ICHO GAZETTE GENETIC

RESEARCH SPECIAL EDITION

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SPECIAL EDITION

Greetings ICHO members and friends! This is an **extraordinary** Special Edition of the ICHO Gazette!

On November 15, 2017, with great anticipation, Curly gene research was scientifically published, and we are ecstatic with the implications of knowing our Curly horses better than ever! In the journal of *Genetics Selection Evolution, 2017 49:85* , the publication is A missense variant in the coil1A domain of the *keratin 25* gene is associated with the dominant curly hair coat trait (Crd) in horse. The direct link is https://gsejournal.biomedcentral.com/articles/10.1186/s12711-017-0359-5 . In this edition of the gazette, we gratefully present Dr. Mitch Wilkinson's untiring support in ICHO Research with his *Curly Coats on Horses Are Caused by Multiple Gene Mutations.* Enjoy this magnificent read, photos, history and great effort from many.

ICHO would like to greatly thank the researchers, contributors, Curly horse supporters, Dr. Gus Cothran of Texas A & M University and colleagues, Dr. Mitch Wilkinson, and our ICHO families for their support.

This Special Edition includes all Genetic Research Articles to date in one Edition. Enjoy!

Joan Henning, ICHO President

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ICHO RESEARCH WEBPAGE

https://www.ichocurlyhorses.com/research.html

ICHO GAZETTE GENETIC RESEARCH SPECIAL EDITION

Welcome to all Curly Friends, Owners and Breeders, the purpose of this special edition is to compile all the Genetic Curly Horse Research articles in one convenient place. This will be a great resource for all Curly Owners and Breeders. It contains information and history of the first found Curly Coat Gene KRT25 and the second isolated Curly Coat Gene SP6 plus the still unknown Curly Coat Genes found in North America and around the world. Balding in Curly Horses and the recent research findings about genetic origins of the Curly lines through PCoA graphs done by Dr Gus Cothran, TX A&M and how each line differs from other Curly lines. There are breeding charts for Curly coat gene breeding's and also preservation breeding.

The link to the ICHO Research webpage https://www.ichocurlyhorses.com/research.html includes all early ICHO Curly Horse research including blood comparisons, skin biopsies, allergy research, Ancestral testing, early Genetic Testing, Gait Gene research and testing and Fiber studies on Curly hair. Our hope is that this Special Edition will help answer all your Curly Horse Genetic Research questions.

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Curly Coats on Horses Are Caused By Multiple Gene Mutations

By Dr. Mitch Wilkinson

Introduction

A great unsolved mystery in the equine world is the origin of curly haired horses in both the domestic and wild horse populations, not only in the Americas, but also parts of Asia.

The present histories of curly horse introductions into North and South America are based on oral legends, dubious speculations, and suppositions based on incomplete information that have been repeatedly stated over several decades to become something that masquerades as truth, but in fact, has little factual basis.

A good example is the incorrect term, "Bashkir", which refers to a region of Russia and is incorporated into the name of one of the three curly horse registries. The speculation of North American curly horses coming from the Bashkir region of Russia came from a 1930's newspaper cartoon that was noticed by early Nevada curly horse breeders. [2]

The legend of an Irish immigrant, horse breeder named Tom Dixon bringing curly horses to Northern Nevada via India in the late nineteenth century has documented evidence in the Nevada State Archives, but the actual evidence is based on a second-hand testimonial that was preserved in the 1950's. There is a possibility that this account may well be factual, but this curly horse introduction could only account for a couple of mutations that contribute to the production of curly coats in North American equines. [2] [5] [18] [4]

For most of the history of curly coated horses in North America, it was thought there was only one coat mutation that imparted a curly coat to horses. Curly enthusiasts spent many hours speculating about a mythical, "old world" breed that somehow found its way to the North American continent and was the direct ancestor of North American curly horses. We now know that there are many possible mutations that can cause curly coats in horses. The fact that curly horses have a wide variation in coat appearance is an indication of the several different types of curly coat patterns, each thought to be caused by a different gene mutation. [1]

The isolation of one gene mutation that causes curly coats in horses has led to the realization that there are other causative mutations within the population. Because of the lack of documented historical evidence, the relationships of one curly coated horse population to another can only be determined by genetic analysis. [1]

The following narrative not only describes a mutation that causes curly coats in a percentage of the curly horse population, but also describes other possible mutations and their effects.

A statement from The President of the International Curly Horse Organization:

The International Curly Horse Organization, ICHO, looks to scientific evidence to define a Curly horse. ICHO is a forward-thinking registry where all Curly horses are welcome. The unique traits of a Curly horse not only deserve thoughtful consideration, but deserve truth. ICHO, since its inception, is distinguished by its use of scientific research and is committed to support and fund Curly horse gene research today and will continue to do so in the future. Currently underway, ground breaking Curly horse gene research makes ICHO unique, and scientific research is a priority to support defining evidence of these marvelous horses. It's with great pleasure to see the support of ICHO materialize in the article by Dr. Mitch Wilkinson. ICHO looks forward to sharing this work with continued support and great anticipation of what is yet to be revealed.

Joan Duesbout Henning, ICHO President

Part I

Curly Genes – Genotypes and Phenotypes

One of several gene mutations that are the causative factors in curly coated horses has been isolated by Dr. Schibler and colleagues in France in collaboration with Dr. Gus Cothran at Texas A&M University.

A scientific paper was published on November 16,2017 in the journal, **Genetics Selection Evolution** reporting the results of genetic studies conducted almost four years prior to the date of publication. [1] https://gsejournal.biomedcentral.com/articles The article was titled: ***A missense Variant in the Coil1A Domain of the Keratin 25 gene is Associated with the Dominant Curly Hair Trait (Crd) in Horse.***

Dr. Schibler's genome sequencing with an Illumina Equine SNP50 Bead Chip used test subjects which included a combination of 70 straight haired and curly haired horses. Horses that exhibited a curly coat type were compared to horses with straight hair from a variety of breeds. The results were used to isolate a mutation that was found to be on a Keratin gene on the 11th equine chromosome pair (ECA 11). This mutation was unique to many of the curly coated horses used as test subjects. The location within the 11th chromosome where the mutation was found is known to code for type I keratins which are the building blocks of hair. [1]

Dr. Schibler's experimental model was based on 51 curly coated horses and 19 straight haired horses from 13 paternal families. Samples of horses from both France and North America were included. To help identify candidate genes, a whole genome sequence was obtained from a presumed heterozygous stallion, **BFC Spartacular Splashes**, and his straight-haired son, Alias Splash. Dr. Schibler identified a missense mutation at **KRT25:p.R89H** as responsible for the dominant curly coat trait. The KRT25 gene is has known association with hair. [1]

Above, BFC Spartacular Splashes owned by Aline & Jeroen Verschuren.

Explanation of some basic genetic concepts as they relate to the KRT25 mutation

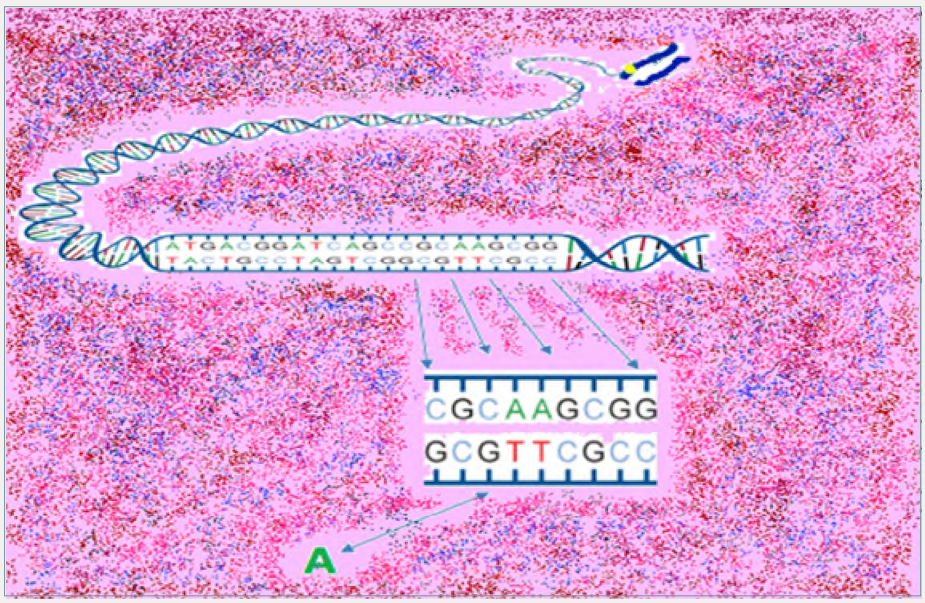
A substitution of a single base pair within the site of a known gene called KRT25 seems to be the causative factor in a large percentage of curly coated horses tested. This mutation has been designated a numerical location on the DNA chain of the 11th chromosome and is mapped as p.R89H. The substitution of a single base in a gene sequence, or **SNP** (Single Nucleotide Polymorphism), is called a **point mutation** when it affects the organism in some way. [9]

Point mutations can, and often do, affect the selection of alternate amino acids which are the building blocks of proteins. There are 20 different amino acids that make up the proteins of all living things. [3] An individual protein can consist of between 50 and 1,000 amino acids that are bonded together in a specific order to create a polypeptide chain. Each polypeptide chain or **protein** has a specific shape which corresponds to its function. The addition, substitution, or deletion of even a single amino acid in the sequence can profoundly affect the resing protein and

consequently affect the resulting tissues that the protein either makes or regulates. [19] [9] [7] [8]

In the case of the mutation isolated within the gene, KRT25, the triplet DNA code for the amino acid, Arginine was changed to the code for another amino acid, Histidine. The code change happened due to the substitution of a Guanine base in the DNA sequence to an Adenine base. This change of a single base in the DNA sequence, or SNP, caused an alternate amino acid to be incorporated into the polypeptide chain. The resulting protein's shape was then slightly altered. The change in shape modified a critical protein function that is essential for the proper assembly of keratin type I and type II complexes in the hair follicle. Keratin complexes are the building blocks in hair formation. A mutation which results in the substitution of one base for another in the DNA sequence and consequently the coding for an alternative amino acid is known as a **missense mutation**. [9] [19]

Adenine(A) is substituted for a Guanine(G)



In the above illustration

An adenine(A) bonds in the DNA chain to a thymine(T), so a change in one base also changes the corresponding base in the other strand of the molecule. In this instance, the original guanine(G) which was bonded to a cytosine(C) was changed to an A – T pair. This minor change in one base pair, out of millions of bases in the DNA chain of an individual animal can, incredibly, exert profound effects. If the change, or SNP, happens in a critical location in the chain that codes for a gene, it becomes a ***point mutation.***

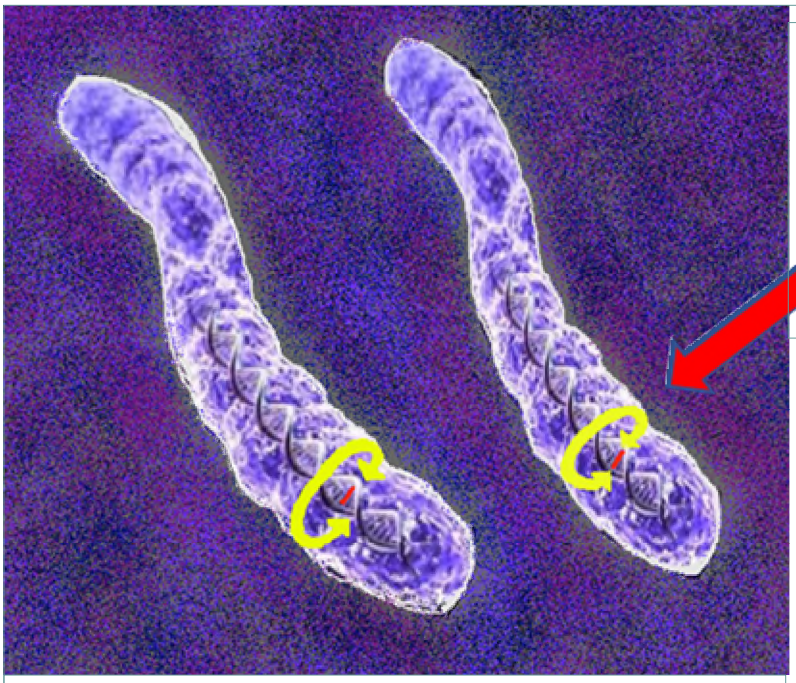
The **missense, point mutation** in KRT25 was found to be present in a little more than half of the curly coated horses that have been tested. [1] [10]

The remaining curly horse test subjects that did not have the KRT25 point mutation present were grouped together by pedigree, location, and physical appearance. These horses were suspected of carrying different gene mutations that resulted in curly coats. [10]

The other, unknown, curly hair producing gene mutations were given designations based on founding sires (**Curly Jim**), founding breeders (**Cook**), type (**Sulphur** and **Spanish Mustan**g) or location (**Patagonian, Mongolian, or Siberian**). Currently, genome sequencing is being conducted by Dr. Cothran and his team at Texas A&M University to isolate other suspected mutations that result in curly coats on horses. This project has been funded by The International Curly Horse Organization on an ongoing basis since 2003. The late Sandy Hendrickson, one of the original founders of the organization, started the project to place the registry on sound scientific principles.

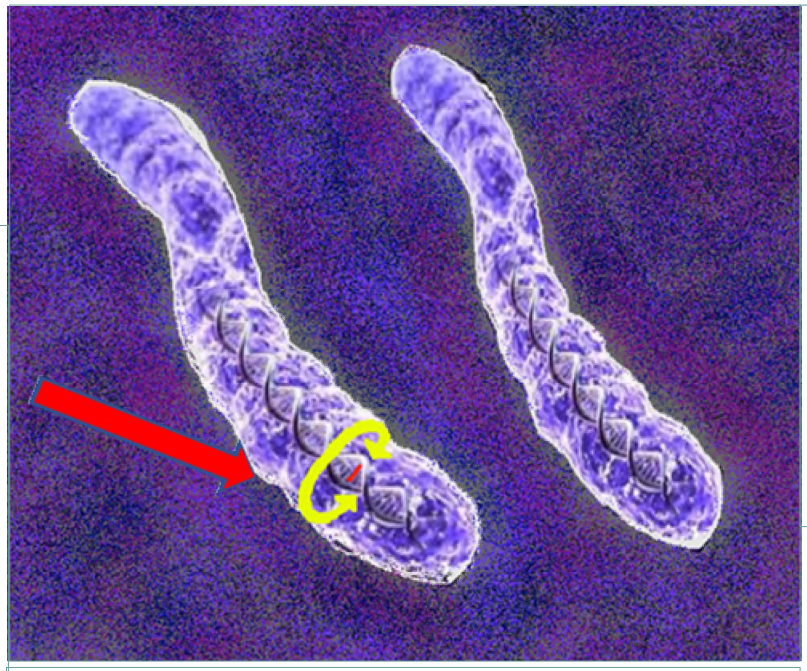
The phenotype, or appearance, of the horses that carry the KRT25 mutation is dependent on whether they carry a **single** copy of the mutation (heterozygous) or **two** copies of the mutation (homozygous) on chromosome 11 which consists of two DNA strands. One strand is inherited from the dam while the other is inherited from the sire. [19] [8] [7]

The KRT25 mutation has a dominant mode of inheritance. This means that an individual horse only needs one copy of the mutated gene to show traits. [3] Most of the curly coat traits are thought to have a dominant mode of transmission. [11]



Left, both DNA strands have the KRT25 mutation. This horse is homozygous for KRT25 mutation. Example: Adenine sire – Adenine dam or AA. An Adenine substitution for Guanine in both DNA chains.

The example above shows a chromosome that is homozygous for the KRT25 mutation. This individual inherited a copy of the mutation from both the sire and the dam. This is chromosome 11 of a horse.



The example to the left shows a chromosome that is **heterozygous** for the KRT25 mutation. This individual inherited the mutation from either the dam or the sire, but not both.

Right, the area where the KRT25 mutation is located on a single DNA strand. The other DNA strand of the chromosome does not have the mutation. Example: Adenine sire – Guanine dam or AG-- an Adenine was substituted for a Guanine in one DNA chain, but not the other.

This is chromosome 11 of a horse.

Each suspected curly coat producing mutation results in horses that have distinctive coat characteristics or phenotypes.

Genes come in different versions, called **alleles.** Alleles determine the phenotype or appearance of the animal. The combination of all the alleles in all the various genes of a horse constitute that horse's **genotype**. [9] [19] [7] [8]

In the language of genetics, the different alleles are assigned a short hand description representing the changes in the triplet code for a gene. Each chromosome in the pair is assigned a nucleotide abbreviation based on the type of base which was either the original or the substituted base.

The short hand version for the different alleles in the case of the KRT25 mutation are represented by: (**AA**) or homozygous for the mutation, (**AG**) heterozygous for the mutation, or (**GG**) for a horse that does not have the mutation. [9] [19]

Examples of horses suspected of carrying only one type of curly mutation – KRT25 - are shown below:

Homozygous

Horses carrying two copies of the **KRT25 mutation (homozygous)** are known to have the tendency to produce sparse mane and tail hair. Although shorter in general, there is individual variation in mane and tail growth, as can be seen from the KRT25 homozygous horses in the pictures below. The mane and tail hair of these horses is curly, but also more subject to summer shedding. These hairs seem to be more easily broken or brittle than normal horse mane and tail hair.

