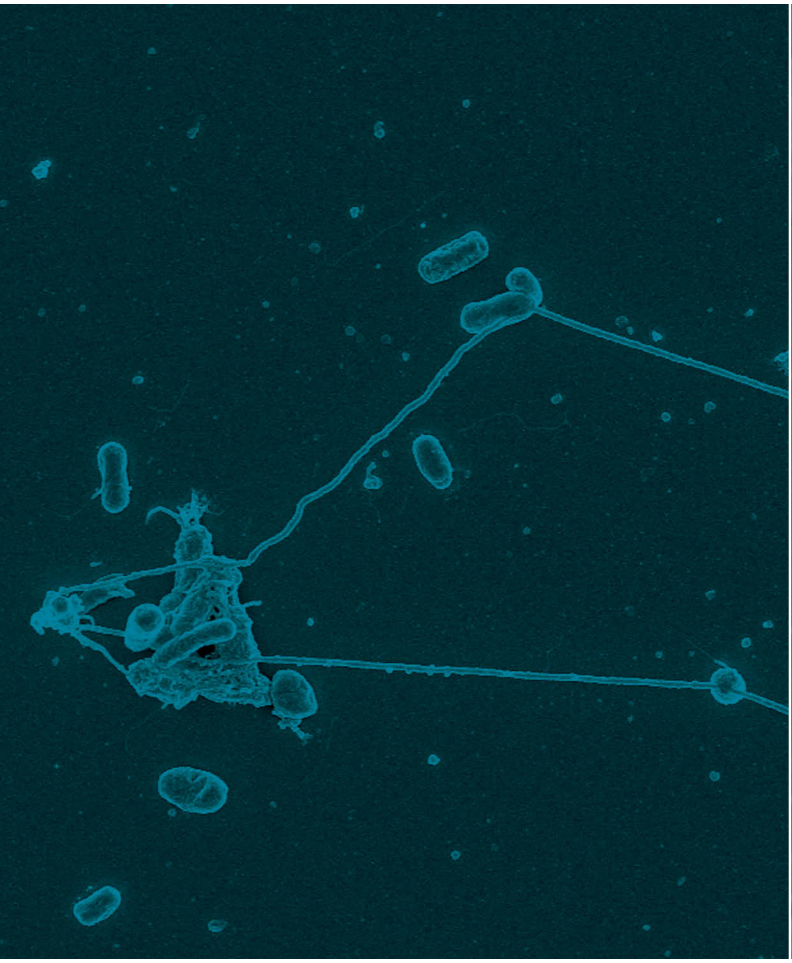
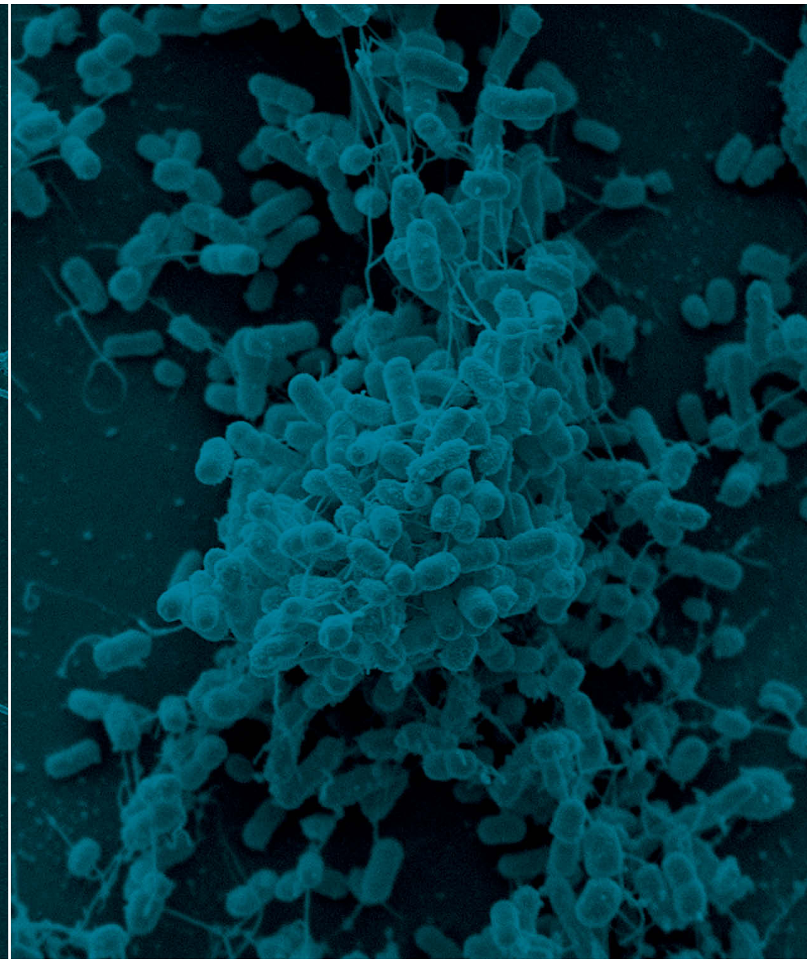




