

EVOLUTION

Bacteria Diversify Through Warfare

ARNHEM, THE NETHERLANDS—It's a civil war in there for many gut-loving bacteria, and the battles between the strains may help explain a microbial mystery: why *Escherichia coli* and other microbes are so genetically diverse. Peg Riley, an evolutionary biologist at Yale University, notes that while two

humans might differ in 0.05% of their DNA, *E. coli* strains vary by

5%—"more diversity than you expect to find [in a single species]," she says. At the recent meeting here of the European Society for Evolutionary Biology, Riley described evidence that a chemical arms race could be helping to drive this genetic diversification by dividing group from group and descendants from ancestors.

The weapons in question are colicins, one of a group of chemical compounds collectively known as bacteriocins, which bacteria use to defend themselves and kill other, closely related strains. These weapons are often deployed in the gut, which houses several dominant strains of *E. coli* in the average mammal. When a new strain begins competing with a resident strain and resources grow scarce, both may release colicins.

"Colicins may be their number one line of defense and offense," says Riley. Designed to recognize specific receptors on other *E. coli* cells, the colicins are transported inside the enemy bacterium and kill it by disrupting cellular functions, for instance by chewing up the DNA.

Each strain escapes harm from its own weapon by producing an immunity protein that turns off its own colicin's killer mechanism. "If it's not their strain of colicin, then they die," explains Riley. "Normally, the bacteria don't have immunity to anything but their own colicin," she adds. But just as a strain of *E. coli* can develop resistance to antibiotics (see below), it can also evolve resistance to its competitors' colicins.

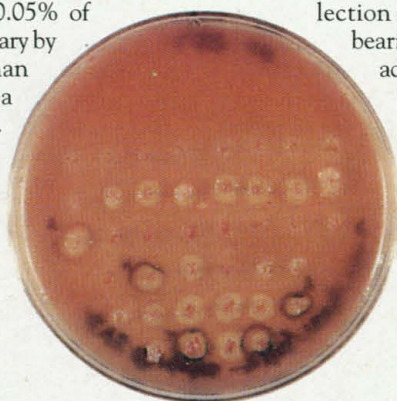
That ability led Riley to suspect that like superpowers in an escalating arms race, the *E. coli* are under constant pressure to develop new defenses and weapons—and that they do so by a seldom-seen form of evolution called positive selection. Most mutations are harmful, and nearly all mutations—whether "good" or "bad"—are simply lost through genetic drift, explains Riley. "We don't have many examples of 'good' mutations that

overcome the power of genetic drift. We suspected that this might be one."

Riley and her colleague Ying Tan tested their idea with several strains of *E. coli* carrying extra immunity genes that give them protection from the colicins of other strains as well as their own. In nature, natural selection should give an individual

bearing the extra gene a huge advantage. "Because it's protected, it won't be swept out of the population by drift. That buys it time" to increase in numbers, says Riley. Indeed, the researchers found that just a single "super-immune" cell put in a flask with 100 million or more ancestral bacteria always ended up invading its competitors.

Riley thinks such a strain's initial advantage might open the way to an additional—if Oedipal—blessing: a second genetic change that alters the *E. coli*'s colicin and turns the strain into a "superkiller" that can eliminate its ancestor as well as other strains. "As the strain increases in frequency [because of the



Mortal combat. Bacterial colonies thrive when exposed to bacteriocins, chemical weapons made by competing strains.

extra immunity gene], the greater the chance that it will evolve this second mutation: a colicin that its ancestor doesn't recognize" and thus can't disarm, explains Riley. Evidence that such genetic changes can happen comes from Japan, where researchers have actually created a superkiller strain via a single point mutation.

Inevitably, says Riley, the advent of a superkiller strain will elicit a response from other *E. coli*, as new strains develop that carry new immunity proteins and new colicins that can overcome the variant strain's defenses. "This kind of experiment shows that such positive selection can act to produce more and more variety," says Riley.

Riley and Tan's findings go far to explain a molecular puzzle that Riley uncovered 5 years ago. In studying the evolutionary history of colicins to determine which were ancestral to which, she found an odd pattern in their DNA sequences: There was always a block, centered on the immunity gene and the end of the colicin gene, with astonishingly high levels of diversity. "I remember thinking, Wow! What could possibly explain that?" she recalls. She now thinks she has the answer: "This is the region that selection is actually acting on" as the *E. coli* evolve.

