher growth rate, do not take over the population. This is due to an asymmetry in the infectivity of the predominant bacteria and the ‘mutant’ bacteria which we call *unidirectional infectivity*. Bacteria that carry RM systems can only be infected by other, infected, bacteria if the latter carry the same set, or a superset of RM systems; only then are the phages sufficiently protected by the methylation pattern that they carry[†]. Here, the predominant bacteria can only be infected by other predominant bacteria, while the mutant bacteria can be infected by other mutant bacteria as well as by the predominant bacteria. This asymmetry is sufficient to expunge ‘mutant’ bacteria from the population; it also explains how bacteria that have acquired novel RM types can take over the population after phages have arisen that have also acquired the corresponding novel methylation patterns (see below).

In the population-based mode we see that the bacteria are polymorphic with respect to the RM systems that they carry. A small number of bacteria are wild-type (red) whereas the other bacteria carry only one RM system (blue, purple, yellow, pink, green). In addition to the polymorphism at the population level the bacteria with different RM systems are distributed over the whole field in small patches. These patches are fairly stationary in time; competition between bacteria that carry the same number of RM systems is neutral.

Only in the centre of the snapshot of Fig. 4(b) a small number of infected bacteria are present (i.e., black). The near absence of phages is a result of the polymorphism of the bacteria which renders them *mutually uninfectable*. Two bacteria are *mutu-*

[†]Note that here we neglect the (small) possibility that phages acquire methylation patterns due to inadvertent methylation.

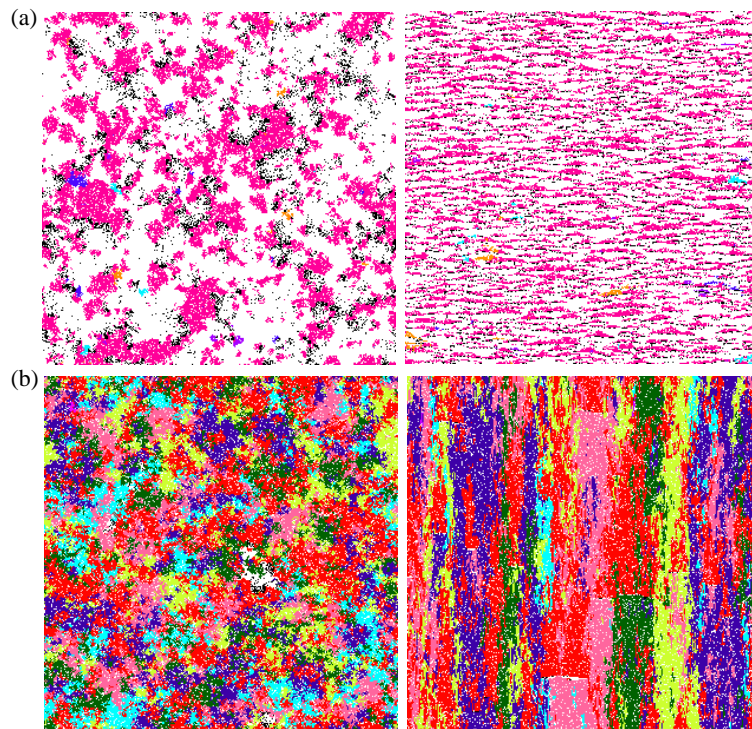


Figure 4. Snapshots and space–time plots (i.e., horizontal cross-sections of the grid of every fifth time-step) of (a) individual-based and (b) population-based diversity. Different colours denote different combinations of RM systems.

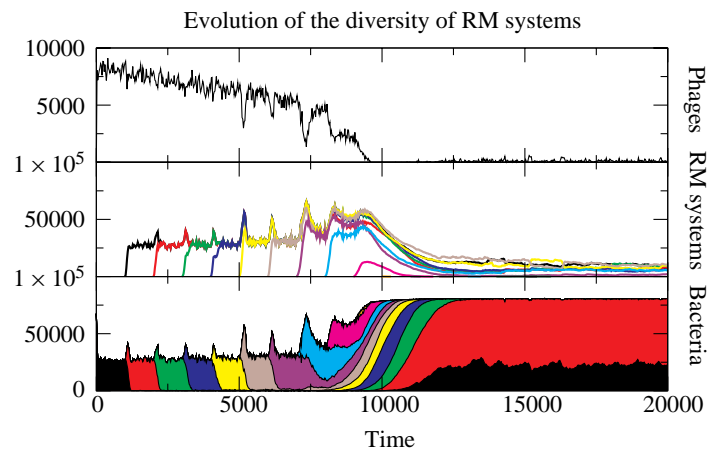


Figure 5. Temporal dynamics of bacteria (bottom panel), RM types (middle panel) and phage population (top panel). The bacterium population is split into bacteria that carry the same number of RM systems. The diversity of RM systems increases until $t = 9000$ and the individual-based diversity is maintained. After the breakdown at $t \approx 10.000$ the bacterium population settles into the population mode with no RM systems or only one RM system per bacterium. The total number of RM types remains high.

