The Color INSIDE

APHA FILE PHOTO

A Paint Horse owner's guide to demystifying the genetics of spotting patterns.

Genetics IQ
Part 1 of 2

By IRENE STAMATELAKYS

Before DNA testing became common—before we downloaded forms and pulled mane hairs and received results via email—Paint Horse breeders relied on collective wisdom and trial and error in their quest to produce those all-so-desirable colored foals. Breeders needed to experiment for years before confirming a horse was a good, great or poor color producer. Foaling time brought lots of pleasant surprises, along with some disappointment and occasional heartache.

While the arrival and development of DNA testing has eliminated a lot of the guesswork, many of us have a limited understanding of the tools science has to offer. Others have a good handle on Paint Horse genetics, but advances and discoveries are happening at such a fast rate that it's challenging to keep up with the latest developments.

With that in mind, the *Paint Horse Journal* has developed a two-part series to increase your genetics IQ. For novices, we'll bring you up to speed on the essentials. For genetics buffs, we'll shed light on some of the latest developments and challenges facing researchers and breeders. And for everyone, we'll attempt to answer the question, "How can these genes enhance my Paint Horse breeding program?"

Genetics Jargon

Before we get into spotting specifics, let's start with some basic genetics terminology you probably learned in biology class. **Chromosomes** can be thought of as strings of **genes**—the building blocks in all living organisms that determine visible traits, like hair color, and non-visible traits, like blood type. A horse has 64 chromosome pairs, inheriting half from the sire and half from the dam.

Each gene has an address—a specific site on a specific chromosome. We call this address a **locus**, with the plural being loci. Quite often, geneticists use the locus name to refer to a gene. And when a gene comes in different forms, those variations are called **alleles**.

For example, the *Tobiano* locus has two alleles: a tobiano allele *(TO)* and a non-tobiano allele *(n)*. Either can occur at the *Tobiano* locus, but each chromosome can only carry one allele. Because chromosomes come in pairs, a horse carries two alleles at a particular locus.

If a gene is **dominant**, like *Tobiano*, the horse only needs one copy of the allele from one parent to have a tobiano spotting pattern. For instance, a **heterozygous** tobiano *(n/TO)* has one *Tobiano* allele; a **homozygous** tobiano *(TO/TO)* has two.

Some genes are **incompletely dominant**. This means that heterozygotes do not look the same as homozygotes. *Sabino 1* is one example, which we'll describe later.

A **recessive** gene is only expressed in the homozygous state. Overo Lethal White Syndrome, for example, is a recessive condition; none of the known spotting patterns are fully recessive.

Finally, **phenotype** is what something looks like on the outside—for our purposes, that's the outward expression of a white spotting pattern in Paint Horses. **Genotype** refers to the horse's genetic makeup. Sometimes a horse can carry a spotting pattern gene, but not express it in a way that's consistent with how we think that gene should look phenotypically.

Now that you've reviewed the lingo, let's take a look at the known mutations responsible for Paint Horse spotting patterns: "The Big Six" and "The Dominant Whites." It's important to note that these genes do not determine whether or not skin cells can produce **melanin**, which is a class of pigment that's responsible for the dark color of skin, hair and more. Rather, these genes influence the migration of **melanocytes** (melanin pigment-producing cells) in the embryo.

The Big Six

Tobiano *(TO)*

CASSIDY COBARR

Inheritance: Dominant

Gene: *KIT* on Chromosome 3

Discovered: 2007

Phenotype: White legs, at least below the hocks and knees; spots are crisply-outlined and frequently cross the topline somewhere between the withers and the tail; head markings like those of a solid-colored horse, with dark eyes; many also have white areas in the mane and tail, giving the tail a two-toned appearance.

Notes: Even though it's a dominant trait, not all tobianos have enough white to meet the minimum requirements for the Regular Registry.

Frame Overo *(O)*

CASSIDY COBARR

Inheritance: Incompletely Dominant

Gene: *EDNRB* on Chromosome 17

Discovered: 1998

Phenotype: In heterozygotes, horizontal white spotting on the neck and body often giving the impression the white markings are framed with colored areas; bald faces and blue eyes are common. It is possible for a horse to carry the *Frame* allele and be solid or minimal white, not meeting the minimum requirements for Regular Registry.

Notes: Homozygous frames—affected by Overo Lethal White Syndrome—are completely white, unviable foals with no or little pigmented skin; a congenital malformation of the GI tract makes the condition fatal.

Sabino 1 *(Sb1)*

COURTESY LAUREN BORK

Inheritance: Incompletely Dominant

Gene: *KIT* on Chromosome 3

Discovered: 2005

Phenotype: White markings on face, lower legs and belly with jagged edges, and roaning on the body. Homozygotes can be completely or nearly all-white but are viable without any known health problems.

Notes: Many Paints have sabino-like patterns that are not caused by *Sabino 1*.

Splash White 1 *(SW1)*

CASSIDY COBARR

Inheritance: Incompletely Dominant

Gene: *MITF* on Chromosome 16

Discovered: 2012

Phenotype: Variable but might include extensive face white, white markings on legs, belly spots and blue eyes; appears as if the horse has been dipped in white. Sorrel/chestnut horses often have more extensive markings than bays.

