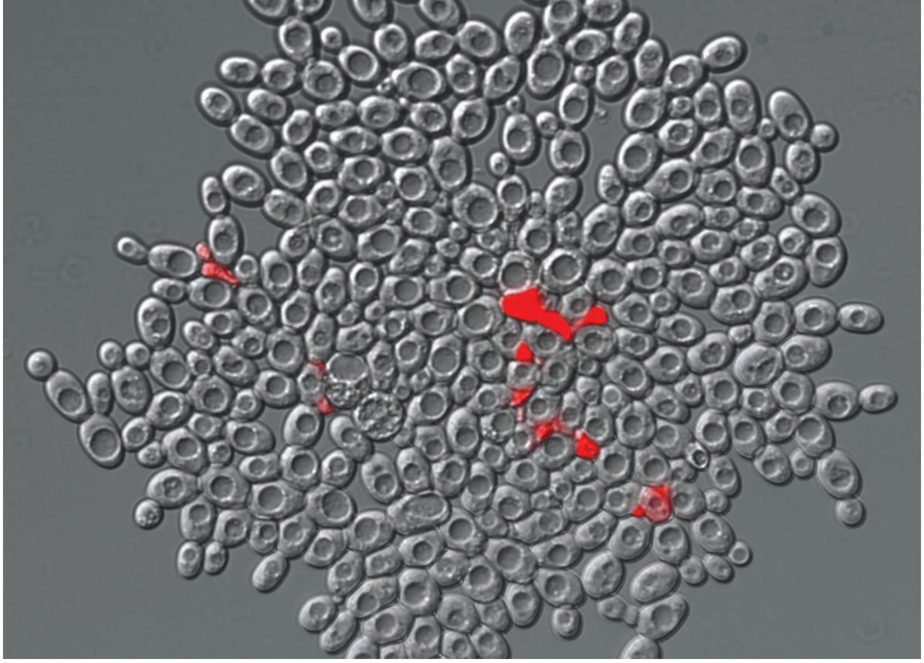
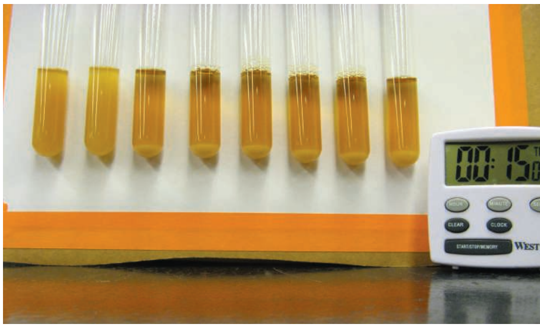




**Bigger, better.** An experiment evolving bigger yeast yielded tubes with more settled yeast through time (*above, left to right*) and resulted in multicellular organisms with dead cells (red) helping reproducing yeast fragment.

EVOLUTIONARY BIOLOGY

# Evolutionary Time Travel

**With clever and challenging lab experiments, researchers are forcing species to become multicellular, develop new energy sources, and start having sex**

In December 2009, evolutionary biologist Michael Travisano was debating with his future postdoc William Ratcliff what to do next in their lab at the University of Minnesota (UMN), Twin Cities. They had just seen a talk on slime molds that delved into what it meant to be a multicellular organism. Under certain conditions, some of these single-cell amoebas can coalesce into masses of millions of cells that act in a coordinated fashion, as if a whole organism.

Inspired by this, Travisano and Ratcliff began musing about how one of evolution's apparently major leaps up the ladder, the jump to multicellularity, takes place. A typical evolutionary biologist might tackle this challenge by comparing fossils or genomes of related unicellular and multicellular species, but the duo had a more daring idea. They decided to try to force the evolution of yeast, normally a single-celled creature, into a multicellular one. "I wouldn't have wagered a large sum of money that this would have worked," Ratcliff recalls. "But if it [did] work, it would be the coolest thing we could think of."

Researchers have long deliberately bred animals, plants, and microbial species for specific purposes—leaner meat, drought-resistant plants, chemical-producing bacteria, and so forth—but what Ratcliff and Travisano wanted to do was probe how evolution itself happens, by forcing it to occur, under controlled conditions, as scientists watch. Despite Ratcliff's reservations, they succeeded in producing multicellularity, at least in a limited form, in just 60 days.

This type of research, known as experimental evolution, has existed almost since Darwin put forth his theories. The approach has risen in popularity over the past few decades, in large part thanks to the pioneering work of Richard Lenski. An evolutionary biologist at Michigan State University in East Lansing, Lenski has for several decades now conducted an ongoing study in which 12 populations of the bacterium *Escherichia coli* live, and evolve, in flasks with limited supplies of glucose for energy. In an extraordinarily long-term effort, Lenski and his lab members have followed more than 50,000 generations of *E. coli* and in so doing gleaned insights into the pace and reproducibility of microbial evolution (*Science*, 25 June 1999, p. 2108). Lenski's work "really highlighted the power" of experimental evolution to other biologists, says Nick Colegrave, an evolutionary biologist at the University of Edinburgh in the United Kingdom.

