MEDICINE

A Weakness in Bacteria's Fortress

Evolutionary biologists are trying to attack bacteria in a new way: by short-circuiting their social life

By Carl Zimmer

AT THE UNIVERSITY OF ZURICH, ROLF KÜMMERLI INVESTIGATES NEW DRUGS TO STOP DEADLY infections. He spends his days in a laboratory stocked with petri dishes and flasks of bacteria—exactly the place where you would expect him to do that sort of work. But Kümmerli took an odd path to get to that lab. As a graduate student, he spent years hiking through the Swiss Alps to study the social life of ants. Only after he earned a Ph.D. in evolutionary biology did he turn his attention to microbes.

IN BRIEF

Researchers from an emerging field called sociomicrobiology believe they have a new approach to fight antibiotic resistance among illness-causing bacteria.

They want to disrupt the processes that allow bacteria to communicate and cooperate with one another.

Evolutionary theory predicts that acquiring resistance to such "antisocial" drugs should be difficult. Not everyone is convinced, however, that this new strategy for developing antibiotics will work.

Photography by Zachary Zavislak

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The path from ants to antibiotics is not as roundabout as it may seem. For decades scientists have studied how cooperative behavior evolves in animal societies such as ant colonies, in which sterile female workers raise the eggs of their queen. A new branch of science—sometimes called "sociomicrobiology"—is revealing that some of the same principles that govern ants can explain the emergence of bacterial societies. Like ants, microbes live in complex communities, where they communicate with one another to cooperate for the greater good. This insight of social evolution suggests a new strategy for stopping infections: instead of attacking individual bacteria, as traditional antibiotics do, scientists are exploring the notion of attacking entire bacterial societies.

New strategies are exactly what is needed now. Bacteria have evolved widespread resistance to antibiotics, leaving doctors in a crisis. For example, the Centers for Disease Control and Prevention estimates that 23,000 people die in the U.S. every year of antibiotic-resistant infections. Strains of tuberculosis and other pathogens are emerging that are resistant to nearly every drug. "It already is a substantial problem," says Anthony S. Fauci, director of the National Institute of Allergy and Infectious Disease. "And there's every reason to believe it's going to get even worse."

The standard response to this crisis has been to slow the evolution of resistance and find new drugs to replace old ones as they grow weak. But this is only a treadmill solution. Bacteria are relentlessly evolving resistance and will continue to do so unless we find a different way to fight them. "Every time we develop a new drug, it fails," says John Pepper, a theoretical biologist at the National Cancer Institute. "So the solution is, 'Quick! Make another antibiotic!' That helps for a few months. But that's just not good enough any more."

Many infectious species of bacteria depend on their collective behavior to make us sick. Sociomicrobiologists are looking for opportunities to disrupt their societies—by interfering with their communication, for example, or blocking their cooperative efforts to gather nutrients. Evolutionary theory predicts that the collective behavior of bacteria should be a ripe target for medicine. Attacking the social life of bacteria may not be a completely evolution-proof strategy. But at the very least, it might slow down the evolution of resistance dramatically.

Sociomicrobiologists have a lot of skepticism to overcome. Although they have presented detailed theoretical arguments and a few promising experimental results, some researchers doubt that their evolution-inspired drugs will be able to stop the rise of resistance. And pharmaceutical companies, which have shied away from antibiotics in general, are not yet ready to push such drugs through the approval pipeline and into the marketplace.

Still, the sociomicrobiologists are getting some attention. The National Institutes of Health has been laying plans for research into antibiotic resistance, and investigators have made the social life of bacteria a top priority. If the work pans out, they will have succeeded in reversing the relation between medicine and evolution. Traditionally an enemy in the fight against bacteria, evolution would become a friend.

THE EVOLUTION OF DRUG RESISTANCE

THE CRISIS of antibiotic resistance has been long in the making. A few years after the first antibiotics were introduced in the mid-1900s, doctors had already discovered some bacteria that could withstand them. At the time, it was not entirely clear what was happening. Today, of course, scientists can probe the evolution of resistance in all its molecular details.

Penicillin, for example, kills bacteria by grabbing onto a protein that helps in building the bacteria's cell membranes. Without this protein, a bacterium will spring a leak and die. In any population of bacteria, a few mutants will be able to defend themselves against penicillin. Bacteria, for example, have pumps to flush toxic chemicals out of their interior. A mutant microbe may produce extra pumps, allowing it to rid itself of penicillin quickly, freeing up its proteins to build its membranes.