Bacteria Need Partners

Bacteria are individuals that always operate in isolation? Not at all, says **Christian Kost** of the **Max Planck Institute for Chemical Ecology** in Jena. In fact, he thinks bacteria frequently can't help but cooperate. His team is using cleverly devised experiments to test this hypothesis.

TEXT **KLAUS WILHELM**

Photo: MPI for Chemical Ecology (2)

Networking bacteria: The electron microscope reveals the tubular connections between genetically modified *Escherichia coli* and *Acinetobacter baylyi* bacteria. The microbes use these tubes to exchange amino acids.

"When I saw it, my first thought was that we must have made a mistake." Shraddha Shitut, a doctoral student at the Max Planck Institute for Chemical Ecology in Jena, looks at her boss Christian Kost and laughs. Kost, in contrast, a biologist and Leader of the Experimental Ecology and Evolution Research Group, was "immediately sure that everything had worked out just fine and we had discovered something fundamentally new."

Still, Shraddha Shitut repeated the experiment over the following days with her colleagues Lisa Freund and Samay Pande. And then once again, just to be sure. "But we always saw the same thing under the electron microscope," she says. The instrument, which can reveal even the tiniest structures measuring a millionth of a millimeter, showed that small channels called nanotubes linked individual bacteria together.

NANOTUBES TRANSPORT NUTRIENTS

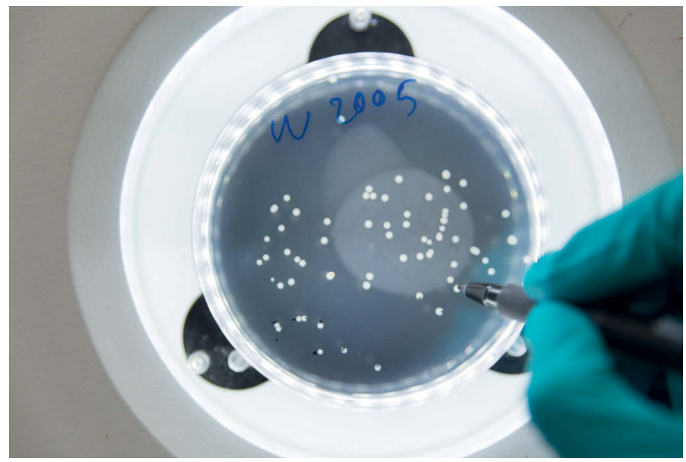
Bacteria use these tubes to reciprocally exchange certain nutrients that are vital for their growth, but that they can no longer produce on their own. Without the missing amino acids, both partners would die. "They depend on each other," says Kost. "In fact, you could say they literally cling to one another for survival."

