Commentary

Chemical warfare from an ecological perspective

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Chemical weapons are recent acquisitions in humankind's ever-growing arsenal of destruction. But bacteria and fungi have been practicing chemical warfare for a very long time. Among the numerous and structurally diverse antimicrobial agents that microbes produce are penicillin by the mold *Penicillium notatum*, many important antibiotics by streptomycetes, a wide range of bacteriocins by *Escherichia coli* and most other bacteria (including the food preservative, nisin, by *Lactococcus lactis*), and killer toxins by the yeast *Saccharomyces cerevisiae*. In this issue of PNAS, Czárán, Hoekstra, and Pagie (1) perform numerical simulations to examine the effects of these interactions on microbial diversity. They come to the surprising conclusion that all this chemical warfare may actually promote biodiversity in the microbial realm. In essence, the authors show that high levels of diversity are maintained by the complex dynamics generated when a version of the "rock-scissors-paper" game (2, §) is played out in a spatial context.

A toxin-producing "killer" microbe is generally immune to the chemical agents it makes. For example, in the case of bacteriocins, the killer constitutively produces an immunity protein that binds the toxin and renders it harmless. Nonetheless, making such toxic compounds is not without costs. These costs include the material and energetic burdens of producing the toxin and maintaining immunity. Also, in some cases, the physical release of the toxin into the environment is lethal to the producing individual. In these cases, the killer is effectively a "suicide bomber" (3), which reminds us again of the disturbing parallels between warfare as practiced by humans and by our most primitive relations. One may wonder, in such cases, how a killer population can survive if toxin production is lethal. The explanation lies in the fact that, in a given generation, only a small fraction of the killer strain actually produces toxin (4).

The presence of antimicrobial agents often selects mutations or other genetic changes that confer resistance. The emergence and spread of antibiotic-resistant pathogens in our communities and hospitals bear unfortunate witness to this evolutionary process (5). Resistant genotypes may often suffer a cost of their own, in the sense that they are inferior competitors to their sensitive counterparts in the absence of the antibiotic or toxin (6, 7). In principle, resistant strains can arise from sensitive types that lose their susceptibility to a toxin (for example, by a mutation that inactivates a receptor to which the toxin binds), or from killer strains that lose the capacity to produce a toxin but retain their immunity to it.

Another important feature of microbial chemical warfare is that one finds a tremendous diversity of toxins, even within a single species. For example, there exist a multitude of colicins with which different strains of *E. coli* kill one another. Molecular studies of the genes that encode these bacteriocins imply a history of strong selection for innovation and change (8, 9), in essence, an evolutionary arms race. Also, toxin production and resistance functions are often encoded by genes located on transmissible, extrachromosomal elements such as plasmids. Once an innovation in chemical warfare arises in one group, it might then be acquired by another. All this implies that the means of chemical warfare among microbes are very labile from an evolutionary perspective.

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Many studies indicate that microbial communities are extremely diverse. For example, one analysis of the reassociation kinetics of the total bacterial DNA in a 30-g soil sample found that it contained some 20,000 common species and perhaps 500,000 rare ones (10). This diversity begs the question of how all of the different species are maintained. Ecologists have long been interested in understanding the forces that maintain diversity, although they have focused mostly on plants and animals, with little attention to microorganisms. Perhaps the simplest, and oldest, explanation is that there must exist as many different resources as there are coexisting species. But this old explanation has been supplanted by both theory and data. The existence of multiple trophic levels can maintain more distinct species than there are underlying resources. For example, two species may coexist on a single resource if there also exists a third species—a keystone predator—that preferentially preys on the superior competitor (11, 12). Two species may also coexist on one resource if its concentration fluctuates in time, such that one species is competitively superior when the resource is scarce although the other is superior when the resource is common (13, 14). Spatial variability in resource abundance, especially when coupled with differences among species in dispersal ability, also can promote biodiversity, in principle allowing an arbitrarily large number of species to persist (15, 16).

In addition to direct competition for limiting resources, which ecologists call scramble competition, organisms sometimes compete by interfering with one another. Besides microbial production of toxins, other examples of interference competition include the production by some plants of compounds that they use to suppress their neighbors, and defense of territories by some animals that may prevent competitors from acquiring resources located therein.

At first inspection, interference competition does not seem to be the sort of process that would help maintain diversity in an ecological community. Consider two species that compete for a single resource. Let us assume that one species is the superior competitor for the resource, but it is sensitive to a toxin that the other species produces. In a physically unstructured environment, such as a well-stirred medium, there may exist an equilibrium where both species are present, but that equilibrium is dynam-

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§Virtual Rock, Paper, Scissors. http://www.2street.com/rock-paper-scissors/.

www.pnas.org/cgi/doi/10.1073/pnas.022641999

ically unstable (4, 17). The equilibrium occurs when the concentrations of resource and toxin are precisely those needed to equalize the net population growth rates of the two species. But if the population of the sensitive competitor is increased slightly, then the effect is to reduce the concentration of resource below the level at which the killers make enough toxin to keep the sensitive population in check. Hence, the perturbation is amplified, and the killer species goes extinct. Similarly, an upward perturbation in the abundance of the killer leads to an excess of toxin, which pushes the sensitive species into a free fall. Thus, owing to the instability of this internal equilibrium, interference competition would not seem to be a force that promotes biodiversity.