Body coat of homozygous KRT25 horses is curly during winter. It has a soft texture and can vary from a wave like appearance to tight curls. [11] [12]

"Duchess"

Domestic curly horse

Tested homozygous

AA

Owned by Donna Hedicke

"Spartacus"

Presumed homozygous

AA

Domestic founding sire

Owned by Sandy Hendrickson

"Jondra Moonwalk"

AA

Owned by the late Andrea Shcaap

"Showing sparse mane and tail"

Heterozygous KRT25

A horse born with only one copy of the **KRT25 mutation (heterozygous)** normally has a much fuller mane and tail. As can be seen from the horses in the pictures below, a full mane and tail are present. The mane and tail hair is subject to shedding in the summer months, but is far less likely to shed completely. Brittleness of hair is still a problem, but the hair seems less brittle than in homozygous KRT25 horses. [11] [12]

Heterozygous wild born curly horse from Nevada

AG

O 'Sparky owned by Angie Gaines

On the right- the summer coat of "Sparlock"

Sparlock is a founding sire of domestic curly horses. Tested AG

Owned by Donna Hedicke

Heterozygous wild born curly horse from Nevada showing winter coat

AG

"Blackie" – owned by author

Both groups of horses shed curly guard hairs and one layer of undercoat during the summer months. Curly hairs are retained on the tail, mane, and ears during all seasons. [6] [3] [11]

"Blackie" summer coat

"Discordant Horses"

Horses Whose Curly Coats are not due to the KRT25 Mutation

One prominent line of horses that also produce curly coats that are **not** due to the KRT25 mutation is known as the **Curly Jim line**. This line of horses is also associated with gaited Missouri Fox Trotters. The popular Curly Jim Line is named after a founding sire of unknown origin named **Curly Jim**. Most of these horses also carry the **DMRT3** mutation which produces gait. [11] [16]

Curly Jim

According to Sandy Phipps, whose family has long been associated with the Curly Jim line of horses, Curly Jim arrived by train with another curly to Mountain View, Missouri in 1956. Both horses were bought at an auction in Tennessee, and no information was available about their backgrounds. The second horse was subsequently killed in a barb wire fence. Curly Jim was trained by a young Johnny Brooks who later became Sandy Phipps brother-in-law. The sorrel stallion was about 3 years old at the time. [14]

Curly Jim was bred to many local mares, but the popular gaited line of curly horses came from the breeding of Curly Jim to a non-curly, grade mare known as the "Bradford Mare". Little information is available about the Bradford Mare. The curly coated foal that was produced from the breeding was a filly named "**Blaze**".

Blaze inherited the non-KRT25 mutation for a curly coat from her father, Curly Jim. When mature, Blaze was bred to a popular Missouri Fox Trotter stallion named, Walker's Marry Lad. **Walker's Marry Lad did not have a curly coat**, but the colt that was produced from the mating did. This colt was named, Walker's Prince T. As a mature stallion, **Walker's Prince T** was the most widely used stallion in the gaited curly line. [11]

Walker's Prince T – widely used curly stallion

Current Research on the Curly Jim Mutation

An intensive scientific investigation is currently underway by Dr. Gus Cothran and his team at Texas A&M University. The study is funded by the International Curly Horse Organization. Its purpose is the isolation of the gene mutation which is responsible for producing curly coats in the descendants of Curly Jim.

A total of ten horses from the Curly Jim line were chosen for the study after their pedigrees were checked by Earlene "Bunny" Reveglia of ICHO. These chosen horses did not have other lines of curly coated horses in their backgrounds. Blood and hair samples were provided by the owners for the study. [11]

Two horses that were found not to have KRT25 (discordant horses) in Dr. Laurent Schibler's investigation had whole-genome sequencing completed in France. A subsequent study of the pedigree of the heterozygous, curly stallion, **Drakvallmons Ite O Maguzu**, and his straight-haired colt, Jak Boreal Maguzu, showed three lines of Curly Jim and one line of Damele. [1] The probability was

very high that the stallion's curly coat came from the Curly Jim lines, since the KRT25 mutation was not found in the curly stallion's DNA. This pedigree information was provided to Dr. Cothran's team by ICHO in a collaborative effort to isolate the Curly Jim gene mutation with Dr. Schibler. [11] [13]

Coat Phenotypes (appearance) of Curly Jim Line Horses

By studying the pedigrees of Curly Jim horses, some of the phenotypes or coat traits can be deduced for homozygous and heterozygous inheritance, even though the actual gene mutation that produces the curly coats has not yet been isolated.

Homozygous Curly Jim Mutation

Horses that carry the Curly Jim mutation that are strongly suspected of being homozygous are known for having tight curls on the body guard hairs. This is known as the "brillo-pad coat". The tight body curls also known as "micro curls" resemble a brillo pad. Manes and tails of these horses have shorter hair, but not sparse hair. It also does not tend to shed in the summer months. [11] [12]

Pictured below is a horse called Kreskin, owned by Jackie Richardson. Kreskin was believed to be **homozygous for the Curly Jim mutation**. His coat appearance, or phenotype, exhibits the tight body curls characteristic of homozygous horses with Curly Jim ancestry. Kreskin's mane and tail hair is thick and full which is common in mature Curly Jim mutation horses.

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"Kreskin"

(above and above right) suspected homozygous Curly Jim mutation.

These three photos show micro body curls on the young stallion and the full mane and tail of the mature stallion which is associated with the Curly Jim Mutation.

Right, Mature Kreskin

with full mane.

Continued from page 17, left, micro curls or "Brillo Pad" curls are tight, short, and course. They make up the body coat in horses with Curly Jim homozygous inheritance.

Heterozygous Curly Jim Mutation

Horses that are suspected of being **heterozygous for the Curly Jim mutation** have full manes and tails and body coats that are curly, but not the tight curls found on homozygous horses. [11] [12]An example is Sir Patrick below:

Sir Patrick

Owned by Jackie Richardson

Above, showing winter and summer coats with full mane and tail -

common in Curly Jim mutation heterozygous horses.

Soft, circular curled coat

Typical winter coat of heterozygous

Curly Jim mutation

"Shooter"

owned by

Terry Schmidt

Part II

In Part II, more types of curly coated horses are presented which do not have the KRT25 mutation. Like the Curly Jim mutation, efforts are underway to discover the genetic mutations that produce these curly coats.

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Part II

Curly Coats on Horses Are Caused By Multiple Gene Mutations

By Dr. Mitch Wilkinson

Other Types of Curly Coated Horses Which Carry Causative Mutations Which Are Not KRT25

The Cook Mutation

Yet another mutation which causes curly coats in horses **not** caused by **KRT25** was given the name "**Cook Mutation**". [2] The Cook family were early breeders of domestic curly horses in Northern Nevada. The Cook horse bloodlines are present in many of today's domestic curly horses. Because the Cook horses were being bred from the earliest days of curly horse domestication, a great many of the domestic curly horses that tested positive for the KRT25 mutation might also simultaneously be carrying the Cook mutation. We will see in Part III how it was deduced that an individual horse can carry two or more curly coat producing genes at the same time. Curly horses that only carry the Cook gene are rare with only a few examples. [2]

The ranch where the family of **Art Cook** lived and bred their horses was located near another family of early curly horse breeders, the Damele family. **Benny Damele** was instrumental in pioneering the domestic curly horse as a breed. [7]

The Cook line of horses can be traced back to the famous Damele stallion, **Copper D**. The Cook stallion was sired by Copper D, and was named simply, **Cook Curly Horse**.

No genetic samples were ever taken of this stallion nor of any first generation

Cook horses. This story of the Cook curly horses was related in an essay by Pat Johnson who knew both the Damele family and Art Cook. Mrs. Johnson bred the Cook line of curly horses for many years. She thought highly of this line of curly horses, but never realized that they carried a different gene mutation. [1] Where and when the Cook mutation entered the domestic curly horse population may never be known. It has been speculated that Copper D could have been carrying two curly gene mutations at the same time and passed the Cook mutation on though Cook Curly Horse to the Art Cook breeding program while not infusing the KRT25 mutation. Another possibility is that Copper D did not pass on a curly gene mutation to Cook Curly Horse, and the curly mutation came from the Damele dam. Many of the Damele mares were wild caught horses from the Austin, Nevada area. It is suspected that the Cook gene mutation may still exist in the wild herds. The Fish Creek HMA which is near both the ranches may have horses that carry the Cook gene mutation.

Art Cook used only one stallion in his breeding program; that was the sorrel colored **Cook Curly Horse**. Art gelded the colts and bred his stallion to his daughters or wild horses near his ranch. Cook Curly Horse was kept in a corral at the Cook ranch, but Art couldn't build the corral strong enough or high enough to keep his stallion in. Cook Curly Horse would periodically break out of the corral, disappear for a few months, and run with the wild herds. Sometimes he would return on his own and other times he was caught in round-ups. [1]

On one of Cook Curly Horse's excursions, he was missing for several years and both Art and Benny thought he had died. Incredibly, Cook Curly Horse was found by Benny Damele breeding some of his mares and was promptly returned to Art.

Art Cook only registered three of Cook Curly Horse's progeny with the ABC registry. One of the colts was named Houdini (ABC P–468). **Houdini** received his name after he escaped from a locked stock trailer. No one could understand how he did it. Houdini was owned by Pat Johnson, and she related that he was extremely intelligent and quite a character. [1]

Houdini's direct progeny named **Nipper** (Ne-Hi Majic ABC 1172) had hair follicle samples taken that showed that Nipper's curly coat was **not due to the KRT25** mutation. [2] [3] [4]

Nipper

Suspected Cook gene mutation, above and below.

Both blood and hair samples were taken of Nipper's foal, Karma. They also showed that the direct progeny of Nipper, Karma, did not carry the KRT25 mutation, but had a very curly winter coat. This line of curly coated horses is currently being sequenced to find the gene mutation responsible. [2]

Karma

Foal of Nipper

Owned by Krista Harvey

suspected Cook gene mutation

Cook gene in wild Nevada horses

Domestic curly horses from Northern Nevada are all partly descended from horses caught from the wild herds of the area. The genetic mutations found in Damele horses and Cook horses all came from the wild herds. Today, many wild caught horses with curly coats in the Northern Nevada region test positive for the KRT25 mutation, but some do not. Below is a photograph of a curly coated horse captured near Elko, Nevada who has a curly coat that is not due to the KRT25 mutation. It is theorized that this horse may be carrying the same mutation as the Cook line of domestic curly horses. Genetic samples of several horses of this type have been collected. Whether this horse, **Elko Nevada**, is carrying the Cook gene or another unknown gene mutation, curly coat producing genes in addition to KRT25 are incorporated in the wild herds found near Elko, Eureka and Austin, Nevada. BLM herd management areas near these locations are still known to contain curly coated horses. [2]

Elko

wild born Nevada mustang

Owned by Angie Gaines

suspected of having the Cook gene mutation, left.

Sulphur Mustang Curly Coated Horses

There is another type of North American curly coated horse which can be found in the Utah wild horse herds, most notably the Sulphur herd in southwestern Utah. These curly coated horses **do not** carry the KRT25 mutation. Curly coated horses are extremely rare in the Sulphur mustang herds, but they do exist.

The Sulphur Herd roams a vast, remote region centered around the Needle Mountain Range. This herd is named after one of the few springs found in the arid high desert, the Sulphur Spring. These horses are descended from some of the first horses brought to the southwest by Spanish colonists and explorers in the late 1500's. To see them in the wild requires a journey of several hours by paved and dirt road from Milford, Utah. [17] [24] [9]

The most striking characteristic of the Sulphur Herd is the very high incidence of **Dun Dilution Factor** in the population. [9] Columbus's second voyage to the New World included semi-feral horses from the Guadalquivir River marsh lands located

near the Spanish village of Almonte in the province of Huelva, Spain. [14] [15] In subsequent voyages, an estimated 500 or more semi-feral horses from the Guadalquivir marshes were brought to breeding farms on the Caribbean islands of Hispaniola, Puerto Rico, Cuba, and Jamaica. These horses brought with them the dominant gene for the Dun Factor. The Spanish called these semi-feral, peasant horses "**Jacas**". Although Columbus complained about the quality of the striped horses, he did not know that these horses were some of the hardiest horses in the world at that time. [12] Their genes and blood lines went into other "better quality" Spanish colonial horses that were imported later in the colonization of New Spain. Along with their resilient, adaptable physiology, these "Jacas" also passed on the high incidence of Dun dilution factor to their decedents in Spanish America. [6] [8]

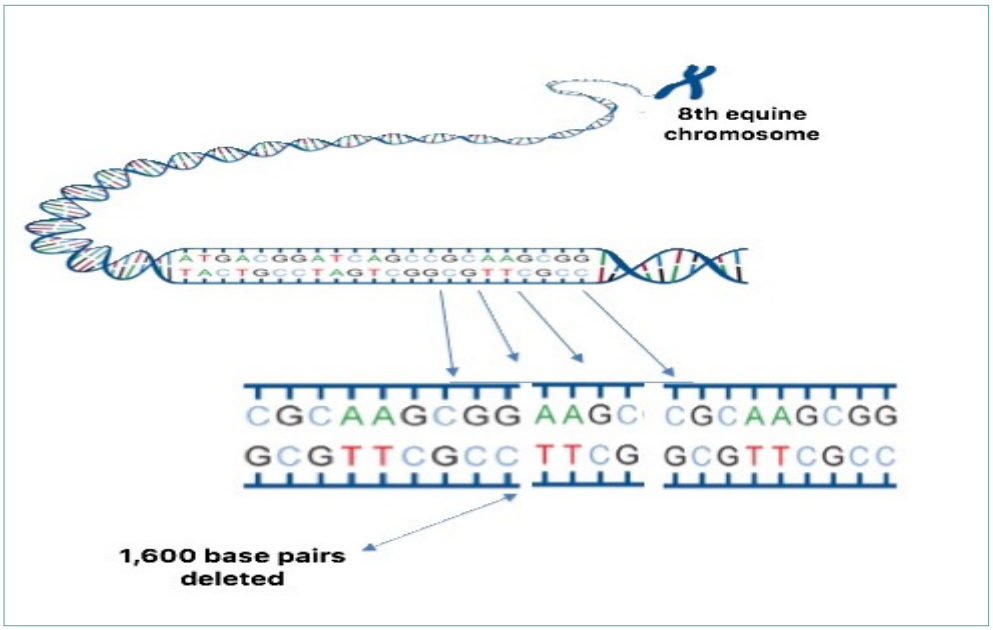
Spanish colonial horses arrived for the first time in numbers to establish a population in 1589. In the decades before this, the southwest was being explored by Spanish expeditions and treasure seekers who were mounted on stallions and geldings, for mares were forbidden to be taken from the Caribbean breeding farms. Coronado's expedition included only two older mares. [8] [11] [16]

In 1589, from central Mexico's newly established horse breeding farms whose parent stocks came from the Caribbean islands, stallions and mares were obtained in large numbers to establish Spain's first southwestern colony in present day New Mexico. Juan de Onate led seven hundred colonists and over 1,500 horses which included 237 mares from central Mexico across the Rio Grande to establish the colony of New Mexico. He was the colony's first governor. However, the exploitation of Natives by the Spanish colonists and the cruelty of the colonial government led by Onate caused a growing resentment among the Pueblo tribes. Juan de Onate was replaced by the Spanish Crown in 1609 for ineptitude, but Onate's successors did little to ease the plight of the Pueblo Tribes. During the next seven decades, Native Americans were being trained in the Spanish art of horsemanship to herd the livestock kept at the Catholic Missions. Finally, the resentment of Spanish domination led to a revolt of the Pueblo Indians in 1680. The revolt was led by a shaman from San Juan Pueblo named, Pope. The **Pueblo Revolt** expelled the Spanish from the area for over a decade. The fleeing Spanish colonists left the majority of their horses behind. Estimates range from 7,000 to 10,000 horses fell into Native American hands. The horses the Spanish left became the mounts for Native Americans and the seeds of the early mustang herds. [11] [6] [8]

A century and a half later in the early 1830's, the Old Spanish Trail passed ne

ar the Sulphur Herd Management Area. There was a brisk trade in horses from Los Angles to Santa Fe. At the time, the southwest was still under Spanish control. Some of the Spanish Colonial horses from Alta California may have escaped during this time and contributed their bloodlines to the Sulphurs, also. [9]

In 2015, Swedish researchers at Uppsala University found that 1,617 base pairs were missing from the 8th chromosome of non-dun horses. In genetic terms, this is called a **deletion**. Horse populations that carry the dun factor, like the Spanish marsh horse ancestors of the Sulphur horses, are usually found around the world in semi-feral, wild horse populations. The researchers wondered why this observation was so consistent? By comparing todays, modern horses to ancient, frozen horse specimens from Alaska and Siberia, they came to the startling conclusion that at one time **all horses had the dun factor** and solid colored horses began by a genetic accident sometime after domestication around 5,000 years ago. It was the preference of human breeding practices which transformed horse populations from being 100% dun dilution to the majority of today's horses having solid colors. [23]



The research also identified the gene which is responsible for the asymmetric distribution of pigment cells called melanocytes which give dun horses their characteristic appearance. The darker markings and stripes on dun horses are called **Primitive Markings**. These markings are used for camouflage. The gene which produces dun characteristics is called the **TBX3 gene**. It is a dominant gene and only needs to be active one of the two chromosomes which make the 8th chromosome pair to produce dun markings. [23]

However, if a chromosome is missing the 1617 base pairs, the TBX3 gene is inactivated in that chromosome. If both of the 8th chromosomes are missing the 1,617 base pairs, the TBX3 gene is completely inactivated causing a solid colored horse is created. [20]

Horses with primitive dun characteristics are found to this day in pockets around the world. The Sorraia River basin of Portugal has the Sorraia horse, Przewalski's horse in Mongolia, the Yakutian horse of Siberia, Norwegian Fjord horses, and Utah's Sulphur horses. [24]

Leg bars on Sulphur Mustang

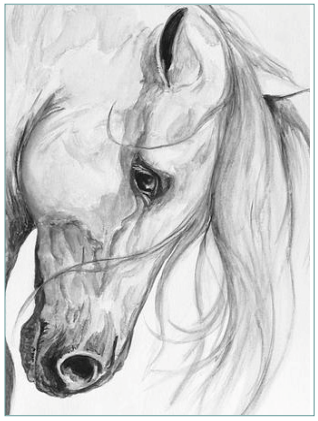
Shoulder and neck stripes on grullo Sulphur mustang

Wild Sulphur Stallion – Sulphur HMA

The continued survival of Sulphur mustangs is in question, but Sulphur mustangs that carry the, as yet, unknown curly coat producing gene are definitely on the verge of extinction. At the present time, there is only one known curly

breeding stallion in the Sulphur HMA. There are three curly Sulphur mustangs known to be in captivity.

