6 The Ecological and Evolutionary Dynamics of Model Bacteriocin Communities

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No one knows whether death, which people fear to be the greatest evil, might not be the greatest good.

Plato, *The Apology of Socrates*

Summary

The use of model laboratory communities, model organisms, and mathematical models has deeply enriched our understanding of the causes and consequences of toxin production in bacteria. In particular, such models have provided much insight into the dynamics of microbial communities with toxin producers. Both experimental and theoretical approaches have suggested that population structure can be critical to the initial invasion of a toxin-producing strain. Furthermore, spatial structure may play a central role in the maintenance of diverse assemblages of toxic and non-toxic strains. Models have also revealed some counter-intuitive predictions, such as the evolution of competitive restraint in communities with toxin-sensitive, toxin-resistant, and toxin-producing bacteria. Toxin production itself is a dramatic form of niche construction, where producing strains alter the chemical nature of their surroundings. Such modification feeds back to affect the ecology and evolution of all community members. Models have helped greatly to clarify the effects of this feedback.

6.1 Introduction

Allelopathy, defined as the suppression or death of one organism due to the toxic chemicals excreted by another organism, is a ubiquitous phenomenon within microbial communities. In bacterial assemblages, the agents of allelopathic interaction are the bacteriocins. Bacteriocins are narrow-spectrum antimicrobial proteins found within nearly every major lineage of Bacteria

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Bacteriocins: Ecology and Evolution
(ed. by M.A. Riley and M.A Chavan)

(Riley and Wertz 2002a, 2002b). Given that bacteriocinogenic (toxin-producing) strains kill closely related non-producing strains, bacteriocins are commonly interpreted to be anticompetitor compounds (Riley 1998; Riley and Gordon 1999). Over the past few decades, there has been much interest in exploring the microbial dynamics of toxic consortia (Adams et al. 1979; Chao and Levin 1981; Levin 1988; Frank 1994; Tan and Riley 1996; Durrett and Levin 1997; Iwasa et al. 1998; Gordon and Riley 1999; Pagie and Hogeweg 1999; Nakamaru and Iwasa 2000; Czárán et al. 2002; Kerr et al. 2002; Czárán and Hoekstra 2003; Kirkup and Riley 2004). Some of these studies have shown that Socrates' insight carries particular salience for communities with bacteriocinogenic members – allelopathy may play a critical role in maintaining diversity in these systems (Durrett and Levin 1997; Pagie and Hogeweg 1999; Czárán et al. 2002; Lenski and Riley 2002; Kerr et al. 2002).

The best-studied case of microbial allelopathy is found in the bacterium *Escherichia coli*, which possesses many toxic strains. In *E. coli*, the gene encoding the toxin (termed a colicin) is housed on a plasmid along with a constitutively expressed immunity gene (conferring protection against the action of the colicin) and a lysis gene (usually expressed under conditions of stress, causing lysis of the cell and subsequent release of the colicin; James et al. 1996). Thus, in *E. coli* (as well as other Gram-negative bacterial species) bacteriocinogenic cells die in the process of releasing their toxins. A plausible interpretation is that the lethal release of toxins kills non-producing competitors, promoting the spread of remaining clone mates that carry the plasmid encoding immunity to the toxin. However, under precisely what circumstances would such lethal production evolve? And in communities with producers, what are the expected population-level consequences?

Models have proven extremely useful in answering such questions. Indeed, much of the current understanding of bacteriocin systems has come through the use of models, taken broadly to include model organisms (such as *E. coli*), model laboratory communities, and theoretical models. The foundational studies of bacteriocin-mediated community dynamics were done with *E. coli* in experimental microcosms (Adams et al. 1979; Chao and Levin 1981), and laboratory communities have continued to provide insight, both in vitro (Tan and Riley 1996; Riley and Gordon 1996; Gordon and Riley 1999; Wiener 2000; Kerr et al. 2002; Massey et al. 2004) and in vivo (Kirkup and Riley 2004; Massey et al. 2004). Such studies are often motivated by one of two related questions: how does toxin production arise? And how does toxicity influence the dynamics of the community?

The latter question has been targeted by several theoretical biologists studying bacteriocin systems. Given the large number of different bacteriocinogenic constituents in natural microbial communities, theoreticians have been nearly singularly motivated by providing mechanisms of diversity maintenance. In the process, theoretical biologists have brought a varied analytical and computational set of tools to the task, including systems of ordinary differential equations (Durrett and Levin 1997; Gordon and Riley 1999),

reaction-diffusion equations (Frank 1994; Nakamaru and Iwasa 2000), pair approximation (Iwasa et al. 1998), configuration field approximation (Czárán and Hoekstra 2003), and agent-based simulation (Durrett and Levin 1997; Pagie and Hogeweg 1999; Kerr et al. 2002; Czárán and Hoekstra 2003).

In this chapter, I will review the contributions of models to a deeper understanding of the causes and consequences of microbial allelopathy. The study of bacteriocin communities has benefited tremendously from a dialogue between theorists and empiricists. I will discuss some of the ways in which the theory has been inspired by and has, in turn, inspired experimental work. Finally, I will identify a few areas where the continued interaction between theoretical work, experimental work and natural history may produce deeper understanding. The following sections are organized according to structural complexity of the model bacteriocin community – starting with the simplest single-producer communities and ending with multiple-producer communities.

6.2 Dynamics in Two-Strain Communities: Getting over the Hump

The simplest bacteriocin community consists of two players: a strain producing the toxin and a strain sensitive to the toxin. For bacterial species such as *E. coli*, toxin production can be costly due to constitutively expressed immunity, plasmid carriage and lethality of production (Riley and Gordon 1999; Riley and Wertz 2002a, 2002b). This cost has been demonstrated in the laboratory, where the producer has a lower growth rate or a higher mortality rate than the sensitive strain (Adams et al. 1979; Chao and Levin 1981; Tan and Riley 1996, but see Dykes and Hastings 1997 for a discussion of Gram-positive producers). Given this cost, if the sensitive strain and producing strain were growing in two separate flasks, then the sensitive strain has the edge. But what happens when both strains are mixed in the same flask?

In well-mixed conditions (such as a shaken flask or a chemostat), bacteriocins released by a producer are evenly distributed throughout the entire community. This means that the per capita effect of the toxin on the pool of sensitive cells scales with the number of toxin producers – the more producers, the higher the per capita mortality rate for the sensitive strain. If there are very few producers in the community, then the impact of the bacteriocin on the sensitive pool will be minimal. In such a case, there will be a net growth advantage for the sensitive strain (as production is costly), and the sensitive strain can displace the producer. Alternatively, if toxin-producing cells are common, then the impact on the sensitive pool can be pronounced. Despite the intrinsic cost of toxin production, a high density of the producer can create a heavy *extrinsic* cost in net growth rate for the sensitive strain. If the bacteriocin is sufficiently toxic, the producing strain can displace the sensitive

strain. Under such cases, there is "strength in numbers" under mass-action conditions: above some threshold, the producer can administer enough poison to overburden its sensitive competitor.

A mathematical treatment of the competition between the producer and sensitive strain is given in Box 1. If the producer is sufficiently toxic, the community is bistable: either the producer excludes the sensitive strain or vice versa, depending on initial conditions (Levin 1988; Frank 1994; Durrett and Levin 1997; Iwasa et al. 1998). This bistability has been confirmed in the laboratory: under well-mixed conditions and constant initial density, a producer displaces its sensitive competitor *only* if above a critical frequency (Adams et al. 1979; Chao and Levin 1981). So, invading producers do have a proverbial hump to get over.

Box 1: A mass-action model of a producer strain and a sensitive strain

Durrett and Levin (1997) use the following system of differential equations to model the community dynamics of a sensitive strain (with density s) and producer strain (with density p):

$$\frac{ds}{dt} = \beta_s (1 - s - p) s - (\delta_s + \gamma p) s \tag{B1.1}$$

$$\frac{dp}{dt} = \beta_p (1 - s - p) p - \delta_p p \tag{B1.2}$$

where β_s and β_p are the birth rates, and δ_s and δ_p are the death rates of the sensitive and producer strains, respectively, and γ measures the per capita toxic effect of producers on the sensitive strain. We assume that

$$\beta_s > \delta_s \tag{B1.3}$$

$$\beta_p > \delta_p \tag{B1.4}$$

That is, each strain's reproductive gains outstrip its losses to intrinsic death. When alone, the carrying capacity of strain i is $1 - \delta_i/\beta_i$ (the carrying capacity approaches the maximum of unity as $\delta_i \to 0$ or $\beta_i \to \infty$). We also require that

$$1 - \frac{\delta_s}{\beta_s} > 1 - \frac{\delta_p}{\beta_p} \tag{B1.5}$$

That is, the sensitive strain has a higher carrying capacity than the producer when each is in isolation.

