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A spatial model of the evolution of quorum sensing regulating bacteriocin production

Tamás Czárán[a] and Rolf F. Hoekstra[b]
[a]Ecology and Theoretical Biology Research Group of the Hungarian Academy of Science and Eötvös University, H-1117 Budapest, Pázmány Péter sétány 1/c, Hungary and [b]Laboratory of genetics, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

Like any form of cooperative behavior, quorum sensing (QS) in bacteria is potentially vulnerable to cheating, the occurrence of individuals that contribute less but still profit from the benefits provided by others. In this paper, we explore the evolutionary stability of QS as a regulatory mechanism of antibiotics production in a spatially structured population, using cellular automaton (CA) modeling. QSg is supposed to regulate the excretion of a bacteriocin (anticompetitor toxin) in a population of bacteria polymorphic for the ability to produce and to be immune to the bacteriocin. Both the social interactions resulting from QS and the competitive interactions resulting from the bacteriocin excretion are supposed to be only effective at the local scale, that is, restricted to the immediately neighboring cells. This implies a rather diffuse kind of group selection. The CA model is contrasted to a model assuming no spatial structure but with otherwise identical assumptions. Our analysis predicts that QS as a regulatory mechanism of bacteriocin excretion is evolutionarily unstable when the competitive interactions between bacteriocin-producing, resistant, and sensitive strains only involve closely related strains which can share the signaling and responding genes involved in QS. However, when the competition is between unrelated strains and the QS alleles can only be carried by the bacteriocin-producing strains, stable QS may evolve provided its costs are small and the critical quorum threshold is neither too low nor too high. *Key words:* bacteriocin, evolutionary stability, quorum sensing. *[Behav Ecol 18:866–873 (2007)]*

Many species of bacteria exhibit quorum sensing (QS), the ability to release and respond to signaling molecules (de Kievit and Iglewski 2000; Crespi 2001; Miller and Bassler 2001). Typically, cells produce an extracellular autoinducer molecule—often a peptide (in Gram+ species) or an acyl homoserine lactone (in Gram− strains)—and simultaneously sense its concentration in their immediate environment. Once the concentration exceeds a threshold, the cell is induced to undertake some action, for example, expressing genes leading to the excretion of specific enzymes, toxins, or other functional products. This type of regulation has been named "quorum sensing" because a widely accepted interpretation holds that it allows the bacteria to sense population density. Many bacterial functions, like biofilm differentiation, swarming, toxin excretion in interstrain competition, and virulence factor production, are only optimally expressed above a certain critical population density. Regulation by QS would allow the cells to express the appropriate behavior only when it is effective, thus saving resources under low density conditions.

We concentrate on one particular bacterial target phenotype, namely, toxin excretion in bacterial interstrain competition. Anticompetitor toxin excretion is known to be regulated by QSg for example in the Gram+ *Streptococcus mutans*, an inhabitant of the dental plaque (van der Ploeg 2005); the Gram− *Pseudomonas aureofaciens* 30-84, a strain used as a "biopesticide" to prevent fungal diseases in wheat (Pierson et al. 1994); and in some other species (e.g., Kuipers et al. 1998; Haas et al. 2002). In most of the published cases of QS-regulated toxin production, the genes of the signaling system (i.e., those of signal production and signal transduction) are all different from the effector genes (i.e., the toxin-producing machinery). The best known of the exceptions is the nisin-producing system of *Lactococcus lactis*, in which the signal and the toxin are the same (nisin) molecule, but most other lactic acid bacteria follow the usual pattern of QS genes being separate from toxin-producing genes (Kuipers et al. 1998). Our models also stick to the generic assumption in this respect.

The evolutionary stability of QS as a social communication system among bacteria is somewhat problematic. If cooperative behavior involves a fitness cost for the cooperating individual, the system becomes vulnerable to cheaters. For example, in a QS population, mutant cells that do not produce the signaling molecules may obtain the benefits without having to pay the costs involved in producing the signal. For recent treatments of evolutionary aspects of cooperative behavior in microbial populations, see Velicer (2003) and Travisano and Velicer (2004). Because of the potential evolutionary instability of QS as a social communication system, Redfield (2002) has proposed that its main function may be the detection of the diffusive properties of the local environment of the cell. Because this would be of immediate benefit to the individual cell, the evolutionary stability problems associated with altruistic behavior would not arise.

Brookfield (1998) and Brown and Johnstone (2001) have analyzed models of the evolution of bacterial QS. Although differing in modeling approach, both have studied the evolution of QS in the context of explicit 2-level selection, where selection at the individual level operates against cooperation, whereas selection at the group level favors QS. Both studies conclude that under fairly broad conditions stable polymorphism may arise between presence and absence of QS.

In this paper, we model the evolution of QS using a cellular automaton (CA) approach. In contrast to the models by Brookfield (1998) and Brown and Johnstone (2001), we do not explicitly assume 2-level selection among individuals and among groups but consider all effects of competiton and QS to occur between neighboring cells. Our analysis suggests that

Address correspondence to R.F. Hoekstra. E-mail: rolf.hoekstra@wur.nl.

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QS is unlikely to evolve as a regulating mechanism of narrow-spectrum anticompetitor toxins in bacteria (effective only against closely related strains) but that it may be evolutionary stable when regulating the production of broad-spectrum toxins.

