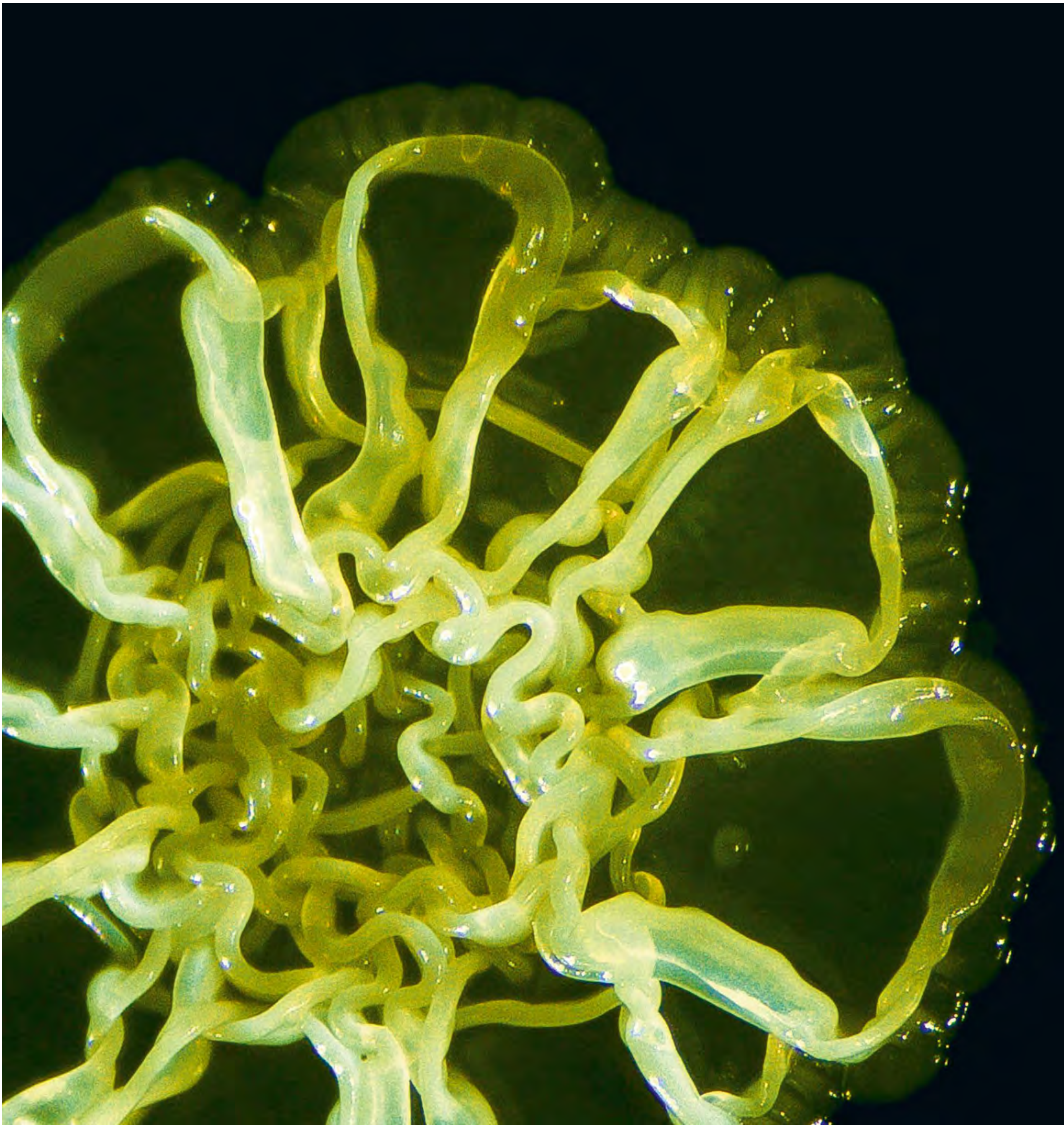

BIRTH OF COLLECTIVES

TEXT: MAGDALENA NAUERTH

Colony of the mat-forming “wrinkly spreader” *Pseudomonas* bacteria. The wrinkled appearance is a consequence of mutations in the genome that lead to overproduction of a cellulose polymer.