Notes: This mutation, the most common splash white variant, appears to be several hundred years old. Viable homozygotes have been found and have more pronounced spotting than heterozygotes.

Splash White 2 *(SW2)*

COURTESY BAR K2 RANCH

Inheritance: Incompletely Dominant

Gene: *PAX3* on Chromosome 6

Discovered: 2012

Phenotype: Variable but might include extensive face white, white markings on legs, belly spots and blue eyes; as if appears the horse has been dipped in white. Sorrel/chestnut horses often have more extensive markings than bays.

Notes: Only found in descendants of a single Quarter Horse mare foaled in 1987; Paints are included. No homozygotes have been reported to date, and it's considered embryonic lethal; breeding two carriers is not recommended.

Splash White 3 *(SW3)*

COURTESY SUSANNA MARINELLI

Inheritance: Incompletely Dominant

Gene: *MITF* on Chromosome 16

Discovered: 2012

Phenotype: Variable but might include extensive face white, white markings on legs, belly spots and blue eyes; appears as if the horse has been dipped in white. Sorrel/chestnut horses often have more extensive markings than bays.

Notes: This variant is extremely rare, found only in two Paint/Quarter Horse lines to date.

The Dominant Whites

All of these mutations are found on the *KIT* gene, located on Chromosome 3. Researchers named them *Dominant White*, although horse breeders might use other terms to describe the patterns. The alleles are dominant, meaning a horse only needs to inherit one copy of the mutation to produce phenotypes that range from slightly increased face and leg markings to sabino-like to completely white. We've bolded the entries for three Dominant White genes known to exist in the Paint Horse breed, and show examples of each below.

Dominant White 5 (W5)

DON TROUT

Dominant White 10 (W10)

KELLY GRAPHICS LLC

Dominant White 20 (W20)

COURTESY KRISTEN MEYERS

The Dominant Whites

Name	Position on *KIT* Gene	Breed
Dominant White 1 (W1)	Exon 15	Franches-Montagnes Horse: White horses trace back to Cigale, a mare foaled in 1957
Dominant White 2 (W2)	Exon 2	Thoroughbred: White horses trace back to KY Colonel, a chestnut with extensive white markings, foaled in 1946.
Dominant White 3 (W3)	Exon 4	Arabian: Spotted horses trace back to R Khasper, a near-white stallion foaled in 1996.
Dominant White 4 (W4)	Exon 12	Camarillo White Horse: White horses trace back to Sultan, a stallion foaled in 1912.
Dominant White 5 (W5) (see photo at left)	**Exon 15**	**Thoroughbred: Horses trace back to Puchilingui, a stallion foaled in 1984 with a sabino-like coat pattern, and his offspring.**
Dominant White 6 (W6)	Exon 5	Thoroughbred: Identified in a single white horse.
Dominant White 7 (W7)	Intron 2	Thoroughbred: Identified in a partially white filly.
Dominant White 8 (W8)	Intron 15	Icelandic Horse: Identified in a partially white horse with mottled phenotype.
Dominant White 9 (W9)	Exon 12	Holsteiner: Identified in a completely white horse.
Dominant White 10 (W10) (see photo at left)	**Exon 7**	**Paint/Quarter Horse: Horses trace back to GQ Santana, a stallion foaled in 2000, and his offspring; expression ranges from nearly all-white to horses with face/leg white and belly spots.**
Dominant White 11 (W11)	Intron 19	South German Draft Horse: Identified in a completely white stallion and three white offspring.
Dominant White 12 (W12)	Exon 3	Thoroughbred: Identified in a single horse with sabino-like pattern.
Dominant White 13 (W13)	Intron 17	Quarter Horse/Peruvian Paso: Identified in a crossbred family with two white animals.
Dominant White 14 (W14)	Exon 17	Thoroughbred: Identified in a completely white horse.
Dominant White 15 (W15)	Exon 10	Arabian: Identified in a single horse with partial depigmentation.
Dominant White 16 (W16)	Exon 7	Oldenburg: Identified in a family with three almost completely white horses.
Dominant White 17 (W17)	Exon 12	Japanese Draft Horse: Identified in a completely white horse with one blue eye.
Dominant White 18 (W18)	Intron 8	Swiss Warmblood: Identified in a single horse with extended white speckled areas.
Dominant White 19 (W19)	Exon 8	Part-Bred Arabian: Horses have bald faces, extended white legs and irregular belly spots.
Dominant White 20 (W20) (see photo at left)	**Exon 14**	**Multiple Breeds: Very common mutation; incomplete dominant trait. Markings might include a wide blaze, leg markings and small belly spots. Has a subtle effect on depigmentation with a stronger effect on bays than sorrels. Living homozygotes have been found and tend to have more white.**
Dominant White 21 (W21)	Exon 17	Icelandic Horse: Identified in a single horse with a sabino-like pattern.

CASSIDY COBARR

The only completely dominant white spotting gene is Tobiano, *which means a horse only needs one copy of the gene to inherit the trait.*

One of the biggest challenges facing researchers and Paint Horse breeders alike is the nomenclature of spotting patterns.

Color Genetics Bookshelf

Want to learn more? Here are two books to add to your equine library.

