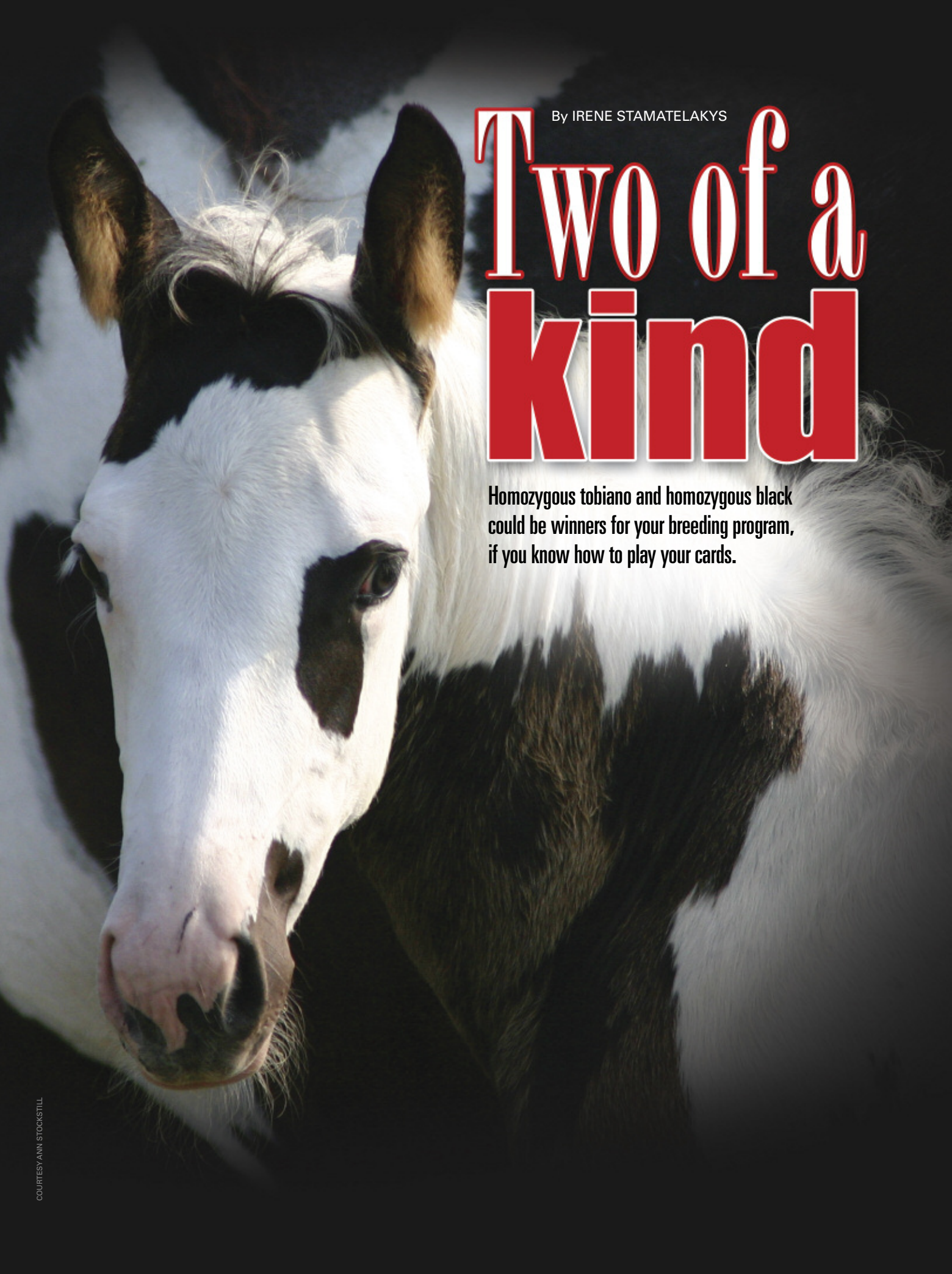
By IRENE STAMATELAKYS

Two of a kind

Homozygous tobiano and homozygous black could be winners for your breeding program, if you know how to play your cards.