MODEL ASSUMPTIONS

The QS and the bacteriocin system

We consider bacterial populations polymorphic for genes controlling the production of, and immunity to, bacteriocin (an excreted toxin that may kill sensitive cells) and for genes controlling the QS system. The locus of "toxin production" and the corresponding "immunity factor" gene constitute the bacteriocin system, whereas the QS system consists of a "signal locus" and a "response module." The toxin gene expresses a protein that, at sufficiently high local concentrations, is toxic to strains lacking the immunity factor (the product of the intact immunity gene). The quorum signal gene codes for an autoinducer molecule, which is excreted into extracellular space. The quorum response module consists of at least 2 closely linked loci, one of which codes for a membrane-bound receptor of the quorum signal. The other is a cytoplasmic signal transfer peptide that is modified by the receptor–signal complex so that it can trigger the transcription of the toxin gene (Figure 1).

The bacteriocin system and the QS system interact through the toxin-producing and the QS response genes, to ensure that in QS strains bacteriocin production occurs only if the quorum signal is of sufficient concentration in extracellular space, that is, at an efficient quorum (local density) of potential toxin-producing cells. This postulate is in line with the consideration that it is economical in terms of metabolic efforts to keep the toxin gene silent until the local density of potential toxin producers exceeds a critical value. The critical density of toxin producers is called the "toxin threshold," at and above which the toxin is of sufficiently high local concentration to kill sensitive individuals in the close neighborhood.

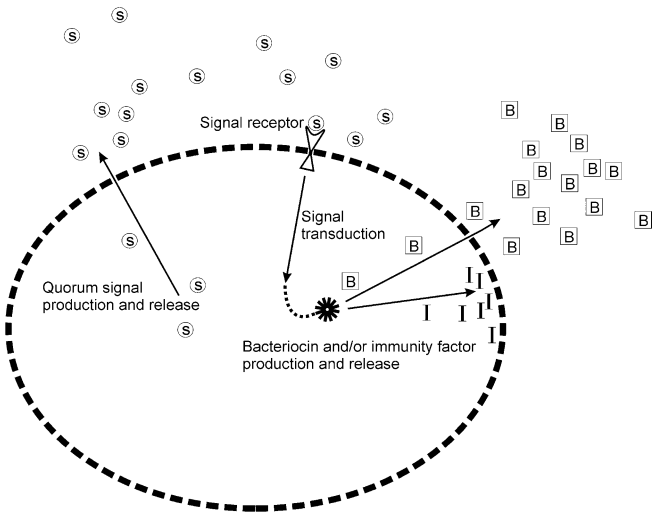

Figure 1
Caricature of the QS system coupled to cooperative bacteriocin production in bacteria. The quorum signal molecule ("S" in a circle) is produced by the signaling gene; the quorum response system (signal receptor + signal transduction machinery) is the product of the receiver module; the immunity factor ("I") and the bacteriocin ("B" in a square) are coded by the immunity and the toxin genes, respectively.

To allow for the QS strains to take the fitness advantage of conditional toxin production, we assume that killer bacteria lacking a functional QS response module produce the toxin constitutively. More precisely, the toxin gene is expressed if either the QS response module is inactive or the QS response module is functional and the extracellular concentration of the quorum signal exceeds a critical level (the "QS threshold") in the close proximity of the cell. For a plausible realization of the simplest possible case of a QS-regulated bacteriocin system, see Figure 1.

Metabolic costs and functional thresholds

Each of the 4 functions (signal production, signal detection, toxin production, and immunity) is assumed to involve a fitness cost due to the metabolic burden of gene expression—a cost that individuals harboring the inactive allele of the same locus do not pay. An obvious constraint on the relative magnitudes of these costs is that the QS system should be significantly cheaper to run than the bacteriocin system because QS is itself hypothesized to be an adaptation reducing toxin costs.

Another assumption is that the toxin gene should be switched on at a local density of potential toxin producers which is just sufficient to kill nearby sensitives. At quorums lower than the toxin threshold, the production of the toxin is just wasting resources. Thus, we assume that the toxin threshold and the QS threshold are equal.

Based on these assumptions, we analyze 2 models (a spatially explicit CA and its mean-field approximation that omit spatial aspects, cf. The Single-Species CA Model and The Single-Species Mean-Field Model) to answer the following question: "Is it theoretically possible that the QSg system has evolved and is maintained as an adaptive regulatory circuit of bacteriocin production?".

We try to answer the question by exploring 2 different scenarios called "single species" and "3 species," corresponding to narrow-spectrum bacteriocins (effective only against related strains) and broad-spectrum bacteriocins (effective to a wide range of unrelated strains).

1. In "single species," no rigid linkage (genetic or functional) between the bacteriocin and the QS loci is assumed. Sensitives, resistants, and killers may obtain and exchange the QS genes by mutation or by recombination (through conjugation or transformation).
2. In "3-species" runs of the simulations, we assume that it is only the killer (K) strain that possesses the QS machinery and sensitives and resistants cannot obtain these genes by horizontal transfer.

THE SINGLE-SPECIES CA MODEL

The CA model is staged on a 500 × 500 square lattice of toroidal topology that sets periodic boundary conditions for the habitat. Each cell of the lattice harbors a single bacterium. All individuals belong to the same bacterial species, that is, they are genetically identical, except possibly at the loci controlling the bacteriocin system and the QS system. With respect to the bacteriocin system, we consider the following genotypes:

toxin-"ON" and immunity-"ON" (killer, K);
toxin-"OFF" and immunity-"ON" (resistant, R);
toxin-"OFF" and immunity-"OFF" (sensitive, S).

The fourth logical possibility (toxin-"ON" and immunity-"OFF") is nonviable because such a genotype would commit suicide well before reproduction, thus having no chance to spread in the community. In the QS system, we have the following 4 genotypes:

signal-"ON" and response-"ON" (signaler–responder, sr);

signal-"ON" and response-"OFF" (signaler, s0);
signal-"OFF" and response-"ON" (responder, 0r);
signal-"OFF" and response-"OFF" (deaf–mute, 00).

