Peruvian Cacao Collection Trip Yields Treasures


ARS (D2286-2)

A village on the bank of Rio Pastaza, in Peru. Two wild cacao populations were found and sampled near the upstream portion of this river.


PEGGY GREB (D2285-1)

Fortunato No. 4 chocolate, a fine-flavor product made from the Pure Nacional type of cacao identified in northern Peru.

Chocolates are always in demand—from Valentine's Day and beyond. In the chocolate world, the fastest growing segment of the industry is fine-flavor, high-end chocolates. Until now, the source of these specialized confections has been largely limited to small regions of Venezuela and Ecuador.

A stand of very old trees, in an unexpected location, has yielded a coveted type of cacao tree. Usually, cacao trees are found along rivers, but these gems were found at a higher altitude than normal, and in Peru instead of Ecuador or Venezuela.

Collection expeditions in 2008 and 2009 through the Amazon Basin of Peru uncovered the exceptional find, along with other distinctive new populations of cacao.

Agricultural Research Service researchers at the Sustainable Perennial Crops Laboratory (SPCL) and the Systematic Mycology and Microbiology Laboratory (SMML) in Beltsville, Maryland, and Peruvian collaborators came away with hundreds of new cacao tree samples from these trips. One of these, discovered by additional collaborators from Marañon Chocolate, was Pure Nacional—an old, very rare, and highly coveted variety that has garnered a great deal of interest from makers of fine-flavor chocolates.

SPCL research leader Lyndel Meinhardt, SPCL geneticist Dapeng Zhang, and SMML mycologist Gary Samuels (now retired) collaborated with the Instituto de Cultivos Tropicales (ICT), a research center in San Martin, Peru, to identify the new varieties of cacao. The researchers are studying 342 cacao specimens collected from 12 watersheds and categorizing the

DNA of the specimens. The group has identified new cacao types with unique flavors that are distinctly Peruvian, which may one day be marketed in the same way as wine—by geographical provenance.

Start from the Beginning

The cacao tree, *Theobroma cacao,* produces beans that provide the raw material to make chocolate. The tree is cultivated in some tropical countries with the right environmental conditions. The origin of cacao is the Amazon region of South America. Like many tropical tree crops, seeds of this plant lose their viability quickly after being harvested. For this reason, varieties or types of cacao must be maintained in living germplasm banks.

"The majority of the material in cacao germplasm banks was collected prior to the 1940s. There are more than 5,000 different varieties of cacao currently in collections around the world," says Meinhardt. "While this sounds like a large amount, most are breeding lines derived from a small number of types, so it actually represents a small fraction of the genetic diversity that still exists in the wild, especially in the center of origin of this species."

To address this limitation, expeditions were begun in 2008 to explore the upper Amazon River area in Peru. The purpose of these trips was to find and collect wild cacao trees and attempt to establish them in a living germplasm bank in Tarapoto, Peru, Meinhardt says. These trips were jointly funded by the U.S. Department of Agriculture and INCARGO, Peru's Ministry of Agriculture.

ARS (D2286-1)

Using a sterile technique, ARS scientist Gary Samuels extracts a sample of living plant tissue from a wild cacao tree on the bank of Rio Marañon in Peru. Fungi in the sample could prove useful as a biological control agent of important cacao pathogens.

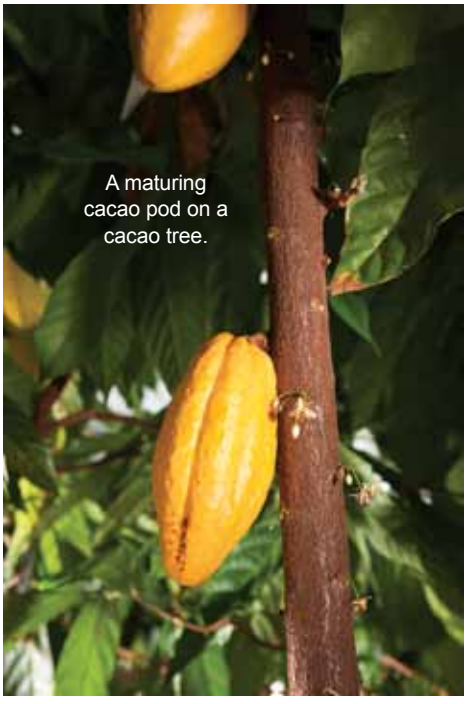
"In 2008, 7 river systems were explored, and 190 cacao trees were sampled. Of the initial 190 trees collected, 128 were successfully reestablished in the germplasm bank. In 2009, 5 more river systems were explored in 2 expeditions and a total of 152 trees were collected and reestablished in the germplasm bank in Tarapoto," explains Meinhardt. "From the 12 river systems explored, we have identified 3 completely new populations of cacao that were not previously known to science." These expeditions collectively represent one of the largest efforts ever conducted to search for wild cacao.