At this summer's 13th Congress of the European Society for Evolutionary Biology in Tübingen, Germany, it was clear that the field of experimental evolution has itself evolved. Biologists today conduct controlled evolution studies with everything from viruses to fish. And as the multicellularity experiment conducted by Travisano and Ratcliff indicates, many are trying to address complex questions such as how evolution fashions major changes in a creature's lifestyle. One team at the Germany meeting reported tackling how sex evolves, for example, whereas another examined how an alga deals with losing access to light, its main source of energy. "Evolution is expanding from a strictly comparative and observational science to an experimental one," says Graham Bell, an evolutionary biologist at McGill University in Montreal, Canada.

Even the field's pioneer admired the ambitious work presented in Tübingen. "As the field grows, people are thinking about more and more specific hypotheses and complex scenarios," Lenski says.

## Dark science

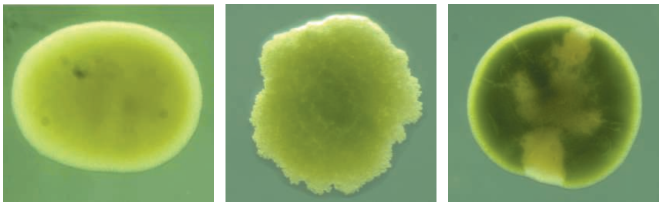
For Bell, the complex scenario was to try to get a plant to grow in the dark. The cells of plants, which survive by photosynthesis, are structured around harnessing light and converting it to chemical energy. What if there were no light? The plant might have to shift from depending on its photosynthetic machinery to another source of energy, perhaps the mitochondria that power most non-photosynthetic eukaryotes. A few parasitic plants and at least one protist have made such a switch, and Bell wanted to see if he could drive that change in the lab. It "constitutes a new way for life" for a plant, he explains.

Bell couldn't test a typical plant—none grows and reproduces fast enough to make such an experiment in evolution feasible. So he turned to a photosynthetic microbe belonging to the plant kingdom, the single-celled green alga called *Chlamydomonas*, often studied as a model system in cell and molecular biology. Bell knew this organism already had some ability to feed off acetate in a pinch and wondered if it could build on that to thrive in the dark. After all, he points out, "you can't evolve something from nothing."

CREDITS: WILLIAM RATLIFF/UNIVERSITY OF MINNESOTA (2)

"It was a big challenge," Lenski says. But "experimental evolution offers a way to see the relevant processes in action."

Bell set up 2880 cultures of *Chlamydomonas* on acetate-laden media and left them in a corner of his lab in constant darkness. Every other month he transferred 5% of the algae-media mix in each culture into a new dish of media. Unless the alga was increasing its population 20-fold monthly, a sign of strong growth, it would eventually be diluted out of existence by the periodic transfers. About 90% of the cultures stopped growing within a year, but a few hundred alga lines kept pace, he reported at the meeting. After about 12 months, he started transferring these algae each month, then every other week, and finally weekly, such that only the fastest growers would survive. "You have to be fairly patient," Bell says.




**Dark days.** Very few algae survived growing in the dark (*right*), but those that did evolved a variety of colors and shapes (*above*).

Five years into the experiment, 241 lines of algal "survivors" are thriving in the darkness that would be lethal to the ancestors. The alga lines vary significantly in appearance. Some form clusters that are circular; others are ragged. Some are green, whereas others are yellow or white. Most can still grow in the light, but a few can't and rely solely on acetate, Bell said: "You have a whole range of solutions to growing in the dark."

Bell and his colleagues are now looking at whether these transformed algae can mate with their original lines, or whether more traits than morphology and metabolism have changed through time. He has a list of genes involved with acetate processing that he will check for enabling mutations. At some point, Bell says he might put the acetate users back into the light to see if they can come to depend on photosynthesis again. The genes needed for photosynthesis have likely degraded, and he wants to know whether the algae can fix the broken genes or whether other genes will be brought into play. With this work, we "gain insights that would otherwise be impossible," Lenski says.

### Showing sex is good

Aneil Agrawal, an evolutionary biologist at the University of Toronto in Canada, and his postdoctoral fellow Lutz Becks are also exploring how a species can go back and forth between certain lifestyles. They're addressing the puzzle of sex. The evolutionary quandary is that requiring two individuals, typically a male and a female, to generate offspring makes reproduction much less efficient than asexual cloning, wherein each individual reproduces. Thus one would expect that even if sexual reproduction evolves, it shouldn't persist should asexual individuals subsequently arise, unless the sexual mixing of genomes provided some significant advantage. Yet in most species, sex predominates.

