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Allelopathy in Spatially Distributed Populations

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In a homogeneously mixing population of *E. coli*, colicin-producing and colicin-sensitive strategies both may be evolutionarily stable for certain parameter ranges, with the outcome of competition determined by initial conditions. In contrast, in a spatially-structured population, there is a unique ESS for any given set of parameters; the outcome is determined by how effective allelopathy is in relation to its costs. Furthermore, in a spatially-structured environment, a dynamic equilibrium may be sustained among a colicin-sensitive type, a high colicin-producing type, and a "cheater" that expends less on colicin production but is resistant.

1. Introduction

A central issue in evolutionary theory is the evolution of altruistic behavior, and how it is influenced and enhanced by aspects of the social structure of populations; an equally compelling and closely related issue involves the evolution of antagonistic behaviors. Bacteria may produce toxic substances, known collectively as bacteriocins, that kill or inhibit the growth of competing bacteria of different genotypes through what is known as allelopathy. In general, bacteria that are capable of producing such chemicals are immune to their action. The colicins, the most extensively studied class of bacteriocins, are produced by the bacterium *Escherica coli* and other members of the family Enterobacteriaceae. For concreteness and ease of exposition we will restrict our attention to the last special case. It should be clear, however, that our analysis applies to bacteriocins in general and to a number of other similar competitive situations.

Most theoretical studies of the competition of colicin-producing and colicin-sensitive bacteria assume that the population is homogeneously mixing to arrive at differential equations describing their dynamics [see Levin (1988) and Frank (1994)]. This approach is appropriate, for example, when the bacteria are grown in liquid cultures. However, experimental studies [see Chao & Levin (1981)] show that the outcome of competition is different when the bacteria are grown in a structured habitat such as a soft agar matrix. In this paper we will introduce a spatially explicit model appropriate to the latter situation and contrast its behavior with that of the ordinary differential equations. The differences we find in the behavior of some of the models parallel those found experimentally. In particular, the evolution of alleopathy is possible only in a structured environment. This has also been demonstrated in unpublished work of Chao (1979), but ours is the first analytical treatment of the phenomena. The demonstration that spatial localization is fundamental to the evolution of a variety of traits is complemented by work by Levin *et al*. (1984), Cohen & Levin, (1991), Nowak & May, (1992), and others. Finally, new theoretical results are presented demonstrating that a stable equilibrium can be maintained among a colicin-sensitive type, a colicin-producing type, and a cheater that expends less on colicin production but is resistant. This points the way to a fascinating experimental test.

2. ODE Approach

To set the stage for the introduction of our stochastic spatial model we will describe how others

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have previous looked at the system through deterministic nonspatial eyes. In this approach one starts by assuming the population of bacteria is large and homogeneously mixing. Let u_1 be the density of colicin-producing and let u_2 be the density of the ordinary, colicin-sensitive bacteria. We assume that β_i is the intrinsic birth rate of type i, and δ_i is its natural death rate, so that in isolation its dynamics are given by

$$\frac{du_i}{dt} = \beta_i u_i(1 - u_i) - \delta_i u_i$$

Note that units have been chosen, without loss of generality, so that as $\delta_i \to 0$ the carrying capacity tends to its maximum, unity. When the two types are placed in competition, the equations become

$$\begin{aligned} \frac{du_1}{dt} &= \beta_1 u_1(1 - u_1 - u_2) - \delta_1 u_1 \\ \frac{du_2}{dt} &= \beta_2 u_2(1 - u_1 - u_2) - \delta_2 u_2 - \gamma u_1 u_2 \end{aligned} \qquad (1)$$

in which the additional term represents the rate at which type 1 poisons type 2.

The system (1) has locally stable boundary equilibria at

$$(1 - \delta_1/\beta_1, 0) \text{ and } (0, 1 - \delta_2/\beta_2)$$

provided

$$\delta_i < \beta_i, \quad \frac{\delta_2}{\beta_2} < \frac{\delta_1}{\beta_1} < \frac{\delta_2 + \gamma}{\beta_2 + \gamma} \qquad (2)$$

There is moreover an interior saddle point $(\bar{u}_1, \bar{u}_2)$ in this case. See Fig. 1 for a picture of what happens when $\delta_1 = \delta_2 = 1$, $\beta_1 = 3$, $\beta_2 = 4$ and $\gamma = 3$. The interpretation of the inequalities in order from left to right is:

(i) the birth rate exceeds the death rate so either type can maintain a population in isolation from the other;
(ii) there is a cost to colicin production, metabolic or otherwise, reflected in a lower carrying capacity in isolation
(iii) the competitive benefit of colicin production is sufficiently large to repel invasion by the wild type of an established colicin-producing community.