Horse Genetics (2013, 2nd Edition) by Ernest Bailey and Samantha Brooks

This is the textbook Samantha uses for her equine genetics class at the University of Florida, but she says anyone who survived high school biology can follow along. It covers the basic genetics of the horse including coat color, parentage, medical and population genetics, cytogenetics, performance, breeding systems and genetic conservation, and new advances such as micro-satellite testing.

Equine Color Genetics (2009, 3rd Edition) by D. Phillip Sponenberg

Now in its third edition, this work is an encyclopedia of equine coat color genetics, with in-depth yet accessible explanations and color photos illustrating each example. It's an excellent resource for the Paint Horse breeder and equine color genetics enthusiast.

The Nomenclature Nightmare

One of the biggest challenges facing researchers and Paint Horse breeders alike is the nomenclature of spotting patterns. Over the years, this system of names used to designate a particular pattern has grown increasingly complex and clumsy.

Samantha Brooks, Ph.D., is assistant professor of equine physiology at the University of Florida. Her genetics research led to the discovery of the *Tobiano* and *Sabino 1* genes.

"The nomenclature is a mess," Samantha said. "It's honestly my pet peeve."

The problem is multi-dimensional. On the one hand, you have researchers finding the mutations and, from a scientific perspective, wanting to follow standard naming conventions so that the names make sense to fellow researchers. On the other, you have stock horse breeders who don't necessarily use the same terms. *Sabino 1* is a good example.

"We struck out on our own when we named *Sabino 1*," Samantha said, "Based on the murine (mouse) nomenclature, we should have called it *W* for the *Dominant White* locus."

The term "dominant white" is not particularly descriptive either. It's easy to imagine the gene produces an all-white horse—not exactly what Paint breeders are aiming for. The name actually refers to a dominant form of white spotting, which ranges from extended face and leg markings to a completely white coat.

Researchers decided to call the gene *Sabino 1* for two reasons, Samantha explains. First, the idea was to better match the terminology used by horse breeders. Second, they didn't want to scare or confuse people with connotations based on the lethal dominant white genes found in mice or the overo lethal white gene in horses.

Things got a little more complex when Bianca Haase, a research fellow at the University of Sydney in Australia, began discovering more genes based on the *Dominant White* locus.

"When Bianca started finding her white mutations, many of those were homozygous lethal, and many of those are completely white, but not all of them," Samantha said. "She found a couple in the series—[some] that were potentially viable as homozygous and some [that were] heterozygous—that looked similar to sabino, but she chose to keep with the *W* symbol based on laboratory mouse research. Perhaps some of those should have been pulled out and called sabino instead of dominant white, for the sake of limiting confusion among breeders of spotted horses."

In the Paint world, the most famous dominant white horse is GQ Santana, who was the founding animal of the *W10* mutation. But other *W* mutations vary greatly in their expression—not all look like GQ Santana; they can range from a nearly all-white horse to a mostly solid-colored horse with a blaze and socks to horses that look like what most horsemen would call sabino.

"One that comes to mind is *W20*," Samantha said. "You'll see a lot of *W20* in the reining horses. It's a very frequent allele, and we know horses can be homozygous for it. They are perfectly healthy, and homozygotes rarely have more than 30 percent white on the body. And yet that allele got a *Dominant White* acronym because it is in the *KIT* gene and [researchers wanted] to stick strictly to murine nomenclature. The scientific convention doesn't take into consideration the issues that nomenclature can cause us. *W20* is one a lot of horse people would call a splash white."

Three mutations designated *Splash White* can be confusing, too.

"My issue there is a scientific one because you have two different genetic loci that have the same acronym: *SW*. That's too confusing," Samantha said. "At the very least, they should have been split up and given two different names. But that is the nightmare that is nomenclature in the scientific community. And then you talk to horse people, and they call it something entirely different."

CASSIDY COBARR

It stands to reason that homozygosity increases the amount of white markings—that seems to be the case for this 2015 cropout colt by A Splash Of Silver (QH) and out of Dun Gone Classy (QH). Both parents are n/SW1 and minimally colored; this loud colt carries two copies of the Splash White 1 *gene.*

GAIL SHAW

While many Splash White *carriers might look phenotypically similar, the three known genes are very different. Hello Big Chex, a 2012 chestnut overo stallion by Big Chex To Cash and out of Custom Princess (QH), carries a single copy of the SW1 gene and is negative for other known white-spotting patterns.*

The Mystical Modifier

It can be curious to ponder why one horse might have more or less "color" than another when both carry the same spotting pattern mutation. Breeders have often wondered about the existence of an "enhancement" gene or perhaps a gene that suppresses white.

"[Enhancement] is a word I don't like," Samantha said, "in part because it's been applied to some known genes where it doesn't belong."

The idea of a modifier gene comes from Appaloosa genetics.

"In Appaloosas, we have two genes that control white spotting," Samantha explained. "We have the main *LP* locus, which is present in horses with anything from a completely white coat with just a few spots to a horse who has Appaloosa characteristics but no body spots. On top of that you add the *Pattern* locus, which we call *PATN1*. It controls the amount of white, from one percent to 99 percent."