Curly Sulphur mustangs have thick manes and tails which remain full during the summer months, much like the Curly Jim phenotype. They also produce body curls that have a distinctive wave like pattern. Spanish head, ear, and eye shape is also noticeable and reminiscent of the Andalusian. [16] [18] [19] [17] [13] [14]



Tapered head shape and distinctive eye morphology of Sulphur Mustangs are reminiscent of the Andalusian.

Sulphur Mustang

The wild caught Sulphur mustang below has an unknown gene for a curly coat and the dominate dun gene giving primitive markings.

Mezcal

wild born Sulphur mare

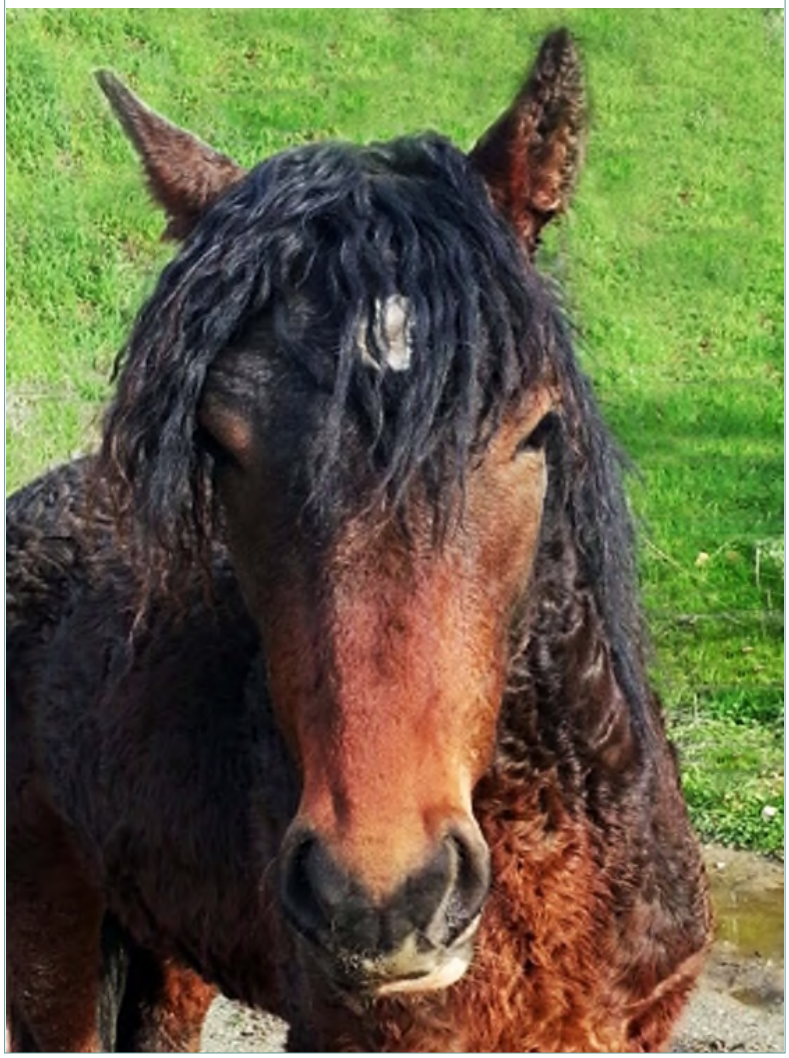
showing winter coat

owned by Dan and Duv Cardenas

The Sulphur mare in the picture above was bred to a non-curly coated Wilbur-Cruce mustang. The resulting foal named, Renegade, had a curly coat. The Wilbur-Cruce sire did not show any curly traits and is unlikely to carry any mutations that could explain the curly coat of the foal. Therefore, the mutation that produced the curly coat in the dam was inherited by the foal and probably has a dominant mode of transmission. [21] [22] 20]

Renegade

Mezcal's foal



Renegade

As a young adult

Showing

Winter coat

Spanish Mustang Curly Coated Horses

Some horses in the Spanish Mustang Registry also have curly coats. These horses almost always have some type of Sulphur Horse connection in their background. Whether the same mutation is common in both populations remains unknown.

Ivy

Spanish Mustang

Showing winter curly coat

Owned by George Kurek

Dizzy Horse Farms

Patagonian Curly Coated Horses

There are curly coated horses in Argentina. In the wild horse herds of Patagonia, a percentage of the horses have curly coats. Hair samples have been obtained from several of these horses and they do not have the KRT25 mutation. Whether

they have one of the undiscovered mutations mentioned in North American curly coated horses will be determined after isolation of other curly genes.

"***The Variation of Animals and Plants under Domestication***" is a book by Charles Darwin that was first published in January 1868. In the book, Darwin cites the work of another naturalist from a generation before concerning his observations of curly coated horses in Paraguay. The horses are given as an example of adaption to a harsh, cold environment. The naturalist who penned his observations in 1802 was a Spanish military officer and engineer named Felix de Azara. Azara stated in his book, *Quadrupeds de Paraguay*, that the curly coated horses were observed near the Rio de la Plata that forms the border of Argentina and Paraguay. Many thought these horses never existed or became extinct. Darwin never actually saw the horses that Azara described. [26]

Felix de Azara

In 2013, Andrea and Gerardo Rodriquez contacted Angie Gaines through the *Curly Mustang Association Facebook.* The couple stated that they were raising curly coated horses that had been captured from the wild horse herds of Patagonia, Argentina. Their ranch was close to a remote village named Maquinchao, but they had internet service.

Hair follicle samples were sent from Argentina to Gus Cothran's lab in Texas. The samples proved **not** to have the KRT25 mutation. In order to run tests to isolate the curly coat causing mutation these horses have;

blood samples must be obtained. It was impossible to ship blood samples from such a remote location without spoilage.

Mario Poli who is a geneticist who works for the Argentine government at the Instituto de Genética "Ewald Favret" in Hurlingham, Argentina. He was contacted in the hope that blood samples from the horses could be processed in Argentina for shipment to the United States. It was a five-hour drive, one way, to Maquinchao to obtain the samples. Mario sent his sister-in-law to gather the blood samples. It took four years to overcome the logistics needed to gather these samples.

By an odd coincidence, the very day the scientific paper about the isolation of KRT25 was published, Mario sent the processed samples to the genetics lab at Texas A&M University for genetic analysis.

curly coated horses

found in wild horse herds located in Patagonia, Argentina

Some special thanks are in order to Duv Cardenas and her in-laws who helped with translations on messages to Argentina.

Siberian Curly Coated Horses

A type of curly coated horses that test negative for the KRT25 mutation are found in Siberia. These horses live near Lake Baikal in southern Siberia near the Mongolian border. The Zabaikalski horses are the traditional horses of the Zabaikalski Cossacks and are being preserved as a national treasure at state run breeding facilities. [2]

Hair Follicle samples were shipped to Texas A&M lab from Siberia. Little else is known about these horses.

Lake Baikal the largest fresh water lake on earth

Zabaikalski Curly Coated Horses

semi-wild Siberian horses,

about ¼ of horses have curly coats.

Zabaikalski Curly Coated Foal

Showing unknown gene for curly coat plus the Lp gene mutation for Appaloosa coloring.

Mongolian Curly Coated Horses

These horses exist in Northern Mongolia. Because of the close geographical location to the Zabaikalski horses of Siberia, they might be related. There are only a couple of pictures in the ICHO data base of Mongolian curly horses. The pictures are shown below.

A friend of a well-known Canadian curly horse breeder, Shelly White, traveled to Mongolia as a part of a non-profit organization called Mongolian Women International. Shelly's friend, Julie Veloo, found five Mongolian curly horses and obtained hair samples that were sent to Gus Cothran's lab at A&M University. All five samples were negative for the presence of KRT25. These curly horses have some other mutation that causes their curly coats. Whether it is the same as the Siberian horses, only time and genetic research will tell. [2]

Mongolian curly coated horses

The Future

Obtaining specimens from remote locations around the world is difficult at best. As we obtain more information about the existence of the different mutations which cause curly coats in horses around the world, we may finally piece together the mystery of curly horses. **As a curly horse enthusiast, keep in mind friends and acquaintances that might be going to some of the locations mentioned in this article who might be able to obtain samples to aid in our search.**

Part III

In Part III, curly coated horses suspected of carrying multiple curly producing genes will be discussed. Future curly research and the conservation of endangered curly horses will also be one of the major topics

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Curly Coats on Horses Are Caused By Multiple Gene Mutations

By Dr. Mitch Wilkinson

Part III

Multiple Independently Inherited Causative Mutations are Found in Individual Horses

Because it was not known until very recently that curly coats in horses were caused by multiple, independently inherited genes, curly horses having different gene mutations were mixed in breeding programs which resulted in individual horses that carry multiple mutations.

The first known horse found to have two independent curly genes was a stallion named Chester. Chester is owned by Angie Gaines in Kaufman, Texas. This horse tested heterozygous (one copy of a gene) for the KRT25 mutation. Chester had been bred to a mare that did not have curly traits. The resulting foal, named Bucky, had a curly coat, but tested negative for KRT25 mutation.

"Chester"

Stallion

Heterozygous for KRT25

Plus

Curly Jim Mutation

Owner: Angie Gaines

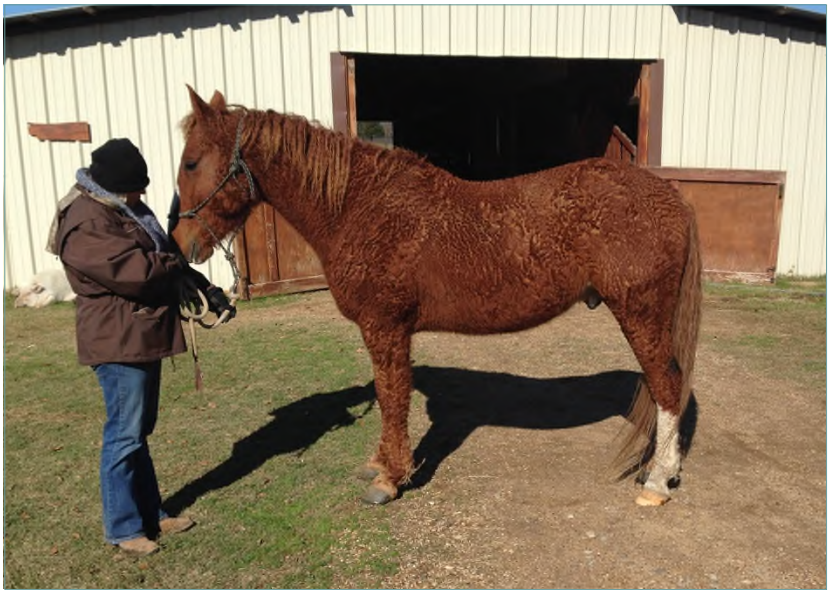
In checking the background of the sire, Chester, it was found that his bloodlines included the Curly Jim line of curly coated horses. Because the colt sired by Chester did not carry the KRT25 mutation, his curly coat must have been due to the Curly Jim mutation which was also carried by the sire. Although the Curly Jim mutation has not been isolated at this time, the conclusion is that Chester is carrying two independent mutations for curly coat, KRT25 mutation and the Curly Jim mutation.

"Blossom"

Left, a non- curly coated mare who was bred to Chester.

"Bucky"

Below, colt of Chester and Blossom suspected of carrying the Curly Jim Mutation, owned by Angie Gaines.



As additional curly producing genes are isolated, horses like Chester and Bucky can be tested to definitively confirm their genetics. It has been speculated that since the mixing of different curly producing genes goes back to early curly breeding programs, many curly coated horses may be carrying more than one curly mutation. The combination of curly gene mutations in individual horses leads to a dazzling number of possibilities of curly traits or phenotypes.

Gaited Curly Horses

In 2012, The International Curly Horse Organization teamed with Texas A&M University and Uppsala University in Sweden in a study of gait in horses. Swedish researchers, led by Lisa Andersson, had discovered a single nucleotide change or SNP in a gene on the equine 23rd chromosome. This change showed a significant association with gaited Icelandic horses when homozygous for the mutation. The gene that was found to be affected by the mutation is known as **DMRT3**. [3]

In the case of KRT25, a guanine was changed to an adenine in the KRT25 gene on the 11th equine chromosome. This produced a **missense mutation** that resulted in a curly coat. In the case of the DMRT3 gene on the 23rd equine chromosome, a cytosine was switched to an adenine. This apparently allowed affected horses to gait when homozygous for the mutation. The mutation which resulted from the switch of a cytosine to an adenine resulted in a **nonsense mutation** which caused the resulting protein produced by the DMRT3 gene to be incomplete by 174 amino acids which are the building blocks of proteins. [3]

In order to understand the difference between a missense mutation and a nonsense mutation, some basic genetic facts must be understood. First, the DNA code is a **triplet code**. Every three bases give information that is needed in the eventual production of proteins which are the building block of life. An example of a short sequence is shown below:

ATG	TTC	GAC	CCC	TGA
Methionine	Phenylalanine	Aspartic Acid	Proline	Stop Codon

Triplet base codons are codes for specific amino acids which are bonded together in the cell's ribosome to form proteins. Proteins can incorporate hundreds or thousands of amino acids in their chains. The codon ATG not only codes for the amino acid, methionine, but is also an **initiation site** where messenger RNA begins reading the sequence needed to build a specific protein; much like a capitol letter at the beginning of a sentence. All good sentences need a period at the end, and **stop codons** like TGA show the messenger RNA where to stop transcribing a particular protein. [6]

In a sense, the DNA code is like a language, but it is a language which can only have 3 letter words. An analogy in the English language might make the process more understandable. If we compose a simple sentence which uses only three letter words, it might look something like this:

The cat ate the rat.

If we substitute the letter "k" for the "c" in cat. The sentence would still make sense. There would just be an alternate spelling of the word "cat". In the language of DNA, there are alternate spellings for specific proteins. **TAT** and **TAC** both code for the amino acid **tyrosine**.

The kat ate the rat.

When another letter is changed in the sentence, for instance, the change of the letter "r" in rat to a "b". The entire meaning of the sentence changes. Instead of the cat eating a small rodent with a tail, the cat is eating a bat, a winged mammal.

The cat ate the bat.

What was just shown is a **missense mutation.** This is the type of mutation that happens in the KRT25 gene to create a structural change in the protein which this gene produced. The changed protein eventually led to a curly coat.

Now, what if we change another letter like the "e" in ate to an "m"? The resulting sentence would look like this:

The cat atm the rat.

The three letters, "atm", are not a recognizable word. This is **a nonsense mutation.** The messenger RNA treats a nonsense mutation as a stop codon and quits reading the sequence at that point. In the case of the DMRT3 gene, 174 amino acids were left off the protein that was produced by genes affected by the nonsense point mutation.

In the short hand notation used by geneticists for alleles (two versions of the same gene), the symbol, "**CC**", is used to denote a "wild type" or normal 23rd chromosome pair in which both chromosomes have cytosine in the associated area of the DMRT3 gene. The symbol, "**CA**", is used where one chromosome of the 23rd pair has the nonsense mutation where an adenine was substituted for a cytosine. Finally, the symbol, "**AA**", is used to donate a 23rd chromosome pair in which both chromosomes are affected by the nonsense mutation which produces gait.

How this changed protein coded for by DMRT3 produced gait in horse was discovered by studying mice which were found to have a similar mutation in the mouse version of DMRT3. It was found in mice that the protein produced by DMRT3 is critical to establishing the circuitry of spinal neurons during fetal development. The nonsense mutation changes the neurons which control stride and coordination of limbs in mice. It is presumed that a similar process happens in equines. [3]

Originally, samples from sixty-one curly horses were sequenced for the Swedish study on DMRT3. A large number of horses descended from the Curly Jim line were included. The Curly Jim horses that were known to be gaited were found to have the DMRT3 - "AA" mutation on the 23rd chromosome. As of the writing of this paper, 137 curly horses have been tested for the variant of DMRT3 which produces gait. The information is kept in the **ICHO Genetic Data Base** and will be an invaluable tool that can be utilized by future curly horse breeders. [5] [4]

The DMRT3 gene is also known as the "**gait-keeper**" gene. When did this gait -keeper gene enter the horse population? In 2016, scientists extracted DNA from the bones of 90 ancient horses. Some lived as far back as 3500 B.C. What they found is that the mutation arose from a single horse that lived roughly between **850 and 900 A.D.** somewhere in England. The DMRT3 gene mutation entered the Icelandic horse population when Vikings brought the prized horses back from the United Kingdom to Iceland and other Nordic countries. The gaits in Norwegian Fjord horses also date from this time. [2]

This brings us to another topic which has been a point of much discussion. Where did the KRT25 missense mutation originate? As we can see from the gait-keeper mutation, horses have been exported and traded from ancient times. But the absence of the KRT25 mutation in known curly coated horse populations found in other locations around the world, leads one to suspect that this mutation was not imported from another location. We may never know for sure, but there is a strong possibility that the mutation happened with a single horse in the American southwest sometime after the Spanish re-introduction of the horse between the late 1500's and mid 1800's. Like all mutations, those that are favorable for animals in a particular environment tend to increase in the population, especially those with a dominant mode of transmission. This is classic Darwinian theory.

If the KRT25 mutation can someday be proven to arise from feral (wild) horse populations in the southwestern United States, it would make these horses uniquely American.

We now know that the North American curly population has curly coats due to several different gene mutations. Some may have originated in North America while others may have been imported from other horse populations. Only by isolating more genes which cause curly coats in equines can curly horse origins be determined, for historical records are lacking or non-existent.

Genes are not "diluted" as they pass from generation to generation. A good example of this is the LP gene mutation which causes a spotted coat pattern in such diverse breeds as Knabstrupper, British Spotted Pony, American Curly, and

the American Appaloosa. It is caused by a mutation on the equine chromosome 1 and has an incomplete dominant mode of transmission. When the LP gene mutation is found with another mutation, "TRPM1", also termed the Pattern 1 mutation, it produces the classic leopard spotting on horses. This gene has been passed down from horse to horse, un-diluted, for the past 25,000 years and possibly longer. [10] Cave paintings in France dating from 23,000 B.C. show horses with the classic leopard pattern produced by the LP gene mutation in combination with the PATN1 or Pattern 1 mutation. [1]

French Cave Paintings of Leopard Pattern Horses

The Lp gene has been a part of the curly population since the earliest days of domestic breeding. It is not known what percentage of the American curly horse population carry the LP gene, but it is a high percentage.