The implication of this analysis is that colicin production is an evolutionarily stable strategy, but so is non-production. In the dynamical system pictured in Fig. 1, if the density of the colicin-sensitive-bacteria is near the equilibrium value one, then the colicin-producing bacteria cannot invade. That is, if they are introduced at a low level then their density will shrink to zero. On the other hand, if the colicin producers are introduced at a large enough level, their density will increase to one and the density of the colicin-sensitive strain will approach zero. In words, selection will only favor genotypes when they are common, rare species cannot invade, and genetic diversity will not be maintained. This situation is "disruptive frequency dependent selection" (see Levin, 1988; Thoday, 1959–1964).

The conclusions of this model have been borne out experimentally by Chao & Levin (1981). Levin (1988) presents a model and simulation results confirming the experimental observations. A similar system has been analysed mathematically by Lenski & Hattingh (1986) for a detoxification polymorphism, but they observe that by a change of sign a disruptive regime would arise.

3. Spatial Model

Given the analysis in the previous section, it is hard to imagine how the colicin producers could arise through mutation in a population of ordinary *E. coli*. Indeed Chao & Levin (1981) have shown experimentally that this will not occur in a culture of bacteria grown in a stirred liquid suspension. This conclusion changes if we look at the situation through the eyes of a spatial model, which is appropriate if we think of bacteria growing in a Petri dish. Rather than keeping track of the physical location of each

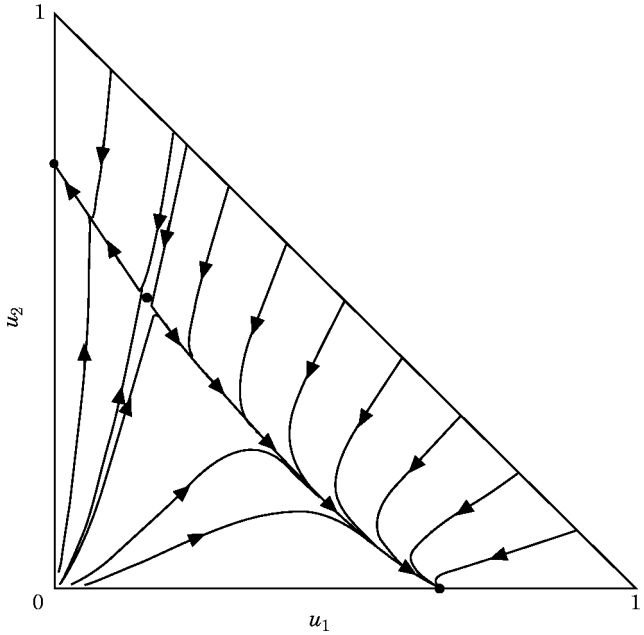

FIG. 1. Solution curves for the mean field ordinary differential equations (1) with $\delta_1 = \delta_2 = 1$, $\beta_1 = 3$, $\beta_2 = 4$, and $\gamma = 3$.

bacterium, we will simplify things and represent space as a grid, specifically $\mathbf{Z}^2$, the points in two-dimensional space with integer coordinates. Chao (1979) introduced a related spatial model in his unpublished Ph.D. thesis and presented some simulation results.

We will describe the state of the system at time t by a function ξ_t: $\mathbf{Z}^2 \to \{0,1,2\}$ where $0 =$ vacant, $1 =$ occupied by a colicin producer, $2 =$ occupied by a colicin-sensitive bacterium. In words, $\xi_t(x)$ gives the state of site x at time t. In our model, time will be continuous, i.e., t is indexed by the real numbers ≥ 0. To specify the evolution of the process we will describe the rate at which various transitions occur. Here, when we say something happens at rate λ, we mean that the probability of an occurence in a short interval of time with length Δt is $\lambda \Delta t$. For more about what this means, see Durrett & Levin (1994a).