The current state of the two-strain community can be expressed as a point (p, s) on the two-dimensional p–s plane (see Fig. 6.B1a, d). Tracking community behavior amounts to following the trajectory of this point over time. The point moves according to Eqs. (B1.1) and (B1.2). One way to get some insight into the point's movement is to draw zero net growth

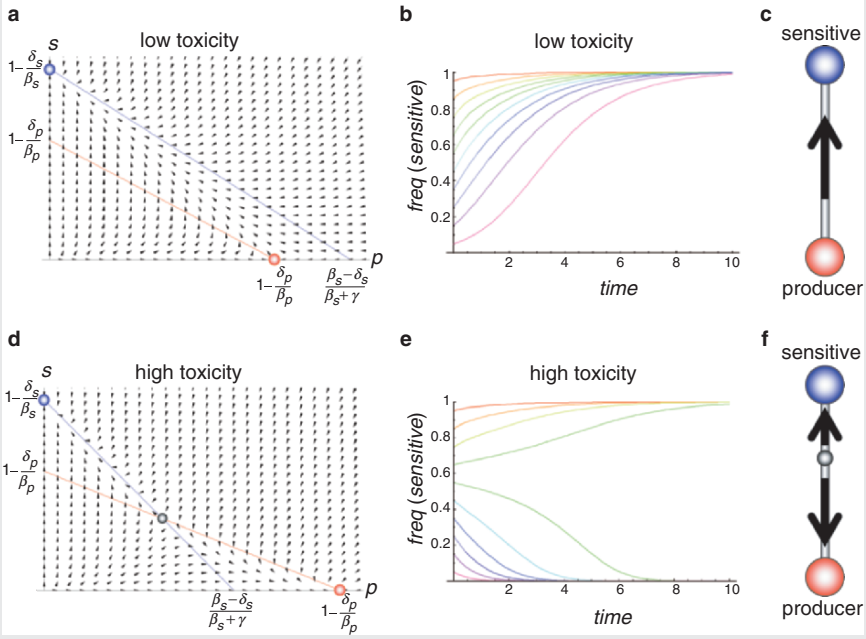

Fig. 6.B1 Exclusion or bistability in a two-strain community. **a** The isoclines for the sensitive (*blue*) and producer (*red*) strains are shown in the p–s plane. The *arrows* give the flow of a point describing the densities of the two strains. When the producer is insufficiently toxic ($\gamma < \gamma_c$), the isoclines do not cross. From nearly all starting positions, the "community point" moves to the equilibrium on the s axis (given by the *blue sphere*). That is, the sensitive strain displaces the producer. **b** Here, we see the same dynamics expressed as the frequency of the sensitive strain over time. Despite the starting conditions, the sensitive type fixes (that is, it approaches a frequency of 1). **c** A symbolic representation of the community dynamics (see Fig. 6.2). The *arrow* pointing from the producer node to the sensitive node indicates that the sensitive strain will outcompete the producer under any starting conditions. **d** When the producer is sufficiently toxic ($\gamma > \gamma_c$), the isoclines cross and a new internal equilibrium (the *gray sphere*) is introduced. This new equilibrium is unstable. In this community, the initial strain densities become important – if the producer is sufficiently abundant relative to the sensitive strain, then the producer will displace the sensitive strain and vice versa. This is a bistable system where both edge equilibria (the *red* and *blue spheres*) are locally stable. **e** Now, the sensitive strain fixes only if frequent enough – otherwise, it goes extinct (and the producer fixes). **f** The symbolic representation shows *arrows* pointing to each node with an unstable internal node in between

Box 1: Continued

isoclines for each strain (the isocline for each strain is a curve where it does not change its density).

The isocline for the sensitive strain (found by setting $\frac{ds}{dt} = 0$) is a line in the p–s plane:

$$s = \left(1 - \frac{\delta_s}{\beta_s}\right) - \left(1 + \frac{\gamma}{\beta_s}\right) p \tag{B1.6}$$

Similarly, the isocline for the producer strain (found by setting $\frac{dp}{dt} = 0$) is:

$$s = \left(1 - \frac{\delta_p}{\beta_p}\right) - p \tag{B1.7}$$

If the point in the plane (giving the strains' densities) is above the sensitive's isocline, then it must move downward (because the density of the sensitive strain is on the vertical axis, and if $s > 0$, then $s > (1 - \delta_s/\beta_s) - (1 + \gamma/\beta_s)\, p \Rightarrow ds/dt < 0$). On the other hand, if the point is below the sensitive's isocline, it must move upward. Simultaneously, if a point is above the producer's isocline, then it must move leftward (because the density of the producer is on the horizontal axis, and if $p > 0$, then $s > (1 - \delta_p/\beta_p) - p \Rightarrow dp/dt < 0$). By contrast, if the point is below the producer's isocline, then it must move rightward.

Therefore, the positioning of the isoclines (whether and how they cross) can yield important information about community dynamics. In this two-strain system, there is a critical toxicity of the producer

$$\gamma_c = \frac{\beta_s\, \delta_p - \beta_p\, \delta_s}{\beta_p - \delta_p} \tag{B1.8}$$

By assumptions (B1.3), (B1.4) and (B1.5), $\gamma_c > 0$. If $\gamma < \gamma_c$ (that is, the producer is not very toxic), then the isoclines do not cross in the positive quadrant of the p–s plane (see Fig. 6.B1a, where the sensitive isocline is in blue and the producer isocline is in red). The arrows in Fig. 6.B1a trace out the potential movement of a point giving the strain densities. Note that the arrows cut the blue line horizontally (because vertical movement of the point corresponds to changes in the sensitive strain, and the sensitive strain does not change its density on its isocline), and the arrows cut the red line vertically (because horizontal movement of the point corresponds to changes in the producer, and the producer does not change its density on its isocline).

As the figure shows, from nearly any starting condition, the community moves to the boundary equilibrium $(0, 1 - \delta_s/\beta_s)$ given by the blue sphere, where the sensitive strain excludes the producer. There also exists an unstable equilibrium $(1 - \delta_p/\beta_p, 0)$ given by the red sphere (introducing sensitive cells into a population of producers at the producer carrying

capacity would lead to the exclusion of producers by the invading sensitive strain). In Fig. 6.B1b, we see that the frequency of the sensitive strain approaches unity despite starting conditions (Fig. 6.B1c shows this behavior schematically). Thus, without sufficient toxicity, the producer always goes extinct in head-to-head competition.

If the toxicity of the producer is above the critical level ($\gamma > \gamma_c$), then both boundary equilibria become locally stable and the isoclines cross at the point $((\beta_s \delta_p)/(\beta_p \gamma) - \delta_s/\gamma, 1 + \delta_s/\gamma - (1 + \beta_s/\gamma)\delta_p/\beta_p)$ in the positive quadrat. This point is an unstable equilibrium (this can be shown locally using linear stability analysis; see the Appendix). From most starting positions, either the sensitive strain displaces the producer or vice versa (see Fig. 6.B1d). Thus, initial community composition becomes important in determining which strain dominates. Generally, if sufficiently abundant, the producer displaces the sensitive strain, otherwise it goes extinct. This is shown in Fig. 6.B1e (and schematically in Fig. 6.B1f). This bistability was demonstrated in vitro with *E. coli* (Adams et al. 1979; Chao and Levin 1981).

Both the mathematical and empirical results discussed above depend critically on the assumption of a well-mixed community. In an ingenious experiment, Chao and Levin (1981) competed a producer and a sensitive strain of *E. coli* in two different habitats: (1) a well-mixed broth-filled flask and (2) an agar-filled Petri dish. They found bistability in the stirred flask (the producer displaced the sensitive strain only when above a critical threshold). However, the producer *always* displaced the sensitive strain in the spatially structured dish (i.e., even if the producer was extremely rare, it displaced the sensitive strain). So, spatial structure had effectively leveled the producer's hump. Why might this be?

