One in a Million, Part 1

— By Christine Hamilton, The American Quarter Horse Journal

An incredible genetic circumstance creates a unique DNA puzzle.

"Excluded."

For the second time Denise Charpilloz had sent in hair from her 2004 foal out of her mare Sharp One for DNA testing and parentage verification. And for the second time, the Veterinary Genetics Laboratory at the University of California, Davis, had excluded not only the stallion Dunbars Gold as the foal's sire, but also Sharp One as the foal's dam.

Dunbars Gold, a 1996 brindle stallion by Two D Nine and out of Outa Chiggers by Outa Utopia. Genetic testing has shown the horse to be an extremely rare chimera, an individual with two DNA types.

Every now and then, people make mistakes when they submit mane (or tail) hair samples for DNA testing on a foal. It usually happens when people accidentally mix hair if they're collecting samples from more than one horse. Although very rare, sometimes mares can actually switch foals or the wrong stallion's semen is unintentionally shipped and used to breed a mare.
All those scenarios result in a DNA test that "excludes" a mare or stallion as a foal's parent. Usually it doesn't take long to figure out what went wrong.

But "I saw it being born!" Denise says. "I didn't mix it up with any other foals; there were no other foals!" And Dunbars Gold's owner, Carole Dunbar, had only one stallion to ship semen from.

AQHA's registration department turned back to the lab's geneticists: could they please give the case another, much closer, look?

**Puzzling Pattern**

The case landed on the desk of Dr. Cecilia Penedo, the lab's associate director of service and genomic research and development.

Dr. Penedo immediately noticed that Dunbars Gold and Sharp One are brindles.

"It's a very rare coat pattern in horses," she says, though it is common in dogs and cattle. "People have not been very successful reproducing this pattern through breeding, and we've never really had much information on the genetics of it because it is so rare."

In fact, of the more than 4.7 million American Quarter Horses registered with AQHA (excluding appendix horses), the registration department has a list of only 15 horses who have exhibited some form of the brindle coat pattern.

Dr. Penedo began reviewing the lab's testing on the horses.

"When I looked up the stallion, Dunbars Gold, I found that we had tested him three times before we established his (DNA) type," Dr. Penedo says.

The first two tests the lab ran were on mane and tail hair samples.
"At that time, there was something odd about his type," she continues. "It looked like the hair samples came from two different animals, like the hair had been mixed together. We requested a second sample and had the same problem."

Convinced the hair samples were getting contaminated, for the third test, the lab requested a blood sample from the horse.

"When the blood sample came in, we got a perfectly good type that would be consistent with one animal," she said.

The blood test results also had some things in common with the hair results.

The lab used the DNA type obtained from blood for the parentage verification on Sharp One's 2004 foal (the first of the stallion's foals to be tested for parentage verification).

But in her review, something else caught Dr. Penedo's attention.

"The oddest thing about the stallion's blood sample results was that the DNA types for sex-linked markers were typical of a female and not a male," Dr. Penedo says. "There was no evidence of a Y chromosome."

Penedo decided to retest Dunbar's Gold.

"We went back to the original hair samples and used a single hair for the DNA test," she says. "And we performed several of these single-hair tests.

"Some of the tests yielded a perfectly good type for a male individual and some a good type for a female individual."

Some of the results also showed two DNA types within a single hair root.

"At that point, I thought, 'This horse is chimeric,' " Dr. Penedo says. Chimerism
In genetics, a chimera is an individual formed from two different cell lines. Scientists believe that it happens when two nonidentical twin embryos (fertilized eggs) fuse into one embryo very early in their development.

Dunbars Gold, a 1996 brindle stallion by Two D Nine and out of Outa Chiggers by Outa Utopia. Genetic testing has shown the horse to be an extremely rare chimera, an individual with two DNA types.

The embryo develops into a normal, complete individual that has two different DNA types. He or she might have kidneys that developed from one DNA type and a heart or skin cells from the other type.

Extremely rare, chimerism has been documented in other species, including cats and humans.

"Dunbars Gold has one cell line that is female and one cell line that is male," explains Dr. Cecilia Penedo. "The cell lines have slightly different DNA types, but both qualify to his sire, so there's not a parentage issue involved. It's almost clear that there were two embryos produced, and they fused."

Although chimeric in his skin and hair, Dunbars Gold's reproductive organs were apparently formed by his male DNA type.
"It's an interesting biological development event. He went on to develop as a completely normal male," Dr. Penedo says.

The lab has tested several of his foals, and they were all produced by his male cell line.

When Dr. Penedo turned her attention to Sharp One, she again made note of the rare brindle coat pattern the mare had in common with Dunbars Gold.

If Dunbars Gold was chimeric in his skin and hair, could there be a link between the equine brindle coat pattern and chimerism? What if the problem the lab was having qualifying Sharp One as the foal's dam was also due to chimerism?

Like Dunbars Gold, Sharp One's DNA type had also been initially established from mane and tail hair samples. However, according to Dr. Penedo, there was no evidence of chimerism in those hair samples.

"I wondered if she could be the opposite of Dunbars Gold," Dr. Penedo says. "If she might show chimerism in her blood but not her hair."

When Sharp One's blood was tested, that's exactly what Dr. Penedo found: two different cell lines (and DNA types) in the mare's blood. But both of her cell lines were female.

The lab then tested Sharp One's body hair taken from the darker and lighter areas of her brindle pattern.

"We were able to identify the two cell lines in the different (body hair) patches," Dr. Penedo says.

When the lab compared the 2004 foal's DNA type to Sharp One's 2003 foal (her only other living foal so far), it found that each came from a different female cell line.
"Unlike Dunbars Gold, she is chimeric in her (reproductive organs)," Dr. Penedo explains. "She is producing two different types of eggs; they can be from one or the other cell line, which is a very interesting situation.

"Once we put the puzzle together, we were able to qualify the foal without any problem," Dr. Penedo says.

**The Pieces Fit**

"We now have evidence of chimerism from two different brindle horses," Dr. Penedo says. "We think that the brindle pattern in some horses could be explained by this very rare event, where two embryos fuse early on in their development and go on to make just one, single individual with different cell lines."

Dr. Penedo is quick to point out that there is evidence of a type of brindling pattern in horses that appears to be inherited, linked to a coat pattern gene, as it is in dogs and cattle.

"We can't say that it is always embryo fusion that leads to the brindling pattern in horses," she says.

"But for the very classic, clear brindling pattern like we see in Dunbars Gold and Sharp One, I wouldn't be surprised if that was caused by chimerism," she continues.

"If it results from embryo development, then there is no genetic control, and you can't really breed for it. Unless there is a gene controlling something that makes it more likely for the fusion to occur, such as something that increases the likelihood of a twin pregnancy."

Dr. Penedo thinks you would only see outward evidence of chimerism in the coat pattern if the two embryos that fused had genes coding for contrasting coat colors.
"For example, if two bay embryos fused, or two chestnuts, you wouldn't see anything," she explains. "I think you'd see the brindle pattern if you had a combination of a base color with one dilution gene. In this case, it was the dun gene. Both Dunbars Gold and Sharp One have dun in their backgrounds.

"It's been a great learning experience," Dr. Penedo adds. "It is shedding a little bit of light on the brindling pattern."

Denise Charpilloz named her foal Sharp Barcoder, aka "Deuce." Deuce is a solid sorrel, and there's nothing chimeric about him. But he is the occasion for an incredible genetic coincidence: If Denise hadn't been trying to breed for the rare brindle coat pattern, these even rarer chimeric individuals would never have met. And geneticists worldwide would not have had this once-in-a-lifetime chance of studying their DNA. What are the odds of that?