"It's super work," says Bruce Levin, an evolutionary biologist at Emory University in Atlanta, Georgia, "and goes a long way toward explaining how that enormous variation in *E. coli* arises and is maintained."

—Virginia Morell

EVOLUTION

Antibiotic Resistance: Road of No Return

ARNHEM, THE NETHERLANDS—They are one of medicine's biggest headaches: bacteria that have evolved resistance to those former wonder drugs, antibiotics. Now it appears that—contrary to everyone's hopes and microbiologists' expectations—these troublesome microbes will remain resistant long after doctors stop prescribing the drugs. That was the grim prognosis offered by Bruce Levin, a population geneticist at Emory University in Atlanta, at the recent meeting here of the European Society for Evolutionary Biology. Drawing on studies of bacteria from a day-care center and in the lab, he said, "I'm afraid I can't be optimistic. We can't go back again" to antibiotic-sensitive bacteria. "The best we can do is slow the pace at which resistance

evolves and increases in frequency."

Researchers had hoped that bacteria that have become resistant to overused antibiotics would "evolve backward," losing their resistance, because the resistant strains wouldn't be able to compete with the sensitive ones once the drugs were removed. "Theoretically, the genes responsible for resistance are supposed to adversely affect the bacteria's fitness," Levin explains. "You're altering a gene's normal function and therefore expect it to have a disadvantage."

But a random survey last year of *Escherichia coli* bacteria collected from a day-care center in Atlanta by Levin and an Emory undergraduate, Bassam Tomah, suggested that the theory may not hold up. In a quarter of the bacteria sampled from the dia-



A match for medicine. *E. coli* is unaffected by six of eight antibiotics.

pers of 25 infants, the researchers found strains of *E. coli* still resistant to streptomycin, an antibiotic doctors have rarely used for the last 30 years. Adding to this puzzle are bacteria in Richard Lenski's long-term evolution study at Michigan State University in East Lansing. These *E. coli* originally carried a streptomycin-resistance gene called *rpsL*, which is known to markedly reduce the bacteria's fitness. Yet, after evolving in an antibiotic-free environment for 10 years, or 20,000 generations, Lenski's bacteria are still streptomycin-resistant. "Why didn't that gene revert to its sensitive state, when it only required the change of a single DNA base?" asks Levin.

To find out, Levin's colleagues Stephanie Schrag and Véronique Perrot allowed laboratory cultures of *E. coli* with *rpsL* mutations to evolve in an antibiotic-free medium for 16 days, or 160 generations. They then competed these evolved bacteria against drug-sensitive *E. coli* and found that they are almost as fit. "That suggests that they evolved a compensatory mutation," says Levin—a second genetic mutation that makes up for the loss of fitness from the first.

Schrag and Perrot, with Levin and Nina Walker, confirmed that suspicion by making their evolved *E. coli* strain drug-sensitive again. They replaced the bacteria's streptomycin-resistant *rpsL* gene with a sensitive version of the gene, then set this genetically altered strain and the resistant strain against each other in another fitness-competition bout. The genetically altered *E. coli* failed miserably—implying that the compensatory mutation reduced its fitness when not paired with the resistance gene.

The interaction between the two mutations would act as a kind of ratchet, preventing bacteria from reverting to sensitivity. "The compensatory mutations establish an 'adaptive valley' that virtually precludes that population of resistant bacteria from returning to drug sensitivity," explains Levin. And that explains why the bacteria in Lenski's lab and possibly those in the children's diapers have not lost their resistance. "Those that revert, that make that one change, are at a disadvantage," explains Levin. The team is now trying to identify the gene that carries this compensatory mutation.

Levin suspects that the same kind of compensatory mutations "will almost certainly be found in other resistant bacteria." But already, the findings have "clear, practical—and rather frightening—implications," says Marlene Zuk, an evolutionary biologist at the University of California, Riverside. "It's not enough to stop using antibiotics; the bacteria aren't going to revert to what they were before"—and antibiotics that have lost their effectiveness won't become powerful weapons again.

