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Killer-sensitive coexistence in metapopulations of micro-organisms

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Many micro-organisms are known to produce efficient toxic substances against conspecifics and closely related species. The widespread coexistence of killer (toxin producer) and sensitive (non-producer) strains is a puzzle calling for a theoretical explanation. Based on stochastic cellular automaton simulations and the corresponding semi-analytical configuration-field approximation models we suggest that metapopulation dynamics offers a plausible rationale for the maintenance of polymorphism in killer-sensitive systems. A slight trade-off between toxin production and population growth rate is sufficient to maintain the regional coexistence of toxic and sensitive strains, if toxic killing is a local phenomenon restricted to small habitat patches and local populations regularly go extinct and renew via recolonizations from neighbouring patches. Pattern formation on the regional scale does not play a decisive part in this mechanism, but the local manner of interactions is essential.

Keywords: killer yeast; bacteriocin; coexistence; metapopulation; cellular automata; spatial model

1. INTRODUCTION: TOXIC KILLING IN MICROBES

In the light of many recent observations and laboratory experiments, toxic interactions appear to be common and ecologically important among micro-organisms. The excretion of antimicrobial compounds effective against related species or conspecifics is known to be widespread among bacteria (Chao & Levin 1981; Dykes 1995; Riley 1998) and yeasts (Tipper & Bostian 1984; Starmer *et al.* 1987; Jacobs & Van Vuuren 1990; Abranches *et al.* 1997; Schmitt & Breinig 2002). In mycelial fungi somewhat comparable phenomena occur as hyphal interference between different species (Berdy 1974). Toxin excretion in *Paramecium* has also been described (Grun 1976●●1●●), and even multicellular metazoans such as sponges have been shown to produce toxic substances against each other (Thompson *et al.* 1985). Antagonistic effects resulting from the excretion of biologically active metabolites are also known in plants (Rice 1984), but the general ecological importance of this so-called allelopathy is unclear.

For obvious practical reasons, the toxins produced by bacteria of medical or industrial importance, and their biochemical mechanisms of action, are particularly well studied. Toxins excreted by bacteria against similar bacterium species or conspecific strains are called bacteriocins. The most well-known types of bacteriocin are the colicins from *E. coli* (Pugsley 1984) and nisins from lactic acid bacteria (James *et al.* 1991; Vuyst & Vandamme 1994). Experimental data based on the analysis of strains in *E. coli* collections (Riley & Gordon 1992) suggest that *ca*. 35% of the strains are colicinogenic. Data on human *E. coli* isolates indicate an even higher (50%) prevalence of colicin production (Achtman *et al.* 1983; Riley & Gordon 1992). The genes coding for the toxin and for the corresponding immunity factor (necessary to avoid suicide in the killer phenotype) are commonly carried by plasmids, both genes located on the same plasmid.

The killer phenotypes of *Saccharomyces cerevisiae* and a few other yeasts have also been studied in depth (Wickner 1992). The best-known killer system in *S. cerevisiae* is controlled by two separate, multi-copy dsRNA virus-like particles in the cytoplasm (Schmitt & Breinig 2002). The genetic backgrounds of bacteriocin production and yeast killing are different, but their effects are very similar in ecological terms: the toxin produced eliminates competitor strains from the habitat. Estimates of killer activity among wild yeasts from various habitats suggest that between 5% and 30% of the strains can kill a standard sensitive *Candida glabrata* strain (Starmer *et al.* 1987; Abranches *et al.* 1997). However, this is almost certainly an underestimate because assays of toxin production are highly dependent on the choice of sensitive strains and the appropriate culture conditions.

The elimination of competitors means either literal killing or severe fitness reduction of sensitive strains, through blocking one or a few specific, vital biochemical reactions in their metabolic pathways or punching a hole into the cell membrane. Whatever actual form it takes, toxin production is a clear case of *interference competition* (Ganter & Starmer 1992)—a type of ecological interaction differing from resource competition in that it involves a direct, active suppression of the rival population by means other than just exhausting the common limiting resources. Active interference with the biochemical machinery of the competitor allows the toxin-producing population to take a disproportionately large share of the common resources at the relatively low cost of toxin synthesis and release, that is, toxin production may ensure a large fitness advantage over sensitive strains. It is possible even without specific 'targeting devices' in micro-organisms, because

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diffusion is sufficient to mediate the toxic effect to the target cells through the common liquid medium of the habitat. This explains, in evolutionary terms, why toxic interaction is common in micro-organisms but not in larger organisms: diffusion cannot deliver a concentrated toxic effect to distances much larger than the body size of a microbe.