PHOTO: MICHAEL SCHWARZ / MPI FOR EVOLUTIONARY BIOLOGY

Cheaters can leave. In the case of the bacteria in Paul Rainey’s lab, that’s exactly what is wanted. In his laboratory at the Max Planck Institute for Evolutionary Biology in Ploen, the evolutionary biologist studies how multicellular life emerges from individual cells. Their findings show that too much cohesion can be counterproductive.

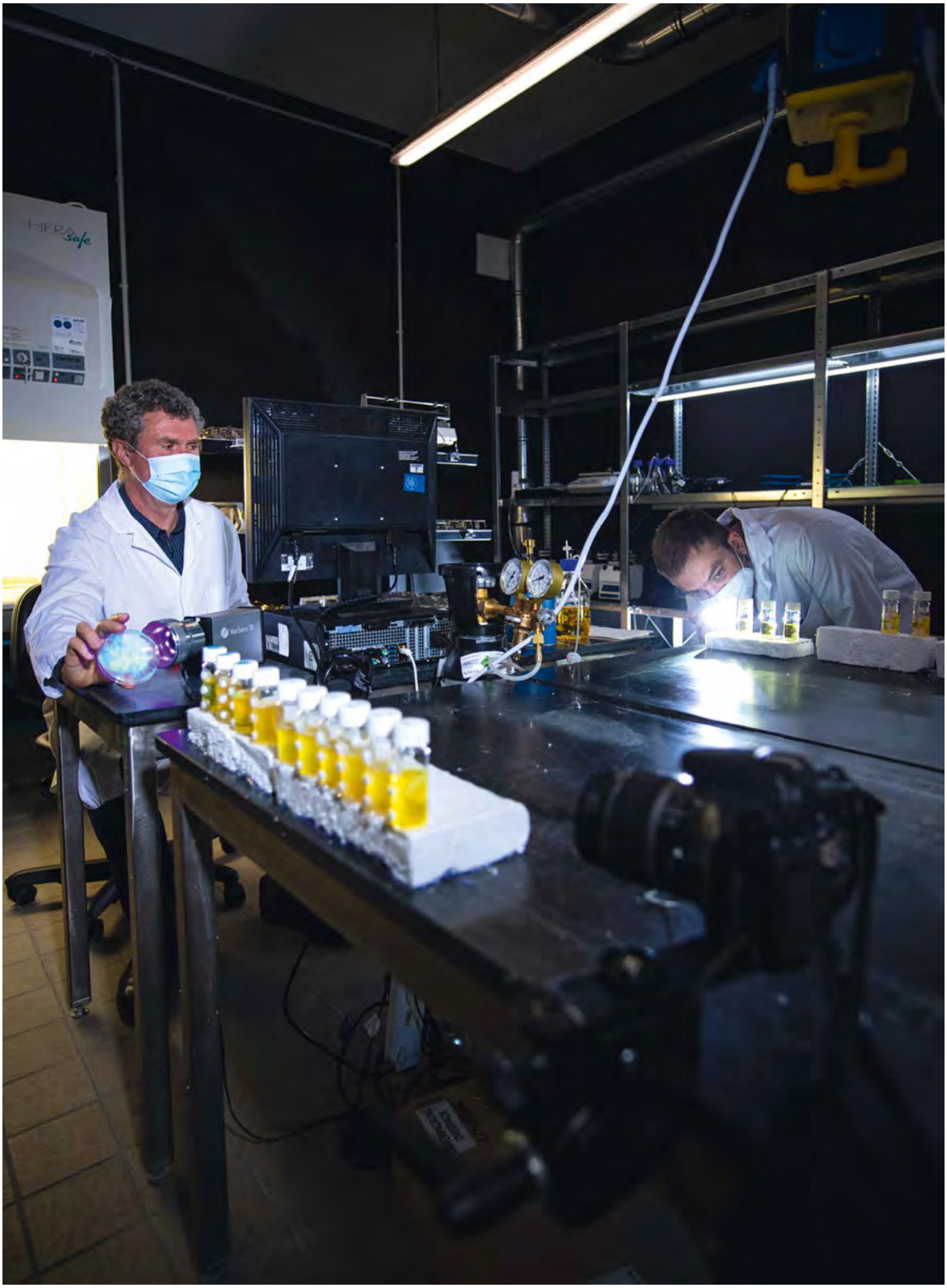

PHOTO: TOM PINGEL FOR MPG

Paul Rainey (left) in the laboratory with Loukas Theodosiou. Rainey is holding a Petri dish containing bacteria that produce a fluorescent pigment that gives its name to the species *Pseudomonas fluorescens*. Microbes floating around in test tubes at different stages of their life cycles.

When life emerged, there was not yet much in the way of cohesion. The first cells were "lone warriors" that had to adapt to the harsh living conditions on Earth 3.8 billion years ago. Just 300 million years later evidence of the first multicellular life emerged in bacteria, famously preserved as stromatolites in Western Australia. The first cells with a nucleus appeared 2.7 billion years ago and multicellular eukaryotic life 1.7 billion years ago. Paul Rainey and his team aim to understand how single cells might have made the transition to multicellularity.

Rainey majored in biology. After completing his studies, the New Zealander decided that he needed a break. For several years, he toured the world with his saxophone as a jazz musician. After returning to New Zealand, he worked as a sales manager for a dairy company. But after a while, he decided to return to his passion of biology and enrolled in university. For his master's thesis, he was supposed to study fungi.

But he soon became aware of a species of the mushroom pathogen, a bacterium called *Pseudomonas tolaasii*. It is able to propel itself forward with the help of its thread-like flagella. Rainey noted that the bacterium was able to adapt to changing conditions in his culture vessels. Some cells lost their capacity to produce the toxin responsible for brown-blotch disease.

His interest in *Pseudomonas* continued to grow. In addition to his actual research projects, he was always conducting side experiments with *Pseudomonas*. This enormous scientific curiosity coupled with perseverance would pay off in the end. But it also sometimes got him into trouble.