"I believe in my staff, otherwise I'd be lost," says Christian Kost. He is a team player – and team play is the focus of his professional career, too. After all, the researcher is studying the social life of bacteria and how they cooperate. The social life of bacteria? Although it seems bizarre, Kost believes this concept holds the key to life in general.

When he talks about his hypothesis, he does so with passion and conviction, backed up by the findings of his cleverly devised and carefully controlled experiments. "It's always better to cooperate than to live on your own," he says. Always? A brief

Photo: MPI for Chemical Ecology (2)

Photo: Anna Schroll



Left-hand page To increase confidence in their data, the researchers must repeat their experiments several times and grow different cell types in separate culture vessels. This results in stacks of petri dishes.

Above Shraddha Shitut (left) counts the bacterial colonies (right) that have grown in the petri dishes to determine how successfully they have reproduced.

pause. "Nearly always! And that's true for all organisms, from bacteria on up to humans."

The 40-year-old scientist is challenging cherished principles. In contrast to common beliefs, he thinks that "bacteria can't help but work together." He believes that the assumption that bacteria always function as independent units is incorrect.

REPRODUCING ON THE DOUBLE

Kost is an evolutionary biologist, so he thinks in the patterns and principles that have shaped the science of life since the times of Charles Darwin. It follows that his hypotheses must stand up to the most fundamental assumption of the theory of evolution: that cooperating organisms reproduce in greater numbers than selfish ones. However, it is virtually impossible to demonstrate this unequivocally under natural conditions.

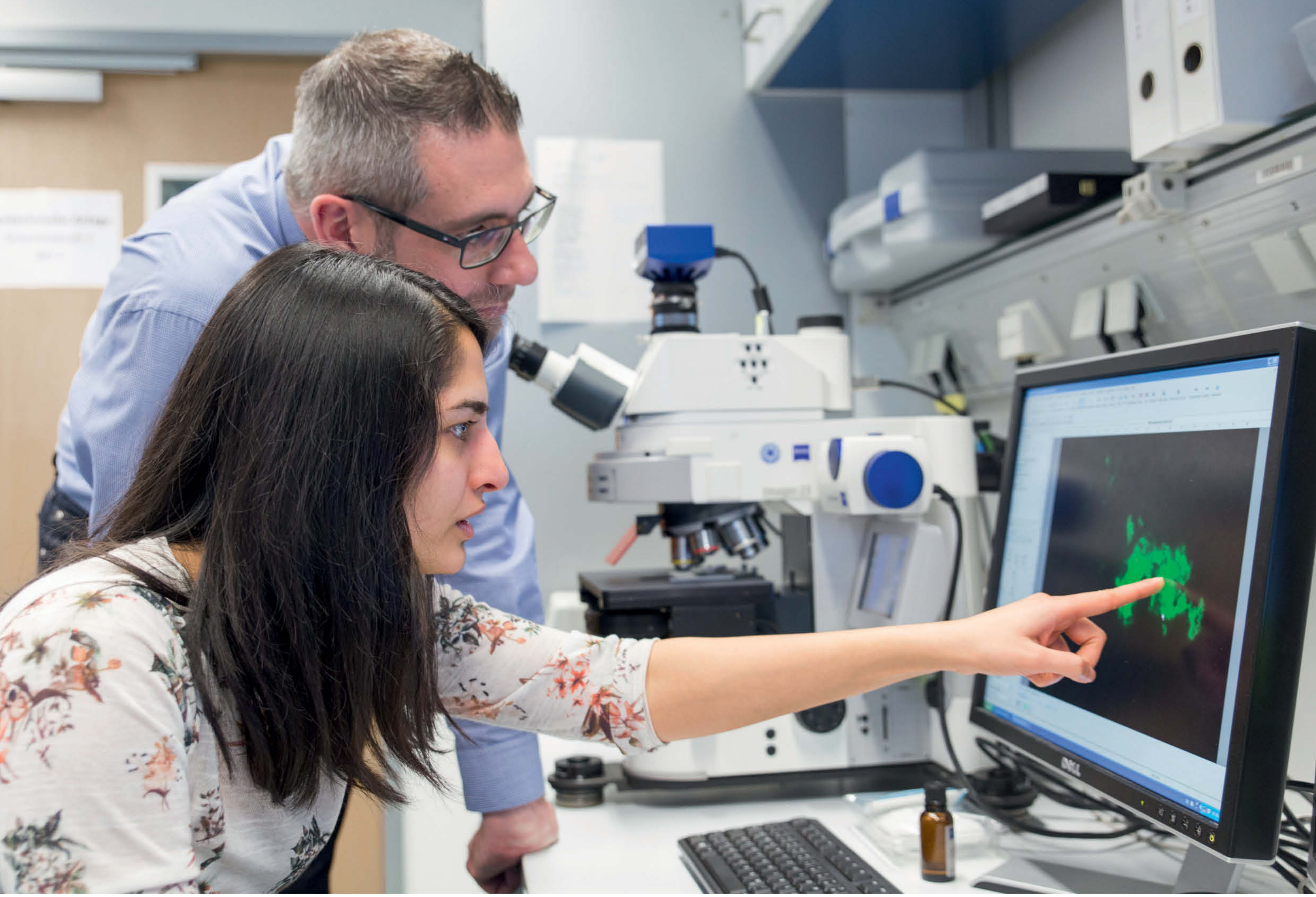
Still, it should be possible to mimic evolutionary processes under laboratory conditions, including the evolution of cooperation, reasons Kost. "Observing the process of evolution in real time and monitoring it scientifically over the course of mere days or weeks – that fascinates me," he says. This type of experiment is possible because some species of bacteria divide every 20 minutes, so they produce offspring very quickly. Moreover, it is also possible to turn off specific genes in a single day, and to control environmental conditions at will.