Normally such a mutation does not provide any evolutionary advantage to a microbe. If a patient takes a dose of penicillin to clear an infection, suddenly those extra pumps can make a huge difference. Bacteria without the extra pumps die, whereas many mutants manage to survive. The survivors multiply, increasing the proportion of mutants in the population. In subsequent generations, the descendants of the original mutants may evolve even better defenses, sometimes by picking up genes from other bacterial species.

For decades new drugs came out of the development pipeline quickly enough to replace the old ones that failed. But now that pipeline is drying up. As the expense of developing new antibiotics has cut into profits, many pharmaceutical companies have bailed out of the antibiotics business and invested instead in more profitable drugs for cancer or hepatitis.

As the crisis deepened, scientists yearned for an antibiotic that would not become obsolete. And sometimes they did find what they believed to be an evolution-proof drug. In 1987, for example, Michael Zasloff, then at the NIH, discovered that African clawed frogs produce a powerful toxin against bacteria in their skin. Zasloff and other researchers soon found that the amphibians were not the only toxin makers. Just about every animal they looked at made small, positively charged proteins that could kill bacteria—a class of molecules that came to be known as antimicrobial peptides.

In journal reviews and in news reports, Zasloff predicted that bacteria would be unlikely to evolve resistance against the drugs. Animals, he pointed out, had been using antimicrobial peptides to kill bacteria for hundreds of millions of years, and yet bacteria today remain vulnerable to the peptides. In 2003 Graham Bell, an evolutionary biologist at McGill University, predicted that Zasloff would be proved wrong. Penicillin and many other drugs had also been discovered being made in nature. But modern medicine delivered them in huge concentrations to patients—thereby creating a tremendous evolution pressure that drove the rise of resistant mutants. As soon as doctors started giving pills packed with antimicrobial peptides to patients, history would repeat itself.

