

# Chestnuts in China

by Rebecca Hirsch

All photos by Sara Fitzsimmons

Sara Fitzsimmons and Katie D'Amico were at Newark Liberty International Airport when they got word of a problem. After months of careful planning, endless meetings and hundreds of emails and phone calls they were finally headed to China to study Chinese chestnuts in the wild. Now standing in line at security just moments from their flight, they learned via an email that there had been a misunderstanding with Chinese officials. The trip might be off.

For Sara, a Regional Science Coordinator for TACF, this was a return visit. She had traveled to China in 2008 with other TACF scientists, locating wild Chinese chestnuts with different levels of blight resistance. This trip would be led by Dr. Bill Powell of the State University of New York College of Environmental Science and Forestry. He would be bringing members of his lab: graduate student Katie D'Amico, technicians Andy Newhouse and Kathleen Baier, and Chinese graduate student Amelia Zhang. The trip was supported by TACF and the USDA Scientific Cooperation Exchange Program. As part of the exchange, Chinese officials had planned the itinerary and made all travel arrangements.

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The goal of the visit was to collect DNA, RNA and metabolites from wild Chinese chestnuts, material that could lead to the discovery of the genes involved in blight-resistance. The American team would first go into the field to collect samples and then extract DNA



The team in the field with their guide, Mr. Chen. From left: Andy Newhouse, Sara Fitzsimmons, Dr. Bill Powell, Katie D'Amico, Amelia Zhang, Mr. Chen.

and RNA at the lab of Dr. Xiangning Jiang, their collaborator at Beijing Forestry University.

Chinese officials viewed the visit differently. They saw it as a chance for the U.S. team to talk about their work with Chinese scientists and officials. The fieldwork was secondary, and the itinerary reflected this, with the fieldwork stuffed into the latter part of the trip.

“That’s not what we wanted,” Bill Powell told the interpreter, Mr. Lin Luogent. “We’re not going to come unless we can do this.” The field days needed to come first so they had time to extract their samples.

Mr. Lin explained that it would be difficult to change the trip now. Each province had its own officials. Each of them would have to be contacted. Bill resolutely held his ground and Dr. Jiang weighed in. He stressed how important it was for the group to get samples. Finally, the interpreter agreed to reroute the trip.

Arriving in Beijing, the group met their hosts, Dr. Jiang and Dr. Wei He. In Dr. Jiang’s lab they unpacked materials and prepared the supplies they would need in the field. Then there was time to squeeze in a little sightseeing. They visited the Olympic Village and dined that evening on Peking duck, a signature Beijing specialty that included a side dish featuring the head of the duck, split in half, cooked to a crisp, and served on a plate.

But their challenges were just beginning. Before leaving Beijing to fly west to the city of Xi’an, their hosts warned that there had been torrential rains in the area, causing mud and rock slides in the mountains that were their final destination. The forecast was for more rain.



Dr. Bill Powell looks on as Andy Newhouse takes a sample from a tree while Katie D'Amico takes notes.

### Into the Mountains

Three species of chestnut trees grow in China: *Castanea mollissima*, *C. seguinii* and *C. henryi*. All are blight resistant, but according to local experts, *C. henryi* is the most resistant, followed by *C. seguinii* and *C. mollissima*.

Groves of chestnuts are easy to find in China, but the American group didn't want cultivated trees. They wanted wild, timber-type trees, preferably six of each species, three healthy trees and three with cankers. That made eighteen trees to find.

The search started with a bumpy flight into Xi'an. They met their driver, a quiet man who spoke no English, but had a fondness for the accelerator of their van. They tore along the rain-slicked highway and shot through long tunnels that threaded the mountains. The driver however, was forced to slow around curves littered with mud and rocks, places where the rains had washed the mountain onto the road.

Farther on, the road narrowed as it cut deeper into the mountains. Rain drummed on the roof as the driver forced the ancient van to its limits around hairpin curves. On one side the land rose straight up. On the other, it plunged into scenic valleys with nothing between the van's passengers and infinity but short stacks of concrete blocks placed here-and-there along the shoulder.

The Chinese countryside was crowded by American standards. The houses they passed had no yards, just farm fields marching up to the door. When they stopped in towns, people crowded around, curious and concerned. Surely they wouldn't travel into the mountains now. Hadn't they heard the roads were dangerous?

### Searching for Large Chinese Chestnuts

The question of how Chinese chestnuts can fight off the blight has long puzzled researchers. The answer lies in a handful of genes that control blight resistance. When these genes are expressed, or "switched on," the information in the DNA is copied into strands of RNA. That expression most likely happens along the margins of cankers, where the tree is trying to wall off the pathogen. If researchers could isolate RNA from the canker margins, it might lead them to genes for blight resistance.

But RNA is a notoriously tricky molecule. Unlike stable DNA, RNA breaks down easily, dissolving into maddening bits that yield no information. To keep the strands intact, researchers typically plunge tissue into liquid nitrogen, freezing samples to a chilly  $-320^{\circ}\text{F}$ . But in the mountains of Shaanxi province, that wouldn't



The washed out road from LanGao to Zoujiaping.

be an option. Instead, the group put their trust in a product that promised “immediate RNA stabilization and protection,” a liquid called RNALater.