The Need for a Registry Based on Verifiable Genetic Testing

In the past 30 years, there has been an explosion of equine genetic research which has led to numerous findings. The cost of genetic testing has continued to decline bringing us to the point of tests that can be done at a very reasonable cost. Throughout the ages when scientific knowledge was not available to horse breeders, the breeders turned to "lines" of horses to help predict breeding outcomes.

The dawn of the 21st century has brought scientific advances that will displace the traditional methods of breeding horses and defining "a breed". For the first time, genetic tests at reasonable costs can tell what gene combinations breeding stock carry in their genomes and what genes particular horses carry as individuals.

The International Curly Horse Organization has been at the forefront of sponsoring research related to curly coated horses around the world. This organization and the dedicated individuals that make up its membership and staff have put scientific facts first. The extensive genetic data base kept by the registry will be a resource for future generations of horse lovers and breeders. ICHO has taken the lead in being one of the first truly modern, scientifically based horse registries.

ICHO offers a Genetic DNA Panel which includes:
17 color tests including Lp and Pattern 1
16 inherited system disorders
and 4 traits including DMRT3 for gait and Curly Coat Gene testing

Tests are also available for KRT25 and SP6 and Ancestry Associations through ICHO. As of the writing of this paper, 174 Ancestry Association tests of both wild and domestic curly horses have been cataloged in the ICHO data base. Every horse that is tested adds to the common knowledge.

Future Research

Some of the gene mutations that are thought to be different in various lines of curly coated horses may turn out to be the same mutation after the different causative genes are isolated. It is equally probable that the existence of new curly causing genes may be deduced from the results.

As the complicated world of curly horse genetics progresses, each piece of the puzzle seems to solve another part of the mystery, while at the same time, opening the door to new questions and lines of inquiry.

Acknowledgements:

The genetic results outlined in this article would not have been possible without the continued research and support of Dr. Gus Cothran and his staff and students at Texas A&M University. Dr. Cothran has diligently worked to solve the mystery of curly horse genetics for over a decade. His scholarly advice and guidance about the contents of this manuscript was invaluable.

Bunny Reveglia, the office manager of the International Curly Horse Organization, has systematically documented genetic data, pedigrees, coat types, and generally coordinated the research into curly coats in horses. Without her dedication, the genetic research into curly coated horses would not have taken place.

The continued support of ICHO board members and their dedication to science has allowed this valuable research to continue. They all deserve some special thanks.

Finally, individual horse owners that have given their support to these projects and most importantly given homes to rare and unique curly coated horses need our thanks. Most notably Angie Gaines of Golden Curls Ranch who has tirelessly given a home to many of our most rare and valuable horses. Duv Cardenas and Krista Harvey who both adopted two of the most endangered types of curly horses and gave them both wonderful homes. Finally, George Kurek and Vicky Ives who helped to track down curly coated horses in the Spanish Mustang Registry.

Although there is still much work to do, without the help of all the dedicated horse owners above, the mysteries of curly coated horses would forever be in shadow.

About the Author:

Dr. Mitch Wilkinson has been a lifelong horse enthusiast. After receiving a bachelor's degree in chemistry and professional dental degrees, he earned a post -doctoral master's degree from Baylor University in biology. Currently, Dr. Wilkinson is Chairman of the Curly Mustang Association and Vice- Chair of the ICHO Research Department.

Direct link to the Curly gene paper is:

https://gsejournal.biomedcentral.com/articles/10.1186/s12711-017-0359-5

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Understanding Curly Horse Coat Genes

From the first moment Curly horses were first spotted, the question has always been, where did they come from and why do they have a curly coat?

Thanks to modern genetic research techniques, two separate Dominant Curly Genes were found, KRT25 and SP6.

A huge thanks to researchers in USA Dr Gus Cothran, France and Germany for finding these genes.

The first gene found, Dominant KRT25, is found in most Curly lines including-

Damele, Native/Warrior, Fredell, Canadian and many mustangs including those from NV and Rock Springs/Salt Wells WY.

Traits of the KRT25 gene includes varying curly coats from marcel wave to C curls and a mane and tail shedding pattern when horses are homozygous for KRT25 (Carry 2 copies of the gene-AA).

A new gene discovered by German researchers, is the SP6 Dominant gene.

Dr Gus Cothran is now testing research Curlies to see who is carrying SP6.

This gene is found in the Curly Jim line of Curlies and current testing confirms this. SP6 is also found in the McKay pony lines.

This line has been crossed with Missouri Foxtrotters for many years and are often called Curly Jim Line Curlies or Gaited MFT Curlies.

Curly Jim was an unknown Curly horse that gaited and is the founding sire of this line, he was never registered in the MFTHBA or Curly registries.

This SP6 gene gives a tight curly coat and the Brillo Pad or Microcurly coat, terms often used to describe the homozygous SP6 Curlies.

This Curly Dominant SP6 gene does not shed mane or tail.

Curlies that carry both KRT25 and SP6 are considered Dual Gened Curlies.

When bred they can pass on either one or both genes.

When a horse has KRT25 and SP6 the shedding patterns of KRT25 overrides the non- shedding patterns of SP6. Many horses with both genes may appear to be homozygous in the breeding shed, when in fact they carry two separate genes, not 2 of the same gene.

The photo shows a tested Curly Jim line top and bottom horse with two copies of SP6, homozygous- TT. This horse also carries Damele lines but did not inherit the KRT25 gene from the Damele lines, he inherited the SP 6 genes from his Curly Jim lines from sire and dam.

INTERNATIONAL Curly Horse ORGANIZATION

https://www.ichocurlyhorses.com/curly-coat-genes.html

Curly Coat Genes

Two Dominant Curly Coat Genes have been isolated, the **KRT25 mutation** and the **SP6 mutation**. These gene mutations give Curlies their Curly Coats. Horses with *Wild Type* KRT25 and *Wild Type* SP6 do not have have the mutation or the Curly coats and are straight haired. It is the mutations in these two genes that give us our Curly coats in the following lines:

KRT25 mutation is found in the following Curly lines: Damele, Native/Warrior (Eli Bad Warrior, Berndt, Hammrich, NTS), Canadian (Cypress, Sjkonsberg, Naismith), Fredell, WY Salt Wells mustangs and most Nevada mustangs.

SP6 mutation is found in the Curly Jim line- which includes Walker's Prince T, McKay line and Circle B ponies. All horses with the SP6 mutation trace back to Curly Jim, an unknown gaited Curly that showed up in Tennessee.

A huge thank you to Dr Gus Cothran of TX A&M and the French researchers for finding KRT25 and to the German research team for finding SP6. Dr Cothran has confirmed the SP6 gene is found in the Curly Jim line through research testing including the McKay ponies and Circle B ponies.

There are also some unknown Curly coat mutations that have not been identified and that includes some Curly mustangs, the Cook line horses and two curly McKay line ponies out of a SP6 mutation heterozygous sire x straight mares that do not have the SP6 mutation. We have more mysteries to solve and continued research is progressing.

Read the Research papers on our webpage

KRT25 research paper

geneticsselectionevolution-curly_gene_krt25.pdf
Download File

German Research Paper SP6 link- https://www.nature.com/articles/s41598-018-24865-3

Walker's Prince T ABC 90 (f) MFTHBA F-12781

was a Curly Jim grandson that sired many gaited Curlies also registered in the Missouri Foxtrotting Registry MFTHBA. He carried SP6 through Curly Jim.

Curly Jim Line

Walker's Prince T* ABC 90 (f) MFTHBA F-12781

Color:	Sorrel
Markings:	Sabino,
Sex:	Stallion
Date of Birth:	7/1967
Gait:	Fox Trot

Curly Jim line is testing for SP6. Curly Jim is the founding sire of this line and SP6 gene passed on by him.

PEDIGREE

Printer Friendly Version

Grandson of Curly Jim Walker's Prince T* **by default SP6 +/-**	Walker's Merry Lad MFTHBA 282, AFTHBA F-105, TWHBEA 482752	Collier's Merry Boy TWHBEA 390778	Merry Boy TWHBEA#350189
			Miss Allen Wilson
		Beauty Sweetheart TWHBEA 431944	Noble Knight TWH 400661
			Walker's Beauty TWH 440947
	Blaze* ABC 135(f) **by default SP6 +/-**	Curly Jim*	unknown
			unknown
		Bradford Mare	Rock-A-Way MFTHBA F-15256
			unknown

Unraveling the Mystery of Curly Coats in the Horse World

by Loretta L. Nielsen, Ph.D.

Horse enthusiasts have long wondered why some horses are born with curly hair instead of the more common straight hair. At long last, the great mystery of curly horses is starting to be revealed!

Recently, two scientific papers were published proving the existence of at least 2 genes linked to curly hair in horses.[1,2] Both of these curly genes are inherited in a dominant manner. By "dominant", geneticists mean that a curly-coated foal only needs to inherit the curly gene from one parent (sire or dam). This foal is called "heterozygous" for the curly gene and has a 50% chance of passing it on to each of their offspring. A foal who inherits the curly gene from both parents is called "homozygous" and all of their offspring will be curly horses.

To greatly over-simplify the findings, among North American Curly Horses one of the newly identified dominant curly genes (*KRT25 variant*)[1,2] was found primarily in descendants of wild mustangs captured and bred by ranchers in North America. The other dominant curly gene (*SP6 variant*)[2] was found in some of these curly horses plus in descendents of a single stallion of unknown origin who produced curly-haired gaited offspring; some of whom were registered in the Missouri Fox Trotting Horse Breed Association (MBTHA)..

Curly horses display many degrees of hair curliness from soft, relaxed waves all the way to tightly wrapped, brillo-like curls. Various degrees of curliness are also obvious in each individual in their eye lashes, ear hairs, mane, tail, and body coat. Breeders have long postulated the influence of many genes on the primary dominant curly gene to explain why curly horses have such a wide diversity of looks (phenotype). For example, I have two brothers from the same parents. One is black with a brillo-type body coat that gets much shorter during the hot summer months, but remains consistent year-round. His full brother is a cremello with very soft and loose waves of curly hair on his main body. The curls in his body coat get tighter and longer during the winter, but are almost none existent in his very short, summer coat.

Over the past few years, scientists from France, Germany and the U.S.A. set out to find the specific piece(s) of horse DNA responsible for putting the curl into horse hair.[1,2] Their focus was the dominant curly gene, as well as another possible gene that seems to cause some curly horse bloodlines to develop various degrees of hair loss and bald patches; common among American Bashkir Curly horses. They used a genetic technique call genome-wide association to identify pieces of DNA that were present in curly haired horses and absent in straight haired relatives. This then allowed them to sequence those specific pieces of the horse genome. In other words, from all the DNA in a horse, they were able to pull out the pieces they wanted to study and then determine the exact sequence of molecular parts that made each piece unique.

One study used DNA samples from 51 curly- and 19 straight-haired French and North American horses descended from 13 different sires. A single, strong signal associated with curly hair was found on equine chromosome 11, in a region that includes the *type I keratin* gene cluster with its 36 genes. Keratin is one of the primary components of mammalian hair. Therefore, a mutation (change) in the DNA serving as a template for

keratin, and ultimately hair, production makes sense. In order to pinpoint the exact culprit, the scientists did an exhaustive comparison of the DNA in a heterozygous curly-haired stallion and his straight-haired son. Only one gene in the *type I keratin* gene cluster, the *KRT25* gene, was sufficiently different between these two horses to warrant further study. The *KRT25 variant* was then confirmed to be a dominant curly gene using DNA from 353 additional horses. However, there were 5 other horses who had curly hair, but no DNA changes in the *KRT25* gene. Closer examination of the DNA sequence of the *KRT25* gene in two of these horses did not find any missed variations of the *KRT25* gene. These curly horses had wild-type *KRT25*. These data provided strong evidence that some horses have a different dominant curly gene controlling the curliness of their hair. In other words, there is more than one dominant curly gene being passed down in North American curly horse bloodlines. Needless to say, this finding is extremely important for all curly horse breeders to know. Thus, the International Curly Horse Association (ICHO) is currently facilitating testing for the genes *KRT25* and *SP6 (see below)*: wild-type versus variant.

Another study used DNA samples from 216 curly- and straight-haired horses with varying degrees of hair loss or no abnormal hair loss at all. This analysis included horses from a wide variety of breeds, including: American Bashkir Curly, Missouri Fox Trotter, American Quarter Horse, Kentucky Mountain Saddle Horse, Danish Warmblood, Oldenburger, Holsteiner, Hanoverian, Duelmen Horse, Black Forest Coldblood, Norwegian, Lewitzer, Friesian, miniature Donkey, Sorraia, Standardbred, Przewalski, Rhenish German Coldblood, Arabian Thoroughbred, Anglo-Arabian, Austrian Coldblood, Swedish Warmblood, and Trakehner-Barb. Using genetic analysis techniques similar to those described above, this group of scientists identified 2 genes on horse chromosome 11 associated with curly hair. They found genetic variants (areas of gene mutation compared with wild-type) for the *KRT25* and *SP6* genes. Horses who had inherited the *KRT25 variant* from one or both parents had both curly hair and varying degrees of hair loss (from negligible to extensive). In contrast, horses who inherited the normal *KRT25* gene with the *SP6 variant* had curly hair, but no bald patches or hair loss. Horses who inherited the mutated genes for both *KRT25* and *SP6* had both curly hair and areas of hair loss. Thus, the *KRT25 variant* gene "overpowers" the normalizing effects of the *SP6* gene with regards to hair loss. This demonstrates how two genes can influence each other to change how a horse looks. It also appears to be the explanation for why registered curly Missouri Fox Trotters consistently have curly hair but no hair loss. They all inherited the *SP6 variant* from a single founding stallion along with the same wild-type *KRT25* gene found in straight-haired Missouri Fox Trotters. Future research will probably identify more mutations in genes with primary control over the extent of hair curliness in different horse breeds and a multitude of secondary genes influencing their expression. Of note, mutant *KRT25* and *SP6* genes have also been associated with curly hair in humans, rats, mice, and cattle.

For more than a decade, ICHO members have collected DNA samples and photographs from curly-coated horses and their straight-haired relatives in order to create a genetic storehouse that was used by scientists in some of this research. The figures below illustrate how difficult it is to distinguish between the phenotypes (hair appearance) of horses carrying the *KRT25 variant* or the *SP6 variant*. Only genotyping (DNA testing) can separate the types. However, there does seem to be an observable

difference in curl tightness for heterozygotes (1 copy of a variant) versus homozygotes (2 copies of a variant and a tighter curl). Of most interest is the last figure showing a curly horse who carries one copy of each variant. According to his owner, his hair loss has always been minimal and decreased as he got older. However, his general appearance gives no clue as to his actual genotype. Recent testing of DNA from other North American Curly Horses in the ICHO storehouse has tentatively identified some curly horses without either variant (neither *KRT25* nor *SP6*). *[As yet unpublished and exciting data for future studies!]*

Undoubtedly, further exploration of the genetics controlling how horses look and perform will yield other exciting revelations in the coming years. This information is especially important for horse breeders to understand. We now have genetic tests that allow breeding along either the *KRT25 variant* or *SP6 variant* curly bloodlines. No doubt, in the future, geneticists will find other genes that affect the curliness or straightness of horse hair. Some of these genes might also cause health problems that should be eliminated from all bloodlines. For example, some Missouri Fox Trotter foals are reportedly born with extensive health problems, such as ulcers in the lining of the gastrointestinal tract - anywhere from the mouth to the anus, in addition to a curly coat. However, based on available evidence, these horses have inherited a not-yet-identified recessive gene; meaning the health problems only occur when they inherit the disease gene from both parents, never just one. So-called "silent carriers" with only one copy of the disease gene are outwardly healthy. This allows the recessive disease gene to remain hidden for long periods of time until two silent carriers are bred together; with a 25% chance of producing an unhealthy foal with 2 copies of the disease gene. Elimination of this not-yet-identified recessive gene is an important goal for improving the Missouri Fox Trotter breed. However, because these foals are so rare and usually don't reach adulthood, it will require a long-term, serious effort on the part of MFTHBA members to document and collect DNA from affected bloodlines for scientists to analyze. The American Quarter Horse Association (AQHA) has already set a good example of how a breed association, horse owners/breeders, and scientists can work together to eliminate devastating diseases from bloodlines once genetic tests are available *(see the AQHA website)*.

References

1. Morgenthaler C, Diribarne M, Capitan A, Legendre R, Saintilan R, Gilles M, Esquerré D, Juras R, Khanshour A, Schibler L, Cothran G. A missense variant in the coil1A domain of the *keratin 25* gene is associated with the dominant curly hair coat trait (Crd) in horse. Genet Sel Evol 49:8,2017. DOI 10.1186/s12711-017-0359-5

2. Thomer A, Gottschalk M, Christmann A, Naccache F, Jung K, Hewicker-Trautwein M, Distl O, Metzger J. An epistatic effect of KRT25 on SP6 is involved in curly coat in horses. www.nature.com/scientificreports 8:6374, 2018. DOI:10.1038/s41598-018-24865-3

Figures

Figure 1. Curly hair coat of a Fish Creek, Nevada, wild mustang stallion (Fishy Boy) proven by genetic testing to be homozygous for the *KRT25 variant* dominant curly gene.

Figure 2. Curly hair coat of a gray American Bashkir Curly Horse gelding (Lilly's Moon Man) proven by genetic testing to be heterozygous for the *KRT25 variant* dominant curly gene. The photo shows his soft, wavy coat and was taken in April in southern California.