Based on those premises, the Jena-based scientists came up with a laboratory experiment. They took the gut microbe *Escherichia coli* that is very popular among scientists and switched off a gene that is responsible for producing an essential amino acid A. A second mutation caused the bacterium to produce an increased amount of amino acid B. The researchers also modified another bacterial strain with a converse set of mutations, such that it produced excess amounts of A, but not B. Both populations were then put together into a culture medium to see how they would develop.

IT PAYS TO WORK TOGETHER

Rather than dying, the bacteria reproduced 20 percent faster than *Coli* strains that could autonomously produce all amino acids. The two mutants that lacked two genes each must therefore have supplied each other with the missing amino acids. This demonstrates that cooperation is advantageous, also in terms of Darwinian evolution. >

Photo: Anna Schroll (2)



As a consequence, those bacterial strains that can produce all metabolites autonomously should be at a disadvantage within bacterial communities. Why? Because producing the full range of amino acids consumes more energy than sharing the workload with other individuals. Ultimately, cells are thrifty by nature – even if it costs them the freedom of living independently.

But how do cooperating microbes exchange nutrients? This is a serious issue, because if they simply release the amino acids into the environment, other microbes that are not investing in the partnership could benefit as freeloaders, putting the whole venture at risk. A direct connection, on the other hand, would be ideal – a closed pipeline for passing nutrients between cooperators.

Again, the researchers switched off genes for the production of certain amino acids, this time using the soil microbe *Acinetobacter baylyi* as well as *Escherichia coli*, because even bacteria of different species can get along. “It’s most probably very widespread in nature,” says Kost. The results of this experiment showed that, as expected, the strains that were cultivated together in the medium grew best.