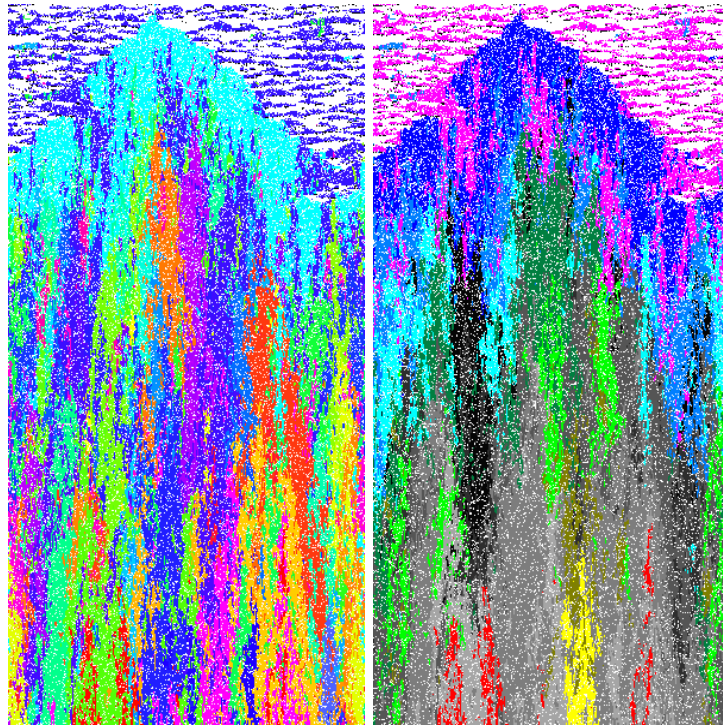


Figure 6. Space–time plots of the breakdown of individual-based mode to population-based mode. In (a) different colours denote different combinations of RM systems. In (b) shades of grey denote different numbers of RM systems per bacterium.

ally uninfactable if each carries an RM type that the other bacterium lacks. In that case, phages produced by one bacterium carry methylation patterns that do not correspond (completely) to the set of RM types that the other bacterium carries. Thus, infections remain localized in single patches, rather than sweep over large areas as in the individual-based mode. The overall result is that the phage population lives primarily on the wild-type bacterium population and only occasionally succeeds in infecting bacteria that carry RM systems following an inadvertent methylation event. At this point, if the number of RM types in the bacterium population is small the phage population is still viable. If the number of RM types is large the phage population is no longer viable and depends on the influx for its preservation. Note, however, that the field size is important; larger fields can more easily maintain viable phage populations for larger number of RM types in the bacterium population.

3.2. Transition between modes. Here, we present the results of a simulation in which we start with a homogeneous wild-type bacterium population that is infected by phages and then we introduce novel RM systems every 1000th time step. The simulation first shows the build-up of RM types in the individual-based mode, and

then the transition into the population-based mode.

In Fig. 5 we plot different characteristics of the simulation. In the bottom panel we plot the size of the bacterium population divided into bacteria that carry 0, 1, \dots , N RM systems. Per RM type, in the middle panel, we plot the number of RM systems that are present in the bacterium population. In the upper panel we plot the total phage population.

The first six RM systems that come into the population become fully integrated into the entire bacterium population. Each new RM type is acquired by a bacterium that already carries a number of RM systems. The novel RM system will give the bacterium an advantage because no phage are yet present that carry the corresponding methylation pattern. These invasions are visible as small peaks in the bacterium population size. When the RM type becomes more abundant in the population at some point a phage will acquire the corresponding methylation pattern as well. This phage will be able to infect the whole bacterium population and the novel RM type no longer gives effective protection against infection. Nevertheless, the novel RM type will continue to fully overtake the bacterium population due to unidirectional infection.

With increasing numbers of RM types present in the bacterium population, however, the transient to full integration of a novel RM type into the population takes longer and longer; the phage population size decreases and the difference in the carrying capacity of bacteria with different numbers of RM systems becomes more pronounced with decreasing growth rates [see also Pagie and Hogeweg (1999)]. In the simulation the seventh RM type never completely succeeds in taking over the population. Although the introduction of the eighth RM system still leads to a substantial number of bacteria that carry all eight RM systems no phage ever acquires all eight modification types.

The space–time plots in Fig. 6 show the breakdown of the individual-based mode in more detail. Into a population that was in a stable individual-based mode with eight RM types we introduced a novel RM type in the middle of the field. Figure 6(a) shows that the patch containing the novel RM type grows to cover the whole field. In the growing patch new combinations of RM types arise continuously.