To specify the model precisely, we begin by describing the interaction neighborhood N. The set N tells us the "neighbors of the origin (0,0)" or the sites that (0,0) interacts with. The neighbors of a general point x are then defined to be $x + N = \{x + z : z \in N\}$. Since the growth and competition of the bacteria occur over short distances two natural choices for N are $N_0 =$ the four nearest neighbors $\{(1,0), (0,1), (-1,0), (0,-1)\}$ or $N_1 =$ the four nearest neighbors plus the four diagonal neighbors $(1,1), (1,-1), (-1,1), (-1,-1)$. In either case we can let f_i be the fraction of neighbors in state i and formulate the transition rates as follows:

birth	rate	death	rate
$0 \to 1$	$\beta_1 f_1$	$1 \to 0$	δ_1
$0 \to 2$	$\beta_2 f_2$	$2 \to 0$	$\delta_2 + \gamma f_1$

In words, each type is born at empty sites at a rate proportional to the fraction of neighbors of that type. The colicin-producing strain dies at a constant rate δ_1, while the colicin-sensitive strain experiences deaths at rate δ_2 plus γ times the fraction of colicin-producing neighbors.

To make the connection between the rates above and the differential equation in (1) we note that if in the spatial model the states of adjacent sites were independent then (for either choice of neighborhood) the density u of colicin producers and the density v of colicin-sensitive bacteria would again evolve according to the differential equations (1).

This model is similar to that studied by Matsuda *et al*. (1987, 1992), and Harada *et al*. (1995) who examined the evolution of social interactions that modify the mortality of neighbors. They studied the model by means of pair approximation, in addition to computer simulations, and discussed conditions for the existence of evolutionarily stable strategies (ESS).

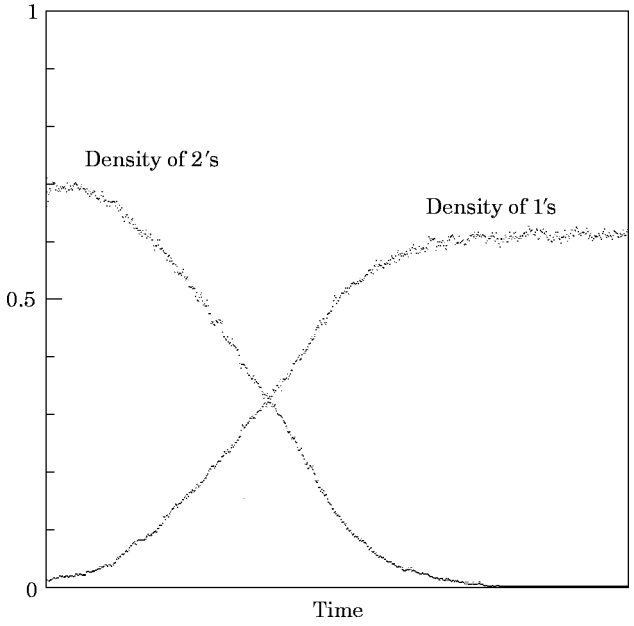

FIG. 2. Density of colicin producers (1's) and colicin-sensitive bacteria (2's) in a simulation of the spatial model with the same parameters as in Fig. 1: $\delta_1 = \delta_2 = 1$, $\beta_1 = 3$, $\beta_2 = 4$ and $\gamma = 3$.

4. Simulation Results

The previous section shows that if we pretend adjacent sites are independent in the spatial model, then we get the system (1) and derive the same conclusions. However, in the spatial model adjacent sites are not independent and the qualitative behavior changes. In this section we will describe the behavior of the spatial model. To be precise we must preface our discussion with a disclaimer. In almost all cases the conclusions stated below are based on simulations and analogies with simpler models, but we do not know how to prove them mathematically.

Figure 2 shows the density of colicin producers and colicin-sensitive bacteria in a simulation of the spatial model with neighborhood set N_0 (four nearest neighbors), and parameters: $\delta_1 = \delta_2 = 1$, $\beta_1 = 3$, $\beta_2 = 4$ and $\gamma = 3$. Here the lattice is 100×100 and to avoid edge effects we have used *periodic boundary conditions*. That is, sites on the bottom row are neighbors of those on the top row; sites on the left edge are neighbors of those on the right edge. We start at time 0 from *product measure*. That is, the states of the sites at time 0 are assigned independently, i.e., by making repeated calls to a random number generator. We started the simulation with colicin producers (1's) at density 0.01 and the colicin-sensitive strain (2's) at density 0.50; but as the graph shows, the colicin producers gradually increase to their equilibrium level while the density of colicin-sensitive bacteria drops to 0. Figures 3 and 4, which show

