Grand experiment. A planting of 12,000 *A. thaliana* seedlings.

Weinig and her colleagues from Brown and North Carolina last year planted inbred lines of *Arabidopsis* that differed in particular genes, such as those coding for proteins that sense day length, both in Rhode Island and in North Carolina. They also planted *Arabidopsis* from Rhode Island in North Carolina and vice versa. Over the ensuing months the researchers monitored when these plants sprouted and when they bloomed. They also noted traits such as the number of branches to see how these plants responded to the conditions in each place. Already, intriguing hints are emerging. The preliminary data suggest that, depending on the season, different genes are affected. The team's goal, Schmitt says, is to understand "what are the causes of selection on particular traits in [the] wild, what are the genes underlying individual traits, and how is selection acting on these genes."

Still others are studying how *Arabidopsis* and its bacterial or fungal pathogens coevolve. Ecological geneticist Joy Bergelson of the University of Chicago, for instance, used sequence data to study the R genes that help the plant recognize bacterial pathogens. Next, her group obtained *Arabidopsis* from various sites around the world and assessed their R-gene makeup. Contrary to what they expected, Bergelson and her colleagues have found that different versions, or alleles, of R genes have persisted in these populations for millions of years. That finding, along with other data from Mitchell-Olds's group, indicates that defense-related genes are not in an evolutionary arms race with pathogens, as current dogma predicts. Instead, suggests Mitchell-Olds, the frequency of the different alleles varies from year to year and population to population, enabling the plant to maintain its defenses against numerous threats.

These insights represent just the beginning of what Mitchell-Olds and others expect to gain by moving *Arabidopsis* studies into the field. "Ecologists may like to work in wonderful pristine places," Mitchell-Olds says, "but this is a system where we can get answers."

–ELIZABETH PENNISI

CREDITS: (TOP TO BOTTOM) MICHAEL PURUGGANAN AND JOHANNA SCHMITT; UNIVERSITY OF CHICAGO HOSPITALS; GREGORY P. COPENHAVER/UNIVERSITY OF CHICAGO

ARABIDOPSIS CENTROMERES

A Journey to the Center of The Chromosome

The *Arabidopsis* genome project is the first to give a detailed picture of the centromeres in a higher eukaryote

As a postdoc at Stanford University in 1994, Daphne Preuss was examining mutagenized pollen grains under a microscope when she saw it: Amid all the dots of lone pollen, four grains were stuck together, tracing the shape of a tetrahedron. Having written her Ph.D. thesis on yeast, where such tetrads are standard and have been the foundation for its genetic analysis, Preuss knew she was looking at something powerful. "I immediately knew this [mutant] was the key to doing all kinds of genetic analysis" in plants, she recalls. "Life would be different."

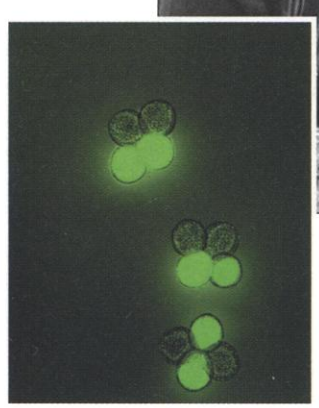
That chance finding launched her career as a plant biologist. Some 6 months after she found it, an electron micrograph of the mutant pollen was on the cover of *Science* (3 June 1994), and Preuss was soon on her way to the University of Chicago, where she directs a lab that runs in large part on the power of her mutant find, dubbed *quartet*.

The lemon-yellow pollen grains in which she spotted *quartet* were from the mustard plant *Arabidopsis thaliana*. What was unusual was that the four gametes were joined. Typically during meiosis in a plant or animal, the two chromosomes within a cell join; recombine, or exchange genetic material; then divide and separate twice into four haploid cells—the gametes. Each gamete, whether pollen or sperm, contains half the genetic complement. But in this newfound *Arabidopsis* mutant, the standard diploid cell produces four adjoined haploid cells—a tetrad, as in yeast. By analyzing these four cells instead of random gametes, geneticists can chart recombination events with unprecedented precision. Preuss realized that this four-in-one mutant could reveal what happens during meiosis in plants as it had in yeast. It would also enable her to define the centromeres, which have been defined in yeast but which remain a black box in plants and animals.

The centromere is a crucial stretch of DNA buried in the knotty terrain at the center of the chromosome. It plays a key role in meiosis, pairing up parental chromosomes and hitching them to protein motors that pull the chromosomes apart before cells divide. The dense, central region of the chromosome containing the centromere is readily visible under a microscope. Yet only in yeast have researchers been able to identify the exact DNA sequence of the centromere.