"We have observed for the first time how cooperative behavior emerged from scratch."

PAUL RAINEY

When Rainey moved to the UK for a post-doctoral fellowship, he observed that in addition to the typical round, smooth colonies, the bacteria in his cultures produced colonies of various other types.

One type was characterized by deep furrows on the cell surface. Rainey therefore called them "wrinkly spreaders". These cells formed a dense mat on the surface of unshaken liquid media. The other type were marked by a fuzzy appearance. He called these "fuzzy spreaders". Like the "wrinkly spreaders", they also formed thin mats – albeit short-lived ones – due to electrostatic interactions among cells.

Environmental complexity promotes diversification

Rainey's tests revealed that the colonies not only looked different but were formed by cells that had different properties. Was that evolution? "At that point, I didn't fully understand the significance of what I was looking at," says Rainey. As his research progressed, it became apparent that the variants appeared only when the culture vessels were not shaken and thus were ecologically complex. Shaking ensures the supply of oxygen in the nutrient solution. In unshaken vessels, the microbes quickly consume the vital gas, creating gradients from high (at the surface) to low (beneath). This is when the wrinkled and fuzzy spreaders came into play: the ability of cells of the different colony types to adhere to one another allows them to form mats at the meniscus and take advantage of the high oxygen content at the surface.

Rainey repeated the experiment many times but always observed the same result. After a few days, a mixture of smooth, wrinkled, and fuzzy cells emerged. In fact, they always appeared in the same order: first the wrinkled ones prevailed; only later did the fuzzy ones appear. "That was the breakthrough: *Pseudomonas* had thus adapted to oxygen deprivation," says Rainey looking back. As exciting as these results were, they did not go down well with his supervisor. After all, his research was supposed to be directed elsewhere. He was therefore forbidden from continuing his experiments. But Rainey continued anyway – albeit in a more discrete manner.