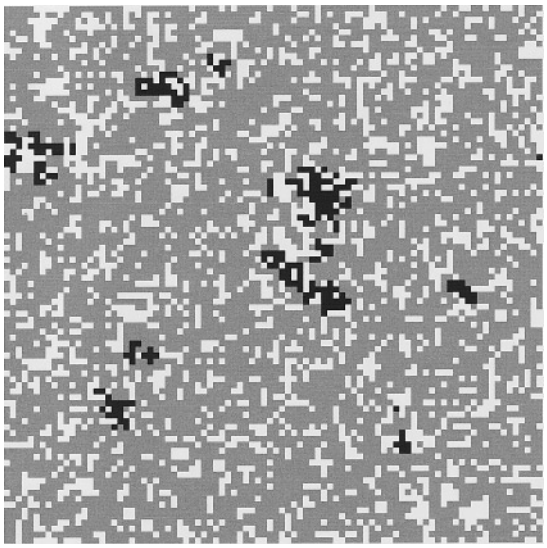

FIG. 3. Snapshot of the spatial model at time 300. Parameters are the same as in Fig. 2. Note that the colicin producers (black) which started at a small density have formed clumps, while the colicin-sensitive strain (gray) occupies most of the space.

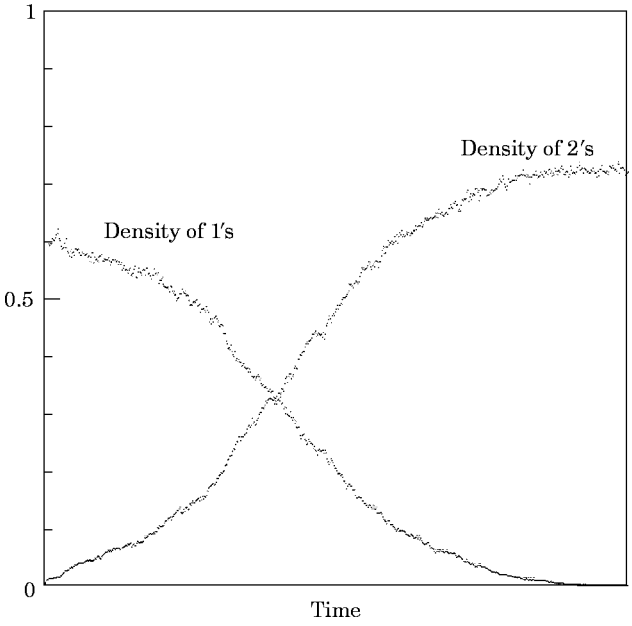

FIG. 5. Density of colicin producers (1's) and colicin-sensitive bacteria (2's) in a simulation of the spatial model with $\delta_1 = \delta_2 = 1$, $\beta_1 = 3$, $\beta_2 = 4$ and $\gamma = 1$. Here 2's win, in contrast to the situation in Fig. 2 (2's win).

snapshots of the process at times 300 and 750, explain how this occurs. The colicin producers first establish themselves in clumps that grow linearly in radius and take over the system.

The victory of the colicin producers in the last example is due to the fact that the colicin induced death rate $\gamma = 3$ is large enough to compensate for the fact that the colicin-producing strain has birth rate $\beta_1 = 3$ vs. $\beta_2 = 4$ for the colicin-sensitive strain. If we reduce γ to 1 the situation reverses and the colicin-sensitive strain is victorious even when it starts from a low density (see Fig. 5). For values of γ between one and three coexistence might be possible but this does not occur: there is a critical value γ_c so that 2's take over when $\gamma < \gamma_c$, while 1's take over when $\gamma > \gamma_c$.