Using the *Arabidopsis* tetrad mutants, Preuss has established where the centromeric region starts and stops on each of the five chromosomes, a first for a complex eukaryote. Now, by building "minichromosomes," she and her colleagues are on their way to pinpointing where and how in that region the proteins attach in meiosis. The research is "blazing trails," says Kelly Dawe of the University of Georgia, Athens, who is developing such minichromosomes in maize.

Preuss's lab and *quartet* have also been indispensable to the *Arabidopsis* genome se-



Quartet. Preuss's chance discovery of a tetrad mutant in *Arabidopsis (left, below)* has enabled her to venture into the center of the chromosomes.

quencing project, started in 1996 with the goal of deciphering the plant's 120-million-base-pair sequence. The fine-scale genetic map her group developed by using pollen tetrads boosted the unprecedented accuracy and completeness of the sequence of this model organism. Not only did the map enable Preuss to define the centromeric region, but it also enabled the six sequencing groups to assign unknown fragments of DNA, especially from the centromeric region, to their rightful places on the chromosomes. "We wouldn't have been able to have done it without her and [postdoc] Greg Copenhaver," says W. Richard McCombie of the sequencing group at Cold Spring Harbor Laboratory in New York.

Into the chromosome centers

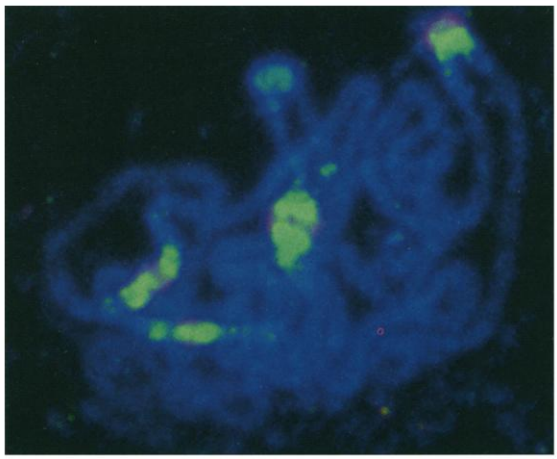
Under a microscope, the cinched region of the centromere is easily one of the most distinguishing features of the threadlike chromosomes. Indeed, cytologists captured the first images of the centromere in the late 1800s. But for all their centrality—literally and figuratively—in dividing cells, centromeres have escaped much dissection. "The centromere has been remarkably elusive to pin down," says Brian Charlesworth, an evolutionary biologist at the University of Edinburgh in the United Kingdom.

For decades both cell biologists and geneticists have attacked the problem of how chromosomes segregate and the role the centromere plays in this process. By the 1940s geneticists were mapping the locations of the dense centromeric regions in yeast and other fungi. This was possible because these organisms packaged their gametes in tetrads, which enabled geneticists to track recombination among the four cells. The centromeres always stayed put on the chromosomes they started out with, in contrast to other recognizable markers. Thus, the centromeres became the baseline for measuring distance to markers on the chromosome arms. From that point on, tetrad analysis became a stock in trade for geneticists, especially as yeast became the favored model system.

In 1980, using tetrad analysis, researchers narrowed the location of the centromere to about a 4000-base stretch of DNA in budding yeast, the first organism to achieve that landmark at that level of resolution. Eventually, as molecular technologies improved, yeast's functional centromere—the precise bases involved in hitching chromosomes to the proteins—was whittled down to a 125-base stretch.

Meanwhile, during the 1960s and '70s, cell biologists had developed staining and other techniques that visually highlighted the centromeric region on the larger chromosomes of plant and animal cells. These techniques also highlighted the corresponding protein structure the centromere meshes with. By that time, researchers were beginning to realize that eukaryotes had devised many ways to segregate their chromosomes—in other words, one centromere did not fit all. But except in yeast, the exact DNA sequence of the centromeres remained elusive, escaping detection even in the massive projects to sequence the genomes of fruit flies and humans.

The problem is that the chromosome centers are jungles of difficult loops and repetitions, or heterochromatin, in contrast to the smooth runs of readable DNA, or euchromatin, on the chromosome arms. As sequencers—who decipher the genetic code of short fragments and then reassemble them in correct order—approach the central regions of the chromosomes, they run into long stretches of nothing but repeated bases, such as ATAT ... AT. These stretches of repetitive DNA are next to impossible to piece together, as they contain few landmarks to orient them. The centromeric region is "the part of the genome that's ignored when it comes to estimating time to 'complete' genome projects," says Gary Karpen, who studies the centromere in *Drosophila* at the Salk Institute for Biological Studies in La Jolla, California.



Terra incognita. The five *Arabidopsis* chromosomes are lit up to show the centromeric regions.