COURTESY ANN STOCKSTILL

In poker, a pair is not much to brag about. Two pairs are just a hair better. But in equine color genetics, a pair—or, even better, two—could be one of the best hands you'll ever hold. We're talking about a sure bet—a pair of tobiano or black genes.

Any Paint breeder will tell you that producing a quality foal that will bring in top dollar is a gamble. In this business, there are no guarantees. But what if you could reduce some of the risk in your breeding program as well as increase the marketability of your product, with fewer solid foals and more sought-after colors?

That's where a horse that is homozygous tobiano and/or homozygous black could give you a competitive edge, in marketing terms. Add that to an already attractive package—good bloodlines and a performance record—and you've got a winning hand.

But before you place your bets, what exactly does "homozygous" mean? What should you know before you make any breeding decisions? And how important is homozygosity to Paint Horse breeders in today's market? We'll answer those questions and more, with a focus on homozygous tobianos and blacks, the two you see advertised most frequently.

Genetics made simple

Simply put, homozygous is an adjective used in genetics to describe having two identical genes for a particular trait. If your high school biology class is a distant memory, let's quickly review some basic genetics.

In his book *Equine Color Genetics*, author D. Phillip Sponenberg, DVM, Ph.D., explains that genes control all chemical processes occurring in living organisms, including traits like hair color.

"In horses, as in all mammals, genes occur on chromosomes," writes Sponenberg. "Chromosomes can be thought of as strings of genes. Chromosomes occur in pairs, and an individual gets one of the pair from the sire and the other of the pair from the dam."

Every gene has an address—a specific site on a specific chromosome. We call this address a locus—plural being loci. Quite often, geneticists use the locus name to refer to a gene.

When a gene comes in different forms, those variations are called alleles. For example, there is a tobiano allele and a non-tobiano allele. Either one can occur at the tobiano locus, but each chromosome can only carry one allele at a time. So a horse will have no more and no less than two alleles for any trait.

This leads us to the definition of homozygous, which Sponenberg describes as "the condition of having two identical alleles at a locus (one on each chromosome)." The opposite would be heterozygous, where the two alleles are different.

For a horse to be black, his genotype must be *Eeaa* or *EEAA* ... As long as the foal inherits one *TO* allele, genetically, he's a tobiano.

As simple as that concept may seem, many people mistakenly use the term, saying "my horse is homozygous." If you know anything about genetics, it should lead you to wonder, "homozygous for what?"

That's because a horse can be homozygous for any number of alleles: Agouti, black, champagne, cream, dun, gray, pearl, red, silver, Sabino 1 and tobiano, just to name a few equine coat color characteristics.

But first, let's take a look at the *Agouti* allele, which plays a critical role in equine color genetics. If your goal is a black foal, and you've drawn the *Agouti* allele, you're out of luck.

The *Agouti* effect

Approximately 20 percent of horses registered with the APHA are bay. If you also include the colors derived from bay—buckskin, dun, bay roan and perlino—almost one-quarter of registered Paints carry and express the *Agouti* allele, symbolized by an uppercase *A*. This dominant allele is responsible for restricting black hair to the points, on horses capable of forming black pigment. Whether a horse is heterozygous (*Aa*) or homozygous (*AA*) for *Agouti*, the result is the same—a horse with black points.

The recessive version of the allele, symbolized by a lowercase *a*, is expressed only in the homozygous state. If a horse is *aa*, then the black hairs—if the horse is capable of forming black pigment—are spread uniformly over the body. About 16 percent of horses registered with the APHA are black, grullo or blue roan.

If you are breeding for a specific color, say black, it is important to know the *Agouti* genotype, or the genetic makeup, of your breeding stock. For example, if a homozygous black stallion is *AA*, then he will always pass the *Agouti* allele to his offspring and only throw bay colors. If he is *Aa*, then there is a 50 percent chance his offspring will be bay or a derivative of bay. If that stallion is *aa*, then he will always pass on the recessive allele, and his offspring could potentially be a uniformly-distributed black-based color, depending on the dam's genetic contribution.