By the 1990s, researchers had put forth more than 20 theories to explain this puzzle. Some experimental work in yeast suggested sex was advantageous in changing environments. And a study in viruses, which swap DNA in a way similar to sex, indicated that sex thrives because it weeds harmful genetic mutations out of a population (*Science*, 28 November 1997, p. 1562). But for the most part, sex's purported evolutionary advantages have gone untested.

Three years ago, Agrawal and Becks decided to look more deeply at the long- and short-term benefits of sex through a series of lab studies, some involving experimental evolution. Researchers had proposed that recombining genes through sex could lead in the short run to fitter offspring. In the long run, the many possible genetic combinations produced by sex mean that there will likely be more genetic variation in the population and greater ability to adapt rapidly—an advantage that could favor the maintenance of sex.

The duo's studies, which center on rotifers, microscopic animals found in lakes and ponds, are showing that it is easy for sex to develop in certain populations but difficult for it to persist. So-called Bdelloid rotifers are famously asexual, but other rotifer species, including *Brachionus calyciflorus*, the one Agrawal and Becks study, sometimes resort to sexual reproduction but only in crowded conditions. (The buildup of a rotifer-secreted chemical induces this sexual behavior in crowds.)

Becks and Agrawal began by testing descendants of wild-caught *B. calyciflorus* rotifer for reproductive fitness, counting the number of eggs offspring produced in conditions that favored either sexual or asexual reproduction. The asexual populations (in uncrowded conditions) produced more than twice as many offspring as the crowded sexual populations, confirming a large fitness cost for sex, they reported in the March *Journal of Evolutionary Biology*. The sexual populations were also not more variable in their fitness, suggesting there was no long-term potential benefit to sex per se. "It showed the big problem we have explaining sex," Becks says.

Next, Becks and Agrawal used experimental evolution methods, tweaking the rotifiers' diet to look at the effect of environment on the balance between sexual and asexual reproduction. They fed one batch of 10,000 rotifers nitrogen-rich algae, a similarly sized batch got less-nutritious algae, and a third batch, broken into subgroups of about 5000 rotifers, were regularly exposed to both. Becks would weekly transfer 1% or 10% of each subgroup from the one kind of alga food source to the other, simulating migration between two environments. At the beginning of the experiment and after 6 and 12 weeks—45 and 90 generations—he tested the rotifers' propensity for sex by exposing a subset from each batch to the sex-stimulating chemical and looking at the eggs produced. (Asexually produced eggs appear solid under the microscope, whereas sexually produced ones seem partially void.) In this way, Becks determined what proportion of the rotifers were able to switch to sexual reproduction.

Rotifers maintained with a consistent food source—whether of high or low quality—produced half as many sexual eggs as rotifers regularly switching between the two kinds of food, Becks and Agrawal reported online 13 October 2010 in *Nature*. And when they followed the rotifers a month longer, they found propensity for sex increased in the rotifers with a mix of foods but declined in the batches where rotifers experienced a constant food environment. In a constant environment, sexual reproduction eventually disappeared altogether, Becks later found.

CREDITS: GRAHAM BELL

Becks and Agrawal wanted to see whether the pattern observed in the simulated migration—more sex in a more diverse environment—held true if the rotifers simply were confronted with a completely novel environment. Over the course of a week, Becks gradually added low-quality food to batches of *B. calyciflorus* rotifers that were used to feeding on high-quality food, transforming their diet. In the first few days of the experiment, as he was changing the food out, the rotifer population started to crash, reaching a low in 12 days. At that time, sexual reproduction began to increase and continued to do so for about 3 weeks, Becks reported at the evolution meeting. Then the trend reversed: The population kept growing but became increasingly dominated by asexually reproducing rotifers, a trend that continued through the final 10th week of the experiment. "We saw this increase [in sex], and then it went down again," he said.

They repeated the experiment for a period of a month and got the same results. In contrast, in control batches kept on a consistent diet, sexual reproduction waned from week one. According to Becks, this experiment and the one with the mix of two food environments suggest that new challenges favor sex but only until a way of coping with that challenge has developed. "In the end, sex was only beneficial during the time that they adapted to their environments," concluded Becks, who is now at the Max Planck Institute for Evolutionary Biology in Plön, Germany. Once the right adaptations had evolved, sex was no longer favored.

