



*An incredible genetic
circumstance creates a
unique DNA puzzle to solve.*

By Christine Hamilton

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.

DON TROUT

One In A Million

“EXCLUDED.”

For the second time Denise Charpiloz 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 Sharp One as the foal's dam.

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 Charpiloz knew there were no mistakes here.

“I saw it being born!” she said. “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 CECILIA PENEDO, PH.D., THE lab's associate director of service and genomic research and development.

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

“It's a very rare coat pattern in horses,” she said (though 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 that have been registered with AQHA (excluding appendix horses), the registration department has a list of only 15 horses that have exhibited some form of the brindle coat pattern.

Her interest sparked, 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,” Penedo said.

The first two tests the lab ran were on mane/tail hair samples.

“At that time, there was something odd about his type,” she continued. “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 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,” Penedo said. “There was no evidence of a Y chromosome.”

Penedo decided to retest Dunbars Gold.

“We went back to the original hair samples and used a single hair for the DNA test,” she said. “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*,’” Penedo said.

**Pronounced “ky-mare-ik”*

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.

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,” Penedo explained. “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 developmental event. He went on to develop as a completely normal male,” Penedo said.



The brindle mare Sharp One, owned by Denise Charpiloz of Vancouver, Washington. The parentage verification of her 2004 foal revealed an incredible coincidence and gave a once-in-a-lifetime research opportunity to geneticists at the University of California, Davis.

WENDI HOSS, BLUE RIBBON PHOTOGRAPHY, WWW.QUALITYPONIES.COM



Sharp One's two coat colors in her brindle pattern radiate from the central swirl on her forehead.



Sharp Barcoder is the 2004 son of two rare chimeric brindle horses. He's a sorrel with no brindling.

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/tail hair samples. However, according to Penedo, there was no evidence of chimerism in those hair samples.

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

When the lab tested Sharp One's blood, that's exactly what they found: two different cell lines (and

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

When 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.

ABOUT THE HORSES

Dunbars Gold

Dunbars Gold, aka "Aurie," is a product of Delos Dunbar's 40-plus years breeding American Quarter Horses. Foaled in 1996 in Buchanan, Michigan, Aurie is by Dunbar's homebred red dun stallion Two D Nine, and out of the palomino mare Outa Chiggers by Outa Utopia.

Delos' daughter, Carole (Aurie's owner), remembers other brindles in Aurie's family. Delos raised Two D Nine's dam, a dun mare named Miss Two Larue by Two Larue, and out of the dun mare Latigo Kiowa (the family's very first Quarter Horse).

Dunbars Gold might have both factors working in him, both chimerism and the genetic predisposition for brindling, Carole said, because there is brindling in his family.

"Two Larue had a sister that was brindle," Carole said.

"And Aurie has an older full brother that we sold as a yearling," she added, (registered as Audacious One). "When the new owners saw Aurie they said, 'You know, the full brother that we have? You can see brindle stripes on him when he's wet.'"

A longtime judge with the American Paint Horse Association, Carole is proud of the stallion and the family of primarily dun horses her father raised, but she didn't show Aurie.

"I said to myself, 'if I show this guy, someone's going to offer my dad a lot of money and he'll sell him!'" Carole remembered with a laugh. "He's incredible. He's as sweet as he is beautiful."

DNA types) in her 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," Penedo said.

When the lab compared the 2004 foal's DNA type to Sharp One's 2003 foal (her only other living foal so far), they found that each came from her two different female lines.

Sharp One

"I couldn't believe that nobody else had bought her!" said Denise Charpillouz of the unique 1999 brindle filly she found on the Internet, way out in Wisconsin. That was back when Sharp One, aka "Salsa," was a yearling.

Since then, Charpillouz has bred Salsa four times trying to repeat her unique brindle pattern; something that she's not sure she can do now that she knows the mare is a chimera. Salsa has had two sorrel colts, one miscarriage and is in foal to the AQHA stallion Major Hearts Desire for 2006.