In Fig. 6(a) we have used different colours to denote different combinations of RM types, with white, red and black denoting empty space, wild-type bacteria and phages respectively. In Fig. 6(b) we have used different colours to denote bacteria with different numbers of RM systems in order to show the transient from individual-based to population-based diversity. Bacteria that carry the ninth RM type in addition to other RM types have shades of a blue–green–yellow colour-ramp; bacteria that do not carry the ninth RM type are depicted in different shades of grey.

Initially, the patch consists of bacteria that carry the ninth RM type; they cannot readily be infected by bacteria from outside the patch because no correspondingly modified phage yet exist. In the patch bacteria start to lose RM types due to mu-

tation and segregation. Because locally phage are still absent the ‘mutant’ bacteria are not yet experiencing the *unidirectional infectability* and are therefore not yet outcompeted by bacteria that carry the full set of nine RM types. The breakdown process becomes irreversible as soon as *mutually uninfected* groups of bacteria arise. Even if, at this point, phages arise that are modified in accordance with the ninth RM type, the presence of mutually uninfected bacteria prevents phages to infect the whole bacterium population in wavy patterns. Instead, they are restricted to infect only small patches of monomorphic bacteria, which quickly leads to their extinction.

The phage population dies out when the patch has grown over the whole field. At that moment the bacteria carry between one and nine RM types. The maximum number of possible combinations with 1–9 RM types is $\sum_{i=1}^9 \binom{9}{i} = 511$. At the time of phage extinction ($t = 900$) 247 different combinations exist, the maximum number of different combinations being simultaneously present in the population is 451 and occurs at $t = 2000$. At this point phages cannot re-infect the bacterium population. Thus, bacteria continue to lose RM systems until the system settles into the population-based mode where bacteria carry at most one RM system. When the number of wild-type bacteria is sufficient for the maintenance of a viable phage population (given the influx; see Section 3.1) the system stabilizes. If phages do not infect the population after the transition the bacteria eventually lose all RM systems which leads the bacteria back to the beginning of the individual-based mode.

Thus, the irregular occurrence of the breakdown of the individual-based mode in the population-based mode, which we found in Section 3, is caused by a chance event, i.e., the arising of mutually uninfected bacteria. The subsequent explosion of polymorphic bacteria only stops when the population-based mode is reached.

4. DISCUSSION

In Section 3 we showed that a population of bacteria that carry RM systems can be in one of two stable modes, both with high numbers of RM types present in the population; *individual-based diversity* and *population-based diversity*. The latter mode is characterized by a heterogeneous bacterium population in which individual bacteria carry at most one RM system, and a phage population that is very small or even completely absent.

In the individual-based mode each bacterium carries a high number of RM systems; they are maximally protected against phage infections. But the phages, which in this mode make up a large population, are insensitive to all RM systems that are present; the protection provided by the RM systems is in fact not functional. Still, bacteria which lose RM systems are outcompeted due to unidirectional infectivity (Section 3.1). Thus, although RM systems are ineffective as protection to phage infections they are maintained in the population.

The finding that two stable modes exist in which the diversity of RM systems is expressed is very robust. We can modify the model for instance by performing global mixing of the bacterium population, incorporating independent mutation of the two genes that code for the endonuclease and for the methylase, or increasing the longevity of the methylation patterns on the phage. All these (structural) changes of the model do not substantially change the results that we report here, i.e., the bi-stability of the two modes, although the two modes are most pronounced in the original model. In the modified models we see that bacteria in the population-based mode can carry more RM systems per bacterium than in the unmodified model but retain the other properties of the population-based mode, i.e., the small number of phages, which are sensitive to most RM types present in the bacterium population. In the individual-based mode more bacteria are present that carry not quite all RM systems but rather one or two RM systems less. Still, bacteria are present that carry all RM systems and the number of phages is very large. The phages show a high degree of insensitivity to the RM types present in the bacterium population.

Studies of natural bacterium populations show that RM systems in bacterium populations are highly diverse and that many bacteria carry RM systems, and often more than one (Wilson and Murray, 1991; Barcus and Murray, 1995). It is, however, not clear if bacterium populations are mostly homogeneous as they are in the individual-based mode (Barcus and Murray, 1995) or heterogeneous as in the population-based mode. Or, in other words, how the diversity of RM types found in individual bacteria compares with the diversity of RM types found at the population level.