Between populations with otherwise similar environmental requirements, the advantage of toxin release may be decisive for the outcome of competition—the toxin producer might be completely dominant over the corresponding sensitive strains in sympatric situations (Adams *et al.* 1979; Chao & Levin 1981). In spite of the asymmetry of dominance relations, the coexistence of toxin-producing and sensitive populations (and sometimes even the exclusion of toxic strains by sensitive ones) has been observed in different natural and artificial microbial communities on different spatio-temporal scales (Chao & Levin 1981; Jacobs & Van Vuuren 1990; Abranches *et al.* 1997), which facts call for an ecological explanation.

2. METHODS: METAPOPULATION MODELS FOR TOXIC INTERFERENCE

We suggest a spatial non-equilibrium mechanism to explain the coexistence of killer and sensitive strains relying on simple and biologically plausible assumptions. The models we used share the following characteristics:

(i) there are two interacting strains, one (the killer strain) dominant over the other (sensitive strain) by means of interference competition;
(ii) the common habitat of the competing strains consists of many discrete patches, each of which offers enough resources to maintain viable local populations of any one of the strains;
(iii) the interference competitive effect is in a weak trade-off with population growth rate: a killer strain grows slightly slower than a sensitive strain;
(iv) the strains are ecologically similar in the sense that, separately placed into the same habitat, they behave alike in terms of local population dynamics (i.e. they follow identical growth trajectories);
(v) if both strains are placed in the same undisturbed local habitat the killer strain always excludes the sensitive one;
(vi) the habitat patches are ephemeral; each patch (local habitat) goes through cycles of externally driven, random local extinctions and recolonizations from occupied patches;
(vii) the extinction rates and the dispersal parameters are the same for all populations.

These assumptions were initially implemented in stochastic cellular automata (SCA); with a little modification of the system, we have derived the corresponding configuration-field approximations (Czárán 1998) to the SCA models.

(a) *Stochastic cellular automata*

The arena of competition is a 100×100 toroidal lattice of local habitats (cells); time is discrete. The local habitat-state transition rules are as follows.

We label the sensitive strain 's' and the killer strain 'k'. Three local abundance states are defined for both strains: absent (0), sparse (s and k) and abundant (S and K). That is, a local habitat can be in one of nine possible abundance combination states (site states) at any point of time: 00, s0, 0k, sk, S0, 0K, Sk, sK, SK. Transitions among these may occur in three ways: (i) colonizations from neighbouring patches; (ii) extinctions due to local catastrophes; and (iii) internal transitions by population growth and competitive interaction. A diagrammatic representation of permissible colonizations and internal state transition routes is shown in figure 1.

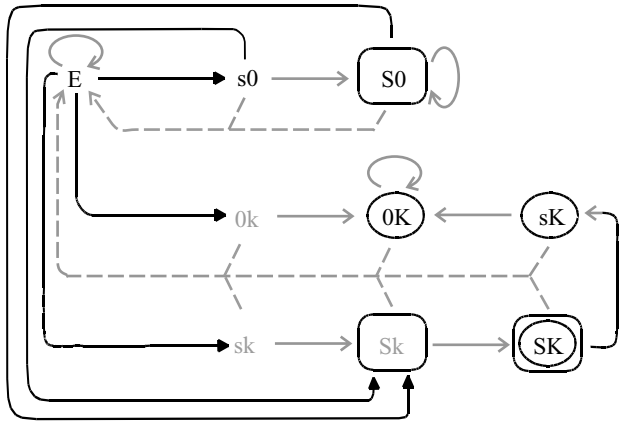

Figure 1. Possible site state transitions of the undisturbed two-strain models. Colonization events (full arrows); internal dynamics (open arrows; population growth and competition); local extinction (dashed arrows); boxed states are dispersive: killer is dispersed (elliptical box); sensitive is dispersed (rectangular box).