Consider a scenario in which producers are very rare in the Petri dish. In such a spatially structured environment, the toxin released by a producer is not distributed to all members of the community. Rather, the sensitive neighbors of producers experience a disproportionately high dose of the toxin. As a consequence, the mortality rate of sensitive cells near toxin-producing cells is higher than that of the average sensitive cell. Given that reproduction also occurs locally, the space liberated near a producer (through the elevated deaths of sensitive cells) is disproportionately available to toxin-producing cells. In this way, small clumps of producers can "toxically clear-cut" sensitive cells at their periphery and radiate outward into a sea of sensitivity (see Fig. 6.1). Since Chao and Levin's pioneering study, the loss of bistability in structured bacteriocin communities has been demonstrated theoretically

through lattice-based simulation (Durrett and Levin 1997) and pair approximation (Iwasa et al. 1998).

Given the above theoretical and experimental results, the prospect for the long-term coexistence of producer and sensitive strains looks fairly grim. In well-mixed habitats, one of the two strains is predicted to displace the other,

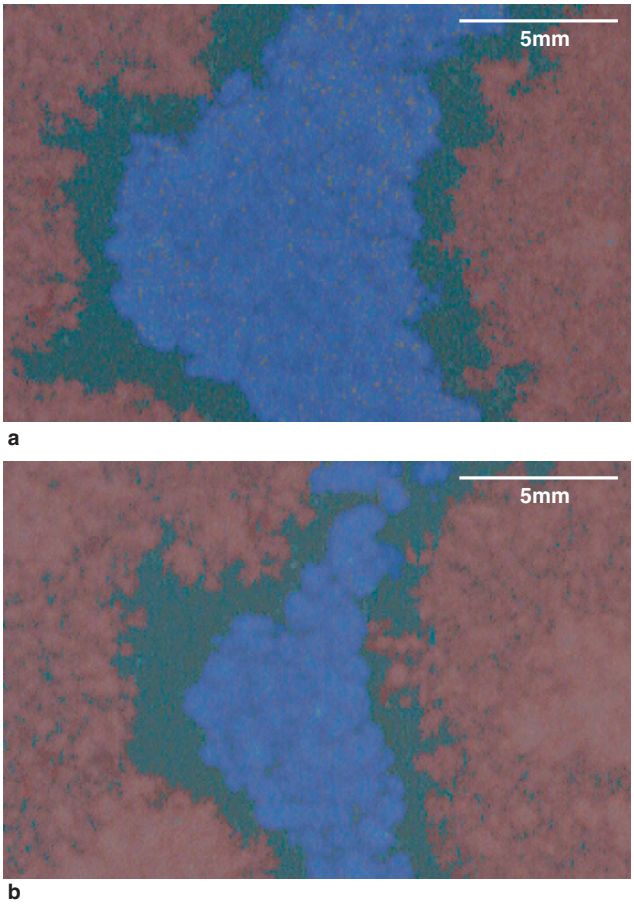

Fig. 6.1 The spatial advance of a producer strain. **a** A photograph of a bacterial community growing on the surface of an agar plate. The bacterial patches highlighted in *red* are producers (E2 colicinogenic *E. coli*) and the bacterial patches highlighted in *blue* are sensitive to the bacteriocin. The cell-free areas between the strains are zones of inhibition, where diffused toxin from the producer has prevented growth of the sensitive strain. **b** A photograph of the same field taken 24 h later (a velvet transfer of the community onto a new agar plate allowed for further growth without disrupting the spatial configuration). The producer patches have closed in on both sides of the sensitive patch. In this way, the producer (which grows to lower density on agar plates) can displace the sensitive strain through local toxic killing

depending on initial conditions. The addition of spatial structure simply tips the scales in favor of the producer. Are there circumstances under which we would expect the two strains to coexist?

Frank (1994) took a reaction-diffusion approach to modeling this two-strain system. He showed that if there is spatial heterogeneity in resource concentration, then both strains can stably coexist. In his model, toxin-producing cells inhabit resource-rich areas (where competition for resources is muted), while sensitive cells dominate the resource-poor areas (where resource competition is intense). Ultimately, Frank's model explains diversity by invoking underlying environmental heterogeneity. Although such spatial heterogeneity is not only plausible but probable, Frank's model does stimulate the following question: is it possible to maintain diversity even in a spatially homogeneous system?

In a recent paper, Czárán and Hoekstra (2003) demonstrate that the answer to this question is "yes". Their model assumes that the microbial community is distributed across many sites; collectively, the sites comprise a "metapopulation". Each site has the same properties (i.e., there is no underlying heterogeneity in this model), and the microbes are assumed to disperse among sites. If a site is simultaneously colonized by both sensitive cells and toxin-producing cells, then the producers will exclude the sensitive cells. However, the authors assume that the fast-growing sensitive cells hit high density (while the producer population is still at low numbers) before going locally extinct (as the producer increases to high density). While at high density, the sensitive cells migrate to other sites (whereas the low density producers do not). Thus, even though the fate of any sensitive strain is local extinction at a site through the toxic killing of a colonizing producer, the sensitive strain can nonetheless persist by embracing a nomadic lifestyle. As long as empty sites are continually being generated (i.e., there is some probability that a community at any given site will crash), the rapidly colonizing sensitive strain can persist globally. Such a model might be especially relevant for explaining diversity in bacteriocinogenic enterics (such as species of *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Serratia*, etc.), where the intestinal tracts of multiple hosts form a metapopulation.

Another explanation of the coexistence of producer and sensitive strains relies on the presence of a third strain of bacteria. We now turn to such three-member communities.

6.3 Dynamics in Three-Strain Communities: Playing Rock–Paper–Scissors

Cells sensitive to a bacteriocin will occasionally experience mutations that render them resistant. In *E. coli*, resistance often involves loss or alteration in a membrane-associated protein that binds or translocates the toxin (James

et al. 1996; Feldgarden and Riley 1998, 1999; Riley and Gordon 1999). Resistance is different than immunity. Producer immunity involves a constitutively expressed immunity protein that binds and neutralizes the producer's bacteriocin, whereas resistance is often engendered by a failure of the non-producing cell to bind or import the toxin in the first place.

Under some circumstances, the resistant strain will have a growth rate intermediate between that of sensitive and producer strains. The resistant strain may grow slower than the sensitive strain because the membrane proteins that bind or translocate bacteriocins often perform other cell functions (e.g., nutrient uptake), and thus their loss or alteration can compromise such functions. On the other hand, the resistant strain will grow faster than the producer when the costs of resistance (e.g., compromised nutrient uptake) are less than the costs of bacteriocin production (e.g., plasmid carriage, constitutive immunity, lethal synthesis). Given this ordering, a sensitive strain will outgrow a resistant strain, a resistant strain will outgrow a producer, and a sufficiently common producer can displace a sensitive type through toxic killing. Such a relationship is analogous to the children's game of rock–paper–scissors (indeed, an easy way to remember this is to look at the first letters of "*r*esistant–*p*roducer–*s*ensitive", although unfortunately, according to the first letters alone, the actual dynamic turns opposite to the *r*ock–*p*aper–*s*cissors game). This non-transitive dynamic has been found to hold for *E. coli* in vitro (Kerr et al. 2002) and in vivo (Kirkup and Riley 2004).

There has been a fair amount of theoretical interest in the dynamics of such rock–paper–scissors communities (Gilpin 1975; Durrett and Levin 1997; Riley and Gordon 1999; Nakamaru and Iwasa 2000; Kerr et al. 2002). In some non-transitive communities, the three players can coexist stably. However, under mass-action conditions, this is not the case for the resistant–producer–sensitive community. Using a system of ordinary differential equations, Durrett and Levin (1997) show that one strain always drives the other two extinct. Actually, the above resistant–producer–sensitive community is a special case of the more general Durrett and Levin model. The sensitive strain is predicted to dominate the well-mixed community (Nakamaru and Iwasa 2000). In the Appendix, we prove that sensitivity is an evolutionarily stable strategy (ESS) for a simple three-strain model.