Pseudomonas bacteria are an ideal model for studying evolution. In the laboratory, unlike in nature, researchers

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can precisely control the living conditions of their experimental organisms. There is also another advantage to working with bacteria. Because evolution proceeds rather slowly, in most populations, the changes in genetic material that influence survival can be observed only over many years. This is not the case with *Pseudomonas*: there is less than one hour between generations. Evolutionary adaptations can be studied as if they were occurring in fast forward. "We were thus able to study evolution in a test tube."

Rainey's experiments made him one of the co-founders of a new sub-discipline of evolutionary biology: experimental evolution. Scientists around the world are now conducting experiments to investigate how organisms adapt to changes in living conditions. This works particularly well in artificial laboratory environments in which researchers are able to precisely control every parameter. Darwin's original theory of evolution by natural selection has thus been scientifically proven many times.

Glue holds cells together

But how do solitary bacteria become team players? First, they must stay together. The genetic analyses of Rainey and his colleagues revealed that as the result of mutation, mat-forming cells produce excess amounts of an adhesive cellulosic polymer. "This polymer acts like a glue that allows the bacteria to adhere to the vessel wall – and to each other," explains Rainey. This allows them to form a mat on the surface of a liquid, where they can take advantage of the higher oxygen content. For the group, this is clearly a great advantage. But for the individual cells? After all, they have to expend energy to produce the adhesive polymer and this comes at a measurable cost to individual cells.

Pseudomonas thus fulfilled the classic definition of cooperation: a behavior by which an individual contributes something to the benefit of others – at cost to self. "This was the first time that the evolution of this behavior had been observed de novo," says Rainey. Cooperation is found in many social colonies in the animal kingdom. For example, worker bees take care of raising their sisters without reproducing themselves. However, because they are genetically similar, they still contribute to the transmission of their genes. This allows the colony to produce new bees. However, for the behavior to take hold, the members of the colony themselves must also reproduce. In spring, a young queen leaves the old hive with part of the colony and starts a new colony. Can the bacterial mats produce offspring? "At first glance, they didn't seem to be reproducing," says Rainey. Without reproduction, no selection is possible: mats of cells are not units of selection. In other words, while cells that make mats replicate, mats themselves are evolutionary dead-ends.

But Rainey and his team did not give up and continued to monitor the system. Over time, further classes of mutant cells evolved within the mats. These no longer produced the adhesive polymer and were able to move freely. They thus benefited from the cohesion of the colony – and a plentiful supply of oxygen – without contributing anything themselves. "In some ways, they are cheating their colleagues. Because they do not expend energy to produce the adhesive polymer, they can multiply more quickly. At the same time, they weaken the cohesion of the mat and gradually cause it to disintegrate," says Rainey.

The prevailing view is that such free-riders are a problem that must be eliminated, or controlled, because otherwise cooperative actions cannot be maintained. However, Rainey and colleagues found that "cheaters" can have an important evolutionary function: under certain ecological conditions, they can help the collective of cells to replicate. "In some ways, the cheater cells play the role of reproductive propagules that multicellular organisms use to reproduce themselves. The cooperating cells of the mat are analogous to soma, or body cells. With this separation between cells that remain in the colony and the cells that disperse, the bacterial mats begin to resemble a multicellular organism," explains Rainey.

Of course, the bacterial mats have a hard time spreading in test-tubes. In nature, the mats might attach to the reeds of a pond. When reeds become free of mats, because mats detach and sink to the ground, the free-moving dispersing cells of other mats can colonize the new niche. It is now no longer individual cells that compete for space and resources but rather mats – they become units of selection. The best-adapted mats are able to displace inferior competitors. "This means that selection no longer works solely at the level of individual cells but also at the level of mats," says Rainey. "Such group-level selection underpins the emergence of complex organisms," explains Rainey.

