# **Untrue** to Type

When plant pollen fertilizes an ovum, the genetic material in the nucleus and the chloroplasts must harmonize. **Stephan Greiner** from the **Max Planck Institute of Molecular Plant Physiology** in Golm, near Potsdam, would like to find out which factors in the chloroplasts prevent the interbreeding of plant species. To do this, he works with a model plant that's not too particular when it comes to the species boundary: the evening primrose.

TEXT **CATARINA PIETSCHMANN**

Thale cress *(Arabidopsis thaliana)* seedlings in a Petri dish. Condensation forms small drops on the lid of the dish. The plant, which belongs to the Brassicaceae family, is an important model organism for plant geneticists.

Photo: David Ausserhofer

Suppose the pollen from a cherry tree could land on a pumpkin blossom, or the pollen from a banana plant somehow find its way to a cactus. Would we get juicy, cherry-flavored pumpkins and prickly skinned bananas as a result?

A fascinating prospect. The earth would be full of bizarre species, and the cuisines of the world would be constantly enriched with new flavors. Evolution, however, has little time for such fantasies. It doesn't mix species at random, but keeps them largely separate – a concept that, over the past 500 million years, has resulted in the existence of approximately 500,000 plant species. And although they all share a common ancestor, there are very few species today that can reproduce with other species.

Nevertheless, something occurs among plants now and again that is extremely rare in the animal kingdom: interbreeding across species boundaries. However, in order for healthy offspring – known as hybrids – to be produced, the two participating species must be extremely similar. Otherwise, the genetic obstacles to be overcome would be too high. For example, despite the fact that they both belong to the Compositae family, pollen from the sunflower doesn't fertilize the dandelion. Genetically speaking, the sunflower and dandelion developed worlds apart from each other. For this reason, they also belong to different genuses.

Sunflowers and dandelions can thus happily grow side by side without anything happening between them. "The signals that show the pollen the way to the ovum are missing," explains Stephan Greiner from the Max Planck Institute of Molecular Plant Physiology. With his Cytoplasmic and Evolutionary Genetics research group at the Max Planck institute in Golm, near Pots-

Photos: David Ausserhofer (top left, bottom), Catarina Pietschmann (top right)

1 The stigma of an evening primrose flower is pollinated with foreign pollen. The petals and stamen were removed before pollination (below). The pollen forms pollen threads that still hang on the stamens of the other flower (above).

2 After the foreign pollination, the flowers are covered to prevent further uncontrolled pollination.

3 In the foil greenhouse, Stephan Greiner checks whether the pollination was successful and the plants are forming seed capsules.

dam, he's hot on the trail of the species barriers – the obstacles to interbreeding between closely related plants.

## BARRIERS ENABLE NEW SPECIES TO FORM

"According to a commonly accepted model, a new species arises when, for some reason, a population of the original species is separated from the rest for a long period of time," explains the biologist. "The reason could be an ice age, a river or even a mountain." The isolated population continues to develop independently, and over the course of future generations, its genes adapt increasingly well to the living conditions in its environment. In an extreme case, for example, it will thrive in a desert while its original species is at home in a bog. If the two populations are reunited – because the ice age has come to an end or the river has dried up – they no longer match. Although they still have very similar genotypes, the activity of some of their genes differs. "The factors for species formation are genetic isolation, a change in habitat and the associated adaptations," summarizes Greiner. Chance can also play a role.

Based on the example of the evening primrose plant, Stephan Greiner is studying the make-up of these species barriers. The focus of his attention is the plastome – the circular genome of the chloroplasts, the small green spheres in plant cells, where photosynthesis takes place. The genetic material in both the nucleus and the chloroplasts is important for the success or failure of a hybrid.

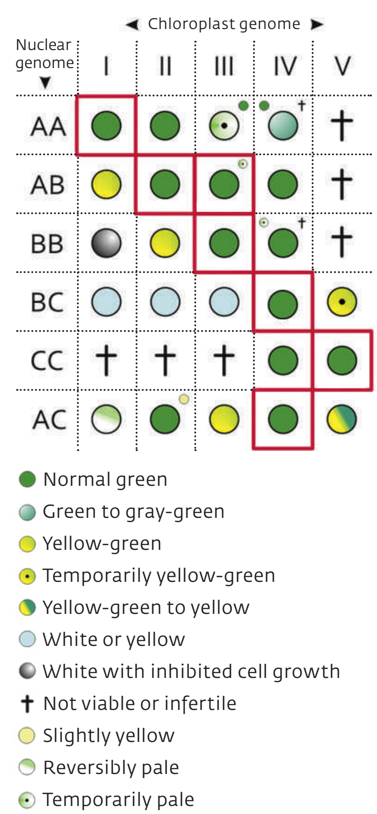
According to endosymbiotic theory, the chloroplasts are the result of an emergency situation: around two billion years ago, most of the living unicellular organisms were faced with a huge problem as the oxygen content of the earth's atmosphere was continually increasing. This was caused by cyanobacteria, which perform photosynthesis – that is, they form carbohydrates from carbon dioxide and water.