(i) *Colonizations*

Empty (00) patches can be colonized from within the Moore neighbourhood (eight adjacent patches) centred on them, but only source habitats containing many individuals (that is, sites in any of the states S0, Sk, sK, 0K or SK) send out colonizers. The simple transition rule of colonization is that Sx sites emit 's' type propagules and xK sites send 'k' type ones, in each case with a dispersal probability d onto each of the eight patches around them. A colonization attempt can have a dynamically relevant effect only on local habitats where the colonizer type is not yet present: we assume that the propagulum is small, and it does not contribute significantly to the dynamics of a previously inhabited patch. A k-type colonization is always successful on patches with no killers present, but for an s-type colonization to be successful, the recipient patch must be empty. That is, s0 and S0 sites can be overcolonized by the killer, but 0k and 0K sites cannot be invaded by the sensitive—this is one aspect of the dominance of 'k' over 's'. Note that the overcolonization of an S0 site implies structured local habitats: Chao & Levin (1981) demonstrate that killers cannot invade an established sensitive population in a liquid culture.

Empty sites can be colonized simultaneously and independently by both types of propagules. For example, if a 00 patch has three source sites: two sK and one SK in its Moore neighbourhood, then the probability that it remains empty is $(1 - d)^4$, which is the likelihood that none of the neighbouring colonizer populations (three K and one S) send propagules there. The chance that this empty patch will be colonized by strain 's' or strain 'k' alone is $d(1 - d)^3$ and $(1 - d)[1 - (1 - d)^3]$, respectively; the probability that the site will be colonized by both species so that it turns to state sk is $d[1 - (1 - d)^3]$.

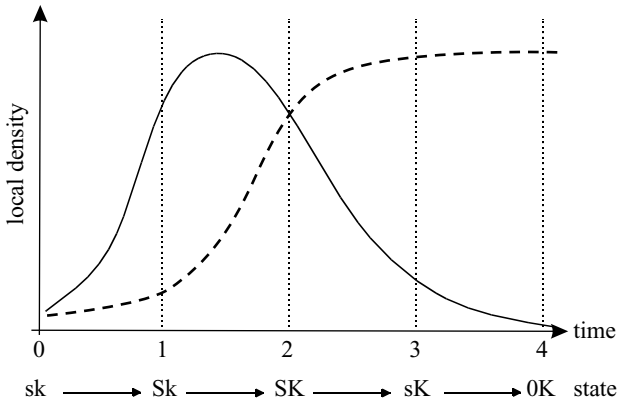

Figure 2. Hypothetical local dynamics on a single site invaded by both the sensitive (solid line) and the killer (dashed line) strain, without disturbance. Local states are: sk: few 's' and few 'k'; Sk: many 's' and few 'k'; SK: many 's' and many 'k'; sK: few 's' and many 'k'; 0K: no 's' and many 'k'.

Equal dispersivity for 's' and 'k' is inherent in the assumption that d is the same for both strains.

(ii) *Local extinctions*

Any patch in a state different from 00 will be reset to the empty state with a probability e, which is the chance of a local catastrophe (disturbance) occurring on a site in a time-unit. This parameter is called the extinction probability; it is essentially the same as the extinction parameter e in metapopulation models.

(iii) *Internal transitions*

Patches neither colonized nor disturbed will either, stay as they were (this applies to 00, S0 and 0K sites), or step forward deterministically each to a specified next state. These internal transitions represent the local dynamics on an undisturbed patch: population growth on single-strain patches, and population growth and competitive interaction on patches with both strains present. 's' and 'k' are both capable of reaching high local abundances in single-strain situations with the same speed (i.e. the transitions s0 → S0 and 0k → 0K require a single time-step for both). The trade-off relation between population growth and competitive ability shows up in the internal state transitions of sites inhabited by both 's' and 'k' (figure 2): the presence of 's' slightly impairs the growth of 'k'.