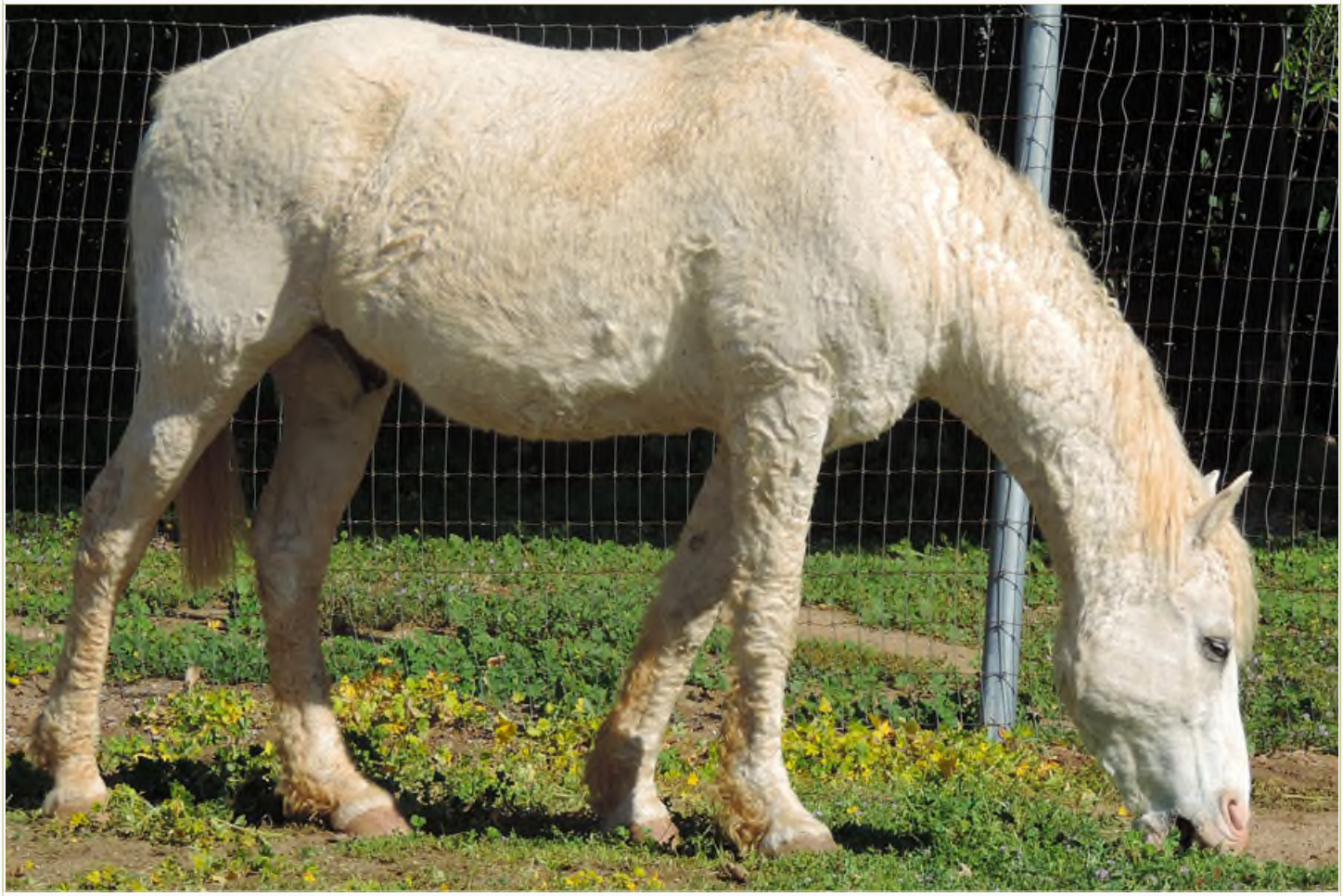


Figure 3. Close-up of the curly coat of a black Missouri Fox Trotter gelding (WDR Diego's Wizard) proven by genetic testing to be homozygous for the *SP6 variant* dominant curly gene. The photo shows the brillo-hair coat in the neck and shoulder area, and was taken in November in southern California.

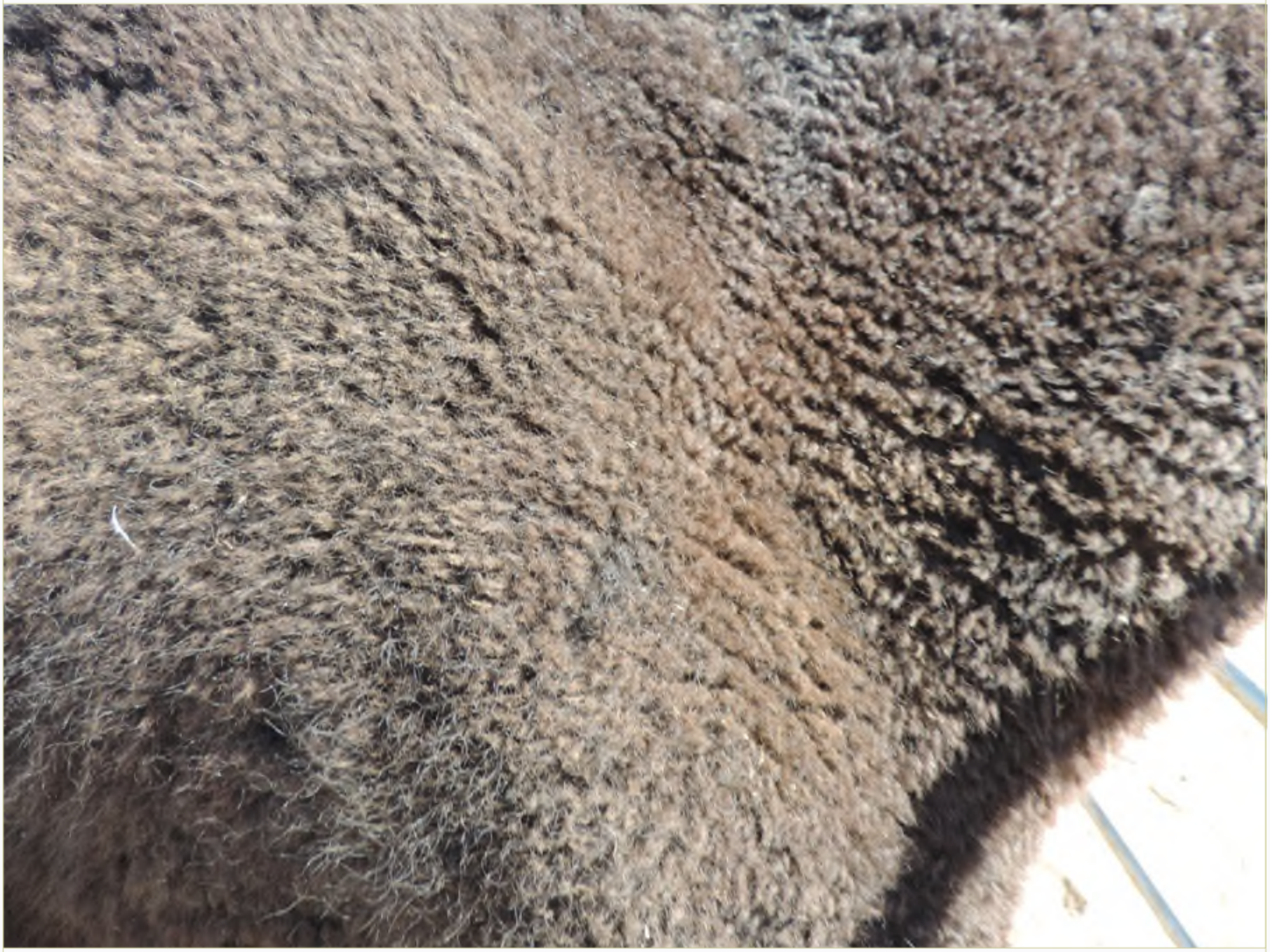
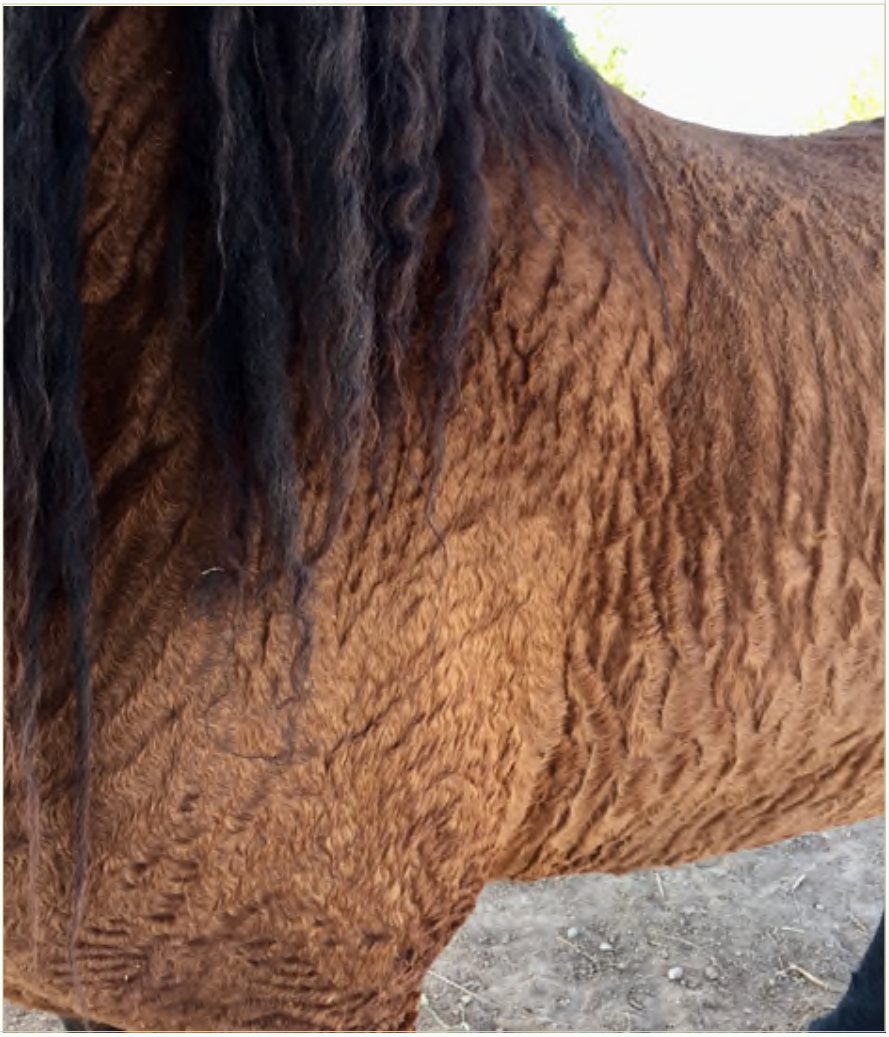


Figure 4. Close-up of the curly coat of a cremello Missouri Fox Trotter gelding (WDR Dragonsmoke) proven by genetic testing to be heterozygous for the *SP6 variant* dominant curly gene. This horse is a full brother of the homozygous *SP6 variant* gelding shown in the previous figure, but has a more relaxed and softer curly coat. The photo shows the hair coat in the neck and shoulder area and was taken in November in southern California.

Figure 5. Curly hair coat of a bay North American Curly Horse stallion (Frostfire's Xeque) proven by genetic testing to be heterozygous for the *KRT25 variant* plus heterozygous for the *SP6 variant* dominant curly genes. This horse is descended from both dominant curly North American mustangs and dominant curly Missouri Fox Trotters.



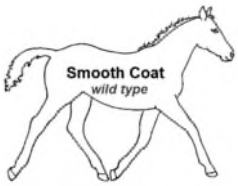
Typical KRT25 and SP6 Crosses

Legend:

Dr Mitch Wilkinson and Bunny Reveglia

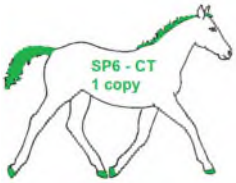
(**AA**) – homozygous for KRT25 – Damele, Native, Canadian, Fredell, WY Salt Wells Mustang and many NV mustang gene

(**AG**) – heterozygous for KRT25 – Damele, Native, Canadian, Fredell, , WY Salt Wells Mustang and many NV mustang gene

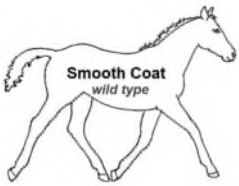


(GG) –wild type – no KRT25 mutation present

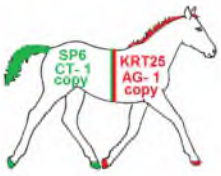
(**TT**) – homozygous for SP6 – Curly Jim gene



(**CT**)- heterozygous for SP6 – Curly Jim gene



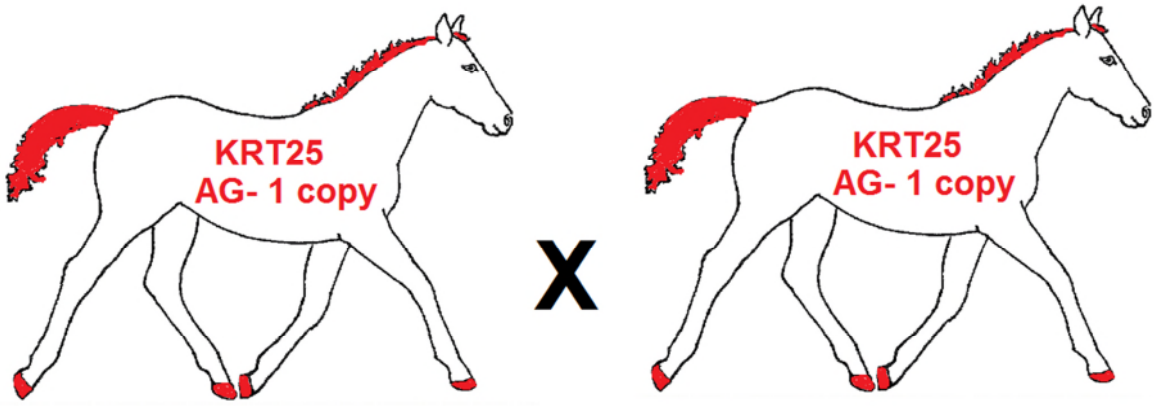
(**CC**) – wild type – no SP6 mutation present



(**AG-CT**) Heterozygous for KRT25 and SP6- Dual Genes

To use this document on typical breeding percentages, the user must know the KRT25 and SP6 status of the potential breeding pair. Hair follicle testing is available through the ICHO office. Once the KRT25 and SP6 status of the breeding pair is known, find the breeding cross that fits. The odds or potential percentages of breeding outcomes are found under each typical breeding scenario.

KRT25 Cross- *heterozygous*



Using a **Punnett Square** to determine the percentages of a mating outcome. A simple cross of two heterozygous horses which have the **KRT25** gene mutation, but **do not** have the SP6.

	A	G
A	AA	AG
G	AG	GG

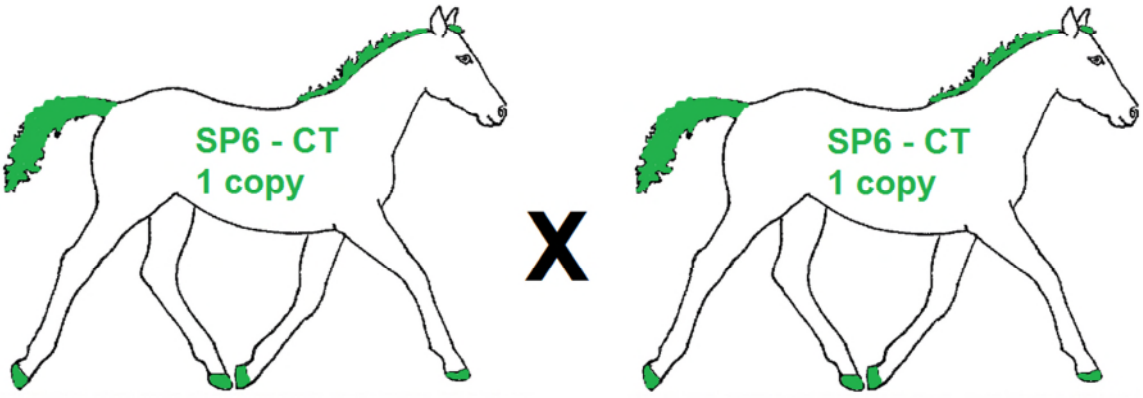
25%	50%	25%
AA	**AG**	**GG**

1/4 odds that the foal will be homozygous for KRT25 with scant mane and tail

1/2 odds that the foal will be heterozygous for KRT25 with mane and tail, but brittle hair

1/4 odds the foal will be born without a curly gene

SP6 Cross- *heterozygous*



Another simple cross of two horses that are heterozygous for **SP6** (Curly Jim), but **do not** have KRT25 in their genetics.