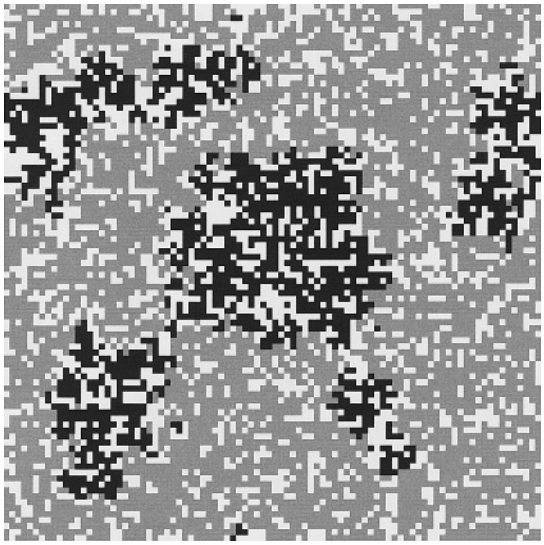

FIG. 4. Snapshot of the spatial model at time 750. Parameters are the same as in Figs 2 and 3. Note how the black clusters of colicin producers have grown and merged. Eventually, by about step 2000, they will have taken over the system and eliminated the gray colicin-sensitive strain.

More generally if we fix $\delta_1 = \delta_2 = 1$, $\beta_2 = 4$, and vary β_1 and γ then we get the phase diagram drawn in Fig. 6. The figure is a free-hand sketch that emphasizes the generic qualitative properties but is not exact. For each fixed value of β_1 there is a critical value $\gamma_c(\beta_1)$ so that 2's take over when $\gamma < \gamma_c(\beta_1)$ while 1's take over when $\gamma > \gamma_c(\beta_1)$. When $\beta_1 = 4 = \beta_2$, $\gamma_c(\beta_1) = 0$. Decreasing β_1 increases $\gamma_c(\beta_1)$ until it reaches ∞ at a point we have labelled β_c. β_c, which is ≈ 1.65 for the neighborhood N_0, is the minimum value of the birth rate needed for a single strain to survive in the absence of the other. When there is a single strain the model reduces to the basic contact process, see Durrett & Levin (1994a).

The situation described here is an instance of Case 2, contingent competition, of Durrett & Levin (1994b). The ordinary differential equations have two attracting fixed points and predict that the winner of the competition depends on the initial densities. In contrast, for the spatial model there is a stronger type that takes over the system whenever it starts with a positive density; that is, there is no dependence on initial conditions as long as those conditions are generic. To be precise, we conjecture that (for the

model on $\mathbf{Z}^2$) the stronger type always wins if there are infinitely many individuals of that type present in the initial configuration. Here, by "wins" we mean that the probability a given site x will be occupied by the weaker type converges to zero as time $t \to \infty$. For related systems where this has been proven rigorously see Durrett & Neuhauser (1994) and Durrett & Swindle (1994).

5. A Three Species System

In the preceding section, the ODE and the spatial model sometimes disagreed on who would win the competition, but both approaches agreed that one type would always competitively exclude the other. In this section we will describe a system in which three species coexist in the spatial model, but in the ODE there is always only one winner.

In particular in the light of the experiments described earlier, it is important to ask whether a "cheater" can invade an environment dominated by a colicin-producing strain, resistant to colicin, by producing less or perhaps no colicin. We find not only that this is possible, but also that the result of the competition may be the persistence of all three types where no two could coexist without the third. To describe the system in words, we assume 1's and 2's both produce colicin, to which they are immune,

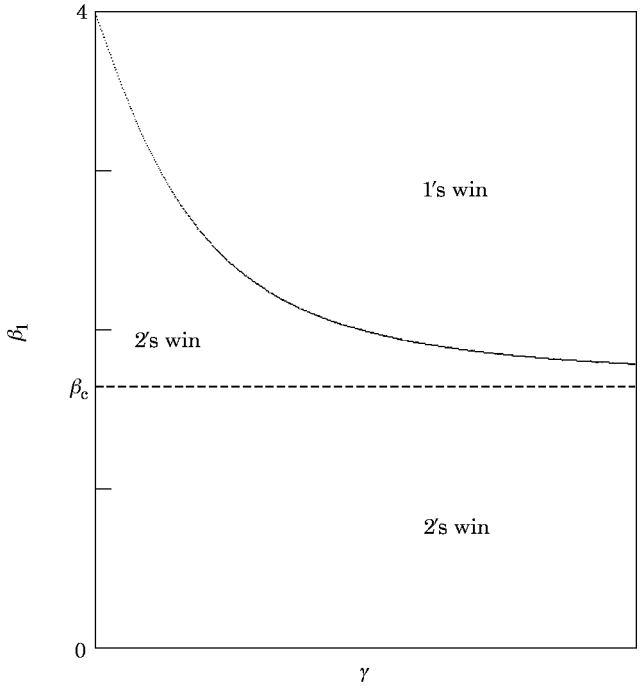

FIG. 6. Phase diagram for the colicin system when $\delta_1 = \delta_2 = 1$, $\beta_2 = 4$ is fixed and we vary β_1, γ.

