

PROFILE: EDWARD BUCKLER

Romping Through Maize Diversity

A computer whiz turned geneticist borrows tactics from Wal-Mart and cattle breeders to manage what may be the world's largest genetic analysis

ITHACA, NEW YORK—On a steamy July morning, Edward Buckler and a crew of technicians, graduate students, postdocs, and a visiting professor from Mexico have fanned out among the 2-meter stalks in a large field of corn here. Bar-code readers in hand, they snip, stretch, or poke individual plants in order to track dozens of traits important to the crop's growth and vitality. Each week, they record the height of every stalk; in early summer, they counted leaves, assessed surface "hairiness," and took small samples of tissue to freeze-dry and send to Germany.

Welcome to the Nested Association Mapping (NAM) project, arguably the world's largest controlled genetic study. It encompasses more than 1000 genetic markers in each of 5000 lines of maize in an effort to elucidate the relationship between genes and physical traits in plants. "It's basically the maize analog of the human HapMap Project, but it is much more powerful and cost-effective," says population geneticist Magnus Nordborg of the University of Southern California in Los Angeles.

Buckler, the 38-year-old plant geneticist running the show, doesn't believe in thinking small. If he gets his way, plane flights will one day monitor tens of thousands of plants daily. "I'd like to know what goes on every hour of every day," says Buckler, a U.S. Department of Agriculture (USDA) researcher based at Cornell University here.

Buckler has capitalized on his combination of computer and biology expertise to develop methods to find genes faster. He and his colleagues have also used existing maize variants to boost the vitamin A content of corn. "Ed seems equally at home in the field pollinating maize as in the lab or developing software or doing theory," says plant geneticist J. Antoni Rafalski of E. I. du Pont de Nemours & Co. (Inc.) in Wilmington, Delaware. Adds James Holland of USDA at North Carolina State University

(NCSU) in Raleigh, "He has single-handedly influenced the plant genetics community to a remarkable extent."

The goal of the massive NAM study is two-pronged. Maize is the number-one crop produced around the world, and NAM will help breeders to exploit its natural variation to improve yields and nutritional value. In addition, Buckler expects to answer a fundamental question: Do a few genes underlie each complex trait, or is there a bewildering array, with each having a minor influence? The answer will not only help plant breeders, but it may also aid biomedical researchers trying to understand the genetics of diabetes, heart disease, and other disorders. "I expect we will learn a lot about quantitative genetics from the maize work, and this will, of course, help us to understand human variation as well," says Nordborg.

So far, it looks like more than a few genes control most traits. That realization will com-

plete attempts to pin down the genetic basis of disease. But with maize, even a 1% improvement in yield translates into millions more tons of food for people and animals, so genes of small effect can make a significant difference. Thanks in large part to Buckler's efforts, "people have changed their thinking, and companies are much more focused on natural diversity" as opposed to adding new genes to improve crops, says USDA plant geneticist Michael McMullen of the University of Missouri, Columbia.

Genetics by second nature

Growing up in Arlington, Virginia, Buckler had unlimited access to a personal computer, on which he designed his own games. To him, genetics is basically life's equivalent of computer programming. "There are not many rules: You get to recombine and to mutate, but you can make incredibly complex things," Buckler laughs, giving his boyish smile: "And it's more rewarding to do genetics than programming."

After high school, he left for the University of Virginia, Charlottesville. He studied early American cultures and became both fascinated with the domestication of maize and appalled at how inefficient agriculture was. "I decided that if I wanted to do something worthwhile, plant genetics was the way to go," he says. With a Ph.D. from the University of Missouri, Columbia, and postdoc experience in statistical genetics at NCSU, Buckler joined USDA in 1998 to work on the Maize Diversity Project, part of the Plant Genome Initiative (*Science*, 23 October 1998, p. 652). Now poised for its second renewal, the project has morphed over time from an emphasis on genome evolution to a massive effort to conquer the genetics of complex traits, with NAM as a key component.

As the Maize Diversity Project matured, Buckler and his colleagues came up with a more efficient way to find genes that influence traits. Researchers typically take two approaches to this task. In one, linkage analysis, they use families—which in corn means plants that can be traced back to the same set of parents. The other approach, association studies, relies on unrelated individuals, be they corn seedlings or people. "What we've been doing is blending the lines between the two" approaches, Buckler explains.



Ed Buckler

In association studies, researchers often look for gene variants that co-occur with a trait, such as golden rather than yellow kernels. But many of the variants they find are false positives, for example, having no effect on kernel color at all. The number of such false positives can be influenced by kinship among individuals and by evolutionary history. For example, when two populations are isolated from each other, their genomes can diverge in such a way that a particular variant might seem to be associated with kernel color, even though it isn't. "That's really a complicated and difficult problem," says John Doebley, a plant geneticist at the University of Wisconsin, Madison. Likewise, when two individual plants are closely related, their kinship can skew any associations detected.