Zasloff challenged Bell to see if bacteria could become resis-

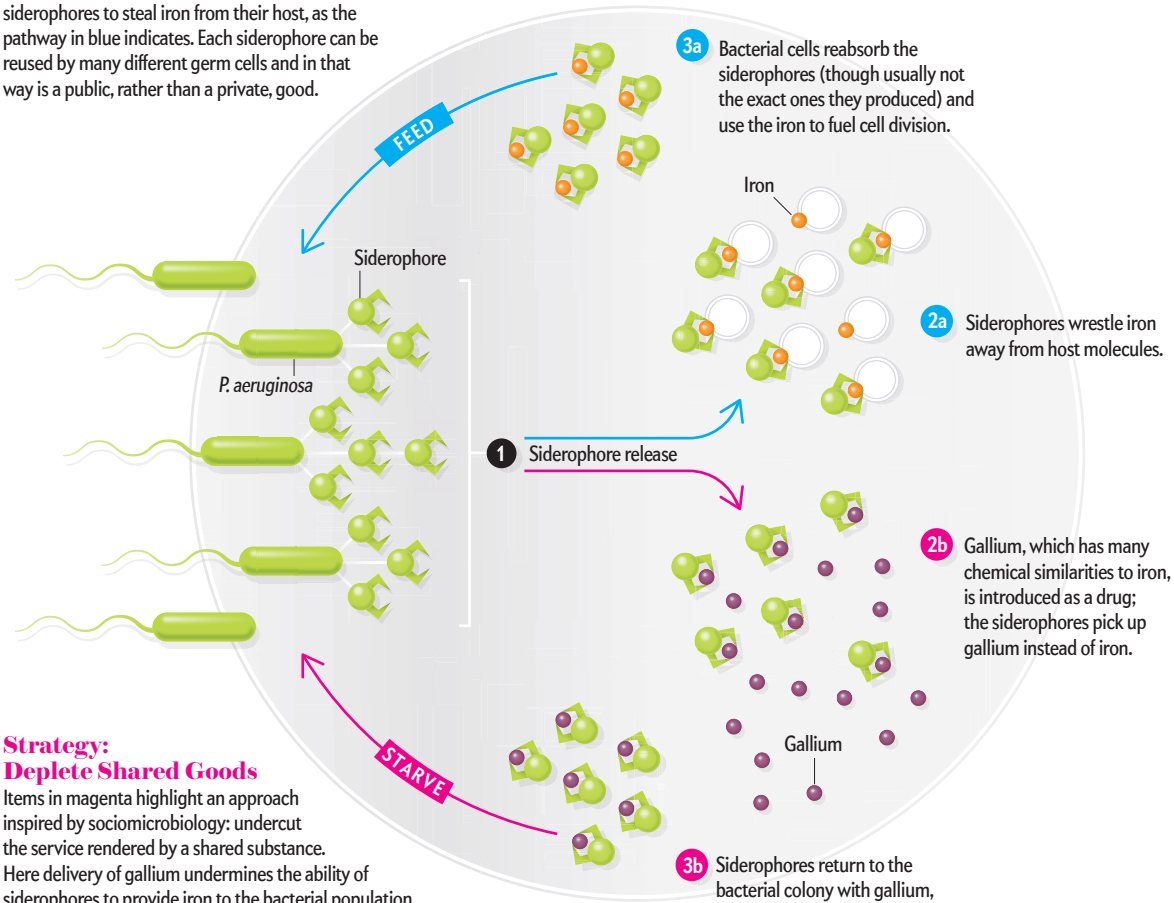
CASE STUDY

Lessons from Evolutionary Biology

Researchers hope to develop more effective antibacterial treatments by interfering with the way various germs communicate and cooperate with one another. Such an approach should trigger less drug resistance, in theory, because no single cell should be able profit by changing the way it responds. One idea, which targets a molecule that *Pseudomonas* bacteria use to scavenge iron, is shown below.

The Target: Communal Nutrient Gathering

Pseudomonas bacteria produce molecules called siderophores to steal iron from their host, as the pathway in blue indicates. Each siderophore can be reused by many different germ cells and in that way is a public, rather than a private, good.



Strategy: Deplete Shared Goods

Items in magenta highlight an approach inspired by sociomicrobiology: undercut the service rendered by a shared substance. Here delivery of gallium undermines the ability of siderophores to provide iron to the bacterial population. Even if a single cell develops a favorable siderophore mutation, that cell will still probably starve because it is likely to take up siderophores made by other bacteria.

tant to pexiganan, one of his best-studied peptides. Bell and his then graduate student Gabriel Perron reared a batch of *Escherichia coli* and exposed it to a low dose of pexiganan. Then they took some of the surviving bacteria to start a new colony, which they exposed to a higher dose of the drug. Increasing the dose over a few weeks, the scientists watched the bacteria evolve to be completely resistant to pexiganan, just as Bell had predicted.

Zasloff immediately acknowledged that Bell had been right. The experiment made him far more cautious about antimicrobial peptides. "If something can happen in a test tube, it is very likely that it can happen in the real world," Zasloff told *Nature*. (*Scientific American* is part of Nature Publishing Group.)

Today we do not know for sure if that is actually true, and we will not unless antimicrobial peptides are eventually approved for use for infections. Currently pharmaceutical companies are running several clinical trials, but 28 years after their discovery, not a single antimicrobial peptide has been approved for use for infections. They are victims of a slow pipeline.

COOPERATION AMONG BACTERIA

CHARLES DARWIN COULD HAVE had no idea that bacteria would become one of the best illustrations of his theory of natural selection. He and other scientists of his day knew very little about how microbes grow. When he presented his theory in *On the Ori-*

Illustration by John Grimwade

gin of Species in 1859, he instead wrote about traits that were familiar to his fellow Victorians, like the fur on mammals and the colors of feathers.

Darwin also wrote about familiar features of nature that had initially made him worry that his idea might be wrong. One of them was that in many species of ants, female workers are sterile. In Darwin's theory, natural selection emerged from the competition between individuals to survive and reproduce. But worker ants, which do not reproduce themselves, seemed to be dropping out of the competition altogether. Their existence, Darwin wrote, seemed to be "actually fatal to my whole theory."

Darwin suspected that a solution to the worker ant paradox lay in kinship. An ant colony is not just a random jumble of strangers. It is more like an extended family. Together a group of related ants may be able to produce more offspring than if they all try to breed on their own.

Darwin's ideas on cooperation have inspired generations of evolutionary biologists to explore them further. That is how Kümmerli got his start as a scientist. He would sequence DNA from ants in different nests, for example, to see how their kinship influenced their behavior toward one another. The research was fascinating but also slow and limited. As Kümmerli got closer to earning his Ph.D., he discovered some evolutionary biologists who were switching from social animals to social bacteria.

