PLANT GENETICS

A 'Shotgun Wedding' Finally Produces Test-Tube Plants

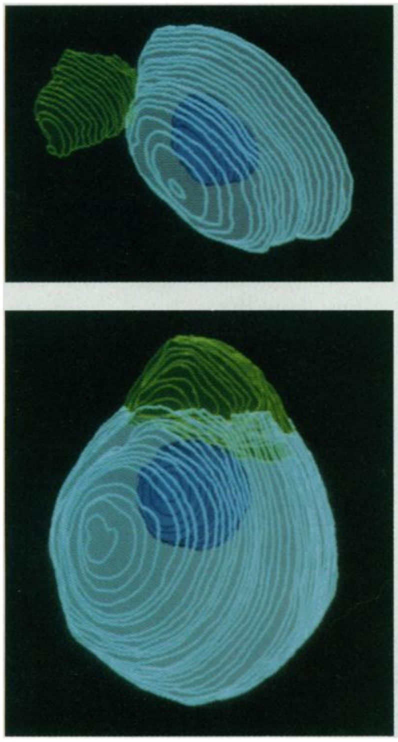
While researchers working with animal cells have spent years exploiting in vitro fertilization to create and study organisms, plant researchers could only look on with envy, for plant gametes stubbornly refused to combine in the test tube. Until now. This month, a group of plant biologists report that they have mated plant sex cells in vitro and grown the result into a fully functional adult plant. That feat, which opens the door to studies of the earliest stages of plant development, could also offer new opportunities for the genetic engineering of crops.

Botanists Erhard Kranz and Horst Lorz at the Institut für Allgemeine Botanik of the University of Hamburg report in the July issue of *The Plant Cell* that they used a brief pulse of electricity to produce what several researchers call a "shotgun wedding": a fusion of corn sperm and egg cells into a zygote.

The technique opens "a very large window in plant development. Until now, no one could isolate a plant zygote," says botanist Christian Dumas of the Ecole Normale Superieure in Lyon, France, whose lab helped document the fusion in a series of electron micrographs. "In vitro fertilization is easy to do in animals—the sperm and the egg are accessible to the experimenter and in a liquid medium. But in plants, the sperm and the egg are encased in tissue."

That obstacle has limited developmental biologists to laboriously pollinating plants by hand, then sectioning the embryo sac (the structure that shelters the embryo) to see what's going on inside. And as a result, "we don't know anything about the molecular events in the early plant embryo," says Ian Sussex, a plant developmental biologist at the University of California, Berkeley.

The barriers to test-tube fertilization of plants began tumbling in the mid-1980s, when investigators first isolated sperm from flowering plants by using a fluid pulse triggered by osmotic gradient to rupture the pollen grains that hold sperm cells. But egg cells, which are held in the immature seed, or ovule, proved more elusive. "The egg is an integral part of the ovule," explains botanist Scott Russell of the University of Oklahoma. To get the egg out of the ovule, adds Russell, "you have to break down connections among cell walls, using enzymes" and painstaking microdissection.


THE PLANT CELL

Short fuse. Top: Plant sperm *(green)* and egg *(blue)* nuclei move together after an electric jolt. Bottom: They fuse in about 1 hour, as shown in diagrams based on micrographs.

But isolating the egg from its biological context actually created another problem, because it also removed the numerous helper cells and other structures that in nature intervene to bring the nonmobile sperm and egg together.

Kranz and his colleagues first used electricity to replace these biological bridesmaids and groomsmen in 1989, adapting a technique researchers had been using to fuse somatic protoplasts—plant cells missing their walls—since the 1970s. Though the technique worked well for the gametes, it still isn't understood just why it was successful, since "at the molecular level, nothing is known about how this works," Kranz says. When the Hamburg researchers apply current to gametes, Kranz says that it sometimes looks as if the electrical pulse creates a large hole in the egg cell through which the sperm enters.

Still it did work, although it took a while to get the technique down. Zygotes from the first attempts, in 1989, ceased growing after dividing to 50 or 100 cells. It took 4 more years, Kranz says, to perfect the method by developing a cell culture containing early embryonic cells from corn plants, which secrete an unknown factor or factors that enhance zygote growth and differentiation.

Using this high-quality cell culture medium, the "shotgun wedding" led to a happy marriage. The wedding photos—a dramatic series of three-dimensional reconstructions based on electron micrographs—clearly show sperm and egg merging over about an hour. The zygotes from these merges developed into fertile corn plants, and genetic analysis indicated success: Each plant had inherited half of its chromosomes from each parent.

The first applications of the technique will probably be in embryonic studies. Kranz, for instance, plans to isolate and identify genes expressed during early embryonic development. Botanist David Cass of the University of Alberta suggests that researchers will want to look at the patterns of tissue differentiation in maize and other plants, possibly even being able to create a "fate map" to see the embryonic origin of certain tissues. For example, says Cass, "in flowering plants, shoot and root apices [tips] are formed in early embryogenesis. We don't understand how. These apical cells are unique and have subtle controls over patterns of development."

Though it will be a boon to the examination of early plant development, electrofusion is not an ideal system for studying fertilization itself. Its power to drive cells together would obscure any natural mechanisms by which sperm and egg might recognize one another and move together. "With this method," says Dumas, "you can't approach the crucial step of gamete recognition." But researchers are already working on nonelectrical alternatives. Alberta's Cass, for example, has been trying to microinject sperm into intact embryo sacs, and he believes his lab may be only months away from successful fertilization.

In a more commercial realm, electrofusion may give genetic engineers a better way to insert genes into crop plants. The currently favored method involves transforming embryonic plant cells by infusing foreign DNA, then trying to grow each cell. Unfortunately, this method doesn't always work: Large numbers of cells are treated, but only a small percentage incorporate the imported genes or retain the potential to grow into plants. Zygotes created by in vitro fertilization may be better targets.

One objection to this approach, however, is that it's slow. Researchers can create only 20 or so zygotes per day, while mass transforming cells permits researchers to work with millions of cells per day. Even though only a small fraction of those cells grow into new plants, the resulting yield is much greater than 20. Geneticists may figure out how to insert genes into electrofusion plants more precisely than they can insert genes into mass transformed plants, giving electrofusion another advantage, but until they do the technique's greatest impact will probably be in the fields of developmental research. But there its effects will no doubt be very fertile.

–Billy Goodman

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