An undisturbed sk patch allows the sensitive population to grow faster initially than the killer (sk → Sk → SK), but later the killer eliminates the sensitive from the patch through interference competition (SK → sK → 0K). Note that 0K is a sink state: all the routes of colonizations and internal transitions on figure 1 end there, which means that the corresponding undisturbed (equilibrium) system converges to the spatially uniform exclusion of the sensitive population.

The parameters of internal habitat dynamics being wired into the state transition rules, the model has two parameters: the probability of neighbourhood dispersal d and the chance of local extinction, e. Both range from 0 to 1, so scanning the two-dimensional parameter space—for regions of extinction, competitive exclusion and coexistence—by simulations at an arbitrarily fine resolution is a matter of computing time.

(b) *Configuration-field approximations*

At the price of adopting two additional, slightly restrictive assumptions while keeping all other postulates of the SCA models unchanged, a semi-analytical approximation (a configuration-field approximation; cf. Czárán 1998) to the SCA system is straightforward to develop. In principle, it is possible to construct the configuration-field approximations for any single- or multispecies model—we have developed them for the one- and the two-species cases, assuming that

(i) the number of local habitats is infinitely large and
(ii) colonizations can take place from any eight sites of the infinite lattice, not just from the eight immediate neighbours of the focal site.

Assumption (i) excludes the contingencies of stochasticity arising from finite lattice size, whereas assumption (ii) filters out the effects of any mesoscale spatial pattern on the dynamics of the system. In this sense, a configuration-field approximation can be used as a reference when determining the actual contribution of the finite-size effect and that of spatial patterning to the qualitative or the quantitative behaviour of the corresponding SCA system.

The configuration-field approximations are derived in electronic Appendix A (available on The Royal Society's Publications Web site).

3. RESULTS: PERSISTENCE AND COEXISTENCE IN TIME AND SPACE

The SCA has been run in four replicates with each parameter combination, each run lasting for 10 000 generations. Systematic simulations across the e–d parameter space reveal that, at intermediate local extinction probabilities ($0.2 \leqslant e \leqslant 0.8$) and sufficient colonization ($d \geqslant 0.2$), the sensitive population can coexist with the killer and, at high enough extinction rates ($0.6 \leqslant e \leqslant 0.8$), the sensitive type even takes over, excluding the killer from the habitat patchwork completely (figure 3*a*). The region of the parameter space where the sensitive survives extends to more than half of the corresponding domain of single-strain persistence (cf. electronic Appendix A; figure 5), even though the only disadvantage of the killer type is that its population grows a little slower in the presence of the sensitive; the solitary growth rates and the dispersal abilities of the two types are the same, and the killer always wins on undisturbed patches.

What roles the possibly emerging mesoscale patterns and the finite size of the habitat universe have in the coexistence of the two populations can be judged from a comparison of the SCA results with those of the configuration-field approximation (figure 3). The qualitative behaviour of the configuration-field model emerges as quite similar to that of the SCA in that the same types of persistence, coexistence and extinction domains obtain in its parameter space. The results are different in quantitative terms, however: the parameter domain of coexistence is much larger in the configuration-field model, at the expense of each persistence domain and the extinction domain. The explanation involves both the finite-size effect and the pattern effect.

First, on the borderline of two parameter domains, one of the populations is always sparse at equilibrium, and that population might go extinct by chance in the SCA model—the smaller the lattice, the easier it is to lose the last cell occupied by the sparse type. As the state of extinc-

(*a*)

colonization (*d*) \ extinction (*e*)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.0		O	O	O	O	O	O	O	O	O	O
0.1	k	k	k	k	O	O	O	O	O	O	O
0.2	k	k	k	k	k	sk	O	O	O	O	O
0.3	k	k	k	k	k	sk	O	O	O	O	O
0.4	k	k	k	k	k	sk	s	O	O	O	O
0.5	k	k	k	sk	sk	sk	s	s	O	O	O
0.6	k	k	k	sk	sk	sk	s	s	O	O	O
0.7	k	k	sk	sk	sk	sk	s	s	O	O	O
0.8	k	k	sk	sk	sk	sk	s	s	O	O	O
0.9	k	k	sk	sk	sk	sk	s	s	s	O	O
1.0	k	k	sk	sk	sk	sk	s	s	s	O	O

(*b*) extinction (*e*)