For most of the other unicellular organisms, which had not mastered this trick, the oxygen released by this process was pure poison. There was only one solution to their problem: they snatched up bacteria that could survive with oxygen, consumed them without digesting them, and lived thereafter in peaceful symbiosis. This is how the mitochondrion emerged. According to the theory, this was the first step in the formation of multicellular organisms and the emergence of animal life. These organisms later also assimilated a cyanobacterium as, through this fusion, the organisms themselves could harness energy from the sunlight.

The first algae emerged in this way, followed later by plants. The mutual adaptation went so far that the partners shared all of the tasks crucial to their existence. The assimilated bacteria became cell organelles, the chloroplasts. They focused exclusively on photosynthesis and left all of the other tasks to the cell. Unused genes were either lost or integrated into the cell's genome. As a result, the chloroplast genome is comparatively small: the genome of the evening primrose chloroplast, for example, consists of around 160,000 letters from the

above: Interbreeding with consequences: The plant on the left is type AA-I. The genome in the nucleus and chloroplasts harmonize. In contrast, the nucleus and chloroplasts in the AA-III plant (right) do not go well together. The leaves are pale with green areas on the edges and along leaf veins.

below: Compatibility/incompatibility chart of the evening primrose: The combinations of letters refer to the nuclear genome types; the Roman numerals represent the different chloroplast genomes. The symbols stand for the different appearances of the hybrid plants. Small additional symbols in a field indicate the variations of a hybrid. The combinations with red edges arise in nature; all of the others originate in the laboratory.




Photos: MPI of Molecular Plant Physiology (2)

1 | Stephan Greiner (left) and Dirk Zerning examine boxes sown with evening primrose in the greenhouse.
2 | Evening primrose seedling, a few days old.
3 | Sven Roigk (left) and Katrin Seehaus transfer the evening primrose seedlings from seed trays to pots and select the plants with the desired appearance.

Photos: David Ausserhofer (3)

» With its 1,000 different varieties, the Max Planck institute near Potsdam holds the largest living collection of evening primrose plants.

genetic code, and contains only around 120 genes. The nuclear genome, in contrast, comprises a billion letters.

In the case of plant hybrids, therefore, not only two, but four sets of genes must fit together: two nuclear genomes plus one chloroplast and one mitochondrial genome. Greiner shows a compatibility/incompatibility chart that contains all of the possible genetic combinations for the evening primrose: three different core genomes (AA, BB, CC) and the corresponding hybrids (AB, AC, BC) have formed, making a total of six. Combined with five plastome variants (I - V), there are 30 different possibilities. The green dots indicate that the hybrids in question are healthy and fertile. The meaning of the black crosses is obvious: here, the end of the line has been reached.

According to the compatibility/incompatibility chart, Plastome I causes the most problems: truly healthy evening primrose plants arise only in combination with the core genome variant AA. Moreover, various gradations exist. In these cases, the chloroplasts don't provide a complete service: problems arise with photosynthesis. In nature, such plants grow more slowly; they produce fewer seeds, and are not as successful as a result.

Along with pelargoniums, the group that includes the geranium, the perennial favorite of amateur gardeners, the evening primrose is the traditional genetic model for biparental inheritance. This means that, unlike in most plants, the chloroplasts are not passed on to the offspring by the maternal line alone, but also through the paternal line. "Another advantage is that we can do something here that doesn't work with other plants. We can swap the chloroplasts between two species." In the first generation (F1), the mixture of the parental genomes is achieved. However, if F1s are crossed with each other – Greiner calls it "selfing" – it's possible to breed the parents out of the hybrids again.

The approximately 130-member family of the evening primrose plant originates from America, and was first introduced to Europe in the 17th century. They grew wild here and are a permanent feature of central-European flora today. Stephan Greiner researches the subsection *Oenothera*, of which there are only 13 species. To the untrained eye, the plants look very similar. However, botanists can distinguish between them based on their flower size, leaf shape, growth, stem color, and branching type.