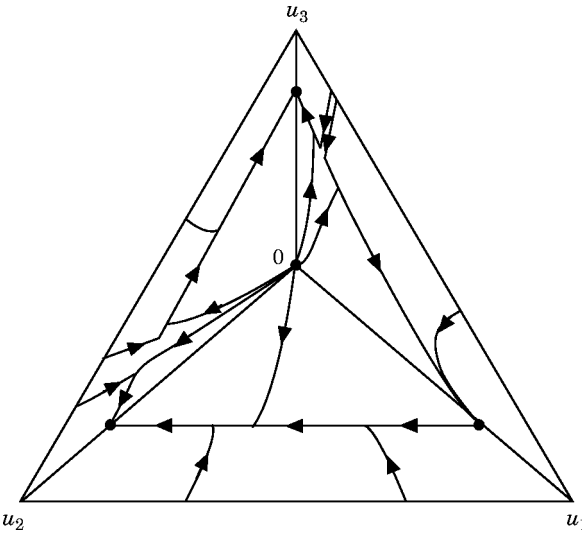

FIG. 7. Solution curves for the mean field ordinary differential equations for the three species colicin system (3) with $\beta_1 = 3$, $\beta_2 = 3.2$, $\beta_3 = 4$, $\gamma_1 = 3$, and $\gamma_2 = 0.5$. Here, the view is from inside the positive orthant looking toward the origin, which is marked with a 0. The letters u_1, u_2, and u_3 indicate the corresponding axes.

and to which 3 is sensitive. The rates for this system are:

birth	rate	death	rate
$1 \to 1$	$\beta_1 f_1$	$1 \to 0$	δ_1
$0 \to 2$	$\beta_2 f_2$	$2 \to 0$	δ_2
$0 \to 3$	$\beta_3 f_3$	$3 \to 0$	$\delta_3 + \gamma_1 f_1 + \gamma_2 f_2$

Here, f_i is the fraction of neighbors in state i and we choose the neighborhood set to be N_0 the four nearest neighbors.

In our concrete example we will set all the $\delta_i = 1$ and

$$\beta_1 = 3,\ \beta_2 = 3.2,\ \beta_3 = 4,\ \gamma_1 = 3,\ \gamma_2 = 0.5$$

Here we imagine that species 1 produces more colicin than 2 does but has the lowest birth rate. The parameters are chosen so that 1's win against 3's while 3's win against 2's. When only 1's and 2's are present the system reduces to the multitype contact process studied in Neuhauser (1992). Since $\beta_2 > \beta_1$, the 2's win against the 1's in this case.

If we write u_i for the fraction of sites in state i and assume that adjacent sites are independent then in general we get the following ODE:

$$\begin{aligned} \frac{du_1}{dt} &= \beta_1 u_1 u_0 - \delta_1 u_1 \\ \frac{du_2}{dt} &= \beta_2 u_2 u_0 - \delta_2 u_2 \\ \frac{du_3}{dt} &= \beta_3 u_3 u_0 - u_3(\delta_3 + \gamma_1 u_1 + \gamma_2 u_2) \end{aligned} \tag{3}$$

If we insert the values for the concrete example then

the picture in Fig. 7 results. In the u_1u_2 plane all trajectories starting with u_1 and u_2 positive are attracted to $(0, \hat{u}_2, 0)$ where $\hat{u}_i = (\beta_i - \delta_i)/\beta_i$. In the three-dimensional ODE there is a surface which connects the two separatrices in the u_1u_3 and u_2u_3 planes, so that above the surface trajectories converge to $(0,0,\hat{u}_3)$ while those below converge to $(0,\hat{u}_2,0)$. These conclusions are true whenever $\beta_1 < \beta_2$ and equilibria exist in the interior of the u_1u_3 and u_2u_3 planes. [Conditions for this can be derived from (2).]