	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
		O	O	O	O	O	O	O	O	O	O
0.1	k	k	k	k	k	O	O	O	O	O	O
0.2	k	k	k	k	k	k	sk	O	O	O	O
0.3	k	k	k	k	k	sk	sk	sk	O	O	O
0.4	k	k	k	sk	sk	sk	sk	sk	O	O	O
0.5	k	k	k	sk	sk	sk	sk	sk	O	O	O
0.6	k	k	sk	sk	sk	sk	sk	sk	s	O	O
0.7	k	k	sk	sk	sk	sk	sk	sk	s	O	O
0.8	k	k	sk	sk	sk	sk	sk	sk	s	O	O
0.9	k	sk	sk	sk	sk	sk	sk	sk	s	O	O
1.0	k	sk	sk	sk	sk	sk	sk	sk	s	O	O

Figure 3. Persistence and coexistence domains for the two-strain systems across the *e*–*d* parameter plane. (*a*) SCA simulation results with 10 000 generations per run; (*b*) configuration-field results. O: both strains die out; k: sensitive dies out, killer survives; s: sensitive survives, killer dies out; sk: both strains survive.

tion is a sink (apart from the initial input, no external introduction of propagules is allowed into the habitat patchwork), the coexistence domain is diminished from all sides by the finite-size effect. For the same reason, the extinction domain expands at the expense of the persistence domains. Second, since the neighbourhood relations of the habitat patches are frozen in the SCA, short-range dispersion can produce small regions of mono-dominant (single-type) persistence at certain parameter combinations. In such a single-type habitat aggregation, most of the propagules emitted by a patch within the clump are wasted, probably landing on local habitats already occupied by the same type. For this reason, the effective rate of colonization is considerably smaller from clumped patches than it would be in a non-aggregated situation (such as the configuration-field approximation), which fact explains why the SCA system goes extinct close to the outer margins of the persistence domains (i.e. close to the extinction domain) of the configuration-field model. The same effect works against the invasion of the sensitive population by clumps of the killer in the SCA, resulting in a larger persistence domain for the sensitive type at the expense of the coexistence domain. That is, the mesoscale pattern emerging in the SCA model at certain parameter combinations seems to help the sensitive strain to eliminate the killer. Figure 4 compares the time-series of the SCA and the matrix model in this part of the parameter space. In sum, the pattern effect decreases: (i) the coexistence domain at boundaries common with the persistence domains; and (ii) both persistence domains in favour of the extinction domain.

The pattern effect proves to be much more pronounced than the finite-size effect with lattices of the size used in the simulations: decreasing the size of the lattice to a quarter of the original (from 100×100 to 50×50) had no qualitative effect on the SCA results—the persistence–coexistence map of the parameter space for the smaller system is the same as that of the larger one in figure 3*a*.

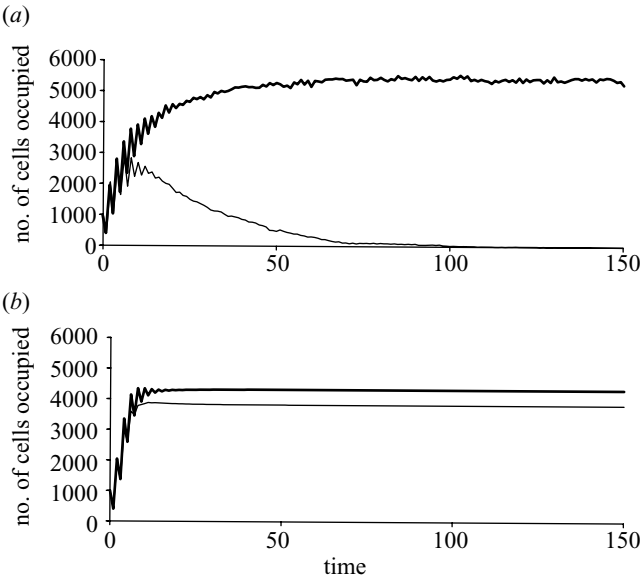

Figure 4. Occupancy trajectories for the two-strain systems at $e = 0.6$ and $d = 0.7$. The thick line represents sensitive and the thin line represents killer. (*a*) SCA simulation; (*b*) configuration-field approximation.