	C	T
C	CC	CT
T	CT	TT

25%	50%	25%
TT	**CT**	**CC**

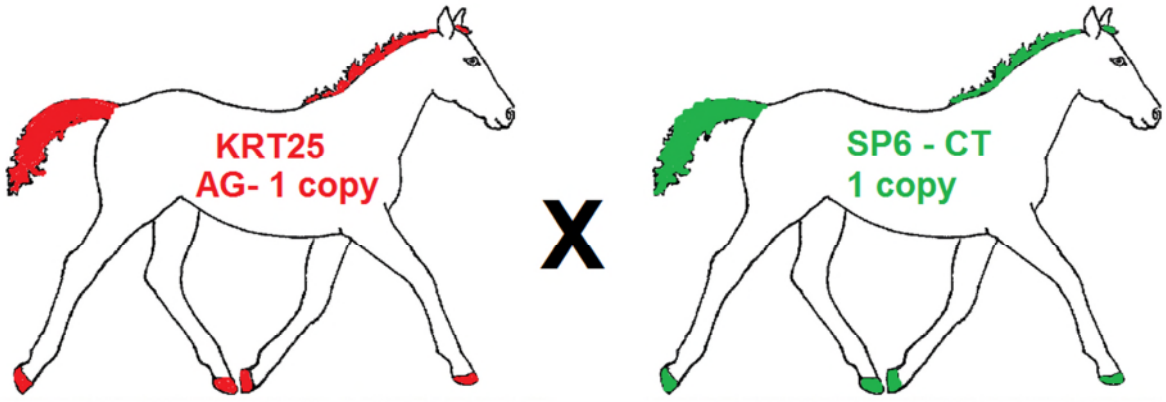
1/4 odds the foal will be homozygous for SP6

1/2 odds the foal will be heterozygous for SP6

1/4 odds the foal will not inherit a curly gene

All results will produce a foal with normal strength hair and full mane and tail

Cross #1



The cross of two heterozygous for each gene: #1 Parent- KRT25 AGCC X #2 Parent- SP6 GGCT- parents only carry KRT25 or SP6 but do not carry both at the same time

Cross:
AGCC × GGCT

	AC	AC	GC	GC
GC	AGCC	AGCC	GGCC	GGCC
GT	AGCT	AGCT	GGCT	GGCT
GC	AGCC	AGCC	GGCC	GGCC
GT	AGCT	AGCT	GGCT	GGCT

25%	25%	25%	25%
AG CC	**AG CT**	**GG CT**	**GG CC**
			Straight

3/4 odds of producing curly foal --- 1/4 odds of producing straight foal

1/4 odds for foal to be heterozygous for KRT25 and curly with no SP6

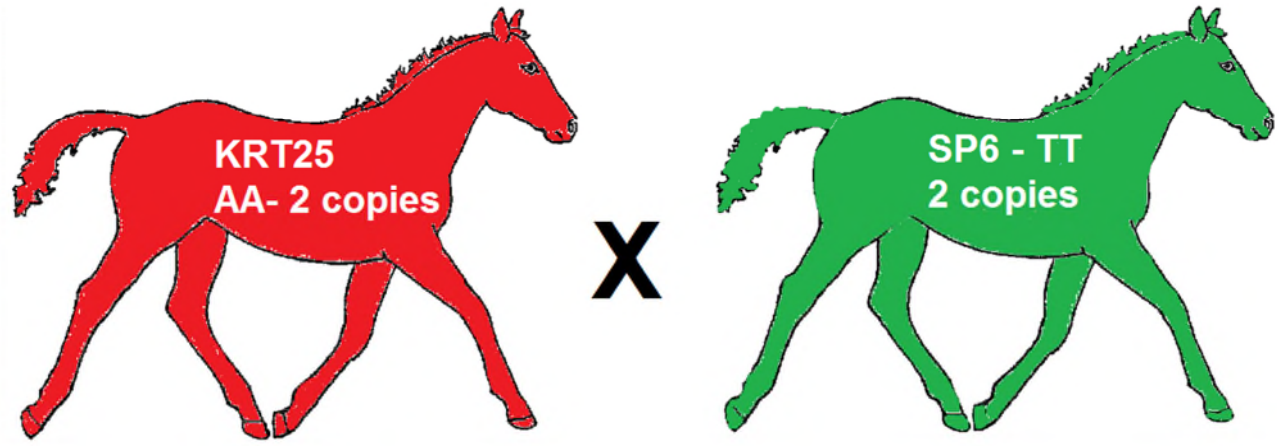
1/4 odds for foal to be heterozygous for SP6 and curly with no KRT25

1/4 odds of foal carrying both KRT25 and SP6 - hetero for both – **dual gene**

1/4 odds of foal not having a curly gene and straight coated

0% odds of producing a foal with sparse mane and tail

Cross #2



#1 Homozygous KRT25 Parent crossed with #2 Homozygous SP6 Parent with each parent carrying 2 copies of a different curly gene

Parent #1 - AA CC (sparse mane and tail)

Parent #2 - GG TT (full mane and tail)

Cross:
AACC × GGTT

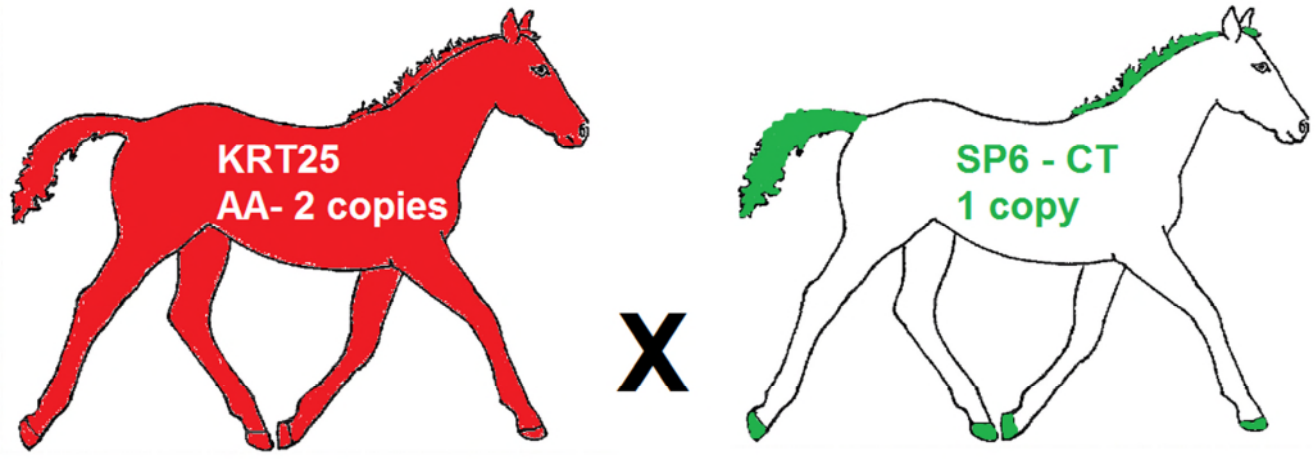
	AC	AC	AC	AC
GT	AGCT	AGCT	AGCT	AGCT
GT	AGCT	AGCT	AGCT	AGCT
GT	AGCT	AGCT	AGCT	AGCT
GT	AGCT	AGCT	AGCT	AGCT

Offspring - 100% - AG CT – Dual Genes- Good mane and tail, but possibly shorter with hair brittle

0 % odds of Straight

0 % odds of foal with sparse mane and tail

Cross #3



#1 Parent Homozygous KRT25 (AACC) x #2 Parent Heterozygous SP6 (GGCT)

Parent#1 AACC (sparse mane and tail) **Parent #2 GGCT (full mane and tail)**

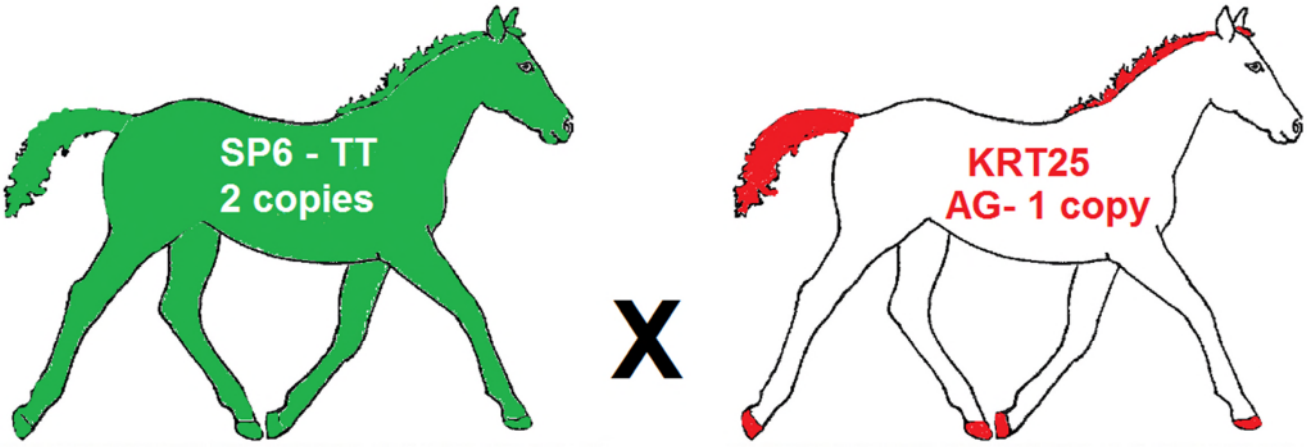
Homozygous KRT25 x heterozygous SP6

Cross:
AACC × GGCT

	AC	AC	AC	AC
GC	AGCC	AGCC	AGCC	AGCC
GT	AGCT	AGCT	AGCT	AGCT
GC	AGCC	AGCC	AGCC	AGCC
GT	AGCT	AGCT	AGCT	AGCT

50% heterozygous KRT25
50% Dual Gened- heterozygous SP6 & KRT25

Cross #4



#1 Parent Homozygous SP6 (GGTT) x #2 Parent Heterozygous KRT25 (AGCC)

#1 Parent GGTT (Full mane and tail) **#2 Parent (medium mane and tail)**

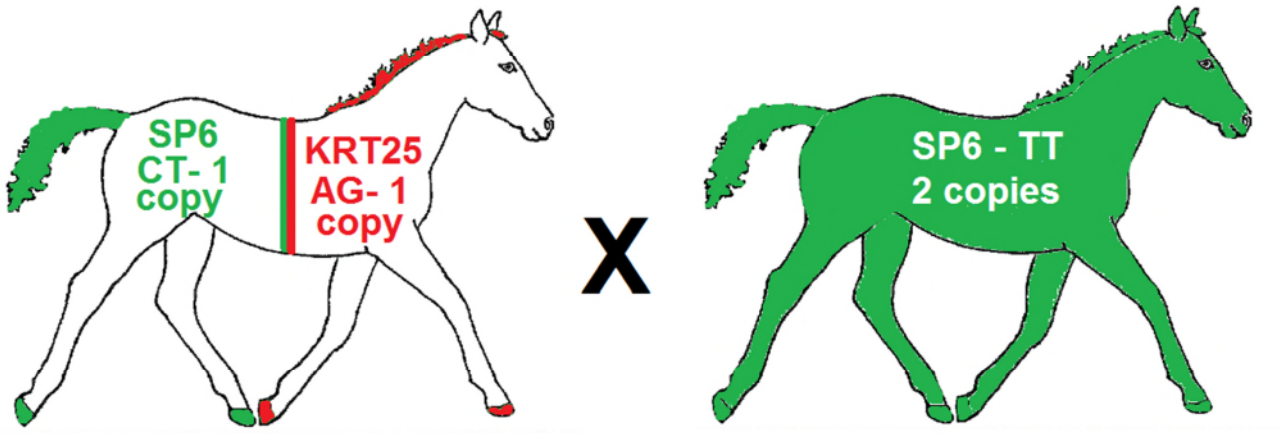
Homozygous SP6 x Heterozygous KRT25

Cross:
AGCC × GGTT

	AC	AC	GC	GC
GT	AGCT	AGCT	GGCT	GGCT
GT	AGCT	AGCT	GGCT	GGCT
GT	AGCT	AGCT	GGCT	GGCT
GT	AGCT	AGCT	GGCT	GGCT

50% Heterozygous SP6
50% Dual Gened- Heterozygous SP6 & KRT25

Cross #5



#1 Parent AG CT- Heterozygous for both genes (KRT25 & SP6) Dual Genes crossed with #2 Parent GG TT- Homozygous for SP6

Cross:
AGCT × GGTT

	AC	AT	GC	GT
GT	AGCT	AGTT	GGCT	GGTT
GT	AGCT	AGTT	GGCT	GGTT
GT	AGCT	AGTT	GGCT	GGTT
GT	AGCT	AGTT	GGCT	GGTT

25% **25%** **25%** **25%** -- or 1/4 odds of each phenotype happening

GG CT **GG TT** **AG CT** **AG TT**

1/4 odds for foal to be heterozygous for SP6 and curly with no KRT25

1/4 odds for foal to be homozygous for SP6 and curly with no KRT25

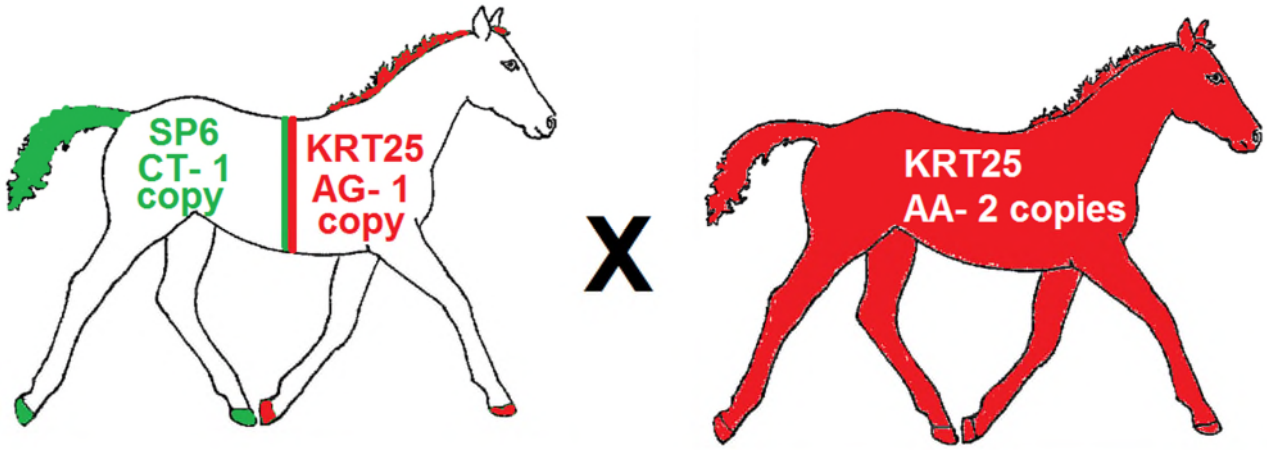
1/4 odds for foal to be heterozygous for both KRT25 and SP6 – **dual gene**

1/4 odds for foal to be heterozygous for Krt25 and homozygous for SP6- **dual gene**

0% - straight offspring

0% - AA or horse with scant mane and tail

Cross #6



The Cross of #1 parent Dual genes AGCT x #2 parent KRT25 AACC homozygous

Cross:
AGCT × AACC

	AC	AT	GC	GT
AC	AACC	AATC	GACC	GATC
AC	AACC	AATC	GACC	GATC
AC	AACC	AATC	GACC	GATC
AC	AACC	AATC	GACC	GATC

25%	25%	25%	25%
AA CC	AA TC	AG CC	AG TC

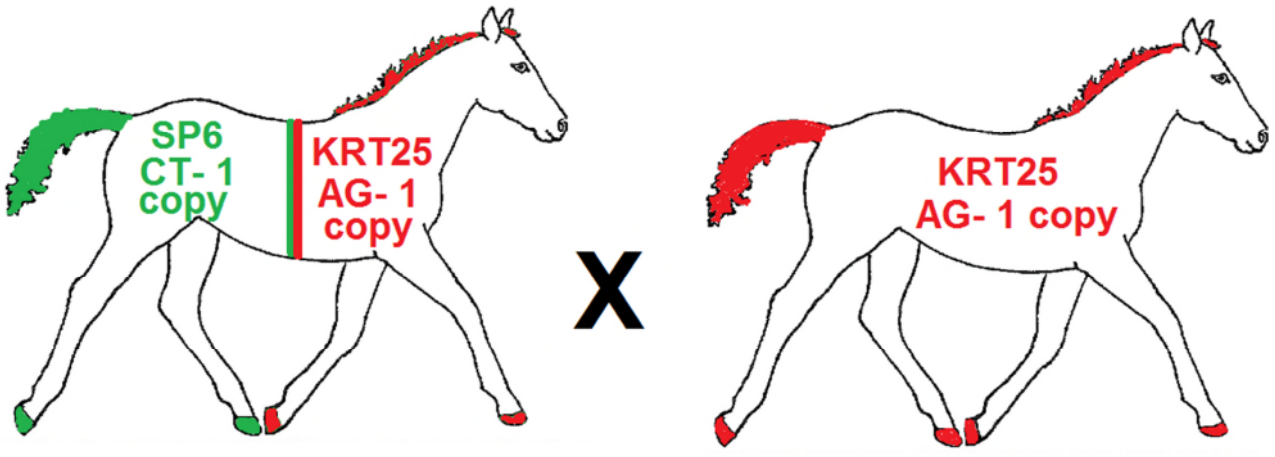
1/4 odds the foal will be homozygous for KRT25 with no SP6 present - scant mane and tail

1/4 odds the foal will be homozygous for KRT25 and heterozygous for SP6 - scant mane and tail

1/4 odds the foal will be heterozygous for KRT25 - mane and tail but brittle hair

1/4 odds the foal will be heterozygous for both KRT25 and SP6 - **dual gene**

Cross #7



The cross of #1 Parent Dual Genes AGCT x #2 Parent Heterozygous KRT25 AGCC

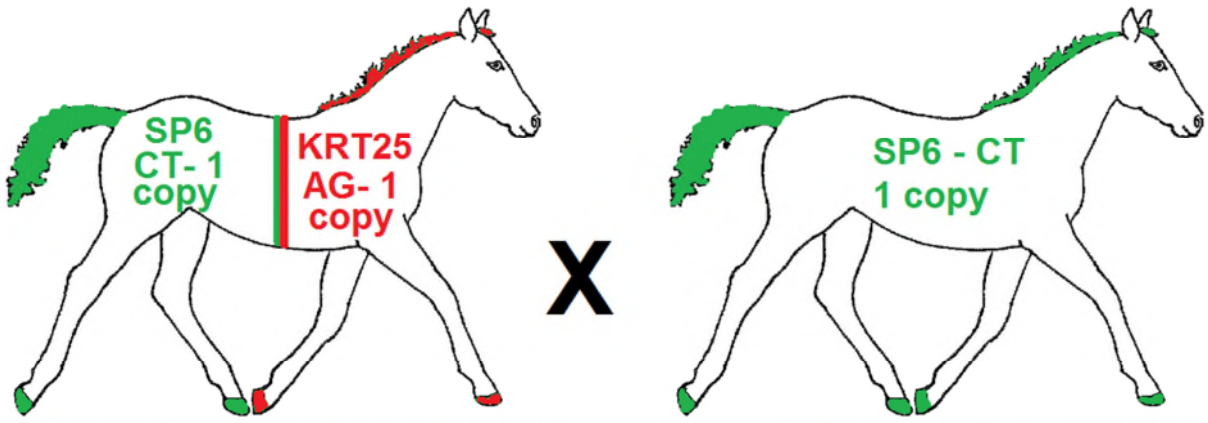
Cross:
AGCT × AGCC

	AC	AT	GC	GT
AC	AACC	AATC	GACC	GATC
AC	AACC	AATC	GACC	GATC
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC

12.5%	**12.5%**	**25%**	**12.5 %**	**25%**	**12.5%**
AA CC	AA TC	AG CC	GG TC	AG TC	GG CC

1/8 odds of AA CC – homozygous KRT25 with no SP6 - scant mane and tail
1/8 odds of AA TC - homozygous KRT25 and heterozygous for SP6 - scant mane and tail - **dual**
1/4 odds of AG CC – heterozygous KRT25 with no SP6 – full mane and tail – brittle hair
1/8 odds of GG TC – heterozygous SP6 with no KRT25
1/4 odds of AG TC - heterozygous for bothKRT25 and SP6 – brittle hair **- dual**
1/8 odds of GG CC - straight