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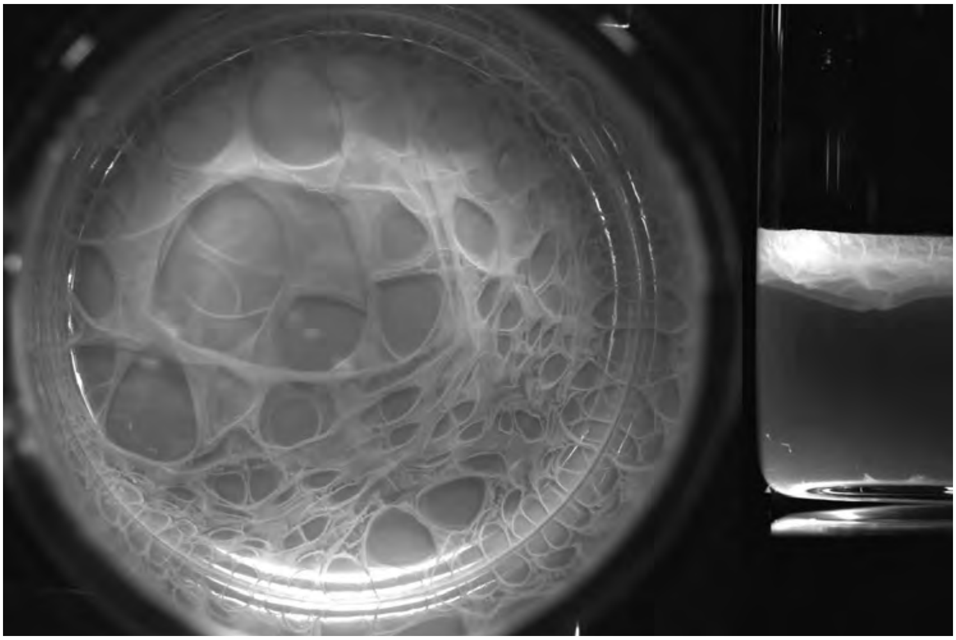

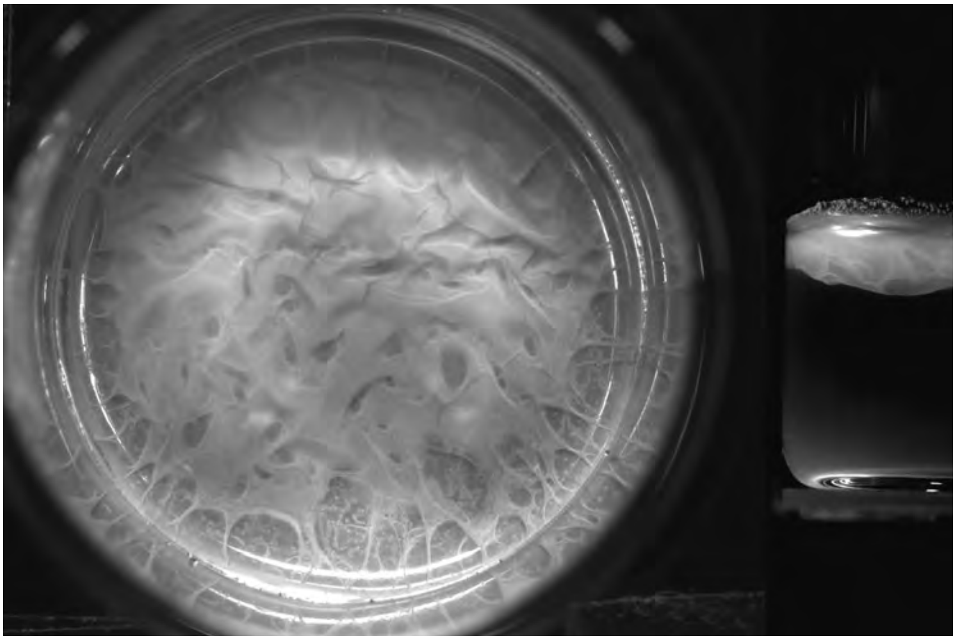

Mats of *wrinkly spreader* mutants developing at the meniscus of liquid medium. Over-production of cellulosic polymer allows cells to adhere to each other, and the edge of the culture vessel, and remain at the air-liquid interface where they are rewarded with a plentiful supply of oxygen. Using a 45° angle mirror, it is possible to record growth of the mat from both the top-down and lateral perspectives. Images were taken at 12 (top) and 24 hours (bottom).