## AMERICAN ORIGINS

The populations, which were separated mostly by ice ages and are converging again, are indicated on a biological map of the American continent. Type AA-I can be found almost throughout North and Central America. BB-III grows only in Florida and a few neighboring states. To the northeast from here, a mixed area (AA-I/BB-III and hybrids) exists with a small enclave of CC-V plants.

One section of Greiner's evening primrose plants are outside in the airy foil greenhouse. Here, too, there are yellow flowers everywhere. But they only appear like this to human eyes. Greiner opens a flower. Inside, it has barely visible stripes. "Butterflies can see in the UV range. For them, these flowers are white with red stripes."

Greiner's research group works with 300 experimental lines. "With about 1,000 varieties, we have the biggest living collection of evening primroses here in Golm," he explains. Some of them grow in pots in the adjacent high-tech greenhouse. In some of them, the yellow flowering branches are completely packed in airy white paper bags – to ensure that they stay "clean," meaning they can't be pollinated by other lines.

Many plant species, including crops like barley, beans, and peas, don't even rely on cross-pollination. Some self-pollinate before they open their flowers; others refrain completely from sexual reproduction and produce seeds without fertilization. The question arises here as to which system, sexual or asexual reproduction, is more effective. "Basically, nobody really knows why sex exists anyway," says Stephan Greiner. "For example, a desert plant is perfectly adapted to extreme drought. If it's cross-pollinated, it's pretty unlikely that its offspring will manage as well in desert conditions." In such cases, the fact that mutations accumulate through inbreeding speaks in favor of sexual reproduction. Sex, in contrast, constantly renews the gene pool.

Part of Greiner's work involves classical breeding experiments: targeted pollination, waiting for the seeds to form, sowing, replanting, and tending. With 300 varieties, that's a lot of work. To this is added the molecular biology work: taking samples, obtaining DNA, reproducing, and analysis.

Another research approach is deliberate mutagenesis, which is the intentional triggering of changes in the genes, which changes enable the chloroplasts to harmonize with the nucleus again. "We have an evening primrose line that is called chloroplast mutator. It has a defective gene in the nuclear genome left: Chloroplasts in a thale cress *(Arabidopsis thaliana)* leaf. Plant cells have different numbers of these organelles, with which they harness energy from sunlight using photosynthesis.

right: The researchers can determine the rate of photosynthesis, in this case in a thale cress leaf, by measuring fluorescence.

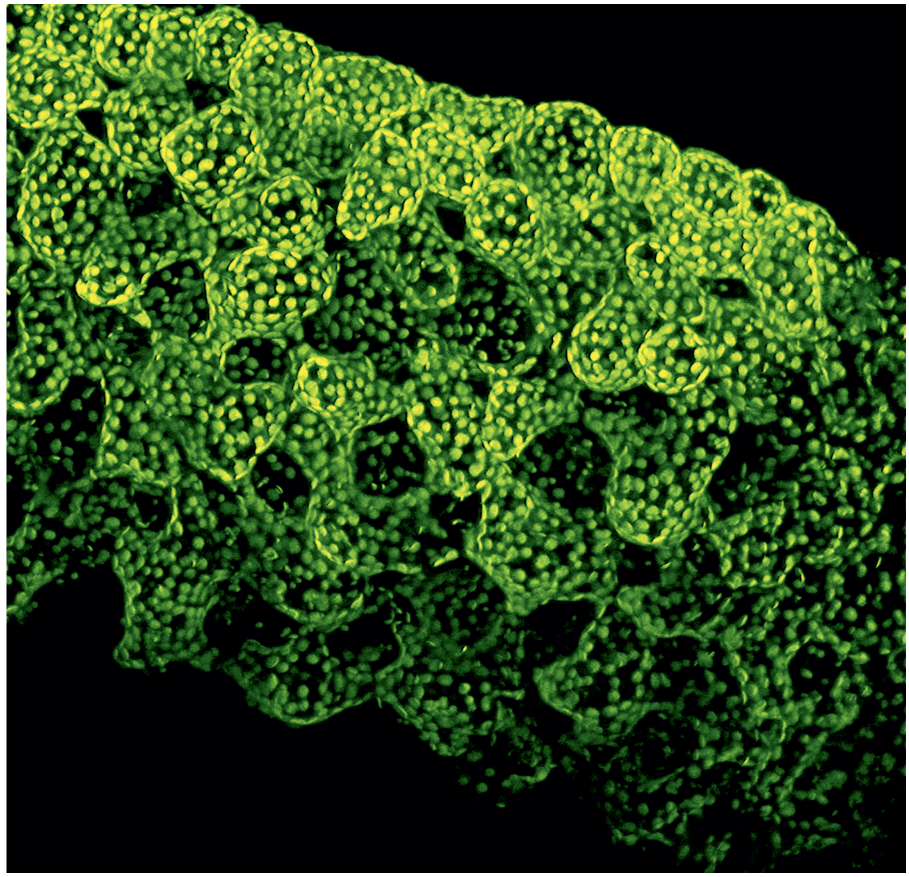


Photo: SPL-Agentur Focus

and thus causes a relatively high number of mutants." From this, the biologist also hopes to obtain information as to why some hybrids have no chance of survival.