One way to visualize the dynamics in this three-strain community is to use a de Finetti diagram. Here, a single point inside or on a triangle carries all the information to deduce the frequencies of the three strains: each of the three vertices is labeled with one of the three strains, and the frequency of each strain is given simply by the normalized distance from the point to the edge opposite to the relevant vertex. For instance, a point on the "sensitive" vertex corresponds to a community fixed for sensitive types, whereas a point on the edge connecting the "resistant" and "producer" vertices corresponds to a community without any sensitive cells, and a point in

the center of the triangle corresponds to a community with equal frequencies of each strain.

In Fig. 6.2a, we give the "boundary dynamics" on a de Finetti diagram for the rock–paper–scissors game (Frean and Abraham 2001; Czárán et al. 2002). We see that any community comprised of only "rock" and "paper" fixes for paper (since paper beats rock), any community of only "paper" and "scissors" fixes for scissors, and any community of only "scissors" and "rock" fixes for rock. In Fig. 6.2b, we show the dynamics when all three players are present – and we see continued cycles. In Fig. 6.2c, we give the boundary dynamics for the resistant–producer–sensitive game (when the producer is fairly toxic). Here, we see that we do not have a simple flow from one vertex to the next on the outside of the triangle. Rather, on the "sensitive–producer" edge we have flow going in both directions – both the sensitive pool and producer pool will exclude the other when they are sufficiently frequent in a two-strain community (see Fig. 6.B1f in Box 1). This is another appearance by the bistability described above. The hump (represented as a small gray point on the sensitive–producer edge in Fig. 6.2c) has reemerged. In Fig. 6.2d, we see that the dynamics are quite different than in the strict rock–paper–scissors game – the sensitive strain excludes the others from nearly every starting condition (see Nakamaru and Iwasa 2000, and the Appendix). Do note that these models assume infinite population sizes, and often the dynamical trajectory can come very close to the producer–resistant edge of the triangle (where the sensitive pool is extremely rare). Thus, in a finite population, one might often observe extinction of the sensitive strain and consequent fixation of the resistant strain (Kerr et al. 2002).

Interestingly, when this same three-member community is spatially structured (e.g., modeled as cells occupying the points of a lattice where reproduction and interaction are localized), all three strains can coexist (Durrett and Levin 1997; Kerr et al. 2002). Due to local reproduction in a spatially structured environment, clumps of each of the three strains form, and these clumps chase one another at their boundaries. Sensitive patches chase resistant patches, resistant patches chase producer patches, and producer patches in turn chase sensitive patches. Thus, all clumps are simultaneously chasing and being chased, and the upshot of this shifting mosaic is that all strains are maintained (see Box 2). By propagating three strains of *E. coli* in a well-mixed habitat (a stirred flask) and a structured habitat (the surface of an agar plate), Kerr et al. (2002) experimentally demonstrated that spatial structure can promote the maintenance of diversity in a bacteriocin community. In a sense, spatial structure in these cases obliterates the "hump" on the sensitive–producer edge of the de Finetti diagram (Durrett and Levin 1997; Iwasa et al. 1998). In Fig. 6.2f, we see simulated dynamics from the lattice-based model described in Box 2. This behavior is much closer to the rock–paper–scissors game of Fig. 6.2b (with the caveat that the arrows flow in the opposite direction).

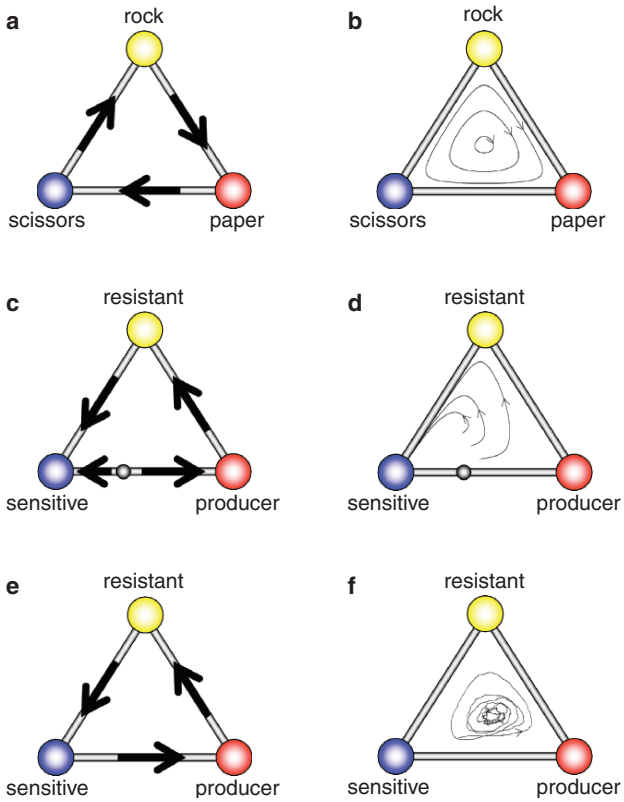

Fig. 6.2 Three-strain community dynamics. In this figure, the de Finetti diagram is used to represent changes in community composition. Each vertex of the triangle is labeled with one of the three competitors, and then the frequencies of the competitors are given by the location of a point inside or on the triangle. To find the frequency of a specific competitor, simply compute the normalized distance from the point to the edge opposite the competitor's vertex. Thus, the closer the point is to any given vertex, the more frequent is the corresponding competitor. The movement of this point traces out trajectories within the triangle that give an illustration of community dynamics. **a** This schematic gives the basic rock–paper–scissors dynamics (e.g., Frean and Abraham 2001). The *thick arrows* on the edges of the triangle give the pairwise competition outcomes. For instance, since rock beats scissors, the point giving frequencies "flows" from the scissors vertex toward the rock vertex (rock "competitors" replace scissors "competitors"). **b** When all three competitors (rock, paper, and scissors) are simultaneously present, the point is inside the triangle. The community dynamics are shown for Frean and Abraham's (2001) rock–paper–scissors model (we set their $P_r = P_s = P_p = 0.7$). The trajectories are closed loops – the frequencies of each competitor oscillate indefinitely (where the amplitude of oscillation depends on starting frequencies) and all three strains are maintained. **c** This schematic gives the resistant–producer–sensitive dynamics in a well-mixed habitat (see Appendix). The *thick arrows* indicate that the sensitive strain will outcompete the resistant strain, and the resistant strain will outcompete the producer. However, along the edge connecting the sensitive and producer vertices, there is a bistability (if producers are sufficiently common, they displace sensitive cells and vice versa – see Box 1). **d** The interior dynamics are noticeably different from the rock–paper–scissors game – the sensitive strain ends up dominating the community. **e** In the spatial version of the resistant–producer–sensitive dynamics, the rock–paper–scissors game reemerges (although the arrows flip because the pairwise competitions reverse when using the same *r–p–s* lettering on the triangle). **f** When all three strains are present in a finite structured lattice, the community cycles into a stable oscillating coexistence (see Box 2)

Box 2: A lattice-based three-strain model

The following approach is a slight modification of the agent-based simulations in Durrett and Levin (1997). A virtual community of sensitive cells, producers and resistant cells occupy the points of an $L \times L$ square lattice with wrap-around boundaries. To start the simulation, every point in the lattice is randomly assigned one of the following states: {**S**, **P**, **R**, **E**}, where **S** represents a point occupied by a sensitive cell, **P** is a point with a producer, **R** is a point with a resistant cell, and **E** is an empty lattice point. The community dynamics are given by an asynchronous updating scheme, in which a random sequence of focal points in the lattice are picked and the state of each focal point is changed probabilistically. For instance, an **S**→**E** transition describes the death of a sensitive cell, whereas an **E**→**P** transition describes the "birth" of a producer. The probabilities of specific state changes of a focal point depend not only on its current state, but also potentially on the states of points in its neighborhood. For instance, a sensitive cell surrounded by toxin-producing cells has a higher probability of death (i.e., the **S**→**E** transition is more likely) than an isolated sensitive cell.

By varying the size of the neighborhood, the scale of ecological processes (such as toxic interaction, competition for space, and dispersal) can be controlled. If we make the neighborhood small, then dispersal and interaction become spatially restricted. For instance, the neighborhood might be the eight nearest lattice points around a focal point (this is called a Moore neighborhood). Alternatively, we might make the neighborhood of a focal point the entire lattice (minus the focal). For such a "Global" neighborhood, the community behaves like a well-mixed system.