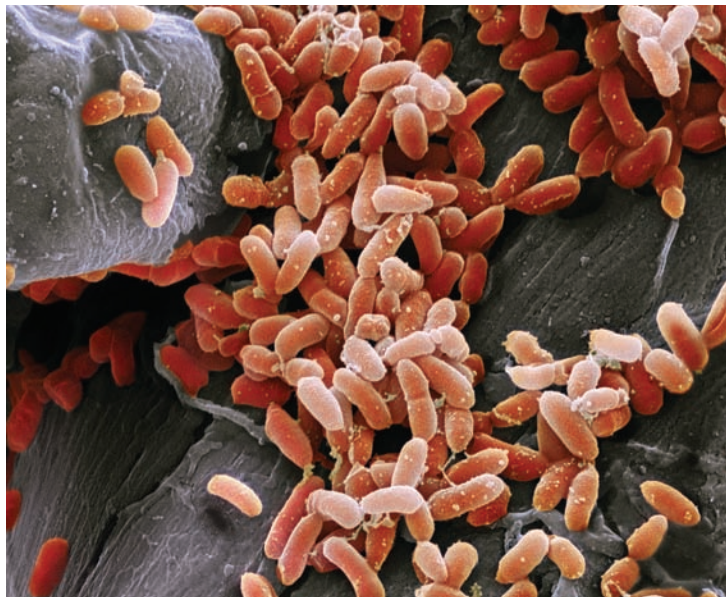
The words "social" and "bacteria" may not be tightly joined in most people's minds, but it turns out that microbes live in intimate communities full of conversation and cooperation. Take *Pseudomonas aeruginosa,* a species that can cause serious lung infections. When one of the microbes invades a host, it sends out signaling molecules. Other members of its species can grab those molecules with special receptors. Releasing and grabbing these molecules is a way for bacteria to say, "I'm here—is anyone else here?"

If the bacteria sense that they do have enough members, they will begin to cooperate to build a shelter. They spray out gooey molecules that grow into a mat, inside of which the bacteria embed themselves. This so-called biofilm can stick to the lining of the lungs or other organs. Nestled deep inside the biofilm, the bacteria are shielded from the attacks of immune cells.

Pseudomonas bacteria also work together to gather nutrients. Bacteria cannot grow without iron, for example, but the human body is a tough place to find it because our cells snap up iron and lock it away in hemoglobin and other molecules. To get an iron supply, each microbe releases molecules called siderophores. The siderophores can wrest the atoms away from our own molecules. "They basically steal the iron," says Sam Brown, an evolutionary biologist at the University of Edinburgh. The bacteria can then absorb the iron-bearing siderophores and use the iron to grow.

The effort is deeply cooperative because each siderophore that a microbe takes in was probably made by one of its millions of neighbors. "One cell will pay a cost that will benefit the whole infection, not that one cell," the NCI's Pepper says. Evolutionary theorists have a name for such molecules: public goods. These molecules are good for the public at large—in this case, the community of bacteria. They are the opposite of private goods, which only benefit the individual bacteria that made them.

Public goods represent a Darwinian paradox. Natural selection should, in theory, wipe them out. Mutants that do not make their own public goods can still use the public goods made by others. This imbalance should put the freeloader at an evolutionary advantage. A mutant that does not make siderophores can still get iron without paying the cost of making siderophores. It should reproduce faster than cooperative bacteria and become more common. And yet it is the cooperators that dominate species such as *P. aeruginosa,* not the freeloaders.



ENEMY GROUP: The collective activities of *Pseudomonas aeruginosa* bacteria, pictured in the electron micrograph above, allow them to trigger hard-to-eradicate infections.

STEVE GSCHMEISSNER *Science Source*

In the mid-2000s a small group of evolutionary biologists began to turn their attention to these intriguing questions about the social life of bacteria. The University of Edinburgh emerged as a leading center for sociomicrobiology, which is why Kümmerli went there in 2007. He did not immediately start running experiments, though. Years of studying ants had not yet prepared him for the hard work of microbiology. Kümmerli and other aspiring sociomicrobiologists had to apprentice themselves in the microbiological arts. They learned how to rear bacteria, how to prevent their stocks from getting contaminated, how to manipulate their genes and how to run experiments. "It took years to learn all the methods," Kümmerli says. "Sometimes we were not taken seriously by the classical microbiologists."

Eventually they got results. They began uncovering tricks that social bacteria use to keep freeloaders at bay. Working with Brown, for example, Kümmerli found that *Pseudomonas* bacteria do not produce a steady stream of siderophores. Instead they churn them out suddenly, in an initial burst. Once the bacteria have created a supply of siderophores, they can recycle the molecules. They absorb iron-bearing siderophores, pull away the iron atoms and then spit the siderophores back out. Thanks to the durability of siderophores, the bacteria do not have to use up much energy making new siderophores to replace old ones. Recycling thus lowers the cost of cooperation. It also helps to cut down the advantage of being a freeloader.

As the sociomicrobiologists discovered more about the social evolution of bacteria, they began to wonder if they could apply their insights in a very practical way: by finding new kinds of drugs to fight infections.

TIPPING POINT

TO AN EVOLUTIONARY BIOLOGIST, all antibiotics in use today are basically the same. Each attacks bacteria's private goods. If a microbe mutates to protect its own private goods, it will outcompete other bacteria that cannot. Sociomicrobiology reveals a different target for stopping infections. "Instead of targeting the individual cells, target their public goods," Pepper says.