CREDIT: PAUL FRANSZ/SWAMMERDAM INSTITUTE FOR LIFE SCIENCES/UNIVERSITY OF AMSTERDAM

In *Arabidopsis*, Preuss and her co-workers were able to find the right place for these "orphaned" stretches by analzying the results of 1000 crosses using the mutant pollen, each cross producing four plants, one from each cell of the tetrad. With tetrads, "we can literally redraw what happened in meiosis," she says. They were able to find recognizable markers linked to these orphan stretches and then examine how often the markers stayed put with the centromeres in the tetrads generated in meiosis. The more often a marker separated from its centromere, the farther down the chromosome arm it was. The more often it stayed with its centromere, the closer in it was. The frequency of these separations, or recombinations, within a foursome was used to calculate distance between markers and centromeres. In this fashion the researchers generated successively finer scale maps of the genome, starting out with roughly one marker for every three megabases and finishing in the region of the centromere with one marker for every 10 kilobases. The frequency of recombination between a marker and its centromere also enabled the Preuss group to delineate where on the map the centromeric region starts and stops on all five chromosomes (*Science*, 24 December 1999, p. 2468). When they found no pattern of crossing over in the tetrads, the researchers knew that they had found markers in the genomic terra incognita of the centromeric region itself.

Among the five chromosomes, these genetically defined centromeric regions vary in length from 1.4 megabases to 1.9 megabases, says Preuss. That's about 7% of the entire genome—a far more precise definition of the *Arabidopsis* centromeric regions than earlier estimates of more than 40% of the genome, says Preuss. Inside these regions as well as flanking them, Preuss and her team found more repetitive sequences, most notably, recognizable sequences of 180 base pairs repeated hundreds of times, on all five chromosomes.

Surprising nuggets also turned up inside the centromeric regions, most notably, a significant number of genes. "That was one of the big interesting things," says Preuss, because the centromere had long been considered relatively barren territory. The analysis has located some 200 genes in the *Arabidopsis* centromeric regions, at least 50 of which are expressed. About 40 of these genes appear only once in the genome sequence. "These are bona fide genes that would have been left out" without delving into the centromeric region, says Preuss.

Creating chromosomes

Having defined the centromeric regions and sequenced most of them, the researchers still need to find the functional part and figure out how it works. Given the diversity of centromeres in different organisms, there isn't a universal code to look for.

To find the functional centromere, Preuss's lab is developing a new tool—experimental minichromosomes that are a stripped-down version of an *Arabidopsis* chromosome. They contain all the essential parts: the centromere; telomeric DNA from the chromosome ends; genes of interest or indicator genes, such as green fluorescent protein; and elements to ferry the package into cells. When assembled, these mini-

chromosomes should function in plants alongside the other chromosomes.

Postdoc Kevin C. Keith in Preuss's lab is now testing pieces of DNA from the *Arabidopsis* centromeric regions to see which ones work in cell division—the hallmark of a functional centromere. Keith is combing through all the bases in the centromeric region, including the 180-base repeats. The process is akin to going through jars of bolts in a hardware store to find the right one, in this case, a stretch of DNA that holds the minichromosome to the cell's protein motors. To do so, Keith is methodically inserting sequences of fewer than 100 kilobases into the minichromosomes to test which ones work as the centromere.

Such minichromosomes are in the works for other organisms, including humans. Apart from their use as a tool to explore chromosomal functioning, they have an applied side in genetic engineering as well. Researchers believe they will provide a controlled means of "stacking" large numbers of genes—say, for pathogen resistance—into an organism that could also be engineered to be eliminated when necessary.

One of Preuss's next interests is to use the minichromosomes to study why chromosomes don't always segregate properly and end up with two or no chromatids in a gamete instead of just one. If the gamete is used in fertilization, the resulting offspring will have an abnormal number of chromosomes, a condition known as aneuploidy. It happens between 1% and 2% of the time in *Arabidopsis*—about the same frequency as in yeast—but far more often in human meioses. The development of minichromosomes over the next 5 years "will open up tremendous resources" for exploring such phenomena, she says.

Preuss isn't the only one who thinks so. This past summer she became a Howard Hughes Medical Institute investigator, only the third plant biologist to be so recognized. To commemorate the occasion, Preuss's lab presented her with a framed collage of the pollen tetrads and the data they've generated. At the center is a photo of Howard Hughes holding a fistful of *Arabidopsis*. It's in bloom. —CHRISTINE MLOT

Christine Mlot is a science writer in Madison, Wisconsin.