PATN1 doesn't produce a visible spotting pattern itself, but it does control the expression of other spotting genes. And to get the desired Appaloosa phenotype, you need both the *LP* and *PATN1*

Free Live Seminar:

"Paint Horse Genetics"

2016 APHA Convention
Richmond, Virginia
February 26, 2016
1:30–2:30 p.m.

If you're fascinated by equine color genetics or want to learn more ways to improve your breeding program through genetic testing, don't miss this unique learning opportunity at the 2016 APHA Convention in Richmond, Virginia. Samantha A. Brooks, Ph.D., assistant professor of equine physiology at the University of Florida, will enlighten attendees with her knowledge and research regarding Paint Horse genetics in this free seminar. Plan to get an education on genetics and all it has to offer the APHA. To learn more or to register online, visit *apha.com/events/convention*.

The SW3 gene is relatively rare; one known carrier (n/SW3) *is TD Celebri Te, a 2009 chestnut overo stallion by TD Kid and out of Te Time Playmate (QH).*

Genetic Testing

Find out exactly which colors and patterns your stallion or mare carries before the breeding season begins. APHA's official genetics testing partner, the Veterinary Genetics Laboratory at the University of California–Davis, offers a Comprehensive Coat Color test for $125. This simple procedure, done with 30 to 50 hairs (with intact roots) from your horse, tests for the presence of red factor, agouti, cream, pearl, champagne, dun, silver, gray, *Tobiano*, *Frame Overo*, *Sabino 1*, *Splash White 1*, *2* and *3, and Dominant White 5, 10* and *20*. Results, sent by email, take seven to 10 business days. Order the test by calling APHA at 817-222-6423, and learn more online at *vgl.ucdavis.edu.*

genes. Samantha notes researchers have not yet documented any *PATN1* effect on a pattern other than *LP*.

"Really, Appaloosa is the product of two loci, not just one," Samantha said. "We attribute it to one, but you have to have two to get the pattern people want.

"The analogy [for Paints] is that *LP* is like *Tobiano* and *PATN1* is your mythical enhancement gene," she said. "The reality is that because Paint Horses work with so many different patterns, we probably don't have an enhancement gene. But why else would you get some horses that are 80 percent white and others that have four socks and one white tuft in their mane, but both are genetically *Tobiano*? You can imagine that there might be a modifying locus that's contributing to that. Part of this variation is simply due to chance. If there was a second gene essential to the expression of a key Paint pattern [like tobiano], I think we would have found it by now.

"There are certainly genes that create more white when added to *Tobiano* and perhaps some that create less white. I like the term 'modifying' because it doesn't imply a particular type of effect. Modifiers are not yet described genetically, and at this point they are kind of mythical."

Samantha's theory is that some of these mythical modifiers are actually white spotting patterns.

"With some of these *W* mutations—particularly the ones that look like splash white or sabino—that gene alone is not enough to bump a horse from solid Paint-bred up to Regular Registry," she said. "Folks have not attributed that gene as a spotting pattern—they've just got this horse with socks and a blaze, when it might actually have a good spotting gene there.

"They tend to get called enhancement genes because any time you add two spotting patterns together, you typically get more white. People are giving the credit to the more notable of the two patterns—let's say *Tobiano*—but they are also ignoring the fact the horse is actually multi-patterned. What people are calling an 'enhancer' is just another spotting pattern that has a distribution that doesn't perfectly align with the APHA threshold for Regular Registry. The horse might possess a perfectly valid spotting pattern that can act and produce color entirely on its own. I would categorize these genes as a more minimal spotting pattern, not an enhancer or modifier."

In other words, that mythical modifier or enhancement gene is probably just another spotting pattern—either known or unknown—that is increasing the amount of white on your Paint.

The Potential to Produce

While a horse's genotype tells one story, the expression of that combination of genes—his phenotype and the extent of his white markings—is dependent on multiple factors, including the interaction of different spotting patterns. It's also possible that environmental or even randomly determined factors play a role as well. We still have much to learn in the area of equine color genetics; the benefits of increasing our knowledge and understanding, however, are clear: improving the percentage of foals born with a desirable color pattern and safeguarding the health of the horses we raise.

Coming up next month, ride along with the *Paint Horse Journal* to the leading animal DNA parentage verification laboratory in the world, the Veterinary Genetics Laboratory at the University of California–Davis. There, we'll interview VGL Director Cecilia Penedo, Ph.D., about the importance of equine color testing, how these tests are conducted and the results of a small, informal study of a randomly selected group of Paints—both Regular Registry and Solid Paint-Bred horses—to see how many carried a known pattern gene, as we continue our investigation on how these genes can be used to enhance Paint Horse breeding programs. 50