After hours of winding roads, the group trekked up Nan Gong Mountain with their guide, Mr. Yu-Zhao Chen, who, like their driver, spoke no English. Nonetheless, under Mr. Chen’s guidance, they were able to find what they were looking for. Here, thousands of miles from where they started, the mountains revealed wild Chinese chestnut trees. Sara and Mr. Chen clambered up and down the slopes, choosing the best trees, and Amelia followed, translating. Once a tree was selected, Andy punched a cork borer into the trunk and pulled out a plug. Bill and Kathleen took turns cutting up the plug and dropping the pieces into tubes of RNALater. Katie sterilized equipment with a bottle of alcohol and a hand-held lighter. They often had to grab onto trees to keep from sliding down.

On the second day, six large *C. mollissima* were left to find. They drove for hours, now crossing a creek, now heading up again, on through the mountains in search of trees. In one place, heavy rains had almost completely washed the road away, leaving barely enough level ground to allow a single vehicle to transit. Everyone got out and walked and the driver inched the van through the narrow pass. In another spot, a rock slide blocked the road. Again, everyone disembarked as the van backed down the mountain. This time, they found a small 20-year-old *C. mollissima* beside the road and took a sample. Unable

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Dr Powell cutting up the stem segments and putting them in the test tubes with RNALater. Andy is in the background using a cork borer.

to continue forward, a dejected Mr. Chen told them of numerous huge *C. Mollissima* that lay beyond the rockslide, reachable by a two-day hike.

### Back to the Laboratory

At last, the group was satisfied with the samples they had collected. It was raining as they started the drive from Ankang to Xi’an. Again, they wound along the mountain roads and slipped into the long, dark tunnels.

It was as they rocketed through one of those narrow tunnels that the van suddenly began to drift. The driver turned the wheel one way, but the van wobbled the other. Something was wrong with the steering. Suddenly the van slewed to the right, slamming into the tunnel wall and bouncing off, thankfully leaving only minor body damage and some shaken passengers. With no services until the next town, they had no choice but to slowly keep going, thankful that the steering had not given out along a steep drop-off. They would stop at the next town and assess the problem. Wobbling back and forth across the road, the van soon attracted the attention of a police officer, who escorted them into town.

There they learned that the twisting and turning of the mountain roads had loosened the fittings that connected



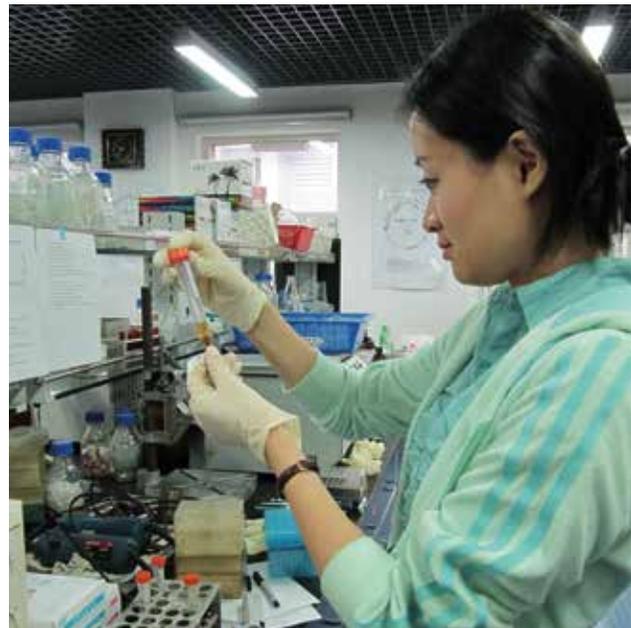
The team hikes up a mountain path through a bamboo grove to to the last collection point.

the axle to the front wheel. While the van was being repaired, the group seated themselves at a local restaurant. After four days of harrowing travel, they were ready to be back in Beijing. They had managed to get all of their samples, but the big question remained: would the RNA still be intact?

In Beijing, the group had two days to do the extractions. Dr. Jiang sent his own students home and graciously turned his lab over to the Americans. They froze tissue in liquid nitrogen and ground it with a mortar and pestle. What had been a chunk of wood became a fine frozen powder. The powder was scraped into tubes, liquids were poured, samples spun in a centrifuge. In the end, they had two sets of tubes. In one set was genomic DNA. It would travel to Penn State University for sequencing, part of an effort to read the Chinese chestnut's genome. The other set was RNA. Because RNA is so fickle, the group carried out an extra step, using an enzyme to copy the RNA back into a stable molecule of cDNA, or complementary DNA.

It was later, back in the U.S., that Bill Powell and his group learned that the RNA had broken down during their trek through the mountains. The samples were too degraded to be of much use in finding new genes. Fortunately, they were intact enough for the researchers to go looking for genes already suspected of having a role in blight resistance. They have used genetic engineering to add these genes, one by one, to the American chestnut's own DNA, and are testing whether they can give blight resistance to the American chestnut.

"It was a great experience," said Bill, speaking of the China trip. "We cloned 6 genes so far, we might clone



Amelia Zhang goes through the protocol to extract metabolites from the chestnut disk samples.

more." He pauses, then adds, "I was pretty amazed, Chinese chestnut trees are not that big. They're not like American chestnut trees."

Sara however still remembers what Mr. Chen had told her in the mountains. Huge Chinese chestnuts are out there somewhere, only a two-day hike beyond the last road. Just waiting.

*You can read more about the China trip at Sara Fitzsimmon's Chestnuts in China blog: [http://www.personal.psu.edu/sff3/blogs/chestnuts\\_in\\_china/](http://www.personal.psu.edu/sff3/blogs/chestnuts_in_china/)*