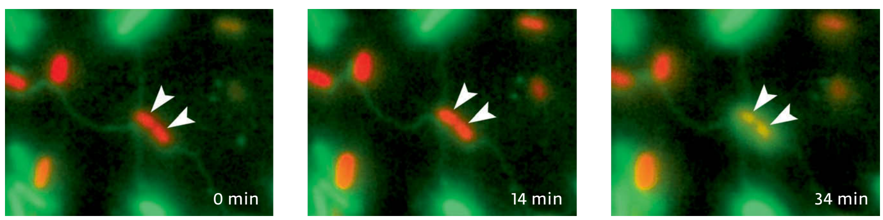
PHYSICAL CONTACT IS NECESSARY

In another test, the cooperating microbes were separated using a filter that allowed amino acids in the medium to pass, but prevented direct contact between the two genetically modified strains. Without contact, none of the microbes were able to grow. “This means the microbes in the partnership need physical contact in order to exchange nutrients,” explains Shraddha Shitut.

Examining images under the electron microscope, Shitut then noticed that, on their outer cell envelope, the *E. coli* bacteria had formed small tubes that were stretching over to the *Acinetobacter* cells: the nanotubes. Presumably one cell taps into the other to access a nutrient, but the partner can also use the channel itself. For motile bacteria such as *Escherichia coli*, producing nanotubes is worth the effort. As soon as they “smell” a potential food source, they presumably move toward it and establish the tube. However, for a microbe such as *Acinetobacter* that can only move passively – for example in flowing water – producing nanotubes is probably not worthwhile, unless a cell that can fill its nutrient gap just happens to be right next to it.

In order to verify their results, the scientists performed another experiment. This time, they supplemented the culture medium with all the required substances, including the amino acids that the genetically manipulated microorganisms could not produce on their own. In this case, the production of nanotubes stopped. “So the formation of these structures obviously depends on how hungry a

Photo: Anna Schroll



Left-hand page A fluorescence microscope helps Shraddha Shitut and Christian Kost study the nanotubes. A green fluorescent dye indicates that the tubes are made from the same material as the bacterial cell membrane.

Above Dyes unveil the exchange between genetically modified *Acinetobacter baylyi* (red) and *Escherichia coli* (green). At the start of the experiment, both species contain the dyes originally administered (left). Gradually, the green pigment moves through a tube from an *Escherichia coli* cell to an *Acinetobacter* cell (middle). After about 30 minutes, the two pigments have become so mixed that the cell appears yellow (right).

cell is," says Christian Kost. And on how many nutrients they release into their environment.

ONLY HUNGRY CELLS FORM NANOTUBES

If enough amino acids are released, there is no need for the tiny tubes, as the cooperators can simply absorb the nutrients they need from their environment. "Because," explains Kost, "forming these tubes likely consumes energy."

Further investigation will be required to determine whether these nanotubular structures are formed solely to enhance efficiency or whether there are other reasons, for example if some types of bacteria use them to parasitize others. Another question that remains unclear is whether the bacteria can actively choose the cells to which they attach, since the microbe at the other end of the tube could potentially contain harmful substances.

Christian Kost interprets the formation of these cooperative communities as evidence of "a principle of self-organization." This can be observed when bacteria are inoculated onto an agar plate. Obtained from algae, agar combined with sugars provides a culture medium for microorganisms.

Once again, the Max Planck scientists in Jena generated double mutants of *Acinetobacter baylyi* and *Escherichia coli*, each strain lacking the ability to produce one particular amino acid, yet producing excessive amounts of another one. This time, however, the bacteria released the amino acids into their environment. The two groups were then pipetted onto an agar plate along with auxotrophic microbes that also required amino acids to grow, but that did not contribute to their production.