CONSERVATION BIOLOGY

Galápagos Station Survives Latest Attack by Fishers

Researchers at the Darwin Research Station put the pieces back together after a festering dispute over fishing quotas turns violent

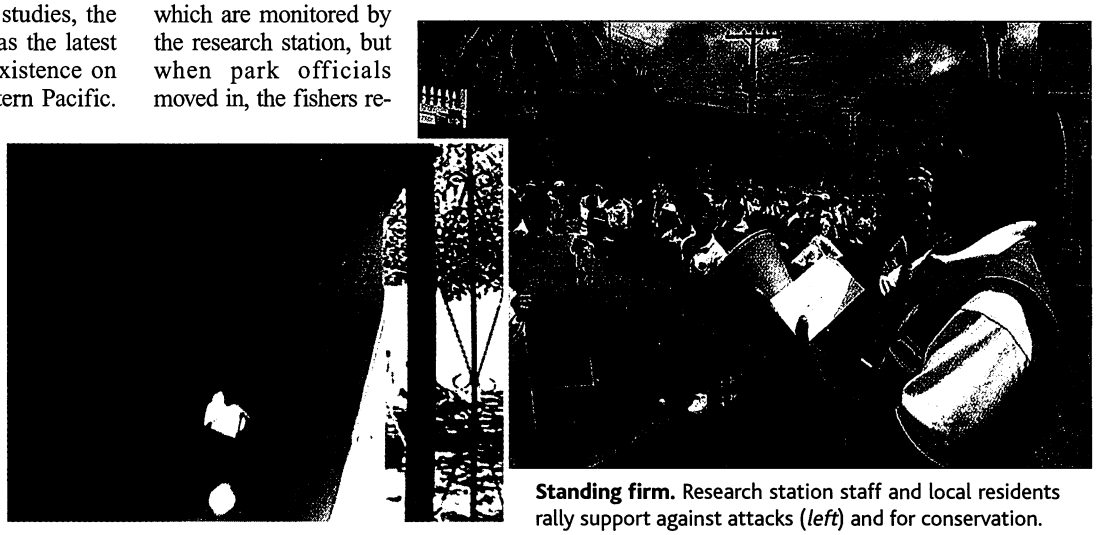
Botanist Alan Tye had a tough first week on the job after becoming acting director of the Charles Darwin Research Station in the Galápagos Islands in November. On Tuesday, he watched police put up barbed wire barricades after the research station on Santa Cruz was threatened with attack from local fishers. On Wednesday the fishers, angry over a quota on spiny lobsters that they feel is too low, hijacked Tye's dinghy during his commute to work. On Friday, Tye learned that Ecuadorian navy special forces had rescued two lab employees who, fearing for their lives, had taken refuge in mangrove swamps on Isabela, one of the station's three island sites.

For Tye and others who have spent years tending to the famed tortoise population and performing other conservation studies, the week of 13 to 17 November was the latest reminder of their precarious existence on this research outpost in the eastern Pacific. In 1995, fishers armed with clubs and machetes took researchers and their families hostage after authorities stopped sea cucumber fishing for the year (*Science*, 3 February 1995, p. 611). In 1997, a park ranger was shot after wandering into an illegal fishing camp. And earlier this year, fishers angry about sea cucumber quotas took several endangered tortoises hostage. Yet the station has survived for 36 years. "We've been through this before," says Tye, who hopes for peace on the 8000-square-kilometer archipelago, which lies 1000 kilometers west of the Ecuadorian mainland. "It's difficult at the time, but the experience throws us together and makes us more determined."

The fuse that set off the most recent conflagration was an annual 50-ton limit on spiny lobsters that local fishers reached barely halfway into the 4-month season. The quota had been set under a 1998 law that requires park authorities to consult with fishers, tourist service operators, and local officials and to draw on scientific advice from the Darwin station (*Science*, 20 March 1998, p. 1857). The law gives the National Park Service the authority to enforce the quotas, which are monitored by the research station, but when park officials moved in, the fishers reacted with what they term a "strike." Unruly bands laid siege to the station and the park service, blocked roads and offices, tore down the island's telephone antenna, and destroyed research records.

The park service bore the brunt of the fishers' anger. On the remote island of Isabela, where the two lab employees and park superintendent Juan Chavez were rescued from the swamps, rampaging fishers carried off computers and scrawled death threats on the walls. "They completely destroyed our office and burned absolutely everything," says park spokesperson Desiree Cruz in an e-mail. They also threatened Chavez's life and trashed his home. In other areas, fishers blocked tourist boats from landing, and a local school official who sided with the fishers threatened high school students who had written letters supporting conservation efforts. "Some of this protest activity approaches terrorism," says Darwin Station ecologist Howard Snell, who also teaches at the University of New Mexico, Albuquerque.

Some research at the station was affect-



CREDITS: PAOLA DIAZ/DARWIN RESEARCH STATION

Standing firm. Research station staff and local residents rally support against attacks (*left*) and for conservation.