With respect to the 4 loci considered, we have $3 \times 4 = 12$ possible allele combinations (genotypes), each paying a different fitness cost. We seed the system with a homogeneous population of deaf–mute sensitives (S00) and let them mutate and interact in a stochastic manner according to the following rules:

1. Each one of the 4 functions (i.e., bacteriocin production, immunity to the bacteriocin, QS signaling, and QS responding) is controlled by an independent locus (or a closely linked group of genes: a functional locus);
2. Each of the 4 functions can be acquired (switched ON) or lost (switched OFF), by mutation or transformation or simply by invasion by a different genotype—more details below);
3. The QS signaling, the QS detection, and the immunity functions are always "ON" if the corresponding allele is present in the genome;
4. Bacteriocin production (if the toxin-producing allele is present in the genome) is always "ON" if QS responding is "OFF" (constitutive toxin production in the absence of a working QS system);
5. Bacteriocin production is conditionally "ON" if the QS responding is "ON" and the number of QS signaler neighbors exceeds the QS threshold;
6. The toxin is effective only above a local threshold concentration—that is, if the number of toxin-"ON" neighbors exceeds the toxin threshold;
7. Each of the 4 functions (the expression of the corresponding "ON" allele) carries an associated fitness cost C, with the ordering of costs

$$C_{\text{toxin}} > C_{\text{immunity}} \gg C_{\text{signaling}} > C_{\text{responding}}.$$

8. Interactions are occurring between random pairs of lattice neighbors. The outcome involves the site of the opponent: the winner replaces the loser with a—possibly mutated—offspring of its own;
9. Toxin-producing (K) bacteria always beat sensitives (S) if they have enough K neighbors to pass the "toxin threshold";
10. If an interaction does not result in killing, the contest is settled on the basis of fitness costs (resource competition), in a stochastic manner: the contestant with the smaller fitness cost has a higher chance to take over the site of its opponent.

We introduce genetic variation into the population by means of "mutations," but the actual biological mechanism of changing one genotype to another is not specified and may involve mutation or recombination following conjugation or transformation. It is assumed that each of the functional loci has a chance to flip to the opposite state (from ON to OFF or vice versa) at reproduction with specific "mutation" rates m_{k-} and m_{k+} at locus k. The mutational changes at the 4 functional loci are independent in all cases except for toxin and immunity loci: to prevent the occurrence of suicidal phenotypes, losing (switching OFF) the immunity function in a killer (K) genotype means losing the toxin gene too.

The output of the CA model is simply the frequency distribution of the 12 genotypes on the lattice. We have sampled the parameter space of the model by changing the crucial input parameters: fitness costs, mutation rates, recombination rate, and the QS/toxin threshold. Simulations over the reasonable parts of the parameter space show that the CA is very robust in terms of the evolutionary fates of the 4 loci studied: apart from relatively small quantitative differences, all runs of the model predict the same. The results are presented as time series for 1) the genotype frequency distributions and 2) the allele frequencies at the 4 functional loci.

THE SINGLE-SPECIES MEAN-FIELD MODEL

On biological assumptions as similar to those of the single-species CA (points 1–10 in the previous section) as possible, we have built the mean-field approximation of the same system. The only difference between the 2 models is that neighborhood interactions are replaced by mass interactions, that is, the population is of infinite size and the competitive interactions are dependent on overall genotype frequencies instead of local ones. Thus, we obtain a nonspatial dynamical system consisting of 12 coupled ordinary differential equations, one for the frequency of each feasible genotype:

$$\dot{x}_i = \sum_j (x_j \cdot \text{Mut}_{ij}) + x_i \cdot \text{Comp}_{ij}(\mathbf{X}, \mathbf{C}, \text{qt}, \text{tt}), (i = 1, \ldots, 12)$$

where $\mathbf{X}$ is the genotype frequency vector with elements x_i, Mut_{ij} is the mutation rate from genotype j to i (calculated from the locus-level rates of mutations m_{k+} and m_{k-} on locus k), and Comp_{ij} is the competition coefficient specifying the overall competitive effect of genotype j on i. Competition has 2 components: resource competition due to differences in fitness costs $\mathbf{C}$ and interference competition due to toxic killing of sensitive (S) strains by killers (K). qt is the QS threshold, in this model, specifying the "total" frequency of quorum-signaling individuals at and above which toxin production sets in in Ksr and K0r genotypes. tt is the toxin threshold, the "total" frequency of actual toxin-producing killer (K) genotypes (both constitutive and quorum signal induced) above which the bacteriocin is effective in the culture. Here we do not specify the actual forms of the mutation and the competition rates—they follow assumptions 1–10 strictly, but they are rather complicated and uninstructive formally, mainly due to the conditional expression of the toxin gene and the resulting variable fitness costs.

The mean-field model can be solved numerically to obtain time series of genotype frequency distributions.

The 3-species CA model

The 3-species version of the CA is the same as the single-species model, except that QS alleles are only allowed in the killer (K) genetic background. Thus, the number of possible genotypes reduces to 6 in the 3-species model: S00, R00, K00, Ks0, K0r, and Ksr.

RESULTS

Mean-field model

The spatially homogeneous, unstructured single-species mean-field model yields rather sobering conclusions. Depending on the actual values of the input parameters (fitness costs of gene expressions $\mathbf{C}$, mutation rates m_k, QS threshold qt, and toxin threshold tt) and on the initial genotype frequency distribution used, either all 4 functions deteriorate resulting in the overwhelming dominance of the S00 genotype (Figure 2B) or it is only the bacteriocin system that persists, with the QS system practically eliminated (Figure 2A).

