Steve Sheppard: Genealogy Toward a Better Bee
Getting Back to Where They Once Belonged[1]
Part I

The bees of the new territories represent in their variation only a part of the natural variation of the bees in the original countries. This is because all the millions of colonies in the New World today descended from a relatively small number of queens brought across the sea in those days.
Freidrich Ruttner, The Hive and the Honey Bee, 1974[2]

by M.E.A. McNeil

They'd been warned away from the border with Russia, but they'd already come nearly 7000 miles for this prize, deep in the Caucasus Mountains, and weren't about to quit because of a six hour drive up precarious dirt roads in a war zone all too recently plagued with robbing warlords. No: they saw some hives, and they saw drones. So there they were, in the apiary of a drunken beekeeper, collecting semen from *Apis mellifera caucasica* when a carload of shirtless men accosted them, shouting.

Steve Sheppard, together with his Washington State University colleague Sue Cobey and grad student Brandon Hopkins, were on a mission to gather germplasm from Old World populations of honey bees. After years of testing both feral and kept bees across the US, Sheppard concluded that New World stock could be enhanced by increased genetic diversity, and he was going about collecting it.

Sheppard is an expert on the genus *Apis*, its 10 or 12 species, and, in particular, on the subspecies of *Apis mellifera* – 26 to 28, depending upon who is counting. "There are a lot of weird subspecies, some with really unique behaviors. There are ecotypes within these subspecies attracted to a particular plant within particular areas," he said.

All of this differentiation took place over millennia. "For the European subspecies this is the theory," he said. "18,000 years ago there were glaciers in Europe. The honey bees were separated in little refugia, and there was restriction to gene flow. In these isolated areas they adapted over time to their ecological milieu, accumulating genetic variations. This is the model for how speciation occurs."

"With species we can use this nice rule that they don't generally interbreed. But that hasn't happened long enough with these subspecies, which of course will interbreed. Subspecies doesn't really have much of a meaning within insects, but in the case of the honey bee I think we have a pretty good case. They reflect 'geographic races', as Ruttner called them. They usually have behavioral differences. And there was typically some sort of barrier to gene flow until humans came and moved them all over. So using subspecies allows us to know what we are talking about."

"Some of the obvious differences are morphology: in general Italian bees are yellow, and Carniolan bees from the Alps are black. The North African bee is two thirds the size of the Italians. There are differences in behavior: if you are a bee that lives in sub-Saharan Africa, there is never a winter, never a time when you're not going to be able to fly, but there are going to be times when there is no food. So the scheme to deal with this is to swarm a lot, and to abscond to find food. Whereas if you are a bee living in northern Poland, there is going to be a winter every year and you had better store up a lot of food. If you swarm and have little colonies, you are not going to make it through the winter. So there is a lot of difference in behavior between these different subspecies. And we now have all kinds of genetic markers to look at this."

Sheppard, a professor at WSU, also heads the Apis Molecular Systematics Laboratory there where much of this work on population genetics and the mechanisms of genetic differentiation is done. He was a member of the international consortium of researchers that sequenced the honey bee genome in 2006 and co-authored a study pointing to the origin of the honey bee in Africa, replacing the commonly held view that it came from Asia.

There has been some confusion over the twin facts that Sheppard is bringing bee genetics in from The Republic of Georgia, in Eurasia, and that his personal germplasm came from Savannah, Georgia – a kind of cultural of refugia that has bred the behavioral characteristics of his gracious, gentle Southern manner.

His great-grandfather, who kept a couple of hundred hives on the floodplain of the Savannah River along the North Carolina border, died when Sheppard was very young. As a boy he grew up playing with the bee equipment left in the workshop on visits to Granny, and he can still taste the memories in tupelo and gallberry honeys.

So, as an undergraduate at the University of Georgia, he took a beekeeping class from Al Deitz and had some 35 colonies of his own. “I started keeping bees as an undergraduate back when it was fun, when there were no mites. I thought the coolest thing in the world was to buy different subspecies and breed my own queens. It's really part of the art of beekeeping,” he said. “I would get Italians, and back then you could get Midnights and Starlines. And you could buy what were sold as Caucasians and Carniolans. Really just black or yellow bees. I used my great-grandfather's extractor.”

Then Sheppard read a chapter in the 1974 edition of *The Hive and the Honey Bee*, “The Races of Bees” by Friedrich Ruttner, a still highly regarded Austrian researcher. At that time morphometrics and behavior were the measures of differences. Ruttner posited that because of the comparatively limited number of European bees brought to other countries, their degree of variation was more narrow than in the parent populations.

