PEGGY GREB (K11920-1)

Who's Who in the Nation's Largest

Citrus Collection?

For most of us, the word "citrus" conjures up images of oranges, grapefruits, lemons, limes—and possibly citrons or blood oranges, if you're a true connoisseur of fruit.

But for ARS horticulturalist Robert R. Krueger, the genus *Citrus* includes at least 16 species, with hundreds of distant relatives, including lesser-known, peelable oddities like pummelos, trifoliate oranges, and kumquats.

Fruits, vegetative tissues, and seeds showing the diversity of the citrus accessions maintained at the National Clonal Germplasm Repository for Citrus and Dates.

Krueger, who is with the ARS National Clonal Germplasm Repository for Citrus and Dates, in Riverside, California, helps look after the nation's largest collection, or genebank, of citrus trees. The trees, as well as their seeds, budwood, and pollen, serve as valuable germplasm—plant tissue containing the essential genetic information needed to start new citrus plants.

The citrus genebank—a University of California-Riverside (UCR) resource that is used cooperatively by the ARS repository—has been gathering accessions for almost a century. Right now its holdings number about 900 specimens, many of which were acquired outside the United States. But it hasn't been known, until recently, just what the collection's real breadth and scope were.

"There were redundancies and gaps in the collection that needed to be sorted out," says Krueger, "and some specimens had even been mislabeled." Mistakes are costly for curators, since a few missing or added letters can change the way a specimen is classified and managed—and possibly how it will be used.

"For example, one accession planted in the field," says Krueger, "was considered to be a Rangpur lime, or *Citrus limonia*. But when you walked past it, you'd think for sure it was a lemon. It looked like a lemon, smelled like a lemon. Eventually I came by some records from the 1950s indicating that the tree actually was a lemon with the scientific name *Citrus limon*. Somewhere along the way, someone had mistakenly attached those two letters to the species name."

A Core Collection

With the help of Mikeal Roose, of the UCR Department of Botany and Plant Science, and former graduate student Noelle Barkley (now with ARS in Griffin, Georgia), Krueger set out to determine the connections among the hundreds of citrus accessions—to see which ones were genetic duplicates and which were novel and distinct.

The project was no small feat. According to national program leader Peter Bretting, who has programmatic leadership responsibilities for all ARS germplasm collections, the set of genetic data they generated was one of the largest ever compiled of its kind.

"Now," says research leader and plant pathologist Richard F. Lee, "we've got a relatively small core subset that represents much of the diversity in the *Citrus* genome. This makes it easier to search for specific genes related to pest and pathogen resistance and to high flavonoid and nutrient levels."

For their work, the researchers used 25 molecular markers, 13 of which they created, to help them track genetic similarities between accessions. The markers—not unlike those used in forensics for determining genetic identity and parentage—helped the researchers identify specimens of unknown origin and differentiate between closely related species.

Of the roughly 900 total accessions, about 400 of unknown, but probable, sexual origin were studied. The others were either known to be crosses from breeding programs or mutations from a parent tree—so they wouldn't differ enough from other accessions to be surveyed.

"About 50 of the 400 accessions represent more than 90 percent of the collection's true diversity," says Krueger. "That's just 13 percent of the collection."

That may sound surprising, but it reinforces a long-held theory among citrus experts that there are just a few naturally occurring forms of citrus in the world and that the rest are hybrids of these ancestral forms. The research also supports the generally held concept that most familiar citrus fruits—including oranges, grapefruits, and lemons—are actually hybrids.

A Chosen Few

Part of this can be explained by the fact that citrus, which is native to Southeast Asia, hybridizes readily.

"Citrus has been domesticated and selected for millennia," says Krueger, "so it's possible that no early ancestors have