To understand these results, first the bacteriocin system has to be studied separately, with the QS system switched off. The 3 feasible genotypes of the bacteriocin system are

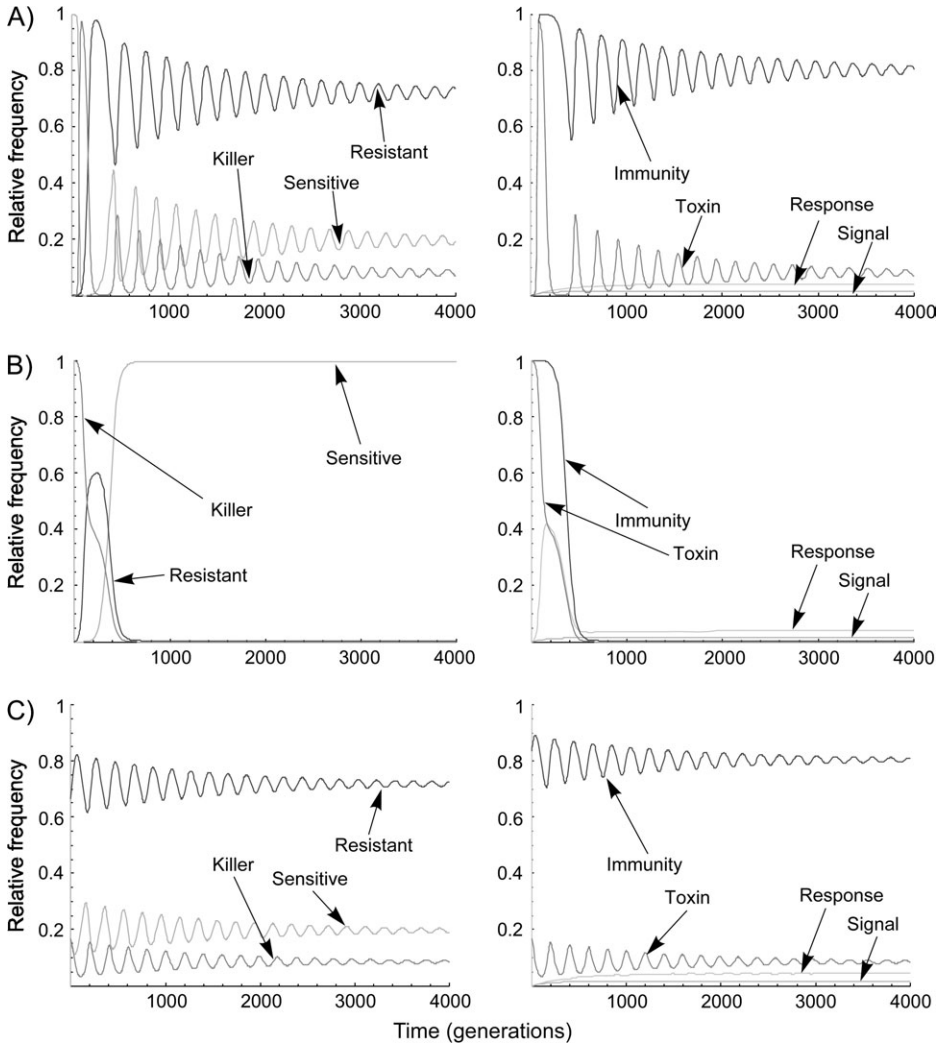

Figure 2
Numerical solutions of the single-species mean-field model. Input parameters—mutation rates: 10^{-4}; basic fitness cost for all genotypes: 100 (arbitrary units); metabolic costs: QS responding 1, QS signaling 3, bacteriocin immunity 10, bacteriocin production 30. Left panels: relative frequencies over time for the genotypes of the bacteriocin system. Right panels: relative allele frequencies over time for QS response. (A) Solution with zero QS and toxin thresholds (qt = tt = 0), initial state: uniform S. (B) Solution with QS and toxin thresholds qt = tt = 0.03 (3% relative frequency), initial state: uniform K. (C) Solution with QS and toxin thresholds qt = tt = 0.03, initial state: 1/6 K, 2/3 R, 1/6 S.

killers (K), resistants (R), and sensitives (S). Based on fitness costs alone, S is the fittest of the 3 types because it does not pay the costs of toxin and immunity factor production. R is inferior to S because it pays the cost of resistance but superior to K which carries the costs of both toxin production and resistance. However, K has the interference competition advantage over S by toxic killing that outweighs its metabolic handicap. Thus, the interaction pattern of the 3 genotypes forms an intransitive cycle like the strategies of the rock–scissors–paper game: K beats S beats R beats K (Czárán et al. 2002). In the spatially homogeneous model, this amounts to neutral oscillations (assuming no mutations and no QS). With positive mutation rates among the 3 genotypes (S, R, and K), the neutrally stable periodic solution of the system becomes damped onto an apparent fixed point due to the net mutational "flow" from all-time frequent genotypes into rare ones. This is why we see damped oscillations on Figure 2A,C.

Figure 2 also shows that the dynamics of the system is hardly affected by the introduction of the QS system, provided that the QS/toxin threshold is set sufficiently low. This is not surprising given that the sole function of QS is supposed to be switching off toxin production at frequencies of K below the threshold in order to save metabolic costs. At toxin thresholds close to zero, this function is useless, so even the small cost of running the QS system is wasted and bacteria expressing it are selected against. This results in a decrease of the QS allele frequencies to their mutation-selection equilibria.

Because there is no way for the killer strain to return from below the toxin threshold in the mean-field system, all the trajectories touching or crossing the threshold end up in the all sensitives (only S) state (Figure 2B). With a reasonably high threshold, most initial conditions lead to this outcome—only trajectories started from within the close vicinity of the coexistent fixed point will actually reach it.