The essay so fascinated Sheppard that he answered an advertisement to go to graduate school at the University of Illinois under Albert Jaycox. In the process of completing his master’s degree on pollination biology, he worked with three subspecies, Italians, Carniolans and Caucasians to compare their pollen collection from soy. “An observation during this work was what led to the thesis for my PhD,” he said. “The dark bees weren't really Caucasians and Carniolans anymore, they were just what people had selected, mainly by color. But they still had some of the original traits. I noticed in the fall, the dark bees drove the drones out viciously within a three or four day period. The Italians in the same yard weren’t doing anything; then a week or two later, the Italians were lackadaisically pushing some drones out, and then the drones would run back in. It was a marked difference.”

His initial thought -- which he said was “naïve but turned out to be not so bad” -- was to compare characteristics of feral bees in the US to those in similar areas in Europe. What he soon found out was that in this country the subspecies had, for the most part, not been studied at all.

His PhD advisor at U.I. was Stewart Burlocker, a population geneticist in a lineage through his own advisor Guy Bush at Harvard, an expert in sympatric speciation, and his advisor Ernest Meyer, the evolutionary biologist responsible for the standard model for how species are formed. Meyer found that species develop not only by barriers to gene flow, like glaciers or mountains (allopatric speciation) but also by availability of a new niche, like forage (phytophatic speciation) – then a revelation. Chronicling these intellectual forebears makes a point: Sheppard studies bees through a large lens.

“The genetic markers that we had at that time, allozymes -- polymorphic proteins – were found with a process called electophoresis,” he said. “Back in the 60s they were the real hot thing for population genetics. They were still quite useful, but now we're talking the early 80s.” He spent a year in the lab adapting the technology to test for markers in bees.

“I didn't have the money as a student to go to exotic places, but I could send a nitrogen container.” He got samples of Apis subspecies from Norway to Czechoslovakia as well as from the US and, in addition, other species from Sri Lanka. Apart from learning that he could get stung by a dead bee, he found, from the standpoint of population genetics, there was less variation in the American subspecies than the Europeans.

By then, available technology allowed him to look at mitochondrial DNA. “Every time I looked at one of the subspecies from Europe I found new genetic variants, things that we had not seen in the US. And by far the Old World had more genetic variants that those in the US.

He could read some stories in the genes, but not all. What bees had initially been imported to the US, when, how many? He knew that the first record of European bees in the New World, the black bee *Apis mellifera mellifera*, dated to 1622 in Virginia – with some slight evidence that the Spaniards had brought them a century before. “I believe, and this is my speculation, that probably no one brought anymore,” said Sheppard. “It was so expensive, and 90% or so would die on the voyage over, it took so long. This was the American honey bee for over 200 years.”

To find out more, he went to the University library and checked out every American Bee Journal from 1861 to 1922 – the dates between the availability of steamship service and the Honey Bee Restriction Act of 1922 that curtailed imports. For six months he scoured the magazines for mentions of importations. He found that the first Italian bees were brought over in 1859. “I can't tell you how crazy people went for these bees,” he said. “They were so sought after that the queens were $100 each in 1860.” A rash of attack ads appeared between two importers stating such things as the other’s bees are “known to be of impure race.”

Other subspecies were brought in as well: *lamarckii* from Egypt, *cypria* from Cyprus, *syriaca* from Syria and *caucasica* from the Caucasus Mountains as well as *carnica* from the Alps, Austria and Slovenia. Some of these were heavily promoted, like *intermissa* from North Africa – then called the Punic bee. It was called “The most wonderful race of bees on earth”, a claim quickly dispelled when they were found to sting readily.

The three that found favor with US beekeepers were the Carnicas, Caucasians and the Italians, which never gave up their position as the most popular. Until the 70s all three were advertised in the bee journals, with Caucasians available through the Sears catalog.

Eight subspecies of the 28 that are known were imported. “For each subspecies that was brought in there were just a few tens or hundreds of queens,” said Sheppard. And the stock was almost certainly not selected from across the parent range, if only for commercial expediency.

“You can have ten times as many colonies in the new place as you had in the old place, but you do not have an increase in genetic diversity. If you bring characteristics over that are in relatively low frequency in your original sample, you can lose those characteristics by chance, genetic drift. That's a bottleneck, and that's just to start with,” he said.