Pure black

Perhaps the more correct term is "homozygous for black pigment," but that would make for some very wordy ad copy. A homozygous black horse is not necessarily black, nor does he throw 100 percent black offspring. The only guarantee here is this horse is not red and will not produce red offspring. Why is that?

Homozygosity for black and/or tobiano can give your horses a marketing edge.

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JEFF KIRKBRIDE, COURTESY CHERYL MILLER

Above: "Ink spots," like those on Chicos Bandelero's coat, aren't proof of homozygosity. Genetic testing is the only way to know—this stallion is homozygous for black and tobiano.

Right: The bay stallion Sacred Assets is homozygous for black and tobiano, so he'll never throw a red-based foal.

To understand this complex concept, you have to understand the workings of the *Extension* locus, and its interaction with the *Agouti* locus.

The recessive version of the *Extension* allele, symbolized by a lowercase *e*, is expressed only in the homozygous state. This is commonly called the "red factor." If a horse is *ee*, then it will be red—sorrel or chestnut or a red dilute, such as palomino or red dun. And if the horse is homozygous *ee*, this will completely mask the *Agouti* genotype. You cannot tell, just by looking, if a homozygous *ee* horse carries one or more *Agouti* alleles, and therefore could produce a bay foal when bred to a black-based horse.

The opposite of red-based, in equine coat colors, is black-based, symbolized by an uppercase *E*. This is also called the "black factor." Whether the horse is heterozygous *Ee*, or homozygous *EE*, the base color of the coat is black. However, several variables can modify that black base color. For example, if the horse is an *Agouti* carrier, the black hair will be restricted to the points, which would make him bay. Or, he could have one or more dilution genes, which would modify the color even further, making him buckskin, grullo, smoky black, or dun—just to list a few possibilities.

For a horse to be black, his genotype must be *Eeaa* or *EEaa*. The only difference is that a homozygous black horse (*EE*) will always pass that black factor to his offspring, guaranteeing that they will be black-based. But it is possible for any black-based horse, for example a bay, to be homozygous black. Simply, the expression of that black *Extension* allele depends entirely on the *Agouti* allele.

Sacred Assets is one such example. The 2005 bay stallion is homozygous for the black gene, as well as the tobiano gene. Owner Cheryl Miller of Kaleva, Michigan, says she does get questions about this.

"I've been asked, 'If he's bay, how can he be black?'" said Miller. "But

bay does come from the black gene." She explains to them that it simply means he'll never throw a red foal.

"There's nothing wrong with sorrel, but there are a lot of sorrels out there," Miller said. "People are interested in getting something different. They don't know there is a homozygous black, that he'll only throw those black-based colors."

Tobiano x 2

The tobiano spotting pattern is a result of the dominant *tobiano* allele, symbolized by *TO*. As long as the foal inherits one *TO* allele, genetically, he's a tobiano. If the parent is heterozygous for tobiano, there's a 50 percent chance for tobiano offspring. If the parent is homozygous for tobiano, 100 percent of the offspring will be tobiano, virtually eliminating the risk of a solid foal. And that is the allure of homozygous tobianos—a 100 percent color guarantee.

But wait! Before you sign that breeding contract, there are a few things you should know.

According to researchers, it is impossible to distinguish visually between heterozygous and homozygous tobianos with complete accuracy; the only way to positively identify a homozygous tobiano is through testing.

Fortunately, the accuracy of tobiano testing improved dramatically in recent years. The old test using blood-protein markers and pedigree analysis was wrong as much as 10 percent of the time. But in early 2008, researchers from the University of Kentucky's Gluck Equine Research Center published the results of a study linking the tobiano gene to a chromosome inversion near the KIT gene. As a result, breeders had a new, more accurate DNA test at their disposal to identify homozygous horses. Even better, the new test was done with hair samples, rather than blood, making it much more convenient.

Samantha Brooks, Ph.D. (now of the Department of Animal Science at Cornell University), whose research gave rise to today's tobiano test, says that a group in Switzerland published a paper validating the new inversion test. There have been zero false positives and negatives to date with the new test, says Brooks.

Although the new test is highly accurate, there's still the possibility a homozygous tobiano could throw a solid foal. How so?