More important, and even more surprising, Figure 2C shows that even if the threshold is relatively high and the initial state is close to the fixed point of the bacteriocin system, QSg does not evolve: the frequencies of the signaler allele and the responder allele do not exceed their mutation-selection equilibria based on mutation rates and fitness costs alone. That is, the QS system itself behaves neutrally in the context of bacteriocin production: apart from selection due to metabolic fitness costs, QS alelles undergo neither positive nor negative selection pressures in the mean-field model. We suspect that the immediate reason for this is that the cheap QS system is just as good a mediator of dishonest signals as it can be of honest ones. We shall return to this interpretation in more detail later.

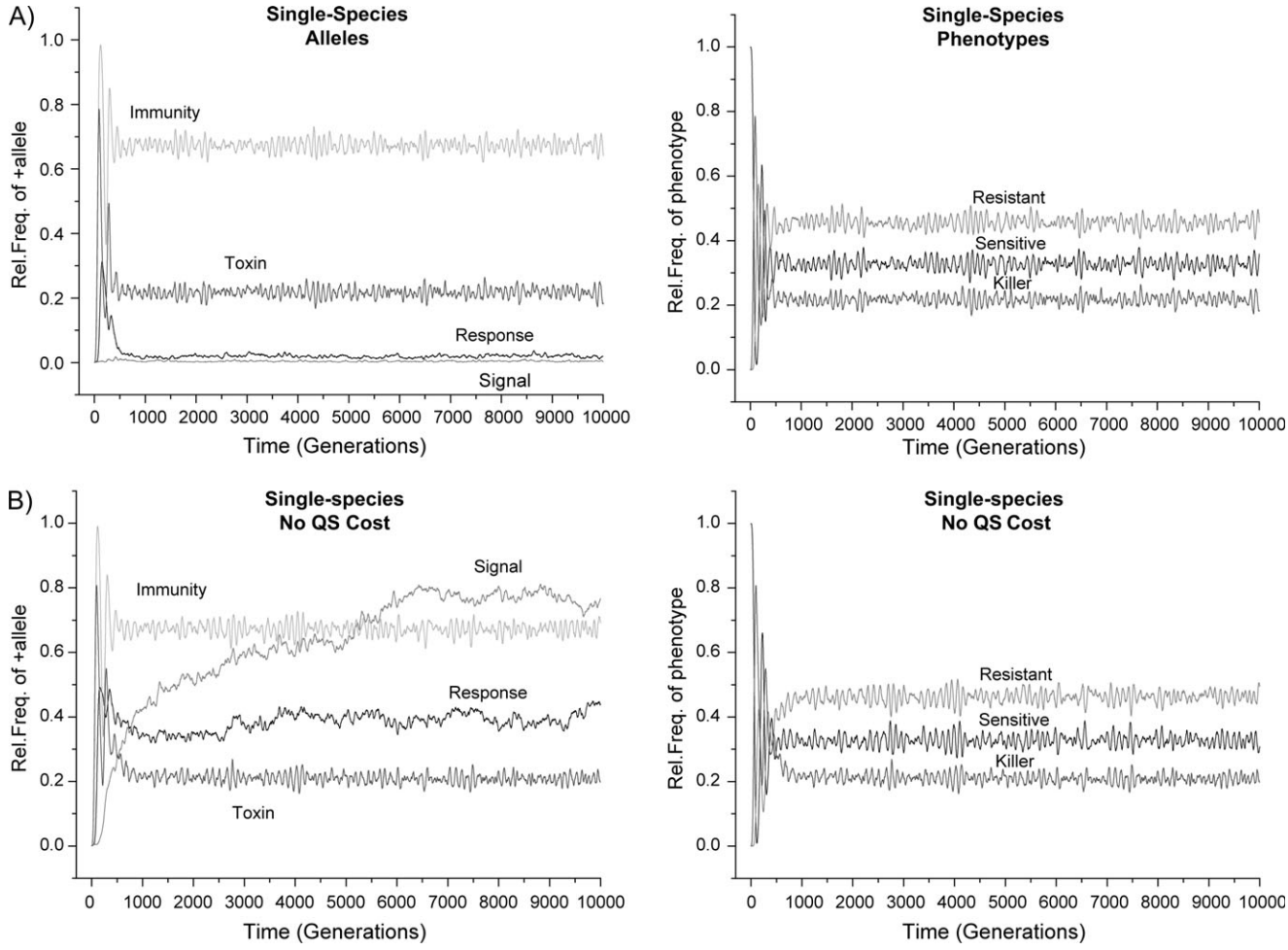

Figure 3
Time series of a typical run of the single-species version of the CA model. Input parameters—mutation rates: 10^{-4}; basic metabolic burden for all genotypes: 20 (arbitrary units), bactriocin immunity 5, bacteriocin production 10; toxin threshold: 3 individuals within the Moore neighborhood of the focal individual. (A) Genotype frequencies for the bacteriocin system with metabolic costs: QS responding 1, QS signaling 2. (B) The same as A, with zero QS response and signal costs. Left panels: Allele frequencies for QS response f(R), QS signal f(S), immunity factor f(I), and toxin production f(T). Right panels: Relative frequencies of the sensitive (Sen), the resistant (Res), and the killer (Kil) phenotypes.

CA: single-species case

Spatial interactions change many of these conclusions. First, unlike the mean-field system, the CA is stable in terms of the relative frequencies of K, R, and S genotypes even without the damping effect of mutations: any mixed initial state (with all 3 genotypes present) quickly converges to a stationary frequency distribution. The equilibrium thus approached is static within the lattice as a whole but very dynamic at the local scale: each site of the lattice experiences the endless [S to K to R to S] state transition cycle. On the mesoscale, we see patches of killers chasing patches of sensitives chasing patches of resistants, etc. (Czárán et al. 2002). Second, unlike the mean-field model, the CA predicts the persistence of the bacteriocin system even at quite high toxin threshold values. Starting from a uniform S population and letting them mutate, in time K type mutants will pop up. They are at a disadvantage compared with the "wild-type" S in terms of fitness costs, yet they still have a positive chance to reproduce. Wherever they happen to reach the toxin threshold frequency locally, they form a "beach-head" patch, from which they invade the whole lattice in a very short time. Thus, local demographic stochasticity maintains killers and leads to the ultimate equilibrium K–R–S frequency distribution.