As a research entomologist at the USDA lab in Beltsville, where he worked for ten years, Sheppard continue to examine introduced populations of bees, including the incursion of Africanized bees. In samples of 700 feral colonies, he and his colleagues found genetic markers from a number of subspecies. An Egyptian mitochondrial marker lived on in 2%. “Those bees were not at all like *lamarkii*, they were like Italians. But they were descendents somewhere back there of a *lamarkii* queen. Mitochondrial DNA has the beauty of never being diluted, so you can see those things that happened in the past. It is also not useful for describing the overall genetic constituency of the organism that you are looking at. So microsatellites are better because they are inherited from both of the parents.” In Arizona, half the feral bees were found with markers from *mellifera mellifera*, descendents of the original importations of black bees – although, again, their appearance or behavior was not the same. Traces from that original subspecies varied by state: in Alabama, it was 25%.

After 1987, when the Varroa mite came in, feral colonies declined precipitously. Over half the colonies east of the Mississippi were believed to have perished. “If you go back to the idea of a genetic bottleneck, losing half your colonies, even if you build up next year to be the same number, you have lost some genetic variability from the half that was lost,” said Sheppard.

Commercial queen breeding also narrows the gene pool. “The whole selection process in itself is reducing genetic diversity, right?” he said. “You don't want the cows that don't give very much milk. So your selection process reduces genetic variability. That's fine, except in honey bees we have to maintain allelic diversity.” Before the Dolittle method of queen rearing caught on, people made splits and had other methods of rearing a few queens. “But nothing like this, nothing where you could take one queen and make thousands of daughters from her,” he said.

In 1993-94 Sheppard visited about 80% of the queen producers that advertised, sampling from their queen mother stock and learning about their practices. In the West, concentrated in a 50 mile radius of Sacramento, most grafted from 10 to 20 breeders. In the Southeast at the time, they often grafted from one best queen. The result was that a third of the managed colonies in the country were replaced with the daughters of 600 mothers – a total of 900,000 queens sold.

When he became a professor at Washington State University in 1996, Sheppard widened his interest in the genetic diversity of American bees. Not every foray was successful. To sample feral colonies, he flew his old plane into wilderness areas and set swarm traps that turned up empty. At the same time, he sent letters to state parks and grounds managers to ask if they knew of any feral colonies. In his study just four years before with the USDA, “They all did. ‘They're in a shed back there, there in that tree over there.’ They write you back and you plan a trip to sample them. I did the same thing in the West, mainly in Washington and Idaho, and got no response in 1997, ten years after the mite. Feral colonies had been really common. It will be interesting to see what the genetics of them are, we don't know right now.”

More recently, his lab has had new tools to go back to look at the US commercial breeding population in 1993-94 and another set of samples from 2004-05. A doctoral student, Debbie Delaney -- now a professor at the University of Delaware – gathered the later samples and used microsatellites to examine both sets. Although there was little difference in the production numbers after ten years -- about 500 mothers produced 870,000 queens nationally -- she found a net genetic loss in the Western queen breeding population. For 10 microsatellite loci there were about 136 different alleles. 10 years later 36 or so of those were gone. A new set of about 20 were found, and some of those included alleles that are found in the Africanized population.

“If you remember that this is going on every year, you realize what's happening,” he said, referring to yet another narrowing of the genetic bottleneck. “Of course this is not the whole story. There are a lot of people with one or two hives who split them, and they mate with whatever is in their neighborhood. There are a lot of large beekeepers who make their own queens. But it is a big part of the story. It might give you the idea that maybe you could do better.”

To find out if they could do better, in 2001-02 Sheppard's lab bought available queens from every American producer that advertised. They screened them for traits such as hygienic behavior, brood area and weight gain, but without regard to color. They selected 12 queens and started maternal sublines in an isolated area with a common drone pool. Varied genetics were introduced through drone mother colonies. "We did that for two or three years, and we made fantastic gains." For example, the bees became highly hygienic.

They distributed the queens in Washington, Idaho and Oregon to local and regional beekeeping organizations and helped them breed from them in their own apiaries. They favored groups that allow the membership to graft from the stock or open the apiary to members' mating nucs. Now the WSU maternal sublines are retired, having served their purpose. "After about six or seven generations you lose the benefit of maintaining those bees separately," said Sheppard.