New Flavors and Disease Resistance?

These new populations could be sources of diseases resistance or could have potential new flavor traits. The fine-flavor chocolate industry is keenly interested in obtaining new and unique flavor sources.

"Our results combined with the needs of the fine-flavor chocolate industry have led to new collaborations that will look at the primary gene pool of cacao. Together with industry we will attempt to gather information on the genetic diversity of wild cacao from all of the countries in South America within the center of diversity," says Meinhardt.

Niche chocolates from South America are not new. The varieties Arriba, from Ecuador, and Porcelana, from Venezuela, are two of the most famous. Arriba has a strong, complex taste that stays on your



A maturing cacao pod on a cacao tree.

PEGGY GREB (D2282-1)

At the Sustainable Perennial Crops Laboratory in Beltsville, Maryland, technician Stephen Pinney (right) and visiting scientist Kun Ji prepare cacao leaf samples for DNA fingerprinting.

PEGGY GREB (D2284-1)

ARS (D2286-3)

During the 2008 collection trip, ARS researchers Gary Samuels (left) and Lyndel Meinhardt (center) and plant pathologist Enrique Arevalo, of the Instituto de Cultivos Tropicales in Peru, examine cacao leaves infected with witches' broom.

tongue for a long time, while Porcelana features a unique light fruit flavor. ARS and ICT are helping Peru create its own niche in the chocolate industry by working with San Martin's Oro Verde cooperative and Marañón Chocolate. Peru's tropical conditions—60 percent of the country is covered in tropical forest—make it ideal for producing exceptional chocolates.

PEGGY GREB (D2283-1)

In Beltsville, Maryland, Gary Samuels examines microscopic structures of fungi collected in Peru.

Witches' Broom Watch

During the 2008 collection trip to Peru, Meinhardt also recorded the incidence of the devastating witches' broom disease (WBD) in wild cacao trees in the upper Amazon region. The scientists studied areas along the Aypena, Charupa, Nucuray, Pastaza, Ungumayo, Ungurahui, and Urituyacu Rivers and determined the overall severity of WBD infection based on the percentage of symptoms on flower cushions, flushes (new stem growth), and fruits.

A team of scientists, including ARS's Zhang, Meinhardt, and Samuels and ICT plant pathologist Enrique Arevalo, found that 14.7 percent of flower cushions and 13.7 percent of trunks were infected, and 9.1 percent of the trees along the Aypena River were infected. The other river areas had similar results.

The incidence of WBD observed during the survey suggests that there is a high level of WBD resistance in these wild Peruvian cacao populations. The scientists are now studying the samples to determine which are best suited for both unique flavor and WBD resistance. WBD can cause yield losses of 75 percent in susceptible varieties.

Friendly Fungi

In addition to collecting cacao germplasm, the team isolated other fungi from disease-free leaves and trunks of the wild cacao trees. This large collection of "endophytic" fungi—fungi that occur in disease-free tissues of all plants—may provide protection against diseases such as WBD either by stimulating the immune system of the plants or through direct parasitism or antibiotic effects against pathogens. Samuels found that several fungal species previously unknown to science were found in the cacao tissues. The potential for biological control using these endophytic fungi is being evaluated at Beltsville by SPCL scientists Bryan Bailey and Ron Collins.

The genome for WBD has been sequenced and it may hold clues for developing control measures to reduce its impact in the future. This was reported in the journal *Biomed Central Genomics* in 2008.

While scientists have the genomes of some cacao populations in hand and are working diligently to improve production and disease resistance, improvements can sometimes lead to unintended consequences, like a change in flavor, according to Meinhardt.

"There are a lot of great chocolate sources; the task is to find them and preserve them before they are lost," says Meinhardt. "Mother Nature has done a great job of creating these exceptional cacao trees."—By **Sharon Durham,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301), Plant Diseases (#303), and Crop Protection and Quarantine (#304), three ARS national programs described at www.nps.ars.usda.gov.

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