From this mechanism, it is obvious that killers are in a desperate need of cooperation among themselves for survival and spread, and they do exploit the benefits of cooperation very efficiently in the spatial setting. Adopting the QS "communication" system could apparently make the cooperation even more profitable to them, saving the toxin costs whenever it would be wasted at densities below the toxin threshold. Based on this expectation, one is tempted to predict that the functioning QS alleles should become associated with the K genotype and go to fixation very fast, but simulations reveal that this is not what actually happens in the single-species scenario (Figure 3). With a moderate cost of QS, the relative frequencies of both QS alleles fall back to a low level determined by their gain and loss by "mutation" and the metabolic burden of their expression (Figure 3A). No sign of selection in favor of the quorum signaler or the quorum responder allele or their combination is observed. One might expect that a very cheap—at the extreme, a completely cost-free—QS system should be adopted by the killer (K) genotype, but this is again not the case. Figure 3B shows that at zero metabolic burden associated with the expression of both components of the QS

Table 1
Contingency table of a simulated genotype distribution

Genotypes	S	R	K	Σ
00	17 160	18 281	10 036	45 477
0r	6634	7833	1259	15 726
s0	33 471	47 819	21 805	103 095
sr	24 076	42 535	19 091	85 702
Σ	81 341	116 468	52 191	250 000

Cell values are the frequencies of the 12 possible genotypes averaged over the last 1000 generations (from generation 9.001 to 10.000) of the run producing Figure 3B. Cramer's *V*-index for the mutual dependence of the bacteriocin system and the QS system is 0.081 for these data, showing a very weak association between bacteriocin production and QS.

Table 2
Relative deviances $b_{ij} = [a_{ij} - E(a_{ij})]/E(a_{ij})$ of genotype frequencies a_{ij} from their expected values $E(a_{ij})$ in Table 1

Genotypes	S	R	K
00	0.16	−0.14	0.06
0r	0.30	0.07	−0.62
s0	−0.00	−0.00	0.01
sr	−0.14	0.07	0.07

$E(a_{ij}) = \sum_k a_{ik} \cdot \sum_l a_{lj} \Big/ \sum_k \sum_l a_{kl}$.

system, the relative frequencies of the functional QS alleles creep close to 0.5—but this is their expected frequency at zero metabolic cost due to the assumption of equal probabilities of gain and loss by "mutation." The frequency of the quorum-signaling allele exceeds 0.5 somewhat because it is used as a cheap way for killers and resistants to convince nearby killer cells carrying the responder allele to produce the toxin and thus clean the habitat from sensitive neighbors for free. Of course this corrupts the reliability of the signal and results in a decrease of the frequency of the responder allele, which, for exactly this reason, stays somewhat below the expected value of 0.5. Moreover, even though the average abundances of the QS genes are quite high, no clear allelic association builds up between bacteriocin production and QS—Cramer's *V*-index suggests a very weak cross-dependence between the bacteriocin system and the QS modules ($V = 0.081$ for the 3×4 contingency table of genotype frequency averages—Table 1—over the last 1000 generations of the simulation results shown on Figure 3B).

Changing the crucial input parameters (fitness costs, mutation rates, and toxin threshold values) between reasonable limits does not affect the main conclusion. In simulations involving a very small cost to the QS alleles (1% of the toxin cost), the alleles for signaling and responding are actually somewhat disadvantageous, reaching a frequency of 20%, with only 10% of the killers possessing the complete QS machinery. Therefore, in the single-species model, QS does not evolve and cannot be maintained for long as a means of communication among cooperating bacteriocin-producing bacteria to save unnecessary toxin costs.

CA: 3-species case

In these simulations, we consider 3 genetically distinct bacterial species, one of which is sensitive (S) and the second resistant (R) to the toxin produced by the third (K). It is only the killer (K) species that can harbor any one or both of the 2 genes responsible for QS. This model is similar to the single-species model with the exception that now the R and S strains cannot contain QS-signaling and -responding alleles and are expected to be unable to corrupt the QS function in K strains. We ask whether this modification of the model leads to an evolutionary stable QS system associated with toxin production.

Let us compare the results of the 3-species model to those of the single-species simulations, with the coresponding parameters given the same values. Figure 4A reveals that at moderate metabolic costs of QS, the system behaves almost exactly like the single-species model (Figure 3A). The sensitive–killer–resistant competitive cycle runs on all localities, without the QS-regulating genes taking off—both return in the long run to their expected levels based on the rates of gain and loss and their metabolic costs. What is different, however, is the case of a zero-cost QS system (Figure 4B). With this assumption, the 3-species model predicts a fully functional QS machinery associated with the toxin-producing species, with almost 100% of the killer strains carrying the QS module. At a marginal QS cost by far the most, but not all toxin-producing individuals harbor both QS genes (result not shown).

Yet another interesting result of the 3-species model is that QS evolves only at intermediate values of the toxin/QS threshold. At low thresholds, the QS system is superfluous because there is a good chance of having a sufficient number of killer strains within almost any neighborhood anyway; therefore, there is no need to issue or to listen to clues in this respect—thus QS is selected against, if it is not for free, and behaves neutral if no QS cost is assumed. At high thresholds, the killer species can spread only along the perimeter of compact patches, but the peripheral cells of a patch have the least chance of being complemented by a sufficient quorum of other killer cells. That is, the probable switch off of the toxin genes on the periphery of a patch blocks the spread of the killer and is thus selected against. Figure 5 demonstrates the loss of QS response genes in killers at a higher toxin threshold.