The next chapter of Sheppard's work has been to reach back to European parent populations to bring in germplasm. He joined long-time Carniolan breeder Sue Cobey of UC Davis and WSU, who was already dedicated to enhancing her stock with European genetics. They struggled for several years to get governmental permission for importation, which had been denied because of the 1922 prohibition. When, in 2004-06 100,000 unregulated Australian queens came in with bees for pollination, it was perhaps the irony of the situation that finally allowed permission for Sheppard and Cobey to bring in semen, which would be more easily tested for pathogens than queens. They have brought in *carnica* semen for three years now, breeding bees in quarantine at WSU.

It has not been an easy mission, a fact apparent to Sheppard, Cobey and Hopkins, when, deep in the Caucasus Mountains, they faced a band of men intent on stopping them. Their elderly translator said, "This is a very bad situation."

"The only language we could communicate in was bad German," said Sheppard. "We were trying to tell them that it was for science. We did have frames of bees and veils, and those guys were shirtless." Which may have given the opportunity to retreat down the mountain – albeit less a few Caucasian alleles.

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Part II of this story recounts the further development of this work.

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[1] This article is derived from interviews with Steve Sheppard on 12/1/11 and 2/14/12, his lecture to the Marin Beekeepers on 2/1/11.

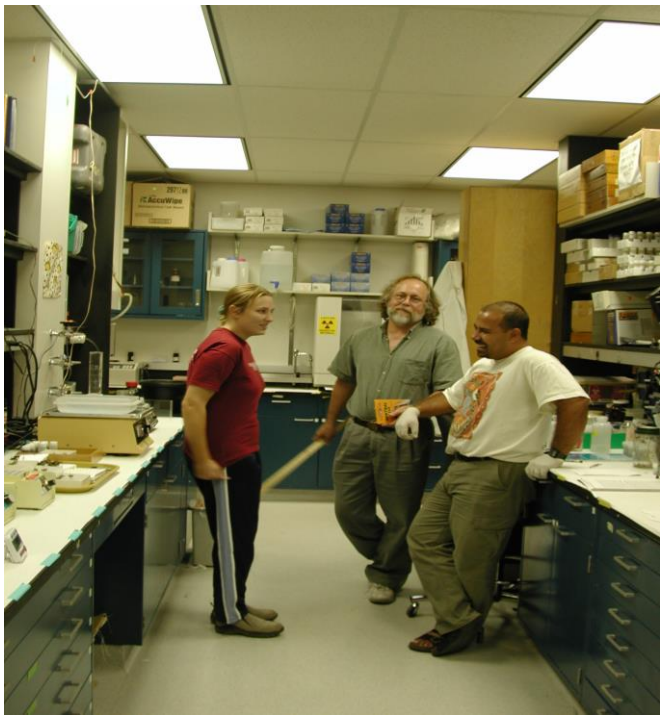
[2] Ruttner, Friedrich, *The Hive and the Honey Bee* 1973 races of these by Friedrich Ruttner, page 19.

Steve Sheppard began keeping bees as an undergraduate in Georgia. He was inspired by Freidrich Ruttner's work on the subspecies of bees to make the subject central to his research. Photo credit: Robert Hubner, Washington State Magazine

Sheppard with colleague Sue Cobey of UC Davis and Washington State University, collecting bee semen from Apis mellifera caucasia in the Republic of Georgia in 2011.

When the lights went out while Sheppard was extracting semen from precious samples of Caucasian bees in Georgia, he worked by flashlight into the night in a makeshift lab at the beekeeper's house. Photo courtesy Steve Sheppard

Beyond being a bee researcher, Sheppard has a ranch in Western Idaho where he grows wheat and raises Icelandic sheep. It is just over the border from Pullman, Washington, where he is an entomology professor at WSU.



As a professor at WSU, Sheppard has mentored many graduate students, among them, shown here in 2001, are Melissa Gardner and post doc Irfan Kandemir. Now a professor in Turkey, Kandemir is working with Sheppard and Cobey on Caucasian bee semen imports. And no, this did not all happen with the big stick that Sheppard appears to be wielding.

Sheppard, third from right, was an editor of the bee research publication Apidologie. Here he is at a meeting in Germany with the international panel of editors in 2009.

Sheppard with a note of cultural dissonance in Almaty, Kazahkstan. (The name for the laundry detergent is from a Turkic root word meaning "snow".) He traveled to the Tien Shan Mountains there in 1999 to sample the genetics of Apis mellifera there.