The beauty of Greiner's research approach is that, because the chloroplast genome is responsible almost solely for photosynthesis, it is possible to see from the plants whether and how this process is impaired. The leaves are either too small, yellow-green in color, or have a conspicuous pattern: white-green stripes or white around the edges. Such segregation patterns indicate that only the impaired chloroplast has been inherited.

It's easy to establish whether and which part of photosynthesis is impaired in hybrids using fluorescence measurements. If the chlorophyll in the leaves works perfectly, a certain volume of the incident light is reflected. From the reflection, conclusions can then be drawn about the state of the chloroplasts.

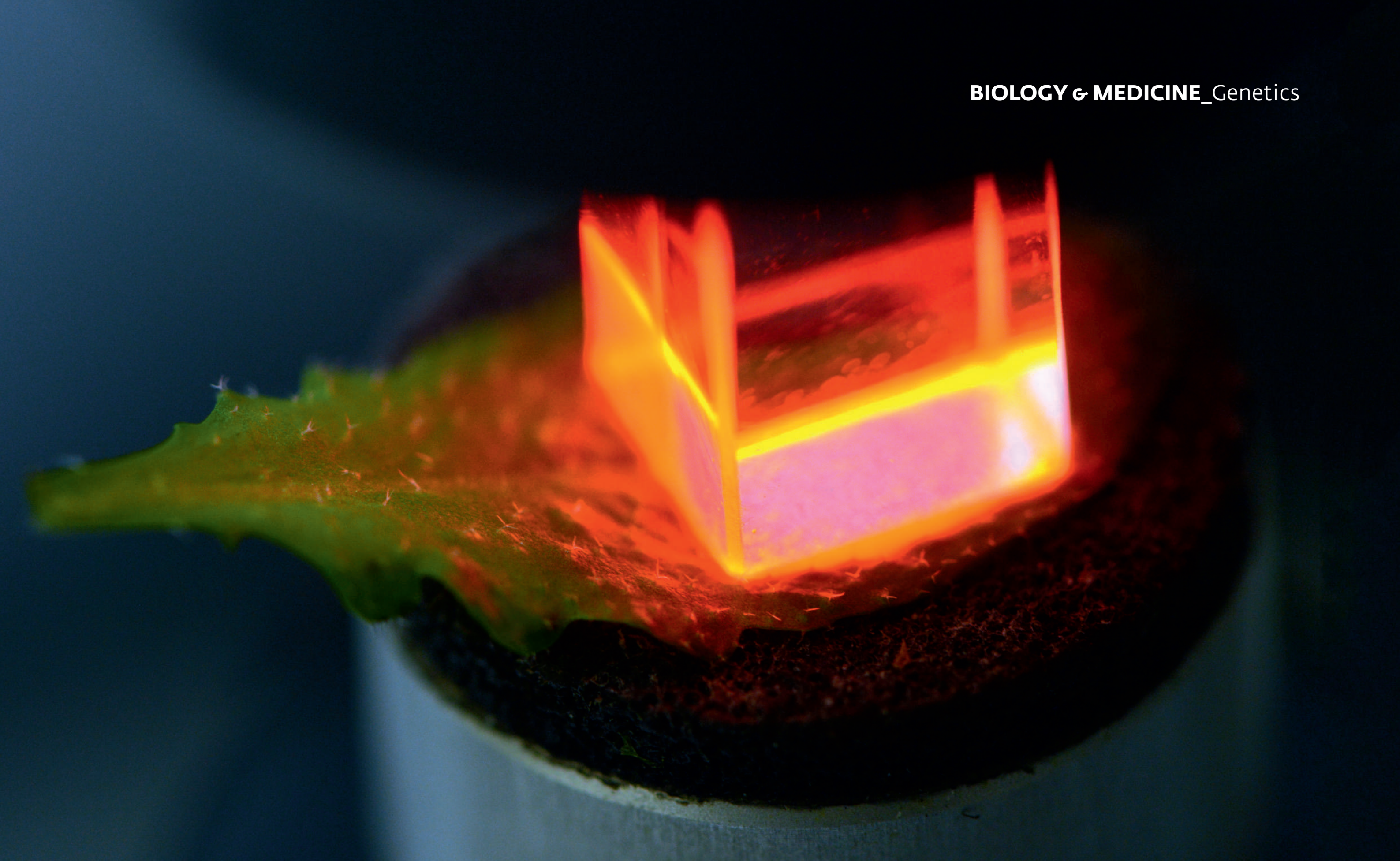
"We are interested primarily in these incompatible hybrids, as the nuclear and chloroplast genome is no longer correctly regulated in them." This occurs when the two genomes have a different evolutionary history. Through the long symbiosis, the nuclear genome and plastome adapted to each other. However, if two different populations are crossed, it's possible that the chloroplasts don't harmonize with the unfamiliar nuclear genome.