Cross #8



Cross of #1 Parent AGCT Dual genes x #2 Parent Heterozygous SP6 GGCT

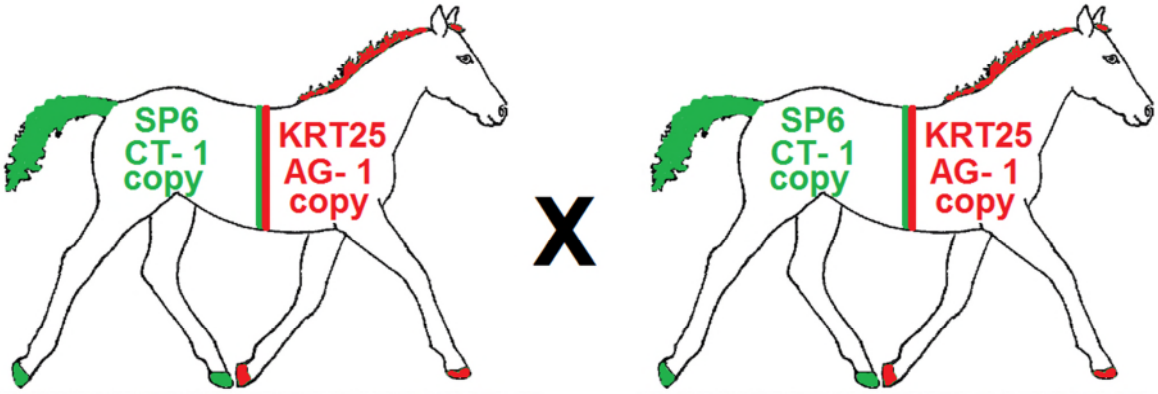
Cross:
AGCT × GGCT

	AC	AT	GC	GT
GC	AGCC	AGTC	GGCC	GGTC
GT	AGCT	AGTT	GGCT	GGTT
GC	AGCC	AGTC	GGCC	GGTC
GT	AGCT	AGTT	GGCT	GGTT

12.5%	**25%**	**12.5%**	**12.5%**	**25%**	**12.5%**
AG CC	AG CT	AG TT	GG TT	GG TC	GG CC

1/8 - odds of AG CC – heterozygous for KRT25 but no SP6 present – full mane and tail –brittle hair
1/4 - odds of AG CT – heterozygous for both KRT25 and SP6 – full mane and tail –brittle hair - **dual**
1/8 - odds of AG TT - heterozygous for KRT25 and homozygous for SP6 – full mane and tail – brittle hair - **dual**
1/8 - odds of GG TT – homozygous for SP6 with no KRT25 present – full mane and tail
1/4 - odds of GG TC – heterozygous for SP6 with no KRT25 present – full mane and tail
1/8 - odds of GG CC - straight

Cross #9



Cross:
AGCT × AGCT

	AC	AT	GC	GT
AC	AACC	AATC	GACC	GATC
AT	AACT	AATT	GACT	GATT
GC	AGCC	AGTC	GGCC	GGTC
GT	AGCT	AGTT	GGCT	GGTT

The cross of two individuals that are heterozygous for both KRT25 and SP6 and carry both genes at the same time. Both Parents Dual Genes- Heterozygous- AGCT

25%	12.5%	12.5%	12.5%	12.5%	6.25%	6.25%	6.25%	6.25%
AG CT	**AG CC**	**AA CT**	**AG TT**	**GG CT**	**AA CC**	**GG TT**	**AA TT**	**GG CC**

1/16 (6.25%) odds of having a straight foal (GG CC)

1/16 (6.25%) odds of having a foal that is homozygous for both KRT25 and SP6 at the same time **- dual gene**

1/4 (25%) odds of having a foal with a scant mane and tail - AA or homozygous for KRT25

1/4 (25%) odds of having a foal that is heterozygous for both Krt25 and SP6 – **dual gene**

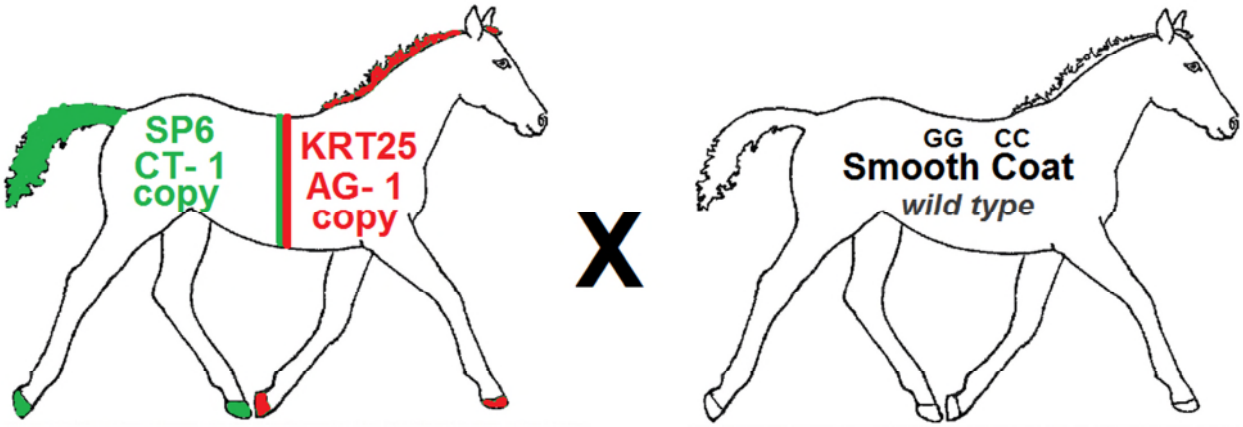
1/8 (12.5%) odds of having a foal that homozygous for KRT25 and heterozygous for SP6 – **dual gene**

1/16 (6.25%) odds of having a homozygous SP6 that does not carry KRT25

1/16 (6.25%) odds of having a homozygous KRT25 foal that does not have SP6

15/16 (93.75%) odds of having a curly foal

Cross #10



The cross of a horse with two genes for curl (heterozygous for both KRT25 and SP6) and a horse with no curly genes- Wild Type- GG CC <u>No mutation</u> (straight) or Smooth Coat.

Parent #1 – AG CT Dual Genes **Parent #2 - GG CC** Straight or Smooth Coat

Cross:
AGCT × GGCC

	AC	AT	GC	GT
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC

(AGTC) with a totally straight mare (GGCC) **(AGTC) X (GGCC)** would produce the following genotypes:

25%	25%	25%	25%
AG CC	**AG TC**	**GG CT**	**GG CC**

So —— 3/4 chance of having a curly and 1/4 chance of having a straight

1/4 odds of having a foal that is heterozygous for KRT25 with no SP6

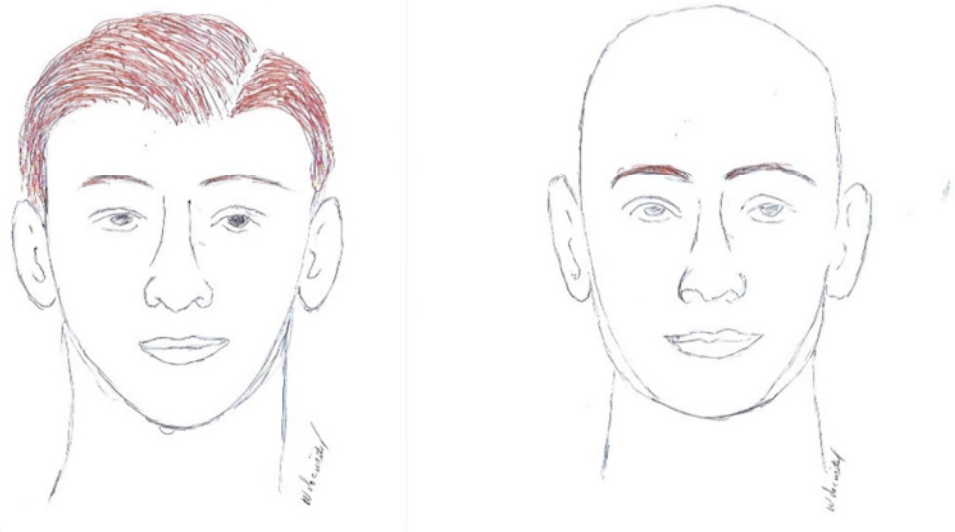
1/4 odds of having a foal that is heterozygous for both SP6 and KRT25 – **dual genes**

1/4 odds of having a foal that is heterozygous for SP6 with no KRT25

Zero chance of having a foal with the AA combination and sparse mane and tail

Epistatic Gene relationships

In classical genetics, if genes A and B are mutated, and each mutation by itself produces a unique phenotype but the two mutations together in the same individual show the same phenotype as the gene A mutation, then gene A is **epistatic** and gene B is **hypostatic**.



For example, the gene for total baldness is epistatic to the gene for red hair. It makes no difference if a person inherits the gene for red hair if he also inherits the gene for baldness. Baldness is epistatic to red hair. Baldness will always "mask" the effects of red hair.

Epistatic genes and hypostatic genes are two separate and independent gene mutations that interact on one aspect of the organism by chance. Epistasis is not **dominance**.

Genetic dominance is an interaction between alleles (two different versions of a gene) at the same gene locus (a specific gene location on a chromosome). Examples: The A allele is dominant over the G allele at the KRT25 locus. Likewise, the T allele is dominant over the C allele at the SP6 locus.

------ so, KRT25 and SP6 are two independent genes at different locations, but the effects of KRT25 mask the effects of SP6 like baldness masks the effects of a red hair gene.

In the case of the two curly genes isolated to date, KRT25 is epistatic (or masks to some extent) the effects of SP6.

Hypotrichosis and Dysplastic Hair Shafts

Hypotrichosis (scant mane and tail) is a condition characterized by sparse hair or a coat that is not as thick or long as normally expected. It is also used to describe hair coats that have defects in the hair shaft or follicles (dysplastic) that lead to a sparser hair distribution compared to normal hair distribution for the species.

KRT25

The missense mutation found in KRT25 is a mutation of a keratin gene which produces a type 1, inner root sheath – specific keratin protein that is essential in the assembly of keratin protein complexes required for the proper assembly of the hair shaft. The hair curl that is produced by KRT25 mutation is a consequence of the abnormal structure of the hair shaft itself. Along with curl, brittleness is also a consequence of the abnormal structure. The brittleness is thought to increase with UV light which is at its peak during the summer months.

Because KRT25 produces dysplastic hair shafts, it is epistatic to SP6 which produces normal hair shaft morphology.

SP6

The missense mutation found in SP6 is a mutation which affects a transcription factor. SP6 produces a **transcription factor**. The transcription factor's role is to produce a protein which controls the initiation and speed of transcription of genetic information from the DNA molecule to the messenger RNA molecule, by binding to a specific DNA sequence. Much like a capitol letter is used to show the beginning of a sentence, the transcription factor protein shows the messenger RNA when, where, and how fast to begin reading that segment of the DNA chain. The function of transcription factors is to regulate - turn on and off - genes in order to make sure that they are expressed (produce critical proteins) at the right time and in the right amount to produce, in this case, a hair follicle.

The slight variation in SP6 from wild type to SP6 mutation produces an inner root sheath of the hair follicle which is ovoid shaped. The **inner root sheath** acts as a mold to form the shape of the growing hair shaft. Ovoid shaped hair shafts curl. Therefore, the hair shaft produced by the SP6 mutation is **not dysplastic.** It has a normal hair shaft anatomy and is not brittle, but instead has an ovoid shape which produces curl.

Article and illustrations by Dr Mitch Wilkinson

Graphic's by Bunny Reveglia- ICHO

Curly Horses

A Mysterious Tale of Two Transcription Factor Mutations

By Dr. Mitch Wilkinson

Curly Jim

In 1956, two young, curly-coated stallions were bought by a man from Mountain View, Missouri, named Vic Clemens. Clemens bought the horses at an auction in Tennessee and had them shipped by train to Mountain View. The horses resembled Missouri Fox Trotter horses in build; they also were gaited. At the auction, Clemens could not find any information about where the horses came from and who were their previous owners. The background of these horses is still unknown today. (11,12)

One of the two curly stallions was subsequently killed after becoming tangled in a barbed wire fence, but the remaining stallion was named Curly Jim. Curly Jim was about three years old at the time, and his training was begun by a 16-year-old local teenager named Johnny Brooks. (11)

The chestnut colored stallion had several owners throughout his life.

"Bill" Ed Tune and subsequently Gurn Hodge also owned Curly Jim. (11 ,12)

Two curly horses arrive by train to Mountain View, Missouri

Curly Jim was pasture bred to many mares. Recent genetic tests have confirmed that a line of curly-coated ponies known as the McKay ponies owe their curly coats to Curly Jim. Due to lack of records, the McKay pony line and its connection with Curly Jim

had been questioned, but tests have confirmed that Curly Jim was the founding sire. (8, 20)

McKay Ponies

Gentleman Jim

Kopper Kid

Besides founding a line of curly-coated ponies, Curly Jim was responsible for a line of riding horses. Curly Jim's line of gaited curly-coated saddle horses became one of the most popular lines of curly-coated horses. In the 1950's and 1960's, Missouri Fox Trotter horses were bred intensively in the region where Curly Jim resided. Since Curly Jim was gaited and had similar conformation to Missouri Fox Trotters, it was only natural that his line became mixed with Missouri Fox Trotters. Due to extensive breeding with Missouri Fox Trotters, the Curly Jim line will forever be associated with the Missouri Fox Trotter breed.

The connection of the Curly Jim line to the Fox Trotter breed occurred through his daughter Blaze. Curly Jim was bred to a grade mare known as the Bradford Mare. The resulting foal was Blaze. Blaze inherited the curly gene from her father and was curly coated.

Blaze was subsequently bred to Walker's Merry Lad, a renowned, straight-haired Missouri Fox Trotter stallion who was standing at stud in the Mountain View, Missouri, area at that time. The breeding resulted in a gaited, curly-coated stallion named **Walker's Prince T**. Walker's Prince T was a curly stallion that was used extensively and bred not only to other curly horses but also to many Missouri Fox Trotter mares. Eventually, there were three stallions who carried the Walker's Prince T name. These stallions not only passed on the unique curly coat of Curly Jim, but also the gait mutation from the Missouri Fox Trotter mares that were bred into the line. (9, 10, 12)

Walker's Prince T

The Pedigree of Walker's Prince T

The original Walker's Prince T was owned by Lester Tunes. Johnny Brooks owned Walker's Prince T (II). Walker's Prince T (II) was a direct descendent of the horse Brooks trained when he was 16 years old, the great-grandson of Curly Jim. (11)

The combination of gait and the unique curly coat which will not shed mane and tail hair during the summer months makes the Curly Jim line of curly horses extremely popular. Interestingly, both traits are due to mutations within genes that produce **transcription factors**. In order to understand the mechanisms involved in producing these two traits, gait and coat curl, we need to investigate what transcription factors are and how they affect organisms.

Transcription Factors

All the information needed for any living thing to live, grow, and reproduce is stored in its DNA. A fundamental question is **how** and **when** the information coded within the DNA molecule is utilized by the organism.

Basically, the information coded on the DNA molecule is transferred to a messenger RNA molecule (**mRNA**) which in turn

transfers the information to transfer RNA (**tRNA**) molecules. The transfer RNA molecules work within a cell organelle called a **ribosome** which produces proteins. Within the ribosome, tRNA acts as an adaptor to convert DNA information to proteins by recognizing the mRNA codon (three base RNA code) at one end and binding to a single, specific amino acid at the other end. Transfer RNA molecules assure the proper order and kinds of amino acids which when bound together in long chains will become **proteins**.

All the physical characteristics of an organism (known as the **phenotype**) are made up of thousands of proteins. There are also water, minerals, and fats, but all the living functions and the basic framework of all living things are made of proteins.

The reading and transfer of the DNA information to mRNA is known as **transcription;** whereas, the assembly of proteins in the ribosome is known as **translation**. (15)

DNA → mRNA → ribosome + tRNA → Protein

Transcription **Translation**

What Transcription Factors Do

Transcription factors help to regulate the timing of the information that is being transcribed from the DNA molecule to the mRNA molecule. Transcription factors are themselves proteins that are produced by **genes** (discrete segments of DNA at specific locations called **loci**). In order for any organism to live, essential proteins must be produced in the correct amounts, in the proper sequence, and at the correct time. This complex interaction of one group of essential proteins regulating another group of essential proteins is where transcription factors take a critical role.

Transcription factors control the rate of transcription of genetic material from DNA to mRNA.

Without regulation the vital processes of the cells would be random chemical reactions which would produce uncontrolled amounts of proteins that would be incompatible with life processes.

Transcription factors bind to the DNA molecule to regulate the rate of transcription, but they do not regulate transcription by themselves. Other proteins like coactivators (speed up transcription) and corepressors (slow down transcription) work with transcription factors to help regulate transcription. This complex relationship is especially important during fetal development when the timing and amounts of proteins help form the developing animal.