Evolutionary theory predicts that bacteria will be less likely to evolve resistance to drugs that go after public goods. Imagine, for example, that researchers were to develop a drug that attacked siderophores. As a result, bacteria would become starved of iron.

Now imagine that an individual microbe acquired a mutation that protected its siderophores from the drug. That mutant would not gain any advantage. Bacteria collectively release all their siderophores into their host, where the molecules get mixed up. When a microbe takes up an iron-bearing siderophore, it is almost certainly not one of its own. As a result, mutants cannot outreproduce their fellow bacteria.

Sociomicrobiologists first developed this argument in the abstract, through mathematical equations and computer simulations. "We devise all these theories and say look, this ought to work if you just try it," Pepper says. "But all that effort is useless if no one is going to try it." Those experiments are now under way. Recently, for example, Kümmerli, Brown and their colleagues tried out a drug that attacks siderophores. Previous research had revealed that siderophores made by *Pseudomonas* grab a metal called gallium just as easily as they grab iron. The researchers wondered if they could use gallium as a drug to starve the bacteria of iron.

To find out, they ran an experiment on caterpillars. They infected the insects with *Pseudomonas* and let the infection run its course in some of the insects, which all died. But the infected caterpillars that were given gallium all recovered.

Having shown that gallium could act as an antibacterial drug, the scientists ran another experiment to see if the bacteria could evolve resistance to it. Evolutionary theory predicted that they should not. "We were quite nervous doing the experimental evolution," Kümmerli says. He and his colleagues knew very well how other promising drugs had been crushed by the power of evolution. "We were just hoping no evolution came up," he adds.

For their new experiment, the scientists reared *Pseudomonas* in a broth that included iron. But the iron was bound up in molecules that the bacteria could not absorb. They needed to use their siderophores to pry the iron away from the molecules to survive. In one set of trials, the scientists exposed the bacteria to some conventional antibiotics. At first, the drugs rapidly slowed down the growth of the bacteria. But after 12 days of exposure, the bacteria became completely resistant to the antibiotics.

Then they ran the experiment all over again, this time exposing the bacteria to gallium instead of conventional antibiotics. The gallium drastically slowed down the growth of the bacteria. After 12 days, the bacteria were just as vulnerable to gallium as they had been at the start. The experiment met the predictions of the sociomicrobiologists. A drug that targeted public goods had prevented bacteria from evolving resistance.

Pepper, who was not involved in the experiment, considers the gallium experiment a major success for sociomicrobiology. "I think it's exactly what was needed as a next step," he observes. "I hope this will be a tipping point for people."

Kümmerli hopes that other scientists will start testing gallium in infected mice and, perhaps in a few years, in humans. Such trials would be relatively easy to run because gallium has already been extensively tested in humans for a number of medical treatments.

POTENTIAL DRUGS

SIDEROPHORES are just one of a number of public goods that sociomicrobiologists are studying as potential targets for drugs. Some bacteria, for example, make us sick by releasing toxins. But they only do so once their population is big enough to deliver a potent wallop. Then they unleash toxins that cause our cells to rupture, spilling out molecules that the bacteria can feed on. Drugs that can disarm toxins may be able to render bacteria helpless without even killing them.

Other researchers are investigating the signals that bacteria send to one another. They are discovering molecules that can jam this communication in various ways, such as blocking the receptors that usually grab signaling molecules. If bacteria cannot communicate with one another, then they cannot cooperate.

Antisocial drugs could have another advantage over conventional antibiotics: instead of wiping out lots of species of bacteria at once, they may be able to narrow their targets. That is because the public goods made by one species are typically only useful to that species alone. Thus, antisocial drugs might be less likely to wipe out good germs along with the bad.

As promising as this research may be, however, some scientists are skeptical that antisocial drugs will avoid resistance. Thomas Wood of Pennsylvania State University and his colleagues have been investigating a few of the most promising of these compounds. And their results are sobering. In an experiment on a drug that interferes with bacterial signaling, for example, they found mutants that could grow in spite of the drug. In other words, the bacteria evolved a way to live without a public good. "I'm not hopeless," Wood says. "I just don't think this one class of drugs is a panacea."

It is possible that Wood's results mean that certain public goods are not truly essential. If that is so, then evolution-based drugs will have to target only the essential ones.

Even if antisocial drugs turn out only to slow down resistance, Pepper says, they will be an important advance. "We're losing this race, and lives are at stake," he declares. "Even if we can just gain an edge against our opponent, that's going to save a lot of lives." SA

MORE TO EXPLORE

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