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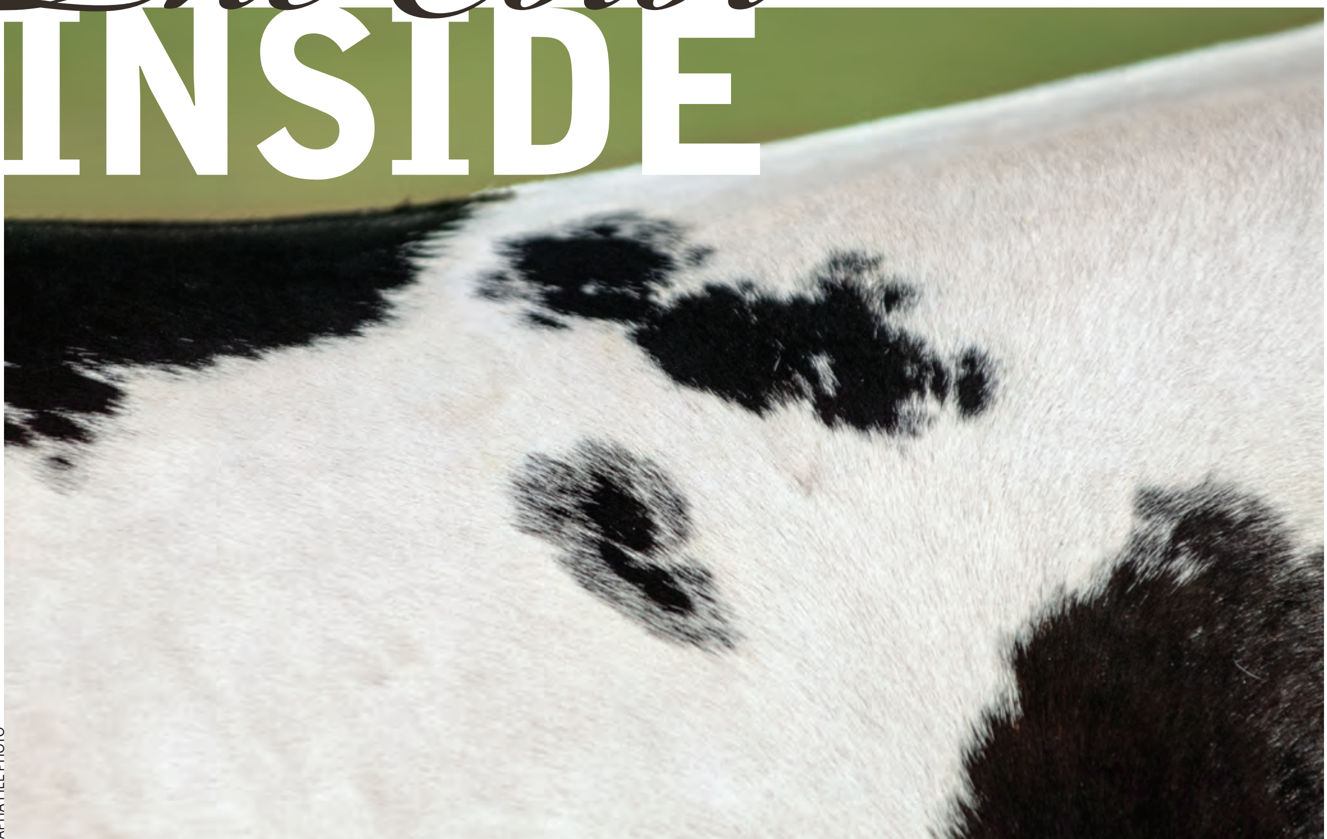
The Color INSIDE



APHA FILE PHOTO

A Paint Horse owner's guide to the power and potential of genetic testing for spotting patterns.

Genetics IQ
Part 2 of 2

By IRENE STAMATELAKYS

Last month, we shed some light on the genetics behind the white spotting patterns common to the Paint Horse breed: *Tobiano*, *Frame Overo*, *Sabino 1*, *Splash Whites 1*, *2* and *3*, and *Dominant Whites 5*, *10* and *20*. We covered the essentials and touched on some of the latest developments and challenges facing researchers and breeders, specifically the naming system and "mythical modifiers."

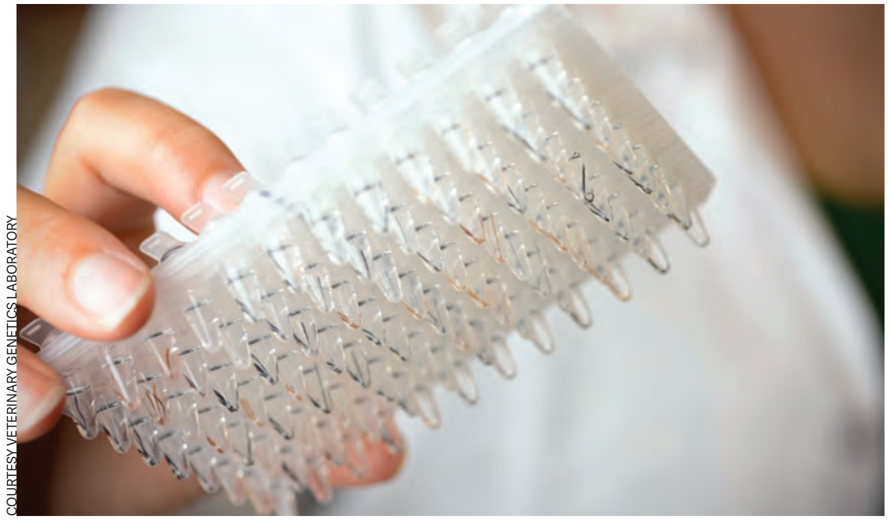
This month, we go inside the Veterinary Genetics Laboratory at the University of California–Davis to learn more about the lab, how DNA tests are conducted and the results of a small, informal study of a randomly selected group of Paints—both Regular Registry and Solid Paint-Bred—to see how many carried a known pattern gene. We'll also take a closer look at the *W20* mutation, which is relatively unknown but highly prevalent in Paints. And we'll discuss the importance of color testing with breeders and researchers as we continue our investigation on how these genes can be used to enhance Paint Horse breeding programs.