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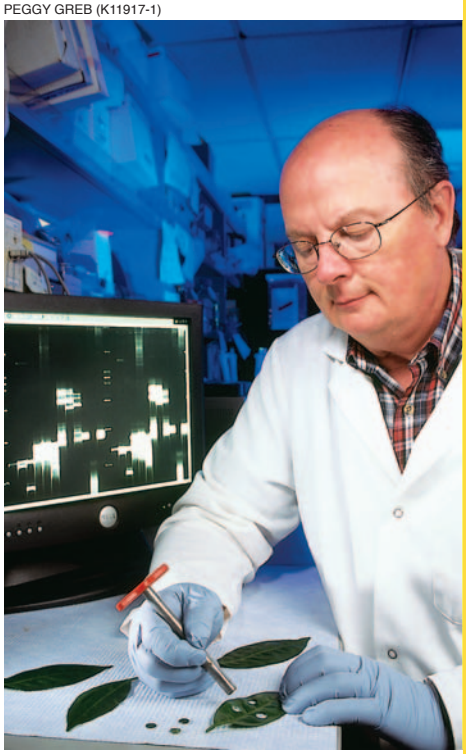

Plant pathologist Richard Lee prepares leaf samples for nucleic acid extraction.

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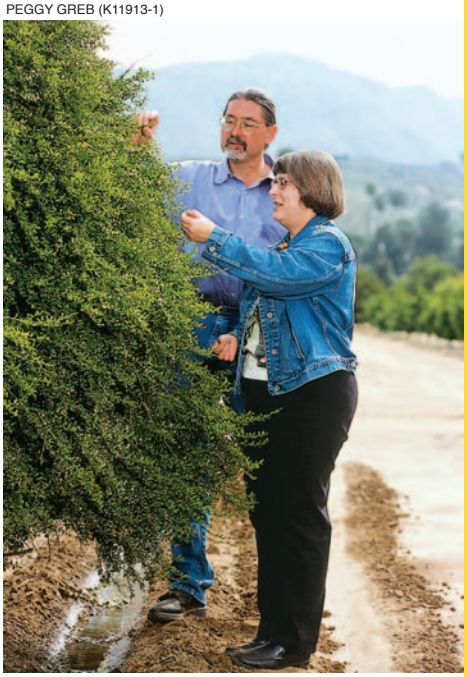

ARS horticulturalist Robert Krueger and Tracy Kahn, museum scientist at UC Riverside, examine leaf structure of a wild citrus relative maintained at the genebank.

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ARS horticulturalist Robert Krueger (left) and Mikeal Roose, a professor at the University of California-Riverside, discuss genetic diversity of a citrus accession called Som Keowan Mandarin.

escaped modification by humankind. But it's worth investing in the plant exploration needed to find out."

Because citrus readily crosses and the resulting plants can be propagated true-to-type through grafting, selective breeding efforts encouraging desirable qualities—like sweetness—have been very successful. But breeding for certain traits can mean others are inadvertently lost.

"Selective breeding has led to advancement of certain 'elite' germplasm lines," says Krueger, "often at the expense of wild, ancestral types."

This doesn't mean that new citrus types won't be developed through conventional breeding and biotechnological breeding efforts. "But citrus breeding efforts probably won't receive new genetic material from the area of origin because of the threat of habitat loss and the political challenges surrounding international exchange of plant materials."

Citrus In Situ

In situ preservation—conserving plants and organisms in their indigenous habitats—offers research opportunities available nowhere else. Like outdoor laboratories, citrus preserves allow the timeless interplay between citrus plants and their pesky pests and pathogens to carry on untouched—providing scientists with important glimpses into the plants' finely tuned, natural defenses.

"But germplasm maintained in this manner is more vulnerable to natural disasters and other events that could destroy valuable genes," Krueger says.

This makes "ex situ" collections, like the Riverside citrus genebank, particularly critical—especially since many of the world's wild citrus stands are at risk from encroaching industrialization. "Citrus's area of origin—northern India, southern China, and nearby Southeast Asia—is experiencing rapid population growth and development," says Krueger.

He hopes to travel to southern China to assess firsthand the amount of citrus diversity still present there. Molecular techniques now available should make the task of recognizing novel citrus specimens much easier. As time allows, he also plans to continue to peel away the evolutionary mysteries still surrounding this favorite fruit.—By **Erin Peabody,** ARS.

This research is part of Plant, Microbial, and Insect Genetic Resources, Genomics, and Genetic Improvement, an ARS National Program (#301) described on the World Wide Web at www.nps.ars.usda.gov.

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Biological technicians Patricia Nielsen (back left), Vicki Newman (back middle), and Jaclyn Sweet (foreground) work to propagate and maintain experimental plants in greenhouses of the National Clonal Germplasm Repository for Citrus and Dates.