DISCUSSION

The mean-field version of our model does not permit the evolution of QS as a regulatory mechanism of bacteriocin production. This may in part result from the absence of spatial structure in this model, which prevents the formation of locally high concentrations of bacteriocin-producing cells, which might profit the most from regulation by QS. However, the results from the single-species CA version of the model suggest another more important explanation. There are 3 conspicuous facts in the outcome of the CA model calling for explanation with respect to the temporal pattern of QS allele frequencies: 1) the responder allele is kept at a low frequency, 2) the quorum-signaling allele spreads over all bacteriocin genotypes, and 3) there is no association between those alleles of the bacteriocin system and the QS modules that are expected to benefit from cooperation. The common reason for these facts is "cheating." The QS system, just like any other form of communication, is exposed to cheating strategies—in our case, to mutant genotypes promising cooperation (issuing the quorum signal) thereby convincing others to cooperate (produce toxin), yet not cooperating when it comes to investment into toxin production. Cheaters are at an obvious advantage although they are rare: toxin production is costly, but the cheap quorum signal is sufficient to induce toxin production in cooperating neighbors, which

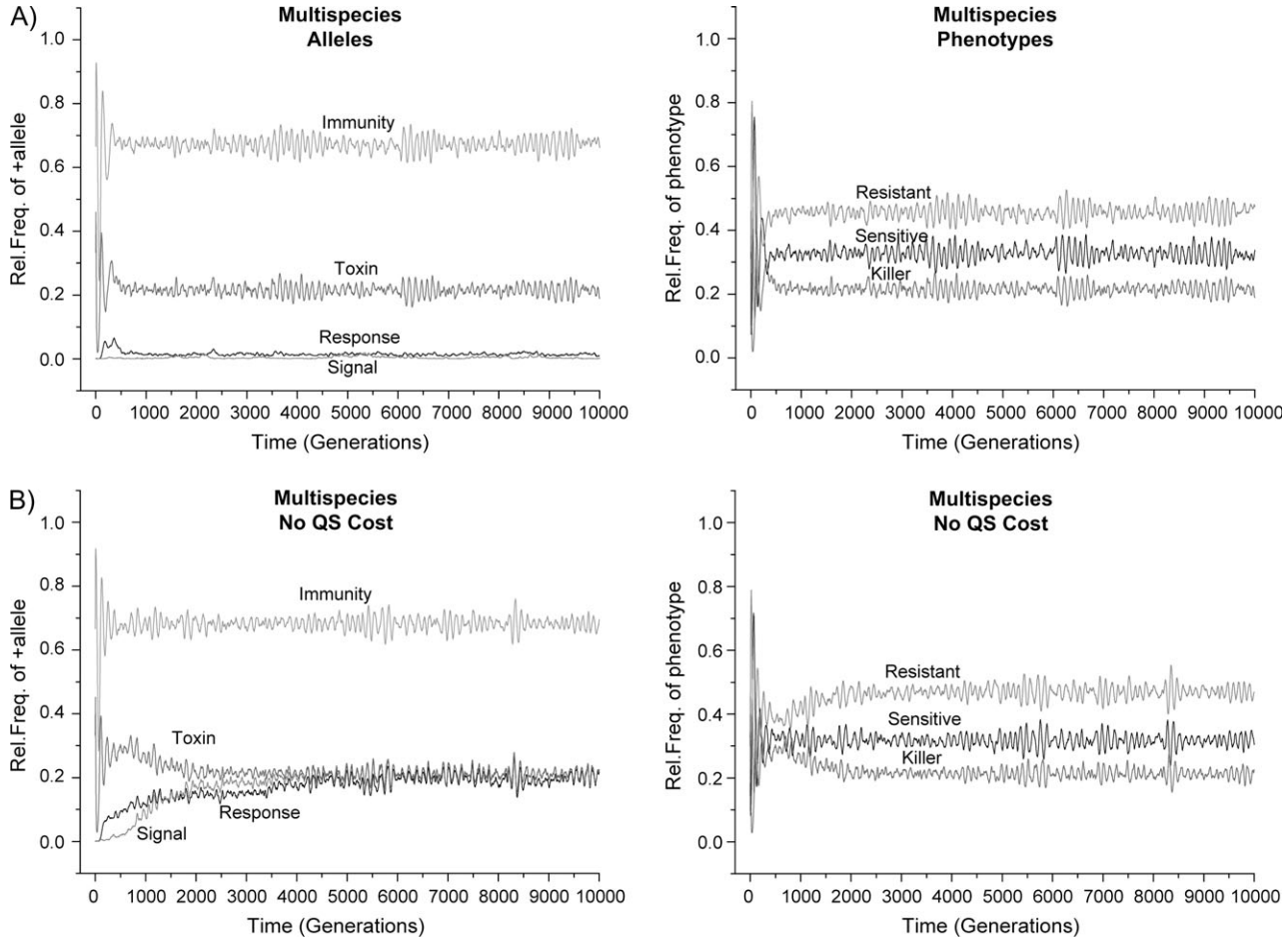

Figure 4
The same as Figure 3, for the 3-species model. Parameters and labels as in Figure 3.

means they have a good chance to enjoy the benefit of the bacteriocin without paying for it. The most successful of cheaters is obviously Rs0, the resistant type which is signaling but not responding (i.e., producing bacteriocin). It is the resistant type that pays the least yet gets most help from others. Rs0, the most efficient parasite is also by far the most frequent genotype in the quasi-stationary states of the simulated communities (cf. Table1). As cheaters become common, their fitness advantage decreases, but the reliability of the QS "communication system" is corrupted by then. Thus, the key to understanding this result appears to be that in the single-species model, killer (K), resistant (R), and sensitive (S)