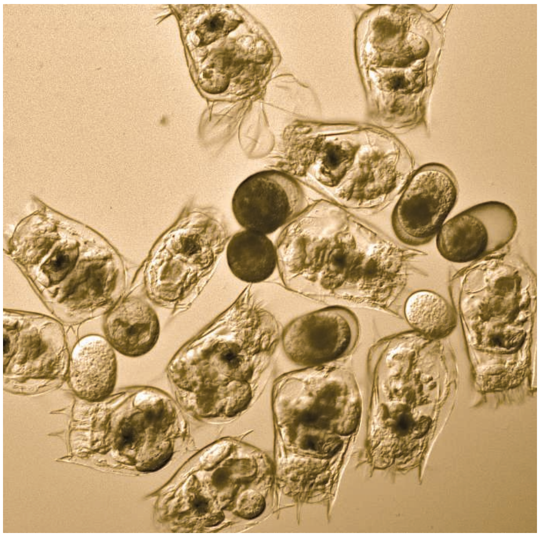
"What's remarkable is that they've developed a rapidly evolving empirical system that allows them to watch the evolution of sex in real time," says Sarah Otto, an evolutionary biologist at the University of British Columbia, Vancouver, in Canada. "By tracking changes in the frequency of sex, the rotifer system promises to allow us to tease apart the mechanisms that promote the loss or maintenance of sex." Lenski adds that "the work allowed them to challenge and support a classic model for the evolution of sex."

### Yeast beasts

Just as the evolution of sex represented a major transition for life, so did the leap to multicellularity. When Travisano and Ratcliff began to consider what experimental conditions would encourage the yeast *Saccharomyces cerevisiae* to go multicellular, they focused on size. One rather obvious hallmark of multicellular creatures is that they are bigger than unicellular organisms. On its own, largeness could offer many evolutionary advantages: a greater ability to access nutrients or avoid being eaten by small predators, for example.

Still, finding the right selective force to make yeast go big was a challenge. Working with their UMN colleagues R. Ford Denison and Mark Borrello, Travisano and Ratcliff first tried exposing cultures of yeast to detergent, thinking that some might form a multicellular entity in which the outer cells would shield the inner ones from the detergent's destructive power. But nothing survived.

Next, they turned to gravity as the selective force, letting tubes of yeast in solution, after being shaken to distribute the microbes evenly, sit quiet for 45 minutes. The researchers then transferred the bottom 1% of the tube's contents to new cultures. Bigger yeast, which would be more likely to settle out, should have a better chance of surviving these transfers. "It turns out that 45 minutes is pretty lenient," Ratcliff says. Almost all the yeast settled out, so there was little selection for larger size.



**Sex mania.** In typically asexual rotifers, the ratio of sexually derived eggs (darker) increases in novel environments but decreases after conditions stabilize.

CREDIT: LUTZ BECKS/UNIVERSITY OF COLOGNE

To speed up the process, they instead gently spun the tubes for 10 seconds in a centrifuge before transferring the bottom 1%. Two weeks later, ever-bigger pellets of yeast were settling to the bottom of the tubes in two of the 10 cultures, Travisano reported at the meeting. Spherical clusters of cells loosely resembling snowflakes eventually dominated all 10 setups. Tests showed that these weren't simply individual cells that managed to stick together, as happens when yeast in brewing beer aggregate. Instead, these clusters arose because dividing yeast cells had lost the ability to separate completely. The researchers ultimately discovered that the yeast in these snowflakes make much less of the enzyme that enables normal separation.

The snowflakes also began reproducing like a multicellular organism. Individual cells in the snowflakes would divide but not detach, enlarging the snowflake. And once the snowflake reached a certain size, it would fragment, releasing a daughter snowflake. At first the fragments were about equal size, but as more and more generations went by, a pattern developed: Snowflakes broke into a smaller and much larger part. That's presumably "so it could produce more offspring by allocating fewer resources for each one," Ratcliff said.

The newly multicellular yeast also evolved a division of labor that facilitated its uneven fragmentation. At first all the cells in the snowflake divided. But after hundreds of generations, some cells in each snowflake stopped dividing and eventually died. These cells in effect were sacrificed for the benefit of the multicellular organism, becoming sites where the snowflakes fragmented, Ratcliff reported. "It's the beginning of specialization," Lenski says.

Some scientists question whether the new complex yeast are true multicellular organisms, but others nonetheless praise the experiments. "The origin of multicellularity with the subsequent evolution of specialized cell lineages represents one of the most important transitions in the history of life," David Reznick, an evolutionary biologist at the University of California, Riverside, says. "I would have never dreamed that it could be possible to study it from an experimental perspective."

By doing so, Ratcliff says, he's gained a better understanding of this transition. "The constraints on the evolution of multicellularity may be lower than we thought," Ratcliff concludes. As a result, he now believes multicellularity arose more often than researchers have realized—most current estimates suggest it has emerged about 20 times in various lineages—but then subsequently faded away.

The attempt to transform yeast into a multicellular species also impressed Lenski. "That's some of the neatest work going on right now," he says. "It makes you think about what is a major transition" in evolution of life.

Indeed, Colegrave says, "experimental evolution is an approach which can in principle be used to address any of the big questions in evolution."

**–ELIZABETH PENNISI**