If we pick an empty point to update, then it becomes filled with strain i ($i \in \{\mathbf{S},\mathbf{P},\mathbf{R}\}$) with probability f_i, where f_i is the fraction of the empty point's neighborhood filled with strain i. If a point occupied by strain i is picked, then it goes to an empty state (a death event) with probability Δ_i. While $\Delta_{\mathbf{P}}$ and $\Delta_{\mathbf{R}}$ are assumed to be constant parameters, $\Delta_{\mathbf{S}}$ is not; the death rate of a sensitive cell is assumed to increase linearly with the fraction of producers in its neighborhood:

$$\Delta_{\mathbf{S}} = \Delta_{\mathbf{S},0} + \tau f_{\mathbf{P}} \tag{B2.1}$$

where $\Delta_{\mathbf{S},0}$ gives the intrinsic probability of a sensitive cell's death (i.e., when there are no producers in its neighborhood) and τ measures the toxicity of the producer (τ is similar to the γ parameter in Box 1). To guarantee non-transitivity in this system, the following is assumed:

$$\Delta_{\mathbf{S},0} < \Delta_{\mathbf{R}} < \Delta_{\mathbf{P}} < \frac{\Delta_{\mathbf{S}} + \tau}{1 + \tau} \tag{B2.2}$$

Box 2: Continued

In words, conditions (B2.2) simply state that there is a net growth hierarchy with the sensitive strain on the top, the resistant strain in the middle, and the producer on the bottom. However, the producer is above a critical toxic level, which yields a non-transitive competitive dynamic.

When this three-strain community is simulated using a Moore neighborhood, all three strains coexist under many different parameter settings. Because dispersal is local, clumps of the three strains form and these clumps chase one another at their boundaries – **S** clumps chase **R** clumps, **R** clumps chase **P** clumps, and **P** clumps chase **S** clumps (see Fig. 6.B2a, b). However, when a Global neighborhood is used, diversity is rapidly lost

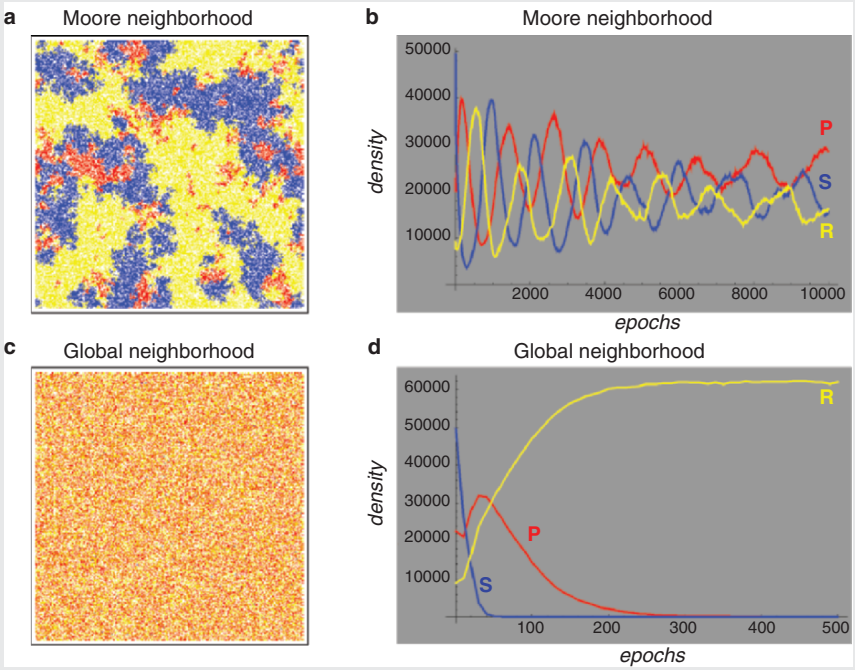

Fig. 6.B2 Lattice-based simulations. **a** A snapshot of a 300×300 structured lattice after 750 epochs (an epoch is $L \times L = 300 \times 300$ updates). Sensitive cells are *blue*, producers are *red* and resistant cells are *yellow*. A Moore neighborhood was used, and the parameters were $\Delta_{S,0} = 1/4$, $\Delta_P = 1/3$, $\Delta_R = 0.312$, and $\tau = 0.65$. Substantial clumping can be observed in this picture of the lattice. These clumps chase one another across the lattice according to the non-transitive dynamic. **b** The population dynamics over 10,000 epochs showing that all three strains persist for long periods of time. **c** A snapshot of a 300×300 unstructured lattice after 50 epochs. A Global neighborhood was used with the same parameters as for parts **a**, **b**. In this case, there is no spatial clumping. **d** Diversity is rapidly lost from the Global neighborhood simulation

(see Fig. 6.B2c, d). In the Global neighborhood, the toxic effects of producers are distributed globally. This can drive the sensitive strain to very low levels (unless the producer is not very toxic). Indeed, because our lattice is finite, the sensitive strain often goes extinct. Once one member of a non-transitive triplet is lost, the final competitive outcome is decided (for the same reason that a game of "rock–paper" would be much less entertaining for schoolchildren than a game of "rock–paper–scissors"). If the sensitive strain exits the community, then the resistant strain simply outcompetes the producer, and we end up with a monomorphic population.

The simulations with a Global neighborhood correspond closely to the dynamics given by the set of mean-field ordinary differential equations (see the Appendix and Fig. 6.2c, d). However, because such mathematical models assume infinite populations (and thus one can have an arbitrarily small density of sensitive cells), the sensitive strain is expected to "hang on" as the resistant displaces the producer, and eventually dominates the community. However, the outcome for the maintenance of diversity is the same: diversity is lost in the well-mixed community. Thus, population structure can be critical to coexistence. This role for spatial structure promoting diversity in a non-transitive bacteriocin community was demonstrated in vitro with *E. coli* (Kerr et al. 2002).

6.4 Evolution in Three-Strain Communities: Survival of the Weakest

Up to this point, we have considered only ecological dynamics in microbial communities. Of course, given their large population sizes and short generation times, it would be inappropriate to ignore evolution. There have been a few theoretical studies that have considered the effects of evolutionary change within a rock–paper–scissors system (Frean and Abraham 2001; Johnson and Seinen 2002). However, there has not been any detailed theoretical or experimental analysis of the evolutionary dynamics within the aforementioned resistant–producer–sensitive system.

As resistance to a bacteriocin arises readily through mutation of sensitive cells, and the cost of resistance is often variable (Feldgarden and Riley 1998, 1999), it would seem reasonable to consider the possibility that the cost of resistance can change evolutionarily. One way to model this situation is outlined in Box 3. An intuitive expectation is that the resistant strain should evolve to minimize its cost (e.g., continually lower its death rate or raise its reproductive rate). What actually occurs in simulations seems bizarre at first glance: in a spatially structured community with producer and sensitive strains, the resistant population does *not* evolve to minimize its cost! Why is this?

Box 3: An evolutionary simulation

In order to introduce evolution in the cost of resistance, we consider a small wrinkle to the lattice-based model in Box 2. Specifically, instead of fixing the probability of death of a resistant cell as a global parameter Δ_R, we allow every single resistant cell to carry its own Δ_R. Within the framework of the model, this Δ_R is the genotype of our virtual resistant cell. When a new resistant cell is "born", a mutation can occur to change the death probability. Specifically, if $\Delta_R(parent)$ is the death rate of a parent, then we assume that the death rate of an offspring is:

$$\Delta_R(offspring) = \begin{cases} \max(\min(\Delta_R(parent) + Z, \Delta_P - \varepsilon), \Delta_{S,0} + \nu) & with\ prob.\ \mu \\ \Delta_R(parent) & with\ prob.\ (1-\mu) \end{cases} \quad \text{(B3.1)}$$

where μ is the probability of mutation and Z is a random variable (for instance, $Z \sim N(0,\sigma^2)$ or $Z \sim Unif(-\phi, \phi)$, where σ or ϕ relate to the amount that the death rate can change due to a single mutation). We assume that the death rate of the resistant cell must always remain intermediate between the intrinsic death rate of the sensitive strain and the death rate of the producer – the positive parameters ε and ν are taken to be small, but are nevertheless included to guarantee that, despite any evolutionary change, the non-transitive competitive structure is maintained.