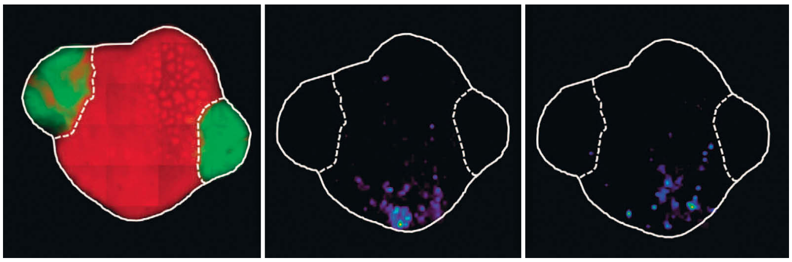
At the onset of the experiment, all cells were perfectly mixed. However, after just 24 hours, says Kost, "we could see a clear spatial distribution." Initially, a small number of double mutants happened to be side by side. Since both types released their surplus amino acids into the environment, double mutants in the immediate vicinity benefited most.

Where non-cooperating bacteria were nearby, the growth rate of the cooperative double mutants was reduced. Over time, auxotrophic cheaters were pushed farther out toward the edge of the colony – like outsiders in human societies. This spatial separation seems to stabilize the system evolutionarily.

Intuitively, one might assume that the system should collapse, because non-cooperating bacteria derive benefits without contributing, thus sucking the cooperators dry. "Not so." says Kost. "Our results show that the colony settles and stabilizes at an equilibrium such that all participants can coexist."

Still, the cooperating partners fare better than the parasitic loners. In reality, they are all dependent on each oth-

Photo: Nature Communications



As shown on the left, selfish bacteria (green) can only exist on the edge of cooperating bacterial colonies (red). High concentrations of the amino acids histidine (middle) and tryptophan (right) occur only where cooperating microbes are located, and not around the selfish cells.

er; freedom in the sense of independence seems to be a truly rare condition. "It doesn't make sense to do everything for oneself. It's always better to divide the labor," says Kost.

EVOLUTION FAVORS COOPERATION

He sees this as only logical in light of the latest research results. The driving force is the loss of functional genes, and gene loss is inevitable because genes face constant mutation pressure. In this process, the probability that a gene will be destroyed is significantly higher than the probability that a new, beneficial gene will emerge. Given this context, bacteria can't get around entering into obligate interactions with each other. They are thus compelled to cooperate.

"Cooperation and the division of labor are powerful principles in evolution," says Kost, and explains that the genomes of more than a thousand species of bacteria have now been decoded. Remarkably, only about 35 percent of those possess *all* genes they need for survival.

"That's only the tip of the iceberg," suspects the biologist, "because to date we have mostly sequenced the genomes of bacteria that can be cultivated in the laboratory, and those represent less than one per mill." It seems that losing genes is no problem for bacteria, because the resulting cooperation requires less energy.

In natural ecosystems, such cooperation may give rise to multicellular units comprising representatives of different species – colorful networks that are more than the sum of their parts, along the lines of "my neighbor, my savior!" The group gains new benefits from the interactions between different microbes. "And the more we study it, the more we realize that this occurs in natural conditions, too," says Christian Kost. ◂

TO THE POINT

- **For many years, bacteria were thought of as purely individualistic organisms, but they are well able to cooperate, thus compensating for gene loss.**
- **Many bacteria are part of a network that even connects them to bacteria of other species.**
- **Cooperation is a basic principle of life and a driving force for the development of biological complexity. Individuals combine to form superorganisms ("holobionts") in which the dividing lines between individuals are blurred.**

GLOSSARY

Auxotrophy: Auxotrophic organisms are unable to produce one or more vital substances for themselves, and must obtain them from their environment instead. Auxotrophy arises when a mutation deactivates a gene required for the production of an essential nutrient.

Evolution: According to the neo-Darwinist theory of evolution, natural selection can only work at the level of the individual. British biologist Richard Dawkins believes selection starts with even smaller units, namely genes. This means that a gene is prioritized for passing on to the next generation if it is of benefit to the individual. It provides its carrier a selective advantage, increasing its evolutionary fitness. However, this does not rule out the possibility of group selection. Under certain conditions, evolution favors whole groups; cooperation between different species of bacteria is an example of this.

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