Welcome to my Laboratory

Originally established in the 1950s to provide parentage verification for cattle registries, UC–Davis' Veterinary Genetics Laboratory expanded its services in the 1960s to include horses, offering identity and parentage testing via blood typing. Today, it is the leading animal DNA parentage verification laboratory in the world, serving as the official laboratory for 120 horse registries, in addition to offering genetic disease and coat color testing to the general public.

While Paint Horse owners could choose one of several private laboratories for equine coat color testing, a significant percentage use VGL.

"For the last three years, we've seen a significant increase in both coat color and disease panel testing done via APHA and a decrease in orders for individual tests," VGL Director Cecilia


COURTESY VETERINARY GENETICS LABORATORY

The University of California–Davis' Veterinary Genetics Laboratory is APHA's official genetic testing partner. Leaders in genetic testing, the VGL offers a variety of coat color and pattern tests along with genetic disease testing and other services like parentage verification.

From the minute a test is ordered, precautions are taken to ensure results are both accurate and timely.

Penedo, Ph.D., said. "Panel testing for either disease or coat color for Paints is only available through APHA and for a relatively low cost. Owners are recognizing the value and taking advantage of these membership services that are clearly important and beneficial to the breed."

From the minute a test is ordered, precautions are taken to ensure results are both accurate and timely. Cecilia says this is a fairly detailed process, with many checks along the way to safeguard accuracy. [See "Extracting DNA" for an in-depth look at the process.]

"The breadth of genetic tests that we have available, the high quality and accuracy of our results, our expertise and good customer service set us apart," Cecilia said. "Being part of the university and the School of Veterinary Medicine allows our resident faculty to be engaged with research, to keep abreast of latest developments and to teach and train students. All this translates to a better service provided to our customers."

VGL's commitment to research is also key to their partnership with APHA. In 2015, the association commissioned a small study looking at white pattern genes in a randomly selected group of horses.

"We tested 298 Paints for all coat-color and white-pattern mutations—19 mutations in all, roan not included," Cecilia explained. "There is still some analysis of the data to be done, but the results give a broad view of coat color variation in Paint Horses."

Of the nearly 300 Paints tested, the following results were noted:

- *Frame Overo* present in 37% of the sample
- *Tobiano* present in 32% of the sample
- *SW1* present in 9% of the sample
- *SW2* present in 2% of the sample
- *SW3* present in 1% of the sample
- *Sabino 1* present in 3% of the sample
- *W20* present in 39% of the sample
- *W10* present in a single horse in the sample
- *W5* not present in the sample
- *Appaloosa* not present in the sample

"The Paint Horse breed is clearly very diverse in color mutations," Cecilia said. "The majority of known mutations can be found in the breed; there are still white-spotting mutations to be identified that we know are present in the breed. More research is needed to resolve some of these patterns. White spotting is a very complex trait, with many genes that cause depigmented phenotypes, variable expression and influence of genetic and non-genetic factors."

W20: Subtle but Effective

Interestingly enough, four out of every 10 horses in the small APHA/VGL study were *W20* carriers. But what do we know about this mutation? Located on the *KIT* gene along with 22 other known white spotting patterns, it was discovered in 2013 and is believed to have a subtle effect on pigmentation. Horses with *W20* might have a wide blaze, leg markings, small belly spots and/or roaning throughout the coat, but the gene might have an even greater impact in amplification of white markings caused by other genes.

"It has been associated with increased size of white markings and with extended amounts of white when combined with other white-spotting mutations," Cecilia said. "It will be interesting to correlate the *W20* results with white-spotting phenotypes for this group of Paints."

Kao Castle, Ph.D., is the director of Practical Horse Genetics, a laboratory in Redfern, New South Wales, Australia. They began offering the *W20* test


CASSIDY COBARR

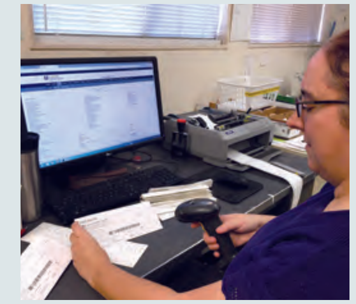
While we commonly think of frame overos that have large patches of white "framed" by areas of color, the pattern can be expressed more minimally, too.

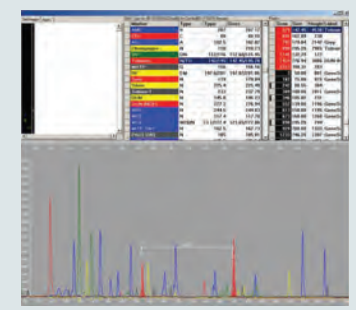

CASSIDY COBARR

While it's thought that homozygosity for a pattern like Splash White 1 *might increase the amount of visible white, that's not always the case. Both of these foals tested as* SW1/SW1 *carriers—homozygous for the gene—but they display vastly different amounts of white.*

In the Roots: Extracting DNA

Ever wonder what happens to your horse's mane hair sample once it arrives at UC-Davis for testing? Veterinary Genetics Laboratory Director Cecilia Penedo walked us through the process.