PHOTOS: MICHAEL SCHWARZ/MPI FOR EVOLUTIONARY BIOLOGY

> **“Specific ecological conditions are required for the evolution of collectives.”**
>
> *PAUL RAINEY*

SUMMARY

Because of their short generation time, bacteria can quickly adapt to new living conditions. Bacterial cultures can therefore be used to observe evolution in the laboratory as if in fast forward.

When oxygen becomes scarce in nutrient solutions, individual bacterial cells produce an adhesive polymer that allows them to form cellular mats. In this way, they can take advantage of the higher oxygen content at the surface.

Some cells save on the costs of collective living. While harmful to the short-term persistence of mats, they can serve as propagules for mat reproduction.

Ecological factors as simple as patchily-distributed resources and a means of dispersal allow the process of Darwinian evolution at the level of collectives to become established.

The results put Rainey on the trail of a new idea. They recognized the central role that ecology can play in effecting major evolutionary transitions, including the transition from cells to multicellular life, the transition of matter to the first self-replicating chemistries, or the evolution of chromosomes from genes. The idea is referred to as “ecological scaffolding” and it solves a long-standing chicken and egg problem.

Evolutionary milestones

Evolutionary transitions begin when lower-level particles join forces. They complete when collectives of particles participate in the process of evolution by natural selection. For this to happen three properties must exist: collectives must be individually distinct and vary, they must reproduce, and they must be able to pass on their traits to offspring. However, early manifestations of collectives invariable lack these properties. As a consequence, arguments that posit their evolution by natural selection are not tenable. So, how do we explain the origin of these most fundamental Darwinian properties (variability, replication, heredity)?

Together with his colleagues Andrew Black and Pierrick Bourrat, Rainey has developed a model that simplifies and generalizes his team’s experimental findings and shows how Darwinian properties can be exogenously imposed (scaffolded) by particular ecological conditions. In terms of the bacterial mats in the pond, this means that. Returning to the pond-reed-mat analogy, the spatially separated reeds on which the mats settle allow for discrete variation between mats. When mats fail, newly available reeds can be colonized by dispersing cells. Dispersal and re-establishment of a new mat is akin to mat-level reproduction. And if the new mat is formed from a single propagule from a parental mat, then the newly formed mat inherits properties from the parent.

“The mats themselves have no ‘intention’ of participating as evolutionary units in the process of natural selection,” explains Rainey, “but ecological conditions cause a Darwinian process to unfold at the level of mats.” Of course, these externally imposed properties must, if evolution is to proceed in an open-ended way, become endogenous features of the new life form. But, as shown in experiments, and more recently via simple and general models, this is well within the scope of possibility. Rainey and his colleagues at the Max Planck Institute observed for example that the bacterial cells evolve a simple genetic switch that enables the transition between adhesive (mat) and non-adhesive (propagule) cells. They thus no longer rely on random mutation but rather have separate life cycles for growth and reproduction. In nature, the mats could eventually acquire the ability to float on the surface of the water. This would free them from the need for restrictive ecological conditions. The bacterial mat would thus resemble multicellular life forms that release their propagules into the water.

With this new way of thinking – one that recognizes the continuity between organism and environment – the researchers have helped shift attention not only to the importance of ecology, but to environments in which Darwinian properties might be exogenously imposed on otherwise unwitting particles. This opens the door for new opportunities to experiment and solve each of the major evolutionary transitions, including the most challenging: the origins of life.

Whether the first cells actually came together like they did in Rainey’s test tubes is an open question. Life has had to adapt to many changes since then. There have also been many different pathways to multicellularity – depending on the environment in which an organism has evolved. Thus, in the course of evolution, multicellularity has evolved independently on numerous occasions. “It is thus plausible that one route resembles that observed in our experiments,” says Rainey.

