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 (T/O) has one Tobiano allele; a homozygous tobiano (T/T/O) has two.

Some genes are incompletely domi-
nant. This means that heterozygotes do not look the same as homozygotes. Sabino I 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 expres-
sion 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” is a class of pigment that’s responsible for Paint Horse spotting patterns in embryos.

**The Big Six**

<table>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
<th>Gene</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobiano</td>
<td>Incompletely Dominant</td>
<td>KIT</td>
<td>Horse</td>
</tr>
<tr>
<td>Frame Overo</td>
<td>Incompletely Dominant</td>
<td>KIT</td>
<td>Thoroughbred</td>
</tr>
<tr>
<td>Sabino I (SII)</td>
<td>Incompletely Dominant</td>
<td>KIT</td>
<td>Thoroughbred</td>
</tr>
</tbody>
</table>

**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)

#### Inheritance:
- Incompletely Dominant
- Gene: KIT on Chromosome 3

#### Overview:
- Phenotype: Various, depending on the level of expression. Ranges from a white horse with mottled phenotype to a horse with a sabino-like coat pattern, and his offspring.
- Name: Researchers named them Dominant Whites.
- Note: Tobiano (T/O) is a dominant trait, not all tobiano mares have enough white to meet the minimum requirements for a Tobiano Registry.

### Dominant White 10 (W10)

#### Inheritance:
- Incompletely Dominant
- Gene: KIT on Chromosome 3

#### Overview:
- Phenotype: Various, depending on the level of expression. Ranges from a white horse with mottled phenotype to a horse with a sabino-like coat pattern, and his offspring.
- Name: Researchers named them Dominant Whites.
- Note: Tobiano (T/O) is a dominant trait, not all tobiano mares have enough white to meet the minimum requirements for a Tobiano Registry.

### Dominant White 20 (W20)

#### Inheritance:
- Incompletely Dominant
- Gene: KIT on Chromosome 3

#### Overview:
- Phenotype: Various, depending on the level of expression. Ranges from a white horse with mottled phenotype to a horse with a sabino-like coat pattern, and his offspring.
- Name: Researchers named them Dominant Whites.
- Note: Tobiano (T/O) is a dominant trait, not all tobiano mares have enough white to meet the minimum requirements for a Tobiano Registry.
One of the biggest challenges facing researchers and Paint Horse breeders alike is the nomenclature of spotting patterns.

**The Nomenclature Nightmare**

One of the biggest challenges facing researchers and Paint Horse breeders alike is the nomenclature of spotting patterns. Over the years, the 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 Sulino 2 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. Sulino 2 is a good example.

“We struck out on our own when we named Sulino 2,” 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 Sulino 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 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 homoyzous lethal, and many of those are completely white, but not all of them,” Samantha said. “She found a couple on the murine mice—some that were potentially viable as homoyzous 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 homoyzous for it. They are perfectly healthy, and homoyzous rarely have more than 30 percent white on the body. And yet that allele got a Dominant White acronym because it is in the W10 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 a lot of horse people would call a splash white.”

Three mutations designated Splash White can be confusing, too.

“My issue is there is a scientific one because you have two different genetic issues 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.”

**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.

“Emphasis [is] a word I don’t like,” Samantha said, “partly 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 any thing 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.**

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/conventions. **FEBRUARY 26, 2016 1:30–2:30 P.M.**

**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/conventions.
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 Paintbred 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. 🚴

Irene Stamatakis is a special contributor for the Paint Horse Journal.

feedback@apha.com

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

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
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.

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.

The breadth of genetic tests that we offer is unparalleled, and the breadth of research we do is equally without bound. 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.

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.

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. “The turnaround time at the VGL is quite short considering all the quality controls that are in place,” Cecilia said. “We tested 298 Paints for all coat-color and white-pattern mutations—19 mutations in all, none of which were 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 Seitenstueck (SST) 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.”

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.

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 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.

 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.

In the Roots: Extracting DNA

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.

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.

The breadth of genetic tests that we offer is unparalleled, and the breadth of research we do is equally without bound. 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.

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. “The turnaround time at the VGL is quite short considering all the quality controls that are in place,” Cecilia said. “We tested 298 Paints for all coat-color and white-pattern mutations—19 mutations in all, none of which were 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 Seitenstueck (SST) 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 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.”

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 mare. It was discovered. 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 overo,” 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 tobiano 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 roan 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.

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 Cassidy, owner of Shadow Creek 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 homozgyous 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 are a star on their heads. One of my babies this year is homozygous for Splash White 1 only and 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.

“W3/W3 was the breed standard in Richmond, Virginia. Saman-tha 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.

Sonny has sired a few multi-patterned foals that are completely white, as has the Shaw’s newest stallion, Hello Big Chez, who tested heterozygous for Splash White 1. This season “Big Chez” 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 Chez. 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, SW2/SW3, W5/W5, W20/W20] that would not be able to produce a foal. The reason? If you are still debating the value of coat color testing, just talk to Cassidy Cassidy, owner of Shadow Creek 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 homozgyous 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.

“These have 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. We had no idea that he could throw any combination of the genes. From 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.”

Paint Horse Journal | February 2016 | 91

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. Saman-tha 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.
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.

Irene Stamateleaks is a special contributor for the Paint Horse Journal.

feedback@apha.com