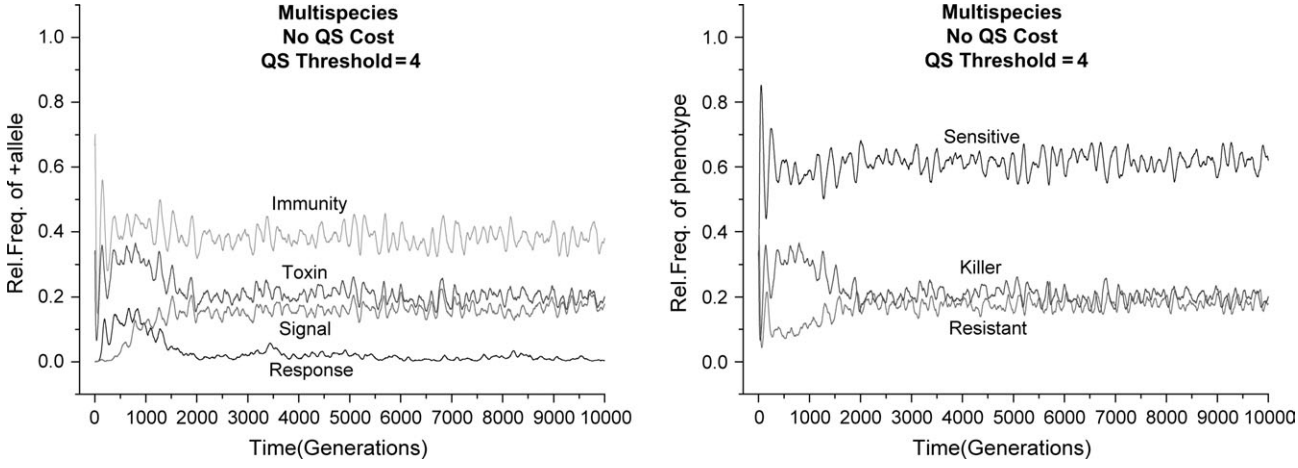

Figure 5
The same as Figure 4, except for toxin threshold, which is 4 in this case. Other parameters and labels as in Figure 3.

strains coexist and that the QS alleles can be present not only in K strains (which can potentially profit from QS) but also in R (and in S) strains, which is causing perversion of the QS function as described above.

In the 3-species model, there is also stable coexistence of K, R, and S strains, but now only K strains can harbor QS alleles. Transfer of signaling and responding alleles from K strains to R and S strains is assumed to be impossible. In particular, signaling R strains cannot occur, which—in our interpretation—have caused the breakdown of the QS system in the single-species simulations. The results show indeed that in this model stable QS can evolve, but in restricted parameter ranges. First, the signaling and response functions of the QS system should be fairly cheap in terms of metabolic costs. Second, the quorum threshold (the local density of K strains required to initiate toxin production) should neither be too low nor too high. At a low quorum threshold, QS does not pay because constitutive toxin production does almost as well, and a high quorum threshold is probably too rarely realized at the perimeter of compact patches of killer strains (where the killing effectively is taking place).

How do our conclusions compare with those of earlier theoretical analyses of the evolution of QS by Brookfield (1998) and Brown and Johnstone (2001)? Both these studies found stable levels of QS for a broad range of parameter values. We find QS to be unstable in the single-species case but stable in the 3-species model, provided the costs of the QS functions (signaling and responding) are small and the density threshold for QS is intermediate. Although using different models, both these studies considered 2-level selection, acting on individual cells and on well-defined groups of cells (colonies), such that colonies containing QS members enjoyed selective advantage compared with colonies in which QS is absent. It is this aspect of group selection that allows a stable maintenance of (an intermediate level of) QS (see also Szathmary and Demeter 1987). In our model, there is also selection at the individual level opposing selection at the level of groups of cells (namely groups formed by the individual cell considered and its immediate neighbors), but this group structure is much more diffuse. Possibly our approach of explicit spatial modeling contributes to the difference in outcome. However, more importantly, in our model the trait that is regulated by QS (toxin production) plays a crucial role in competition between strains. In fact, it is the factor that in a spatial model maintains the coexistence of killer, resistant, and sensitive strains (Czárán et al. 2002). The single-species model allows all strains (not only the toxin producers) to possess QS alleles. It is this aspect that appears to be the main reason why QS does not evolve: resistant strains can corrupt the QS system by adopting the QS signal production, thus inducing killers to start toxin production in neighborhoods lacking a quorum of killer cells. In the 3-species model, where we prevent resistant and sensitive strains to possess signaling alleles, QS does evolve in part of the parameter space, supporting the explanation above. We conclude that our model and the game-theoretical models by Brookfield (1998) and Brown and Johnstone (2001), although differing in many details, do not contradict each other.

A prediction of our model is that QS is not expected to regulate the production of toxin that is only aimed at closely related sensitive strains but that it well may evolve as a regulatory mechanism of the production of broad-spectrum toxins that can kill a wide range of unrelated strains. Indeed, we have not been able to find examples of the type of narrow-spectrum bacteriocins considered in our model to be regulated by QS. The well-studied (narrow spectrum) colicins are regulated by the SOS regulon, as is the case in the induction of bacteriocin production in several other Gram− bacteria (Pugsley and Oudega 1987). On the other hand, many examples exist of broad-spectrum toxins in Gram+ bacteria that are regulated by QS (e.g., Kuipers et al. 1998; Haas et al. 2002).

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