"The fact that there are so few brindle horses is why there have not been any studies done at all until this one (case)," Charpillouz said. "I don't think the genetics surrounding the occurrence of brindling in horses has been figured out entirely."

Regardless, Charpillouz loves her unique mare by Force One and out of the red dun mare Sonny's Coppertone by Sonny Supermann.

"I love the beautiful, swirling, marbled effect of the brindle pattern," Charpillouz said. "People are attracted to it; it stands out in a crowd. It is different; it is rare; it is special."

"My question now is," she added, "does she have an ovary on one side that produces one of the (DNA types)? Or are there eggs (from both types) in each ovary?"

Charpillouz might be the only horse owner on the planet who can wonder that about her mare.

"Unlike Dunbars Gold, she is chimeric in her (reproductive organs)," Penedo explained. "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."

The Pieces Fit

"WE NOW HAVE EVIDENCE OF CHIMERISM FROM TWO DIFFERENT brindle horses," Penedo said. "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."

CONFUSING COLOR

"The best way to think about it is, most chimeras are brindles, but not all brindles are chimeras," said Phillip Sponenberg, D.V.M., Ph.D., noted author and equine color geneticist from Virginia Tech's Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, Virginia.

"Chimeras are mixtures of two individual animals," he continued. "Those animals usually end up being striped, like a brindle horse.

"However, there does seem to be a coat color pattern (in horses) that is brindle that is not related to chimerism," he continued. "There is a gene floating around that can bring about the brindling coat pattern."

Sponenberg thinks that it might have to do with the gene that causes the "sooty" coat color.

"I suspect that the brindling gene reorganizes the sootiness into stripes," he said. "For example, if you look at horses that are bay brindles, they still have the black points, so the brindling doesn't affect the lower legs. It just seems to reorganize the body color.

"I think that for the (brindling to be expressed), the horse has to have both the gene for the brindling, as well as the right background color to demonstrate it on. That's why I think they're so hard to breed for."

The point is you can't tell that a horse is chimeric just by looking at it.

"That's where (genetic) testing is very useful," he pointed out.

COURTESY OF PHILLIP SPONENBERG, D.V.M., PH.D.



A horse exhibiting the sooty coat pattern, where black hairs countershade a base color, giving the coat a sooty appearance. Sponenberg thinks that the inheritable brindle coat pattern in horses works to reorganize the sootiness into stripes.

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 said.

"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 continued.

"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."

Penedo thinks that 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 explained. "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 background.

"It's been a great learning experience," Penedo added. "It is shedding a little bit of light on the brindling pattern in my mind."

Denise Charpilloz named her newly registered foal Sharp Barcoder, aka "Deuce," and he'll soon head to his new owners, Stacy and Alex Payne in McMinnville, Oregon.

Of course, Deuce is a solid sorrel and there's nothing chimeric about him. But he is the occasion for an incredible genetic coincidence: If Charpilloz 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? ❏

Christine Hamilton is a field editor for The American Quarter Horse Journal. To comment, write to chamilton@aqha.org.

Learn More

Here are some Internet sources on equine color genetics:

- Equine Color Genetics information page, compiled by Daralyn Wallace: <http://hometown.aol.com/raunfalcon/colrpage.htm>.
- Horse Colors: <http://greenfield.fortunecity.com/dreams/799/hc/horsecolors.htm>.
- HorseColor.com: <http://www.horsecolor.com/>.
- Cremello & Perlino Educational Association Web site: <http://www.doubledilute.com/>.
- The Veterinary Genetics Laboratory at the University of California, Davis, performs a variety of horse coat color tests and offer information on the genetics on their Web site at: <http://www.vgl.ucdavis.edu>.
- For more on brindle horses specifically, look at J. Sharon Batteate's Web site at: <http://members.aol.com/brindleho>.

Check out these books as well, most available online through Quarter Horse Outfitters at www.quarterhorseoutfitters.net.

- "Equine Color Genetics," by D. Phillip Sponenberg
- "Horse Genetics," by Ann T. Bowling
- "Horse Color Explained," by Jeanette Gower