When a resistant population is simulated without other competing strains, it evolves to minimize the cost of resistance (average Δ_R evolves to the minimum value in the range allowed). However, when evolution occurs in a three-strain community with local dispersal and interaction (using a Moore neighborhood), the cost of resistance does not evolve to its lowest level (see Fig. 6.3). It pays off to exercise competitive restraint in this non-hierarchical community because such restraint aids the enemy of your enemy (which, in turn, harms your enemy and thus aids you). An extremely interesting direction for future experimental work involves exploration of these counterintuitive spatial evolutionary dynamics within non-transitive systems.

The reason is given by the adage "the enemy of my enemy is my friend". In a spatially structured habitat, each strain exists as a set of clumps. These clumps are simultaneously chasing other clumps, and being chased. Now, if a mutant arises within a resistant clump that has a much reduced cost, then this mutant will start to outcompete both its fellow resistant types and any nearby producer cells. In fact, the resulting mutant clump will chase bordering producer clumps more rapidly. If the mutant has extremely low costs,

then the mutant clump can chase a bordering producer clump to extinction, which puts these mutants face-to-face with a sensitive clump (an interaction in which they do not fare well). In this way, by continuing to lower costs, a resistant lineage may "improve itself to death". The fact that many such clumps simultaneously exist across a large spatial arena means that the drive within clumps to reduce the cost of resistance is checked by the enhanced probability of clump extinction. Strains that exercise restraint (i.e., maintain relatively high costs) persist by default as their less restrained cousins burn themselves out.

In Fig. 6.3, we see the maintenance of a non-minimal cost of resistance in a spatially structured three-strain community. On the other hand, if the resistant strain evolves alone in a spatially structured habitat, it does evolve to minimize its cost (Fig. 6.3). In a structured non-transitive community, a higher cost of resistance retards replacement of producers by resistant cells.

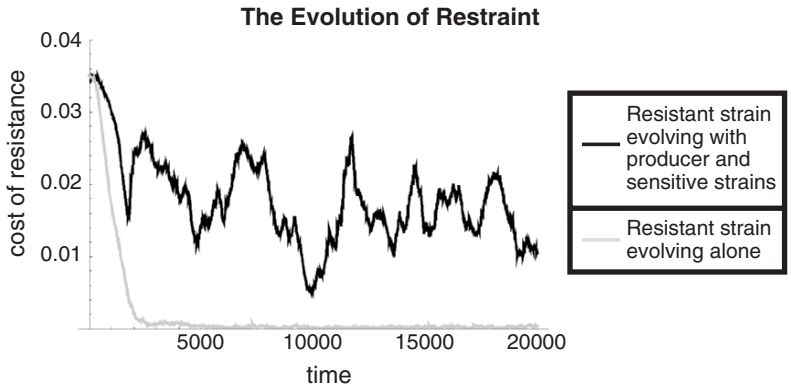

Fig. 6.3 The evolution of competitive restraint. Shown are the results of a lattice-based simulation (described in Box 2) allowing for the resistant strain to evolutionarily change the cost of resistance (see Box 3). The parameters used are $\Delta_{S,0} = 1/4$, $\Delta_P = 1/3$, $\tau = 0.55$, $\varepsilon = 0.004$, $\nu = 0.025$, $\mu = 0.001$, and $Z \sim \text{Unif}(-0.02,0.02)$. The death rate of the resistant strain (Δ_R) can evolve. The average cost of resistance is simply $CR = \bar{\Delta}_R - \Delta_{S,0}$ where $\bar{\Delta}_R$ is the average death rate of the evolving resistant strain. The minimum value that CR can obtain is ν. A proxy for cost of resistance is $CR' = CR - \nu$. All else being equal, the resistant strain is expected to evolve to minimize its cost (i.e., we expect $CR' \to 0$). The *black trajectory* is the average cost of resistance (CR') in a 300×300 square lattice with a Moore neighborhood, where the resistant strain shares the lattice with the producer and the sensitive strain. Here, we see that the cost of resistance does not evolve to its minimum, but rather remains at higher levels (that is, the average death rate of the resistant strain is evolutionarily maintained at a value higher than its obtainable minimum). As a control, the *gray trajectory* shows evolution of the cost of resistance when the resistant strain is evolving alone in 107 × 107 lattice (the lattice size was shrunk so that the average density of resistant cells was roughly the same between simulations). In the case shown (and for several other simulations at a variety of lattice sizes), the solitary resistant strain immediately evolves to minimize its cost. Thus, the presence of producer and sensitive strains in a spatially structured habitat selects for competitive restraint in the resistant strain

By liberating the enemy of their enemy, these costly lineages liberate themselves (Tainaka 1993, 1995; Frean and Abraham 2001; Johnson and Seinen 2002). This phenomenon has been dubbed "survival of the weakest" (Frean and Abraham 2001).

6.5 Dynamics with many Strains: Universal Chemical Warfare

Naturally occurring microbial populations contain several different bacteriocinogenic strains (Gordon et al. 1998; Riley and Gordon 1999; Riley and Wertz 2002a, 2002b). Each distinct producer can be sensitive to the toxin produced by a different producer within its own (or closely related) species. In addition, resistance (sometimes to multiple toxins) can be generated through mutation and is very common in natural populations (Feldgarden and Riley 1998, 1999). What are the dynamical consequences of many interlacing games of rock–paper–scissors being played out simultaneously? How is such diversity maintained? It turns out that by inspecting such convoluted microbial chemical warfare, we gain some insight into mechanisms maintaining diversity (Lenski and Riley 2002).

There have been a few models that have considered multiple bacteriocin producers with cross-killing abilities. Pagie and Hogeweg (1999) model multiple producers within a lattice-based simulation framework. They find that within a spatially structured system, multiple producers can stably coexist. Further, the type of coexistence depends on the cost of resistance against toxins. If this cost is low, then the community enters into a "hyperimmunity" mode where most cells will be resistant to many different toxins, but few cells will produce very many toxins. However, if this cost is high, the community displays a "multitoxicity" mode, where cells are resistant to fewer toxins and tend to individually produce more toxins. Interestingly, the shift from "hyperimmunity" to "multitoxicity" is rather abrupt as the cost of resistance increases. Czárán et al. (2002) build on this earlier model of multiple producers, incorporating horizontal gene transfer and recombination between strains. Under many circumstances, they find that their lattice of multiple producers transitions through a "multitoxicity" mode and settles into a "hyperimmunity" mode.

Spatial structure is an important ingredient in these models – diversity drops dramatically in a well-mixed environment (Pagie and Hogeweg 1999). This role of spatial structure has been validated in a few experimental studies of multiple producers (Tait and Sutherland 2002; Massey et al. 2004). So far, most experimental work on multi-producer communities has been limited to pairs of interacting strains. It will be interesting to follow experimentally the dynamics of larger numbers of microbial players in order to see how well the predictions of the simulation models play out.

The study of communities with multiple producers will be especially exciting in light of recent observations suggesting that bacteriocins excreted by

one producer can act as inducers of bacteriocins in other producers (Kuipers et al. 1995; Kleerebezem et al. 1997, 2004; Tait and Sutherland 2002; Gillor and Riley, unpublished data). Interestingly, such cross-induction can reintroduce bistability into the spatial dynamics of a two-strain system (Gillor et al., unpublished data). Specifically, cells of a rare invading producer (call the invading strain **A**) will induce neighboring resident producer cells (call the resident strain **B**) to produce toxin, which in turn will further induce the invader. This local escalation of chemical warfare can favor the common producer strain, as it effectively "surrounds" the invading strain with its toxin. This means that a population of producer strain **A** can exclude invading strain **B**, and a population of producer strain **B** can exclude invading strain **A**. It will be intriguing to see if this potential return to bistability occurs in spatially structured multiple-producer laboratory communities.

6.6 Discussion

Lewontin (1982, 1983) has suggested that the metaphor of adaptation (in which organisms that best fit preexistent niches are selected) should be replaced with a metaphor of construction. Lewontin's idea is that organisms, through their physiology, behavior and development, alter their world and thus influence the very form of their niche. That is to say, niches are not simply "out there" waiting to be filled, but rather are (at least partially) made via the effects organisms have on their abiotic and biotic surroundings. In Lewontin's view, the organism becomes a co-author in its own evolution and ecology. This process has been labeled niche construction (Odling-Smee et al. 1996, 2003; Laland et al. 1999, 2000), or alternatively ecosystem engineering (Jones et al. 1994, 1997). The production of bacteriocins within microbial communities is a potent form of niche construction – a producing cell alters the toxin concentration of its surroundings, shifting strain composition toward immune and resistant types.