In contrast to the behavior of the ODE, the spatial model shows coexistence, at least for a long time. See Fig. 8 for a simulation of the process on a 200×200 grid with periodic boundary conditions. Here we started in an initial product measure in which the states $i = 1, 2, 3$ each had density 1/3 and plotted the observed density of the three species every 1000 units of time out to time 50000, which represents more than ten periods. After an initial transient the densities are always at least 15–20%, so none of them seems in danger of hitting zero.

6. Summary

The evolution of allelochemics, as many other characteristics that involve intraspecific or interspecific interactions, is mediated in a frequency-dependent context that relies heavily on spatial localization. As Levin (1988) has shown experimentally, in a well-mixed environment the costs of colicin production always outweigh the benefits when the colicin producers are rare; but in a structured environment, the situation may shift to favor the production of colicin. We have investigated, through a series of models, the conditions for the evolution of colicin.

In a mean-field model (in which homogeneous mixing is assumed) there are two stable states: one with only colicin producers present and one with only the colicin-sensitive strain. This confirms the experimental observation that in a well-mixed environment, colicin producers cannot evolve from low frequencies. However, in this setting colicin production is also an evolutionarily stable strategy; if it is represented in the population at a sufficiently high level, the population will proceed to fixation for colicin production. These results were demonstrated empirically and by simulation by Levin (1988), while the work of Lenski & Hattingh (1986) provides analytical justification for a very similar model.

In a spatially structured environment the situation is fundamentally different: colicin production can invade from low densities provided that the additional mortality it imposes on the colicin-sensitive type is above a critical threshold value, determined by the cost of producing colicin. An interesting extension of the reasoning can occur when more than two types are present. In particular, two types that produce colicin at slightly different rates (or with different toxicities) can coexist with a third, colicin-sensitive, type in a dynamic equilibrium. This occurs when one of the two colicin-producing types (A) has a subcritical benefit to cost ratio but a higher intrinsic birth rate than the other colicin-producing type (B).

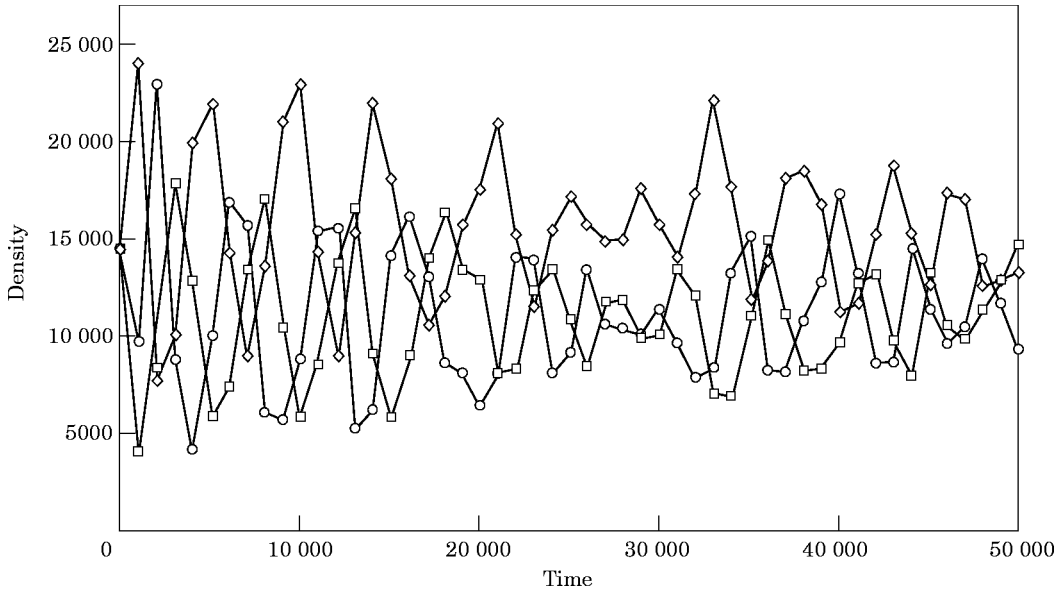

FIG. 8. Densities vs. time in the three species colicin system. Parameters are the same as in Fig. 7. Key: —□—, species 1; —◇—, species 2; —○—, species 3.

Thus, in head-to-head competition, $A > B > C > A$ where C is the colicin-sensitive type. Unlike other systems in which non-transitive hierarchies arise, coexistence is not possible in the well-mixed version of the system; but it is easily achievable in the spatially explicit version.

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