"Expression of the tobiano phenotype [external appearance] is variable and sometimes does not conform to the definitions we like to give it," said Brooks. "Mother Nature is good at proving us wrong as soon as we say something is 'always' a certain way."

It is possible for a horse to have the tobiano genotype but not the phenotype, as we have defined it. In other words, you could get a tobiano that doesn't meet the minimum requirements to make it eligible for the APHA's Regular Registry.

"I have seen some of these minimal tobianos," Brooks said. "There may be other genes modifying the extent of white. For example, the *MC1R* [melanocortin 1 receptor] and *Agouti* genes both have effects on the extent of white in patterns, including tobiano. Thus, blacks tend to have less color, chestnuts more. Modifying genes aside, [minimal tobianos] have the same potential to transmit the *TO* gene as any other *TO* horse."

Bet for value

By now, it's pretty clear why homozygosity has its appeal, at least

Facing the facts

Myth: Homozygous tobianos give you a 100 percent color guarantee.
Fact: This actually depends on your definition of "color." The foal will definitely inherit a tobiano gene, but occasionally it's expressed only minimally. It's possible that the foal won't have enough white hair over unpigmented skin in a qualifying area to be eligible for APHA's Regular Registry. Although such a foal would be registered as a solid Paint-bred, genetic testing could confirm the foal carries the tobiano allele, which it could pass on to offspring.

Myth: "Paw prints" are proof a tobiano is homozygous.
Fact: They are called paw prints, bear paws, ink spots and cat tracks—small, round, colored spots, often surrounded by a roaned halo, popping up in the white areas of a tobiano. While they are frequently seen on homozygous tobianos, they do not a guarantee that the horse really is. Only genetic testing can accurately confirm a horse is a homozygous tobiano.

Myth: A homozygous black horse is always black.
Fact: "A horse's final color results from the interaction of 11 generally independent processes," writes Sponenberg. Because multiple alleles affect coat color, a homozygous black may not necessarily be black in color. Depending on the complete genotype, this horse could actually be bay, grullo, smoky black or even gray, just to give a few examples.

Myth: Breeding to a homozygous black horse guarantees you'll get a black foal.
Fact: The only guarantee when breeding to a homozygous black horse is you won't get a "red" foal, meaning sorrel or chestnut. A homozygous black is *EE*, meaning it has two dominant alleles for black-pigmented hair. It will always pass one to its offspring. A sorrel or chestnut is *ee*, meaning it has two recessive alleles and does not have black hair. Depending on the foal's complete genotype, it could be any of the black-based colors, but never sorrel or chestnut.

Myth: Any tobiano or black horse could be homozygous for that gene.
Fact: To get a homozygous tobiano, both parents must be tobiano. The same rule applies for a homozygous black horse. Both parents must be black-based and have an *E* allele to pass on to the foal.

LEA, COURTESY ANN STOCKSTILL

Although color should not be the sole basis for a breeding decision, choosing a stallion homozygous for tobiano and/or black—such as Summerschicobandito (above) or Strait Texas Kaliman (right)—can help reduce the unpredictability of the resulting foal's color.

COURTESY NEAL AND PATTY BOUMA

when it comes to the tobiano and black genes.

"Tobiano homozygosity helps keep the color on the babies, and that's what people are looking for," said Miller. "Obviously, homozygous tobianos aren't the only ones that produce color, but they are the only ones that guarantee it."

This, she says, is especially important to owners with solid Paint-bred and Quarter Horse mares. However, in today's market, she feels homozygosity for tobiano or black is a helpful marketing tool, but no longer the only criteria mare owners consider.

"There are enough stallions out there that bloodlines and performance in the show pen are also important. When you add the homozygosity, it helps get your stallion into the market and move up the ladder."

Ann Stockstill of Winona, Texas, stands Summerschicobandito and Chicos Bandelero, both of whom are homozygous tobiano, homozygous black stallions. She says that homozygosity is a strong selling point.

"The basic concern is the guarantee of a colored foal," Stockstill explained. "Most of my clients want a black tobiano foal."