Indeed, this toxic niche construction is one way to form a non-transitive competitive dynamic. Specifically, with regards to growth rate, the strain on the bottom of the totem pole (the producer) kills the strain at the top (the sensitive), thus creating a loop in the competitive interactions. Such non-transitivity has been found in other systems as well, including side-blotched lizards (Sinervo and Lively 1996), sessile marine invertebrates (Buss and Jackson 1979), and yeast (Paquin and Adams 1983). Theoretical work on non-hierarchically organized communities has shown that such interactions can promote the maintenance of biodiversity (Huisman and Weissing 1999; Huisman et al. 2001). Non-transitivity may be an important ingredient in the persistence of diverse bacteriocin communities, but it seems to require a partner to get the job done. This partner is population structure.

Because niche construction is ultimately frequency-dependent, toxin producers competing with sensitive cells in a well-mixed environment face a

dynamical hump to get over (Adams et al. 1979; Chao and Levin 1981; Levin 1988; Durrett and Levin 1997; Iwasa et al. 1998). In unstructured habitats, the signature of this hump is present in the resistant–producer–sensitive community, changing the dynamics from a straightforward "rock–paper–scissors" to a "one-winner" outcome (compare Fig. 6.2a, b to c, d). Population structure (e.g., spatial structure) can effectively eliminate the hump (Chao and Levin 1981; Durrett and Levin 1997; Iwasa et al. 1998) and restore the game of rock–paper–scissors. In this spatial game, players stably chase each other around a structured arena as clumps, with balanced gains and losses occurring at the boundaries (Durrett and Levin 1997; Kerr et al. 2002). Kirkup and Riley (2004) demonstrated that the same non-transitive dynamic occurs in the mouse alimentary tract, a spatially structured habitat. In addition, spatial structure is an important ingredient in the coexistence of multiple-producer strains (Pagie and Hogeweg 1999; Czárán et al. 2002).

It is worthwhile to highlight the nature of the explanations of biodiversity maintenance offered by the above models. While biodiversity in the system can result from exogenous heterogeneity in the underlying substrate (Frank 1994), many of these models describe diversity resulting from *endogenous* processes. That is, diversity is a product of the way non-transitive interactions play out in a spatially structured world. In this sense, diversity "flows from within" the system. Part of the recent interest in spatial ecology (Durrett and Levin 1994a, 1994b; May 1999; Bolker et al. 2003) derives from an interest in understanding how global patterns result from local processes (Hassell et al. 1994; May 1999; Wootton 2001). This idea of system self-organization is the natural outgrowth of localized niche construction, where the effects organisms have within neighborhoods scale up to influence the form of the entire community.

Models have been indispensable in the study of bacteriocin community self-organization. Part of this success has depended on the sustained interaction between those exploring theoretical models and those experimenting with model communities in the laboratory. For instance, Chao and Levin (1981) described frequency-dependence in sensitive–producer communities of well-mixed *E. coli*, and then Levin (1988) analytically demonstrated the bistability. As another example, Chao and Levin (1981) demonstrated that the spatial structure afforded by soft agar poured in a Petri dish could eradicate the frequency-dependence and then Durrett and Levin (1997), using cellular automata, confirmed these empirical observations (see also Iwasa et al. 1998). As yet another example, Durrett and Levin (1997), using lattice-based models, predicted that spatial structure would be required for long-term coexistence of the resistant–producer–sensitive community, and this was empirically confirmed 5 years later by Kerr et al. (2002). There has been mutual benefit by maintaining an active dialogue between theoretical and empirical work.

And such dialogue will certainly facilitate future understanding of these communities. There are several questions ripe for exploration. What are the

evolutionary dynamics in resistant–producer–sensitive communities? Will we actually observe a form of "survival of the weakest" in laboratory communities? What are the ecological and evolutionary dynamics of communities with multiple bacteriocin producers? What are the dynamics of diverse bacteriocin communities in natural settings? What effects will cross-induction (another form of niche construction) play in these dynamics? Models will most certainly continue to play an important role in exploring such issues, and through the study of model systems, the next set of questions will begin to emerge.

Acknowledgements. I thank Milind Chavan, Carla Goldstone, Federico Prado, Peg Riley and Karen Walag for many useful comments on previous versions of this chapter.

Appendix

Sensitivity is an ESS in the Well-Mixed RPS Game

Consider the following set of differential equations describing the dynamics of sensitive, producer and resistant strains (see Durrett and Levin 1997, and Box 1):

$$\frac{ds}{dt} = \beta_s (1 - s - p - r)\, s - (\delta_s + \gamma p)\, s, \tag{A.1}$$

$$\frac{dp}{dt} = \beta_p (1 - s - p - r)\, p - \delta_p\, p, \tag{A.2}$$

$$\frac{dr}{dt} = \beta_r (1 - s - p - r)\, r - \delta_r\, r. \tag{A.3}$$

This system has an equilibrium at $(s,p,r) = ((\beta_s - \delta_s)/\beta_s, 0, 0) = (\hat{s}, 0, 0)$ where only toxin-sensitive cells exist. Consider a perturbation to this equilibrium, $(\hat{s} + \varepsilon_s, \varepsilon_p, \varepsilon_r)$, where all ε values are very small. The dynamics of the perturbations are given by:

$$\frac{d\varepsilon_s}{dt} = (\beta_s(1-2\hat{s}) - \delta_s)\varepsilon_s - ((\beta_s + \gamma)\hat{s})\varepsilon_p - (\beta_s \hat{s})\varepsilon_r - \beta_s \varepsilon_s(\varepsilon_s + \varepsilon_p + \varepsilon_r) - \gamma \varepsilon_s \varepsilon_p, \tag{A.4}$$

$$\frac{d\varepsilon_p}{dt} = \left(\beta_p (1 - \hat{s}) - \delta_p\right)\varepsilon_p - \beta_p\, \varepsilon_p \left(\varepsilon_s + \varepsilon_p + \varepsilon_r\right), \tag{A.5}$$

$$\frac{d\varepsilon_r}{dt} = \left(\beta_r (1 - \hat{s}) - \delta_r\right)\varepsilon_r - \beta_r\, \varepsilon_r \left(\varepsilon_s + \varepsilon_p + \varepsilon_r\right). \tag{A.6}$$

Linearizing the system about $(\varepsilon_s, \varepsilon_p, \varepsilon_r) = (0,0,0)$, we have

$$\dot{\vec{\varepsilon}} = \mathbf{J}\vec{\varepsilon}, \tag{A.7}$$

where

$$\vec{\varepsilon} = \begin{bmatrix} \varepsilon_s \\ \varepsilon_p \\ \varepsilon_r \end{bmatrix}, \tag{A.8}$$

and the Jacobian is

$$\mathbf{J} = \begin{bmatrix} \beta_s(1-2\hat{s}) - \delta_s & -(\beta_s+\gamma)\hat{s} & -\beta_s\hat{s} \\ 0 & \beta_p(1-\hat{s}) - \delta_p & 0 \\ 0 & 0 & \beta_r(1-\hat{s}) - \delta_r \end{bmatrix}. \tag{A.9}$$

The eigenvalues of **J** give the local stability of the equilibrium ($\hat{s}$,0,0). Since **J** is a triangular matrix, the eigenvalues line the diagonal. Because we assume

$$\beta_i > \delta_i \text{ for all } i \in \{s,p,r\}, \tag{A.10}$$

and

$$\frac{\delta_s}{\beta_s} < \frac{\delta_r}{\beta_r} < \frac{\delta_p}{\beta_p} < \frac{\delta_s+\gamma}{\beta_s+\gamma}, \tag{A.11}$$

all of the eigenvalues are negative, which means the equilibrium ($\hat{s}$,0,0) is locally stable and thus toxin sensitivity is an evolutionarily stable strategy (an ESS). The other fixation equilibria,

$$e_2 = \left(0, \frac{\beta_p - \delta_p}{\beta_p}, 0\right), \tag{A.12}$$

$$e_3 = \left(0, 0, \frac{\beta_r - \delta_r}{\beta_r}\right), \tag{A.13}$$

are locally unstable (this can be shown using linear stability analysis as well). Lastly, under assumption (A.11), there is another equilibrium:

$$e_4 = \left(1 + \frac{\delta_s}{\gamma} - \left(1 + \frac{\beta_s}{\gamma}\right)\frac{\delta_p}{\beta_p}, \frac{1}{\gamma}\left(\frac{\delta_p}{\beta_p}\beta_s - \delta_s\right), 0\right), \tag{A.14}$$

which also is unstable. Thus, sensitivity to the toxin is the only ESS in this system. Indeed, from nearly any starting point, the sensitive strain will displace the other two strains (Nakamaru and Iwasa 2000).