4. DISCUSSION

In relation to the theoretical problem of the coexistence of interference competitors with a dominance hierarchy in equilibrium situations, we draw the following general conclusions.

(i) The regional coexistence of ecologically similar, but locally exclusive interference competitors is possible in a surprisingly large part of the biologically feasible range of dispersal and local extinction parameters, provided the dynamics of the system is non-equilibrium on the local scale. A low probability of the

occurrence of local, asynchronous disturbance events is sufficient to maintain coexistence.

(ii) Unlike in metapopulation models, and the corresponding mean-field approximations published earlier (Caswell & Cohen 1991; Caswell & Etter 1993), the coexistence of the dominant (killer) and the subordinated (sensitive) types is not dependent on differences in dispersal abilities: in fact the probability of neighbourhood dispersion was the same for all types in all the models studied. The only feature favouring the subordinated type was a slight trade-off between local population growth rate and competitive ability.

(iii) Even if the trade-off relation is very weak, the subordinated strain enjoys the full advantage of local disturbances: the model assumes that the growth rate of the sensitive population is a little larger than that of the killer only if they co-occur on a habitat patch, otherwise the growth rates are equal.

(iv) Comparison of the SCA results to those of the configuration-field approximations shows that, at moderately large lattice sizes, there is ultimately no considerable finite-size effect.

(v) The effect of mesoscale pattern formation can change the results in a quantitative sense: in the SCA models, at intermediate disturbance probabilities, the spatial aggregation of local habitat states owing to short-range dispersal helps the subordinated population to outcompete the dominant regionally.

These conclusions are fairly general, in the sense that they apply to any competitive metapopulation system obeying the same rules of local interactions and colonization. One specific field of its application is the ecological problem of the widespread coexistence of killer and sensitive strains in micro-organisms. Regular, asynchronous local habitat destruction could well be common in microbial communities, whether the local habitats are, for example, host animals or plants, fallen fruits or cactus rots. All such local habitats or habitat patches are temporary, capable of maintaining small local communities for a certain period of time, then disappearing and re-emerging again. This implies non-equilibrium dynamics on the local scale, interrupted by local extinctions, reset by local colonizations—and hence metapopulation dynamics on the regional scale.

Note, however, that the models we applied here are based on the condition that there are only killer and sensitive strains. This seems mostly true for killer yeasts, which usually defend themselves against their own toxin by producing an immunity factor, which is a slightly modified and intracellularly produced toxin molecule. The toxin and the immunity factor are basically coded by the same genes and the metabolic pathways producing them are almost completely identical (Wickner 1992). It is not always true in bacteriocin systems, however, which often use resistance factors to prevent suicide. Resistance is due to mutant membrane-bound transport proteins not accepting the toxin as a substrate. Thus, the toxin and the resistance 'factor' are coded by different genes—although often located on the same plasmid—which allows decoupling of toxin production and resistance by mutations. Of course, a mutant having lost the functionality of the immunity factor gene alone is not viable: such a mutant commits suicide by producing a toxin that it is not resistant to. Losing the toxin gene while retaining resistance may be a viable mutation, however: the result is a resistant strain that does not, itself, produce the toxin. Assuming a metabolic cost to toxin production, the resistant form may be more efficient in resource competition than the killer phenotype. The possibility of the rise of a resistant mutant may result in qualitatively different dynamics, because once the resistant population takes over, sensitive strains also get a chance to spread: they do not even pay the cost of resistance and may thus be superior to the resistant mutant in resource competition. This means that a killer-resistant-sensitive system may constitute a circle of competitive dominance: killers are dominant over sensitives by interference competition (toxic killing), but resistants take over the killer strains and sensitives take over the resistants through resource-competitive exclusion. This offers a completely different mechanism for maintaining the coexistence of genetically different strains (Durrett & Levin 1998; Pagie & Hogeweg 1999; Frean & Abraham 2001; Kerr *et al.* 2002), which we have explored elsewhere in more detail (Czárán *et al.* 2002). Unlike the metapopulation system we discussed here, coexistence in the intransitive competition cycle critically depends on localized dispersion.

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