With Jianming Yu, now at Kansas State University in Manhattan, Buckler has found ways to incorporate both history and kinship into his analyses, eliminating many false associations. For example, to take account of how closely individual plants are related, Yu, Buckler, and their colleagues used genetic markers to assess kinship, then borrowed mathematical tricks used by cattle breeders to analyze giant pedigree matrices. The resulting "unified mixed model" method greatly reduced the number of false positives, they reported in the February 2006 issue of *Nature Genetics*. Buckler estimates, for example, that about 9 million DNA variants, or SNPs, would show up as linked to flowering time using the old approach. The new method narrows that to only a few thousand.

The method has also yielded natural gene variants that enrich maize in vitamin A. Buckler, Torbert Rocheford of the University of Illinois, Urbana-Champaign, and their colleagues measured the amount of the vitamin in hundreds of lines of corn that vary in kernel color—the more orange, the more vitamin A. Using association studies, they pinned down the gene variants responsible for producing more vitamin A precursors (*Science*, 18 January, p. 330). Without this method, "we would have had a lot more junk to deal with," says Buckler. Now researchers with HarvestPlus for Africa and elsewhere are using Buckler's genetic markers to breed those variants into maize varieties, thus boosting vitamin A without introducing foreign genes.

Bigger is better

Once Buckler started doing association studies, he hungered to make them more powerful. For NAM, he and his colleagues



Maize maze. This experimental cornfield in upstate New York will help researchers pin down the genetic basis of traits such as kernel color (inset).

picked 25 unrelated "parent" lines of maize, including popcorn, sweet corn, tropical and temperate varieties, plus long-used commercial strains, representing the full range of diversity in this species. Buckler, Cornell colleague Stephen Kresovich, Holland, and, later, McMullen bred each line with the much-studied (and now draft-sequenced) B73 maize. From each "parent," they created a "family" of 200 new lines, for a total of 5000 lines. Thus this single study includes "families" available for linkage analysis, as well as a large, diverse population of 5000 lines for association studies. The next biggest genetic study involves mice, uses just eight strains, and has the ultimate goal of creating 1000 new strains (*Science*, 25 July 2003, p. 456).

NAM also presented an enormous data-crunching challenge. At the time, "it was not obvious to me how gene-phenotype association information could be jointly analyzed across the 25 cross-populations," Holland recalls. "Ed conceived of the analysis that would efficiently achieve that." Nor was Buckler daunted by the challenge of generating thousands of new maize lines and recording how individual plants grew. His response to his colleagues' concerns: Borrow methods from an operation that daily tracks tens of thousands of items—Wal-Mart. He outfitted his team with the same portable bar-code scanners that Wal-Mart uses for taking inventory and had them tag each plant.

Now that the hard work is done, anyone can grow out the seeds of the NAM lines or traipse through the project's fields to measure variation in their favorite trait, such as starch content. Then, using the project's analytical tools, they can home in on the genes affecting that trait.

Buckler calls the NAM project a "field of

dreams," and, as in the movie of the same name, it's attracting attention and copycats. Dozens of private and academic researchers have ordered NAM seeds from USDA to start their own fields. Cornell's Rebecca Nelson and graduate student Jesse Poland are assessing the genetic basis of disease resistance in a NAM field next to Buckler's. Says Poland: "It's an incredible resource."

Yet even in the dream fields, pinning down genes will not be easy. "Association mapping is not without its problems," says Rafalski. Human geneticists have millions of markers to help navigate the human genome, and they still struggle to find gene variants connected to disease; maize researchers have only about 1100 markers. And picking out which associated variants are the most promising is always a challenge, he adds.

However, Buckler is still thinking big. He has \$1 million from the U.S. National Science Foundation to partially sequence each parent NAM maize strain, which will yield many more markers. He has also set his sights beyond cornfields: He wants to apply genomics to USDA's vast archive of germ plasm—seeds and other tissues from all plants. Breeders can order any of some 600,000 crop varieties from USDA, but it's often hard to know which varieties will improve a crop the most. To begin to find out, Buckler is starting with the grape germ plasm on file, assessing 10,000 SNPs and, to a limited extent, their association to relevant traits. He's hopeful money will come through next year to assess the SNPs in the entire USDA collection. "That Ed is an idea person is as much of an understatement as you can say," says McMullen. "He's always proposing new ideas, and even before we can do the experiment, [the project] will be bigger."

—ELIZABETH PENNISI