But does homozygosity automatically generate higher breeding fees? Not always, says Stockstill.

"I think that a stallion can command a higher breeding fee if he is both homozygous and has an impressive performance record," she said. "Shipping semen to foreign countries is more in demand if the horse is homozygous also."

Test prep

Of the major U.S. laboratories, three offer the *Tobiano*, *Extension* (also called Red Factor) and *Agouti* tests using hair samples.

Laboratory	University of California, Davis	University of Kentucky	Animal Genetics, Inc.
Gene	*vgl.ucdavis.edu*	*ca.uky.edu/gluck/AGTRL.asp*	*horsetesting.com*
Tobiano	$25	$45**	$25
Extension	$40*	$45**	$25***
Agouti		$45**	$25***

*University of California, Davis, only offers the *Extension* and *Agouti* tests together for $40.
**University of Kentucky offers three tests, ordered at the same time for one horse, for $35 each.
***Animal Genetics, Inc. offers the *Extension* and *Agouti* tests together for $40.

Tobiano results are reported as:

N/N	No evidence of altered sequence detected. Horse is not Tobiano.
N/TO	One copy of altered sequence. Approximately 50 percent of the offspring will inherit Tobiano.
TO/TO	Two copies of altered sequence. Horse is homozygous for Tobiano. All offspring will inherit Tobiano.

Agouti results are reported as:

A/A or A/a	Black pigment distributed in point pattern. The basic color of the horse will be bay or brown in the absence of other modifying genes. A has no effect on red pigment (*ee*).
a	Only recessive allele detected. Black pigment distributed uniformly. The basic color of the horse will be black in the absence of other modifying genes.

Red Factor (_Extension_) results are reported as:

e	Only the red factor detected. The horse can be assumed to be homozygous for red (*ee*). The basic color is sorrel or chestnut, but depending on genes at other color loci, the horse could be palomino, red dun, gray, cremello or white.
E/e	Both black and red factors detected. The horse can be assumed to be heterozygous for the red factor (*Ee*). It can transmit either *E* or *e* to its offspring. The basic color of the horse will be black, bay or brown, but depending on genes at other color loci, the horse may be buckskin, zebra dun, grullo, perlino, gray or white.
E	No red factor detected. The horse can be assumed to be homozygous for black pigment (*EE*). It cannot have red foals, regardless of the color of the mate. The basic color of the horse will be black, bay or brown, but depending on genes at other color loci, the horse may be buckskin, zebra dun, grullo, perlino, gray or white.

Source: UC-Davis Veterinary Genetics Laboratory (*vgl.ucdavis.edu*)

And it helps sell horses in a troubled economy, says Stockstill.

"In the market today, my clients in other countries are wanting double homozygous horses with good pedigrees and prefer that they have sires and dams with performance records," she said.

Neal and Patty Bouma of Choteau, Montana, acquired their first homozygous tobiano stallion in 1995. Today they stand Strait Texas Kaliman, who is homozygous for both the black and tobiano genes.

"Black and white still sells better and for more money than any other color," said Neal Bouma. "The pedigree is very important too."

Guaranteed color and top performance bloodlines are a combination they believe is essential for success.

"The demand for double homozygous horses is still high and they sell for at least double and even up to four or five times as much as a colored Paint," said Bouma. "We are selling more horses for more money than we ever have. I think it is because of our bloodlines, the way we promote them and the homozygous factor combined."

Drawing two pair

What are the chances of drawing two pair? Well in poker, the probability of being dealt two pair is 4.8 percent. And in Paints? Well, let's put it this way. If you don't try, you never will.

"When you breed to a Quarter Horse, you take away the possibility of having a homozygous tobiano baby," says Stockstill. If this is to be in the cards, then you've got to follow the genetic rules and breed tobiano to tobiano and black-based to black-based.

Bouma is hoping for a few double homozygous foals this spring.

"That would be our ultimate goal," he said. "The odds are low to get it all, but you can't get it if you aren't trying. I have never shot a bull elk sitting in my living room either. You have to be rolling the dice or playing the lottery to win."

Now that you know the rules, you are ready to play. PHJ

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