References

Adams J, Kinney T, Thompson S, Rubin L, Helling RB (1979) Frequency-dependent selection for plasmid-containing cells of *Escherichia coli*. Genetics 91:627–637

Bolker BM, Pacala SW, Neuhauser C (2003) Spatial dynamics in model plant communities: what do we really know? Am Naturalist 162:135–148
Buss LW, Jackson JBC (1979) Competitive networks – non-transitive competitive relationships in cryptic coral-reef environments. Am Naturalist 113:223–234
Chao L, Levin BR (1981) Structured habitats and the evolution of anticompetitor toxins in bacteria. Proc Natl Acad Sci USA Biol Sci 78:6324–6328
Czárán TL, Hoekstra RF (2003) Killer-sensitive coexistence in metapopulations of microorganisms. Proc R Soc Lond Series B-Biol Sci 270:1373–1378
Czárán TL, Hoekstra RF, Pagie L (2002) Chemical warfare between microbes promotes biodiversity. Proc Natl Acad Sci USA 99:786–790
Durrett R, Levin S (1994a) The importance of being discrete (and spatial). Theor Popul Biol 46:363–394
Durrett R, Levin SA (1994b) Stochastic spatial models – a users guide to ecological applications. Philos Trans R Soc Lond Series B-Biol Sci 343:329–350
Durrett R, Levin S (1997) Allelopathy in spatially distributed populations. J Theor Biol 185:165–171
Dykes GA, Hastings JW (1997) Selection and fitness in bacteriocin-producing bacteria. Proc R Soc Lond Series B-Biol Sci 264:683–687
Feldgarden M, Riley MA (1998) High levels of colicin resistance in *Escherichia coli*. Evolution 52:1270–1276
Feldgarden M, Riley MA (1999) The phenotypic and fitness effects of colicin resistance in *Escherichia coli* K-12. Evolution 53:1019–1027
Frank SA (1994) Spatial polymorphism of bacteriocins and other allelopathic traits. Evol Ecol 8:369–386
Frean M, Abraham ER (2001) Rock-scissors-paper and the survival of the weakest. Proc R Soc Lond Series B-Biol Sci 268:1323–1327
Gilpin ME (1975) Limit cycles in competition communities. Am Naturalist 109:51–60
Gordon DM, Riley MA (1999) A theoretical and empirical investigation of the invasion dynamics of colicinogeny. Microbiology-SGM 145:655–661
Gordon DM, Riley MA, Pinou T (1998) Temporal changes in the frequency of colicinogeny in *Escherichia coli* from house mice. Microbiology-UK 144:2233–2240
Hassell MP, Comins HN, May RM (1994) Species coexistence and self-organizing spatial dynamics. Nature 370:290–292
Huisman J, Weissing FJ (1999) Biodiversity of plankton by species oscillations and chaos. Nature 402:407–410
Huisman J, Johansson AM, Folmer EO, Weissing FJ (2001) Towards a solution of the plankton paradox: the importance of physiology and life history. Ecol Lett 4:408–411
Iwasa Y, Nakamaru M, Levin SA (1998) Allelopathy of bacteria in a lattice population: competition between colicin-sensitive and colicin-producing strains. Evol Ecol 12:785–802
James R, Kleanthous C, Moore GR (1996) The biology of E colicins: paradigms and paradoxes. Microbiology-UK 142:1569–1580
Johnson CR, Seinen I (2002) Selection for restraint in competitive ability in spatial competition systems. Proc R Soc Lond Series B-Biol Sci 269:655–663
Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386
Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as physical ecosystem engineers. Ecology 78:1946–1957
Kerr B, Riley MA, Feldman MW, Bohannan BJM (2002) Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. Nature 418:171–174
Kirkup BC, Riley MA (2004) Antibiotic-mediated antagonism leads to a bacterial game of rock-paper-scissors in vivo. Nature 428:412–414
Kleerebezem M, Quadri LEN, Kuipers OP, de Vos WM (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol Microbiol 24:895–904

Kleerebezem M, Bongers R, Rutten G, de Vos WM, Kuipers OP (2004) Autoregulation of subtilin biosynthesis in *Bacillus subtilis*: the role of the spa-box in subtilin-responsive promoters. Peptides 25:1415–1424
Kuipers OP, Beerthuyzen MM, Deruyter P, Luesink EJ, de Vos WM (1995) Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal-transduction. J Biol Chem 270:27299–27304
Laland KN, Odling-Smee FJ, Feldman MW (1999) Evolutionary consequences of niche construction and their implications for ecology. Proc Natl Acad Sci USA 96:10242–10247
Laland KN, Odling-Smee FJ, Feldman MW (2000) Niche construction, biological evolution and cultural change. Behav Brain Sci 23:131–175
Lenski RE, Riley MA (2002) Chemical warfare from an ecological perspective. Proc Natl Acad Sci USA 99:556–558
Levin BR (1988) Frequency-dependent selection in bacterial-populations. Philos Trans R Soc Lond Series B-Biol Sci 319:459–472
Lewontin RC (1982) Organism and environment. In: Learning, development, and culture. MIT Press, Cambridge, MA
Lewontin RC (1983) Gene, organism, and environment. In: Bendall DS (ed) Evolution from molecules to men. Cambridge University Press, Cambridge
Massey RC, Buckling A, Ffrench-Constant R (2004) Interference competition and parasite virulence. Proc R Soc Lond Series B-Biol Sci 271:785–788
May R (1999) Unanswered questions in ecology. Philos Trans R Soc Lond Series B-Biol Sci 354:1951–1959
Nakamaru M, Iwasa Y (2000) Competition by allelopathy proceeds in traveling waves: colicin-immune strain aids colicin-sensitive strain. Theor Popul Biol 57:131–144
Odling-Smee FJ, Laland KN, Feldman MW (1996) Niche construction. Am Naturalist 147:641–648
Odling-Smee FJ, Laland KN, Feldman MW (2003) Niche construction: the neglected process in evolution. Princeton University Press, Princeton, NJ
Pagie L, Hogeweg P (1999) Colicin diversity: a result of eco-evolutionary dynamics. J Theor Biol 196:251–261
Paquin CE, Adams J (1983) Relative fitness can decrease in evolving asexual populations of *S. cerevisiae*. Nature 306:368–371
Riley MA (1998) Molecular mechanisms of bacteriocin evolution. Annu Rev Genet 32:255–278
Riley MA, Gordon DM (1996) The ecology and evolution of bacteriocins. J Indus Microbiol 17:151–158
Riley MA, Gordon DM (1999) The ecological role of bacteriocins in bacterial competition. Trends Microbiol 7:129–133
Riley MA, Wertz JE (2002a) Bacteriocin diversity: ecological and evolutionary perspectives. Biochimie 84:357–364
Riley MA, Wertz JE (2002b) Bacteriocins: evolution, ecology, and application. Annu Rev Microbiol 56:117–137
Sinervo B, Lively CM (1996) The rock-paper-scissors game and the evolution of alternative male strategies. Nature 380:240–243
Tainaka K (1993) Paradoxical effect in a 3-candidate voter model. Phys Lett A 176:303–306
Tainaka K (1995) Indirect effect in cyclic voter models. Phys Lett A 207:53–57
Tait K, Sutherland IW (2002) Antagonistic interactions amongst bacteriocin-producing enteric bacteria in dual species biofilms. J Appl Microbiol 93:345–352
Tan Y, Riley MA (1996) Rapid invasion by colicinogenic *Escherichia coli* with novel immunity functions. Microbiology-UK 142:2175–2180
Wiener P (2000) Antibiotic production in a spatially structured environment. Ecol Lett 3:122–130
Wootton JT (2001) Local interactions predict large-scale pattern in empirically derived cellular automata. Nature 413:841–844