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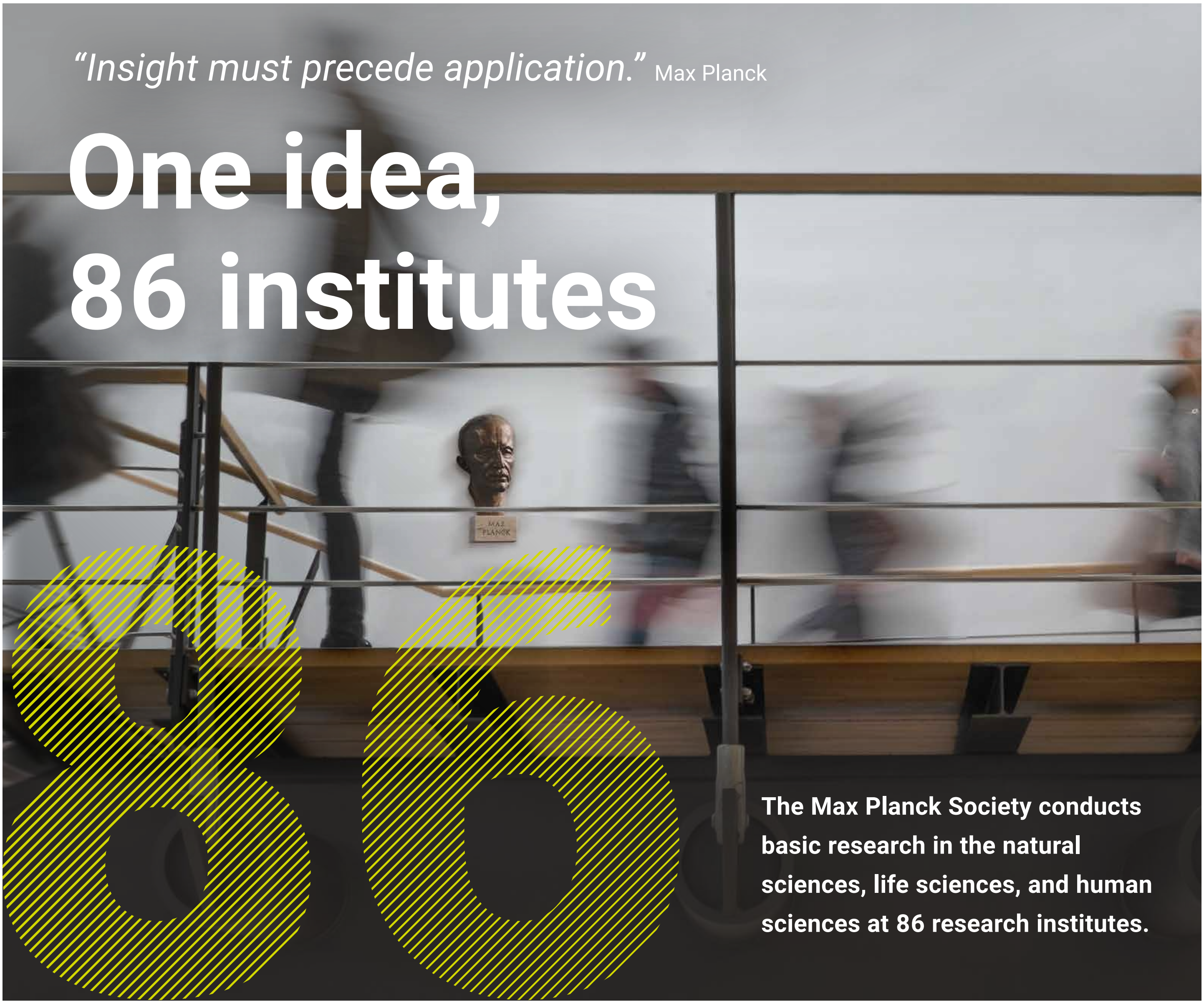
"Insight must precede application." Max Planck

One idea, 86 institutes



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