1. Hair samples delivered to the laboratory are given a unique VGL case number used to track each sample in the system. All information available for the sample (case number, horse ID, registration number, sex, date of birth, name and registration number of sire and dam, client ID, etc) is logged into the computer database.
2. Samples are batched according to tests ordered. DNA is extracted from hair roots from each sample in a batch.
3. The DNA extracts are subject to the Polymerase Chain Reaction (PCR) process, a laboratory technique used to make copies and to amplify the specific target DNA sequences for each test. For parentage analysis, the targets are the 21 markers used for this purpose. For the diagnostic tests, such as coat color and genetic disease, samples are put through the appropriate assays as ordered. All diagnostic test panels include ID and sex markers that allow further tracking of each sample to ensure the correct sample is being tested. All diagnostic tests are run in duplicate; for each case, two independent DNA extractions are done and their DNA types are matched.
4. DNA typing is done by capillary electrophoresis to separate the fluorescence-labeled PCR products and allow us to visualize the outcomes of the PCR tests. DNA types are determined using computer software developed by the VGL.
5. DNA types are placed in the VGL database and analysts review results, prepare the information and send reports to clients (registries or individual owners). Reporting of results is done electronically.

"The turnaround time at the VGL is quite short considering all the quality checks that we do before releasing a result. The majority of reports are sent to APHA within three to four business days from receipt of sample, unless there is a need to retest. If results are needed for prospective sale or breeding contracts, owners should submit samples as early as possible," Cecilia advised.

"One of the advantages of coat-color panel testing is that Paint breeders have more complete information about their horses and are better equipped to make informed decisions."

MEG MARTEL

Researchers think W20 might impact the expression of other white spotting genes. Oh Im Awesome [AUS], for example, carries SW1 and W20 and displays more ragged edges to his markings than what's typically found on horses with only SW1.

commercially about six months after it was discovered.

"It's well-known that if you have a frame overo stallion, and you are looking to breed foals with lots of white markings, you should look for solid mares with a fair bit of white—a blaze and decently-high socks," Kao said. "That's a pretty good description of a horse with *W20*."

Curious to see the effects of the mutation, Kao requested a photo of any horse that tested positive for *Frame Overo* and *W20*. The main challenge was finding horses that carried just the two genes because so many were multi-patterned.

"One of the first things I noticed was horses that were frame overo and also had *W20* seemed to be the more loudly marked frame overos," Kao said. "With the relatively small numbers that have come through, it looks like it does increase the odds of getting a loud frame overo with a nice amount of white. But it's definitely not a rule. I have seen two instances where a horse has been positive for *Frame Overo* and *W20* and just had very minimal markings."

While she hasn't noticed a significant difference on tobianos carrying *W20*, Kao found two horses that carried a *Splash White* mutation and *W20* that looked different from a typical splash white overo.

"There seemed to be a little bit more white, and it changed the nature of the markings," she said, describing the edges as more ragged and roany than a traditional splash white pattern. She also thinks *W20* boosts the amount of white on a horse with *Sabino 1*.

As luck would have it, a *W20* test led to a rare discovery.

"I had someone send a sample for a filly who was homozygous for *Sabino 1* that was completely white, and the owner insisted that I test for *W20* as well," Kao recalled. "I said there really is no point—the two are alternatives to one another. If your horse has two *Sabino 1* alleles, it can't possibly have a *W20*."

But the owner insisted, and much to Kao's surprise, the filly carried *W20* as well.

"There has been a genetic recombination within the *KIT* gene," she explained. "In this case, *Sabino 1* and *W20* are on the same chromosome together and are being inherited together. So there now is *Sabino 1* without *W20* and *Sabino 1* with *W20*, and those horses are pretty obviously sabino. It's really an odd one. Research-wise, it was a bit of luck.

"The mutation rate in the *KIT* gene in horses must be unusually high. I don't think there are any other animal species where there are so many mutations in the *KIT* gene. It's very interesting from a genetics or research standpoint."

Should You Test?

If you are still debating the value of coat color testing, just talk to Cassidy Cobarr, owner of Shining C Grulla Horses in Wolfe City, Texas. A firm believer in genetic testing, she tests all the horses in her breeding program; Cassidy learned the hard way a few years ago about trusting hearsay when she got a surprise from a mare she was told was homozygous for the *Tobiano* and black genes.

"I went ahead and bred her to my solid grulla Quarter Horse stallion who is heterozygous black and homozygous dun, and I thought I would be guaranteed a grulla tobiano baby," Cassidy said.

To her surprise, the mare foaled a red dun tobiano/splash white filly with a bald face and blue eyes.

"The only way that was possible," she said, "was if the mare was not homozygous for black and carried other spotting genes. So I tested her, and sure enough she came back positive for *Splash White 1*. It's an example of how genetic testing can also surprise you sometimes, even when you think you have it all figured out.

"I have a couple of mares in my program that I've tested who carry *Splash White 1*, and all they have is a star on their heads. One of my babies this year is homozygous for *Splash White 1* and only has four stockings and a bald face, which is really common; I also have a colt who is really obvious that he's homozygous splash because he was so excessive compared to his parents, who were very minimal."

Cassidy says testing is worthwhile compared to the investment needed to produce a foal.