## WHEN CHLOROPLASTS AND NUCLEUS DON'T HARMONIZE

Inadequate cooperation between the nucleus and chloroplast appears to be an important species barrier. However, this is not attributable to photosynthesis itself. "Cyanobacteria have been harnessing energy from sunlight for two billion years, so they've had enough time to optimize photosynthesis. Therefore, it's unlikely that anything in this has changed within a relatively short period of one million years."

No, it appears to be more a matter of fine-tuning, the regulation of the complicated process. "Photosynthesis depends on water – evening primrose plants in Florida have more water for photosynthesis than the plants in California." That could be the crucial point. Greiner's team has already found two factors that influence the success of hybrids: an enzyme for the regulation of chloroplast proteins and a sub-unit of photosystem II – one of the complexes that provide energy for the plant.

The evening primrose has been a part of Ulm-born Stephan Greiner's life since his student days at Ludwig-Maximilians-Universität in Munich. Although the classical genetics of this family of plants had already been well researched at the time, a molecular model did not yet exist. This therefore became the topic of Greiner's doctoral thesis. "It was back-breaking work," he remembers. Together with a colleague, he developed processes for releasing DNA, RNA and the organelles from the plant – no easy undertaking, as evening primrose plants have so many mucilaginous substances that cutting them up produces a sticky, filamentous mass. "Many botanists had worked on it before we did, but none of them managed to come to grips with this chewing gum. Using our methods, we were able to analyze the evening primrose's five plastomes and create genetic maps." However, his doctoral supervisor re-



tired, and there was no further interest in the model. So many years of research, and all for nothing?

The fact that Stephan Greiner now researches at the MPI in Golm owes to a fortunate coincidence. In 2009, he decided to make a clean break and took an office job with a Munich-based patent law firm. "The job wasn't bad and it was well paid," he says smiling. "It would have been fine." However, after six months in the maze of patent rules and regulations, he received a telephone call. Ralph Bock, one of the three directors of the MPI in Golm, was interested in Greiner's Oenothera system and invited him to a meeting.

"I was actually through with science, but the research group impressed me. They're fully familiar with all of classical genetics, as well as chloroplast biochemistry. Very few can claim that." And so Greiner bade farewell to Munich and the law firm, and moved to Brandenburg in mid-2009. The 33-year-old now supervises six doctoral students and is completely snowed under with work.

His current aim is to identify factors that reflect the genetic adaptation of plants. "We will then be able to identify whether a factor has changed completely by chance or under the pressure of natural selection – that is, whether, for example, it causes a plant to adapt to the desert climate." As far as the evening primrose is concerned, Greiner is optimistic: of 30 possible combinations, as the compatibility/incompatibility chart shows, 19 yield incompatible plants. Thus, the search field is manageable, but big enough to enable him and his team to draw generally valid conclusions.

Could we also learn something about species barriers between animals from his research? Greiner laughs. "It's not directly transferrable, as animals don't have any chloroplasts. However, incompatibilities between the nucleus and mitochondria play as important a role in animals as they do in plants."

Greiner's research will definitely have an impact on plant breeding. Once the species barriers have been identified, ways of overcoming them can be found. "If it's just a matter of one or two factors, it could be done through gene exchange." Innovative hybrids would also be possible. Definitely not cherry pumpkins or prickly bananas, but high-yielding grain varieties that might survive in the Sahara.

But do we actually want transgenic plants? For a planet that will soon have ten billion people to feed, this question is practically rhetorical. However, that's a different story altogether. ◂

**TO THE POINT**

- In addition to the cell nucleus genome, the chloroplast genome is an important factor for the formation of species.
- The regulation of the genes in the chloroplast genome influences the interbreeding capacity of different evening primrose lines.
- Hybrids of different evening primrose lines can't adapt photosynthesis to natural conditions very well. As a result, the plants remain weak and form fewer seeds.

Photo: David Ausserhofer