—Virginia Morell

PALEONTOLOGY

Does Evolutionary History Take Million-Year Breaks?

The history of life is one continuous upheaval, or so strict Darwinists would have it. Species come and go continually, as creatures either adapt to a changing environment and ever-shifting competition and evolve into new species, or become extinct. But lately, a small group of paleontologists has been asserting that evolution sometimes takes a holiday. In the fossil record of hundreds of millions of years ago, they point to examples of entire communities of marine animals that remain snared for millions of years in something close to stasis, then plunge into a brief frenzy of extinction and new species formation.

Claims of such "coordinated stasis" have galvanized the paleobiology community into a frenzy of its own as researchers try to test the idea by studying how other animal communities fared over tens of millions of years. The first results to come in are "a mixed bag," concedes paleontologist Carlton Brett of the University of Rochester in upstate New York, who, with Gordon Baird of the State University of New York, Fredonia, first proposed the concept of coordinated stasis in 1992, based on 400-million-year-old marine fossils from New York. "The pattern we have seen is holding up well in our rocks," he says, but "that pattern is perhaps toward the extreme end of a range." Indeed, most studies of similar fossil records have found little evidence for prolonged periods of evolutionary stasis.

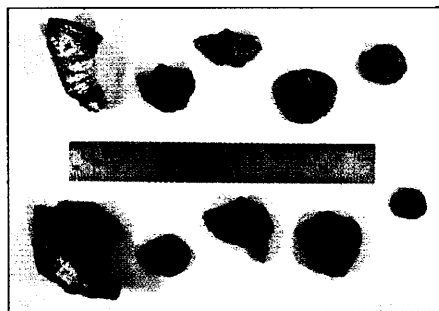
Yet confirmation of even occasional episodes of coordinated stasis in the fossil record could have major ramifications for understanding evolution. One proposed explanation for the stasis is that the species in the static ecosystems interacted so tightly that there was no room for change. If so, the more fluid ecosystems of recent times, in which individual species react independently to evolutionary pressures, may not be the evolutionary norm. The brief upheavals of accelerated evolution said to begin and end the periods of stasis are more widely accepted, but just as intriguing, hinting at little-understood evolutionary dynamics (see sidebar).

The classic case of coordinated stasis comes from the animals that lived in ocean-bottom

muds during the early Silurian to middle Devonian periods, about 440 million to 380 million years ago. Those muds hardened into fossil-bearing shales that are now found in Ontario, New York state, and Pennsylvania. Studying the rocks nearly a century ago, paleontologist Herdman Cleland noted that the array of fossil species, including the mollusklike stalked brachiopods, corals, mollusks, echinoderms such as starfish, and trilobites, seemed to change very little over many millions of years.

It was not until the early 1990s that Brett and Baird, drawing on fossil specimens collected over 20 years, quantified the stability that Cleland had reported. They identified 14 intervals, generally running 3 million to 7 million years each, during which 60% or more of species persisted with little change. Within each interval, extinction, speciation—the formation of new species—and immigration of species from outside the now-vanished ocean basin were all more or less on hold, until the interval ended in a period of drastic turnover lasting just a few hundred thousand years.

The herky-jerky pattern hearkens back



Where's the evolution? Not much happened to these common marine species over a nearly 10-million-year interval (bottom to top) roughly 400 million years ago.

to the revolutionary concept of punctuated equilibrium proposed by Niles Eldredge of the American Museum of Natural History in New York City and Stephen Gould of Harvard University in 1972. They argued that species tend to persist unchanged for millions of years before abruptly giving rise to a new species,

instead of evolving gradually. Coordinated stasis "is punctuated equilibrium at a higher level, the ecological level of the community," says Douglas Erwin of the National Museum of Natural History in Washington, D.C. But while there is finally some strong evidence for the reality of punctuated equilibrium (*Science*, 10 March 1995, p. 1421), many paleontologists have had a hard time swallowing the idea that all the species in a community could be held in check at once.

Even more startling was the explanation for coordinated stasis advanced by Paul Morris of the Paleontological Research Institution in Ithaca, New York, Linda Ivany of the University of Michigan, Ann Arbor, and Kenneth