"It just really takes the gamble out of the color when you breed something that's not homozygous," Cassidy said. While not all of her buyers care about coat color genetics, some do. "My clients that are looking for a breeding prospect—to them it's very important, so I like to have that available. It also helps me price the animal because rarity plays a big factor in price."

Australians Gail and Rod Shaw of Woodstock, New South Wales, have been breeding and showing Paint Horses for 30 years. They quickly embraced genetic testing when it became available.

"In 2003, we purchased Artful Gunner," Gail said. "At the time there wasn't any genetic testing for *Splash White*, only *Frame Overo*. 'Sonny' did test positive for *Frame Overo*, and all our solid Paint mares tested negative.

"When testing for *Splash White* became available, we found out that Sonny carries *SW1* and *SW2* as well; at the time, we didn't know that this was common for Gunner-bred horses. We had no idea that he could throw any combination of the genes. From then on, we have color tested just about all our foals."

Sonny has sired a few multi-patterned foals that are completely white, as has the Shaw's newest stallion, Hello Big Chex, who tested heterozygous for *Splash White 1*. This season "Big Chex" sired a foal that is all white out of a multi-patterned mare. They think the foal may be homozygous *SW1* and plan to test when he's older.

"I always tell potential breeders to get their mares genetically tested prior to breeding to Sonny or Big Chex. Just because they are mostly solid in their coat patterns doesn't mean that they do not carry some color genes," Gail said.

From a health perspective, there are other compelling reasons to test all breeding animals for white pattern mutations—and that goes beyond avoiding matings between *Frame Overo* carriers.

"I recommend that breeders avoid matings between horses carrying *SW2* or *SW3* and between horses that have *W5* or *W10*," Cecilia said. "There is no hard evidence based on outcomes from such matings, but we expect them to cause health problems. The prediction is that these could result in homozygous foals [for example, foals with genotypes *SW2/SW2*, *SW3/SW3*, *W5/W5*, *W10/W10*] that would not be

COURTESY GAIL SHAW

Horses carrying multiple pattern genes seem to often express greater white markings. Artful Gunner is such an example: he carries the SW1, SW2 and Frame Overo genes and has sired foals carrying different combinations of those genes.

Genetic Testing

Find out exactly which colors and patterns your stallion or mare carries before the breeding season begins. APHA's official genetics testing partner, the Veterinary Genetics Laboratory at the University of California–Davis, offers a Comprehensive Coat Color test for $125. This simple procedure, done with 30 to 50 hairs (with intact roots) from your horse, tests for the presence of red factor, *Agouti*, *Cream*, *Pearl*, *Champagne*, *Dun*, *Silver*, *Gray*, *Tobiano*, *Frame Overo*, *Sabino 1*, *Splash White 1*, *2* and *3*, *and Dominant White 5*, *10* and *20*. Results, sent by email, take seven to 10 business days. Order the test by calling APHA at 817-222-6423, and learn more online at *vgl.ucdavis.edu.*

COURTESY SUSANNA MARINELLI

Though there's still more to discover, knowledge and understanding of your Paint's genetics can help you make more informed decisions with regard to breeding.

viable or those that could have severe clinical problems.

"It is possible that even combinations of these [compound heterozygotes]—for example, DNA genotypes *SW2/SW3*, or *W5/W10*—could be deleterious," she explained. "Fortunately, *SW3*, *W5* and *W10* are infrequent or rare enough to not cause breed-wide concern, unlike OLWS. One of the advantages of coat color panel testing is that Paint breeders have more complete information about their horses and are better equipped to make informed decisions about mate selection. This will minimize risks of producing affected foals that are aborted or have to be euthanized after birth."

The Breeder's Toolbox

Genetic testing for white spotting patterns has come a long way. Until 2008, the only commercial test available analyzed blood samples for genetic markers for *Tobiano* but could not be used to officially prove homozygosity. Today, testing of about 40 mane hairs with intact roots can reveal with near-certainty if a horse carries the genes for nine different spotting patterns—an amazing tool available to the Paint Horse breeder. And yet, many questions remain.

For example, some horses sport an identifiable pattern—what we might call sabino or splash white—but don't test positive for any of the known white pattern genes. What causes those patterns? And what are the other factors, perhaps genetic or environmental, that influence the quantity and placement of white? Why are some horses solid or minimal white, and others who carry the same mutations are loudly marked?

And finally, what impact do white spotting genes play on a horse's health? One such example is deafness, found with some frequency in frame overos and splash whites.

In 2009, a small study evaluating deafness in American Paint Horses was published, shedding some light on the question. K. Gary Magdesian, D.V.M., a professor of medicine and epidemiology at the University of California-Davis School of Veterinary Medicine and author of the *Journal's* monthly "Vet's Bag" column, was the lead researcher. He continues to investigate the genetic link between white spotting patterns and deafness and has nearly completed a follow-up study. Stay tuned to future issues of the *Journal* for complete results and analysis.

Though we still have a great deal to learn about white spotting patterns in horses, greater knowledge and genetic tests available today offer breeders valuable tools to increase the percentage of foals born with desirable spotting patterns. At the same time, this information also plays an important role in equine health and welfare and in responsible breeding by limiting or preventing embryonic or neonatal loss. Paint Horse breeding will always involve an element of surprise, but genetic testing for white spotting patterns can help improve the odds that those surprises will be good ones. 50

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