The picture changes, however, if we add more biology to the model. The model explored numerically by Czárán *et al.* (1) includes three main features that are missing from the simpler model summarized in the preceding paragraph. First, the authors allow the species to interact in a spatially structured environment (on a two-dimensional surface) rather than under a mass-action regime. In elegant experiments with bacteria, Chao and Levin (4) showed that the conditions for invasion of a killer strain were much broader in a structured environment than in an unstructured one. Under mass-action, a small population of killers cannot invade an established population of sensitive organisms. This failure occurs because the killers must pay the costs of toxin production and immunity, but the benefits—the resources made available by killing sensitive organisms—are distributed at random. Moreover, when killers are rare, the reduction in growth rate experienced by the sensitive strain (owing to extra deaths) is smaller than the reduction felt by the killer strain (owing to its costs), and the killer population therefore goes extinct. However, in a structured environment, such as on the surface of an agar plate, the strains grow as separate colonies. The toxin diffuses out from a colony of killers, thus killing sensitive neighbors. The resources made available accrue disproportionately to the killer colony owing to its proximity, and therefore killers can increase in frequency even when initially rare. However, spatial structure alone does not permit coexistence between killer and sensitive strains. In fact, even the unstable internal equilibrium disappears in a structured environment (18).

Next, Czárán *et al.* introduce a third kind of strain into the model, one that is resistant to the toxin but cannot itself produce the toxin. Resistant strains can occur in most, if not all, of the microbial cases noted at the outset. The authors assume there is a cost of resistance (6), and that this cost is less than the combined costs of toxin production and immunity borne by the killer (19). Owing to this third member, the pairwise interactions among the strains have the nontransitive form of the rock-scissors-paper game (Table 1). The killer strain beats the sensitive strain, owing to the toxin's effect on the latter. The sensitive strain out-competes the resistant strain, because only the latter suffers a cost of resistance. And the resistant strain wins against the killer, because the latter bears the costs of both toxin production and immunity, whereas the former pays only the cost of resistance. In an unstructured environment, this game allows periodic cycles, in which all three strains coexist indefinitely but the abundance of each one fluctuates (1, 2). In a spatially structured environment, this game permits a quasi-stable global equilibrium, one in which all three strains persist with nearly constant global abundance, although different local regions oscillate out of phase with one another (20).

Table 1. Chemical warfare among microbes as a nontransitive, three-way game similar to the ''rock-scissors-paper'' game

Strain below	Wins against	Loses against
Killer	Sensitive	Resistant
Sensitive	Resistant	Killer
Resistant	Killer	Sensitive

Finally, Czárán *et al.* incorporate evolution by allowing as many as 14 distinct systems of toxin production, sensitivity, and resistance, along with the genetic processes of mutation and recombination that can alter these traits and their associations. The permutations of these systems permit several million different strains. Putting all these factors together—chemical warfare with multiple agents and many evolving strains in a spatially structured environment—Czárán *et al.* ask: How much diversity is maintained? And how is that diversity distributed?

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Czárán *et al.* find two distinct quasi-equilibrium conditions, which they call the ''frozen'' and ''hyperimmunity'' states. Many strains coexist in each state, but they differ strikingly in how functional diversity is distributed across strains. In the frozen state, all of the toxins are maintained globally, but the vast majority of colonies are single-toxin killers. That is, each colony makes one toxin, to which it is also immune. Given 14 different toxin systems, it follows that 14 strains make up most of the population. By contrast, in the hyperimmunity state, many colonies produce no toxin, many others make one, still others produce several toxins, but only a few make most of the potential toxins. Resistance shows a very different distribution, with the vast majority of colonies being resistant to most or even all of the toxins.

Which of these two outcomes is achieved depends on initial conditions. If the evolving system begins with the entire population sensitive to all toxins, then the frozen state results. This state comes about because of assumptions that ensure computational simplicity, but which seem biologically improbable. In particular, the model assumes: (*i*) all 14 toxins engender exactly the same costs of production and resistance; (*ii*) two colonies reach a standoff if each one produces a different single toxin, there being no other way to break the tie; and (*iii*) a colony that makes one toxin prevails over a colony that makes two other toxins, owing to the higher costs experienced by the latter. Thus, the evolving population becomes filled with the various single-toxin killers, but any double-barreled killers that appear cannot invade. The hyperimmunity state is reached if the system starts with sufficient diversity that most colonies already have multiple killer and resistance traits, such that standoffs resulting from ties are unlikely. Early in these runs, evolving colonies tend to accumulate killer functions, along with their associated immunities. But, as time progresses and killers come to dominate, the toxins lose their efficacy and many colonies give up toxin production while retaining resistance. Besides depending on initial homogeneity, the frozen state is also precluded when rates of genetic recombination are sufficiently high.

Czárán *et al.* suggest that the hyperimmunity state may correspond to different strains within a species, whereas the frozen state might apply to a multispecies community. However, we are skeptical of the relevance of the frozen state, because it depends on ties and, moreover, on the rather arbitrary assumption that ties are broken in favor of lower costs instead of higher toxicity. This reservation aside, Czárán *et al.* have shown that chemical warfare among microbes can promote substantial diversity in the mechanisms of killing and resistance.

As a 1960s anti-war poster said, ''War is bad for children and all living things.'' Sadly, war happens. At least in the microbial realm, the almost infinite ways of waging chemical warfare may actually maintain some of the tremendous biodiversity that exists.

We thank Simon Levin for inviting our commentary. Our collaborative research is supported by a grant from the National Science Foundation (DEB-9981397).

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