The model predicts that the bacterium population is either strongly limited by phages (i.e., the individual-based mode) or not (i.e., the population-based mode). In the first case all phages are modified with respect to all RM types that are present in the population while in the second they are modified to only a very few RM types or not modified at all. Also in this respect the available experimental data is not conclusive. Although phages occur in many microbial communities the extent to which bacteria are limited by the phages is often unclear (Havelaar *et al.*, 1986; Proctor and Furhman, 1990). Although many phages are insensitive to many RM systems by means other than having acquired methylation patterns (Kruger and Bickle, 1983; Sharp, 1986), still, many phages are also sensitive to several RM systems (Hantula *et al.*, 1991; Korona *et al.*, 1993). Most importantly, however, experimental data do not specify to what extent phages in natural communities are insensitive (by being resistant or modified) to RM systems that are present in those communities.

Given the available experimental data it is at this moment not possible to designate natural bacterium populations to be in the individual-based mode or in the population-based mode. Moreover, if the growth penalty that is imposed on bacterium hosts by RM systems is very small the transition from the individual-based mode to the population-based mode may take a long time. Natural bacterium pop-

ulations could thus be found to be in a transition state, i.e., high numbers of RM systems per bacterium while phages are absent. This state can only be understood in the context of the individual- and the population-based mode.

An important observable of the system is how the (in)sensitivity of phages to different types of RM systems relates to the RM types of the RM systems that are actually present in the system. Currently, the experimental data are inconclusive in this respect; the data originate generally from bacterium populations and phage populations that are sampled separately. When such data becomes available a subsequent comparison between the extent to which phages have developed resistance to RM systems and the extent to which they carry additional modifications which correspond to other RM types would give additional insights in the (evolutionary) interaction pressures between the phages and bacteria.

4.1. Cooperation across levels. In multi-level evolutionary models such as the ones described above it is not easy to determine who gains by cooperating with whom and what the evolutionary outcome will be. Recently we have studied the evolution of the diversity of colicin plasmids in bacterium populations (Pagie and Hogeweg, 1999). We reported that the behaviour of the model could also be described in terms of either an individual-based mode or a population-based mode. In the colicin model the colicins appeared to cooperate with each other at the expense of the bacteria, rather than acting as a 'mere' sum of gains and losses at the level of bacterial competition. The colicin model, however, was not bi-stable like the RM model discussed here; the system settled in one of the two modes, depending on the growth penalty parameter of the colicins and the number of colicins in the bacterium population. In the RM model the phage population acts as a third level in the system by means of which the functionality encoded by the RM systems comes about. The fact that the RM model has one more level than the colicin model, apparently brings about the bi-stability of the system.

Independently, two groups proposed an alternative mechanism for the maintenance of RM systems in terms of selfishness [Kulakauskas *et al.* (1995), Naito *et al.* (1995), but also see O'Neill *et al.* (1997)]. The selfish character of RM systems results from post-segregational host killing. However, RM systems that incorporate post-segregational host killing will at first raise the death rate of the bacteria that carry these RM systems. In a competition with wild-type bacteria, bacteria that carry these selfish RM systems will be outcompeted; post-segregational host killing does not increase the stability of RM systems in this situation (Monod, 1992).

Nevertheless, in our model segregation of RM systems from bacteria does increase the probability of a breakdown from the individual-based mode to the population-based mode. In the individual-based mode a higher ratio of bacteria that carry fewer than all RM systems increases the probability that mutually un-infectable bacteria will be generated (Section 3.2) and that the individual-based mode will break down. Because post-segregational host killing will lower the rate at which bacteria arise that have lost one or more RM systems (they are killed)

it may prevent the breakdown of the individual-based mode and thereby maintain high levels of RM systems per bacterium. In the model, lowering the rate at which bacteria lose RM systems (μ_d) indeed increases the stability of the individual-based mode somewhat. In the individual-based mode, although bacteria seem maximally protected against phage infection, it is only the RM systems and phages that prosper, not the bacteria.

4.2. Conclusion. The system that we studied here, i.e., RM systems in a bacterium population that is infected by phages, has characteristics of other systems in which individuals can build up a diverse set of responses or actions to guard against parasites or predators, or to compete with conspecifics. A general question in such systems is if a diverse ensemble of interactions arises between the antagonists, and to what extent individuals should strive for individual ability at high cost, or choose to live cheap and dangerous but differently.

We have shown that in our model the two extreme cases turn out to be two stable states. In fact, that the two modes are stable does not depend on the cost of carrying RM systems. Only for unrealistically high growth penalties is the individual-based mode not stable. Thus, the 'cost-benefit optimization' of an individual bacterium is only a minor factor in determining the fate of the bacterium. We hypothesize that systems like the RM systems in bacterium–phage communities, such as MHC molecules in vertebrate immune systems or the toxic defence repertoire of plants, may also exhibit the bi-stability of an individual-based mode and a population-based mode. Although individuals may prefer maximal individual ability this is certainly not the only, or most agreeable evolutionary avenue.

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