As we will see, mutations within two genes that produce two critical transcription factors produced the iconic traits of coat and gait associated with the Curly Jim line. (6, 15)

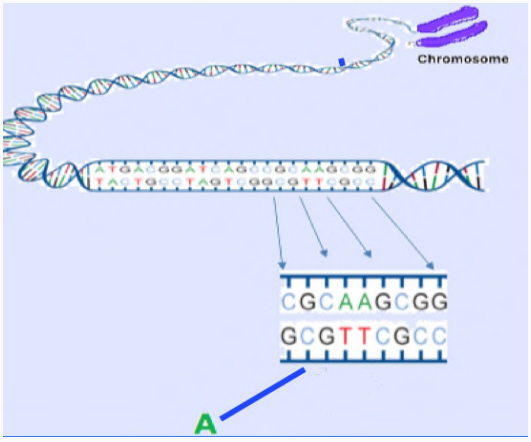
SP6 and DMRT3 Genes

In **August of 2012**, the prestigious science journal, *Nature*, published an article by an international equine genomics group which included Dr. Leif Andersson from Uppsala University in Sweden and Dr. Gus Cothran from Texas A&M University in the U.S. This article showed that in part, gait in horses is due to a **nonsense mutation** in a gene known as **DMRT3** which is located on the equine 23rd chromosome. The DMRT family of transcription factor proteins made from the DMRT3 gene have a unique and distinct binding mechanism for DNA to help regulate the timing of information transferred to mRNA. In the case of the mutation that causes gait, the DMRT3 gene had a misspelling of the DNA code. This

misspelling of the DNA sequence involved a single base pair which is known as a **SNP**. (14)

Misspellings happen on a regular basis as DNA replicates. On average, a misspelling occurs in one out of every **300** base pairs. This is one mechanism that allows **mutations** to occur in nature. Most of the time, the misspellings occur in areas of the DNA that are not being used, but every so often, the misspelling occurs in an area of the DNA chain that codes for a protein. This area of the DNA chain is known as a **gene**. Even then, the DNA can compensate due to multiple spellings of amino acids. Remember, DNA uses a three-letter base code for each amino acid within the protein sequence. Usually, when the altered spelling is translated to mRNA, it will still produce the same or slightly different protein. (15)

In this case, the normal base cytosine or C was substituted with an adenine or A. This produced a triplet code that was by chance a codon for the mRNA to stop reading the DNA molecule. This is known as a **stop codon**. When the mRNA encounters a nonsense mutation, an unreadable triplet base code**,** or in this case, a code to stop reading the DNA chain prematurely, it stops transcription of the DNA chain which results in a defective and truncated protein.



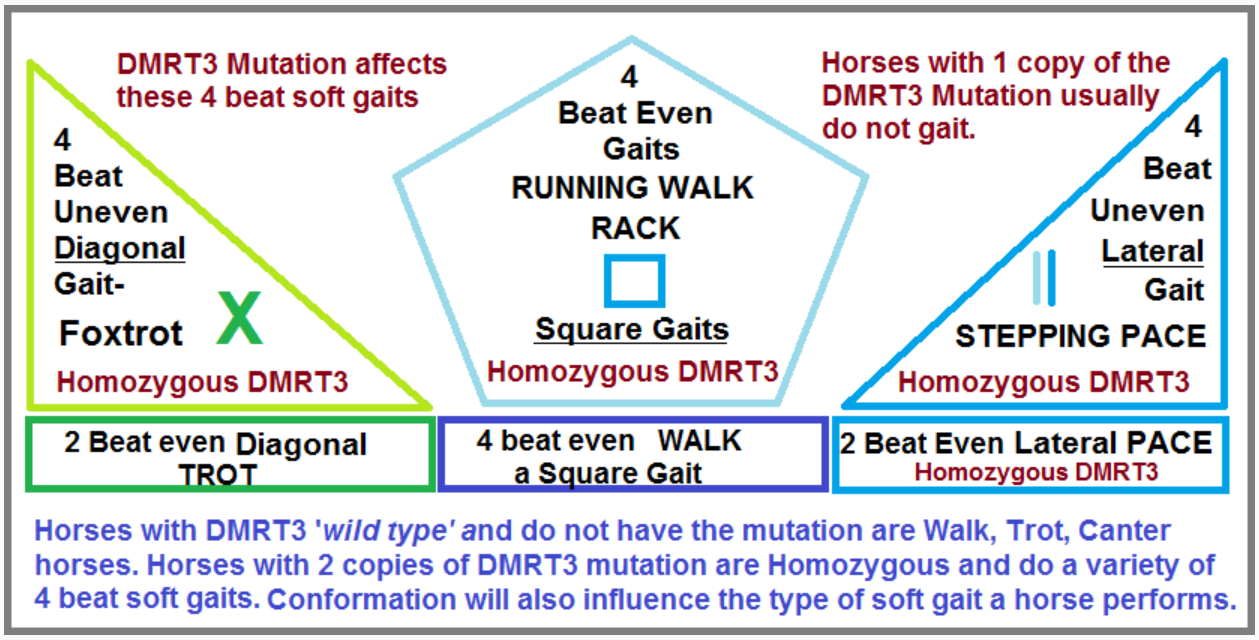
23rd equine chromosome with A substituted for a C resulting in a premature Stop Codon

The misspelling or mutation within the DMRT3 gene caused 174 amino acids to be left off the protein which is an essential transcription factor needed in the development of spinal neuron circuitry during fetal development. The shortened DMRT3 transcription factor causes a defect in the horse's spinal nerves which allows the horse to gait. (14)

This discovery of the mutation within the DMRT3 gene is the first step in understanding gait in horses. There is a large variety of gaits in equines. Other mutations which are thought to combine with the DMRT3 mutation to produce the different gaits are yet to be found.

In the shorthand notation of genetics which shows the base substitutes at specific spots or **loci** where the two different spellings of the gene occur, the DMRT3 mutation is shown with an **A** for the mutated gene and **C** for the wild type or non-mutated version. Each version of the gene is known as an **allele**. (15)

Around 140 curly coated horses have been tested for gait. Most have been from the Curly Jim line. Horses that have tested AA and have received the mutation from both parents show gaited characteristics. Horses that test AC or have only one copy of the DMRT3 mutation can have a softer ride, but that trait is inconsistent. These horses generally do not gait in tested curly horses. Earlene "Bunny" Reveglia has made a very useful chart describing the DMRT3 gene's influence on gait in horses.



DMRT3 Gait Chart- Diagonal Gaits*- the diagonal 2 beat trot on the left (2 diagonal hooves hit the ground at same time) and the DMRT3 mutation soft uneven gait 4 beat diagonal Foxtrot top left (each hoof hits independent of the other diagonally).*

Square Gaits*- Center is the 4-beat square gait walk (each hoof hits the ground independently with equal spaced timing). Center top is the DMRT3 mutation soft 4 beat gait the Running Walk and Rack; they are also square gaits. (Each hoof hits the ground at separate evenly spaced internals).*

Lateral Gaits*- On the right, the 2 beat Pace is also the result of the DMRT3 mutation (Two hooves hit the ground at the same time on one side of the horse, right side, left side with a suspension in the middle). The right top is the result of the DMRT3 mutation and is a 4-beat uneven lateral Stepping Pace gait. (Each hoof on the lateral side hits independent of the other on one side, then other side.)*

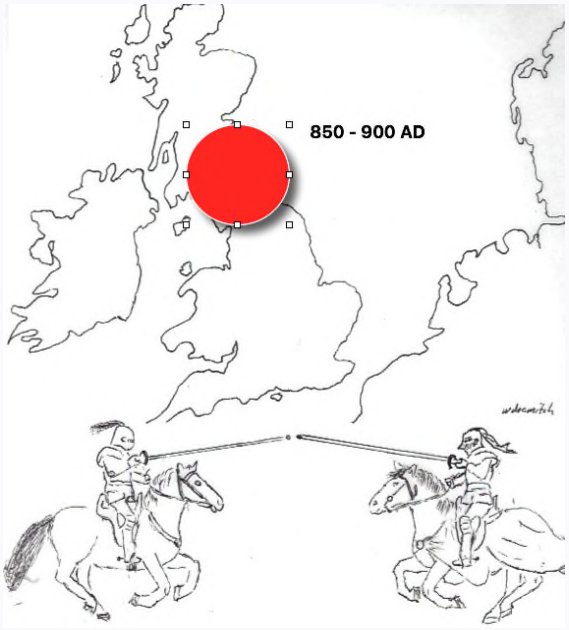
Horses with Wild Type DMRT 3 do the walk, trot, canter, gallop. Horses with two copies of DMRT3 mutation do a variety of soft gaits that is also dependent on their own conformation and ability. Horses with only 1 copy of the DMRT3 mutation generally do not do the soft 4 beat gaits. (7)

It is important to note that "gait" in the Curly Jim line is entirely independent from curly coat producing genes. Gait and curl are different inheritances on different chromosomes.

In 2016, a scientific study was published about the origin of gaited horses. The paper concluded that the DMRT3 mutation occurred with the birth of a single horse that lived somewhere

between 850 AD and 900 AD in England. One of that horse's parents had a misspelling of the DMRT3 gene during the division of the cells that would become sperm or egg cells for reproduction. Having a gait would probably not be an advantage in nature; that is why gaited horses are very rare in the wild horse populations around the world. The horse that was born with gait in England during the middle ages did not come from elsewhere, it was born due to a chance genetic mutation in medieval England. The preference of riders for gaited horses guaranteed the mutation's continuance through the ages. (16,18)

The Curly Jim line like all gaited horses has a direct link with a unique horse born long ago in medieval England.



The DMRT3 gene mutation originated in medieval England.

In the future, the complete genetic picture of gait in equines will be known, but for now, the mutation in the DMRT3 gene on

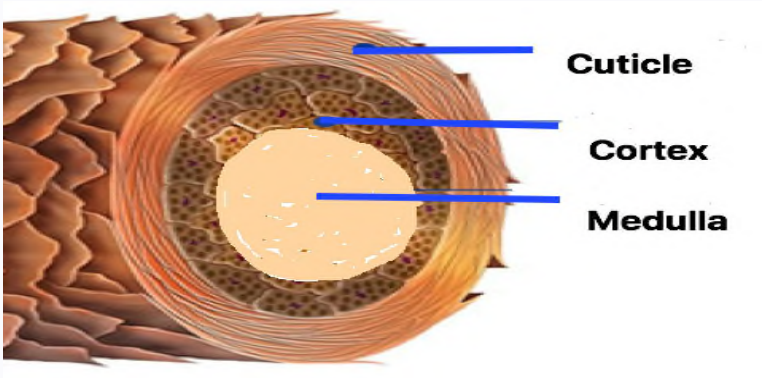
the 23rd equine chromosome is the first step in our understanding all forms of gait.

Isolation of the KRT25 Mutation

In **November 2017**, the scientific journal, ***Genetics Selection Evolution*** published the results of a scientific study that was conducted four years prior to publication. Dr. Laurent Schibler and his team in France at the University of Paris in coordination with Dr. Gus Cothran at Texas A&M University in the U.S. had isolated the first curly gene. This gene mutation that produced curly coats in horses was a mutation within a keratin gene.

The missense mutation found in KRT25 is a mutation of a specific hair producing keratin gene in the **11th equine chromosome** which produces a type 1, inner root sheath specific keratin protein that is essential in the assembly of alpha keratin protein complexes required for the proper construction of the hair shaft. A **missense mutation** is a DNA misspelling which is still readable by the mRNA, but which produces an alternate amino acid within the protein chain. This produces a slightly different protein. (1)

The hair curl that is produced by KRT25 mutation is a consequence of the abnormal structure of the hair shaft itself. Along with curl, brittleness is a characteristic of the hair shaft's abnormal structure. The brittleness is thought to increase with UV light which is at its peak during the summer months. (8)

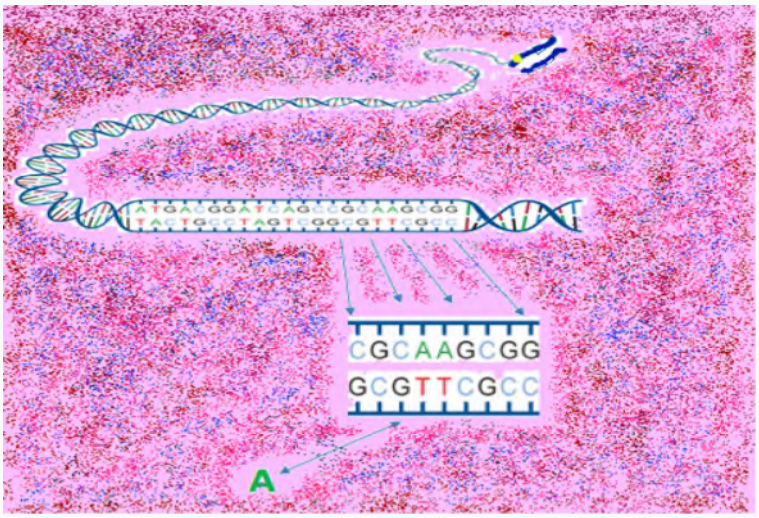


Cross section of hair shaft

The strength of any hair shaft is due to the sulfur bonds present in the middle layer called the **cortex**. The keratin within the cortex contains large amounts of the amino acid cysteine, which allows the keratin molecules to bind together in a helix shape when forming sulfur bonds. In the case of the KRT25 mutation, the hair shaft is bent or curled, but at the expense of strength. (13)

A recent study by German researchers found that hair produced by the KRT25 mutation in many cases lacked a medulla. The exact function of the medulla is not known, but the lack of a medulla shows the unusual structure associated with KRT25 mutation produced hair. (19)

This **missense mutation** found within the KRT25 gene is a consequence of the misspelling of a single base pair of the DNA chain or SNP. Whereas the normal KRT25 gene at the locus(location) of the mutation normally has a guanine or G, the mutated version has an adenine or A. (1)



Substitution of an A for a G in the 11th equine chromosome within the KRT25 gene

When a mutated version of the KRT25 mutation is passed on to the foal by both parents, the foal is said to be **homozygous** for the mutation. This is shown in the genetic shorthand as **AA**. The AA combination produces horses with sparse brittle manes and tails.

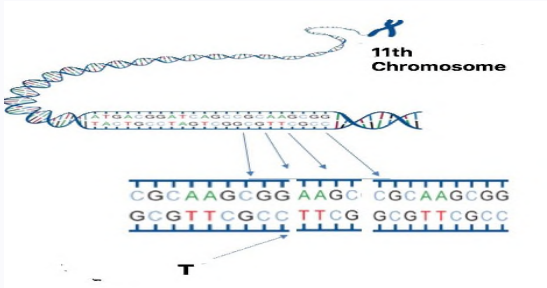
Subsequent testing of a large number of American curly horses demonstrated that the KRT25 mutation was found the majority of horses tested but testing of the Curly Jim line of horses revealed that the KRT25 mutation was not present in this population. (20)

Discovery of the SP6 Mutation

In **April 2018**, Dr. Ottmar Distl and his team in Germany published an extensive study which included not only histological studies of curly horse hair, but also the isolation of a second curly horse gene mutation in a gene which produces a transcription factor protein. The gene is known as **SP6**. The SP6

gene is found on equine chromosome #11 like KRT25, but this mutation is within a gene which codes for critical proteins during fetal development. The paper was published in the scientific journal ***Scientific Reports*.** (19)

It was found that the Curly Jim line's coat was produced by a mutation in the SP6 gene in which a misspelling of the DNA occurred. This was again a **missense mutation** where a thymine base or **T** was substituted for a cytosine base or **C**. This type of mutation was also a SNP which is the misspelling of a single base in the DNA chain.



*11th equine chromosome showing substitution of a thymine(T) for a cytosine(**C**) in the SP6 gene*

Unlike the KRT25 mutation which happened within a keratin producing hair gene, the SP6 gene mutation happened within a **transcription factor** producing gene which then produced a slightly altered transcription factor protein. The slight variation in the protein produced by the mutated version of the SP6 gene was thought to affect the shape of the forming hair follicles during fetal development. Instead of being round, the **inner root sheath** or hair molding component of the follicle was oval in shape. Oval shaped hairs curl as they grow. Brittleness was lacking in hairs produced with the SP6 mutation; the result was a strong, curly

coat. This curly coat was distinctly different from the one formed by the KRT25 mutation. (19)

The SP6 gene produces two similar transcription factor proteins, epiprofin and **SP6 transcription factor**. These two transcription factor proteins are critical during fetal formation of teeth, limbs, lungs, and hair follicles. A large change in these important SP6 proteins can have devastating effects for the animal, but a very small change resulted in a horse with a curly coat. (6)

Gaited horse with heterozygous SP6 mutation

Gaited horse with homozygous SP6 mutation

Horses of the Curly Jim line that are homozygous are associated with an unusual winter, body coat which has been described as micro-curl or brillo-pad. The curls are extremely tight and curl upon themselves. (8)

Micro curls or "Brillo Pad" curls are tight, short, and coarse. They make up the body coat in horses with Curly Jim homozygous inheritance.

Although KRT25 and SP6 are both curly producing genes on the 11th horse chromosome, they are distinctly separate genes that are inherited separately and independently. They can be **inherited simultaneously** in the same individual.

It was found by Dr. Distl's research group that the effects of KRT25 mutation do affect the characteristics of the SP6 mutation when both are found on the same individual. Because KRT25 affects the basic structure of the hair shaft itself and SP6 is more involved with the shape of the hair as determined by the inner

root sheath of the follicle, an oval shaped hair shaft can be brittle if KRT25 is also present. This effect is termed **epistasis**. (6)

An example often sited to illustrated epistasis is shown below:

Epistatic Gene Relationships

In classical genetics, if genes A and B are mutated, and each mutation by itself produces a unique phenotype but the two mutations together in the same individual show the same phenotype as the gene A mutation, then gene A is **epistatic** and gene B is **hypostatic**.

A classic example of the epistatic effect of one gene over another is found in albinism. The system of genes that determines skin color, hair color, and eye color in humans and animals is independent of the gene responsible for **albinism** (lack of pigment). The gene for albinism is an **epistatic** gene, and the genes for brown hair and other color traits are hypostatic to the albinism gene. The individual still inherits the genes for brown hair and blue eyes, but these genes can't be expressed due to the presence of the gene for albinism.

Epistasis

The genes for brown hair and blue eyes are still present but cannot be expressed due to the presence of the gene for albinism.

Epistatic genes and hypostatic genes are two separate and independent gene mutations that interact on one aspect of the organism by chance. Epistasis is not **dominance**.

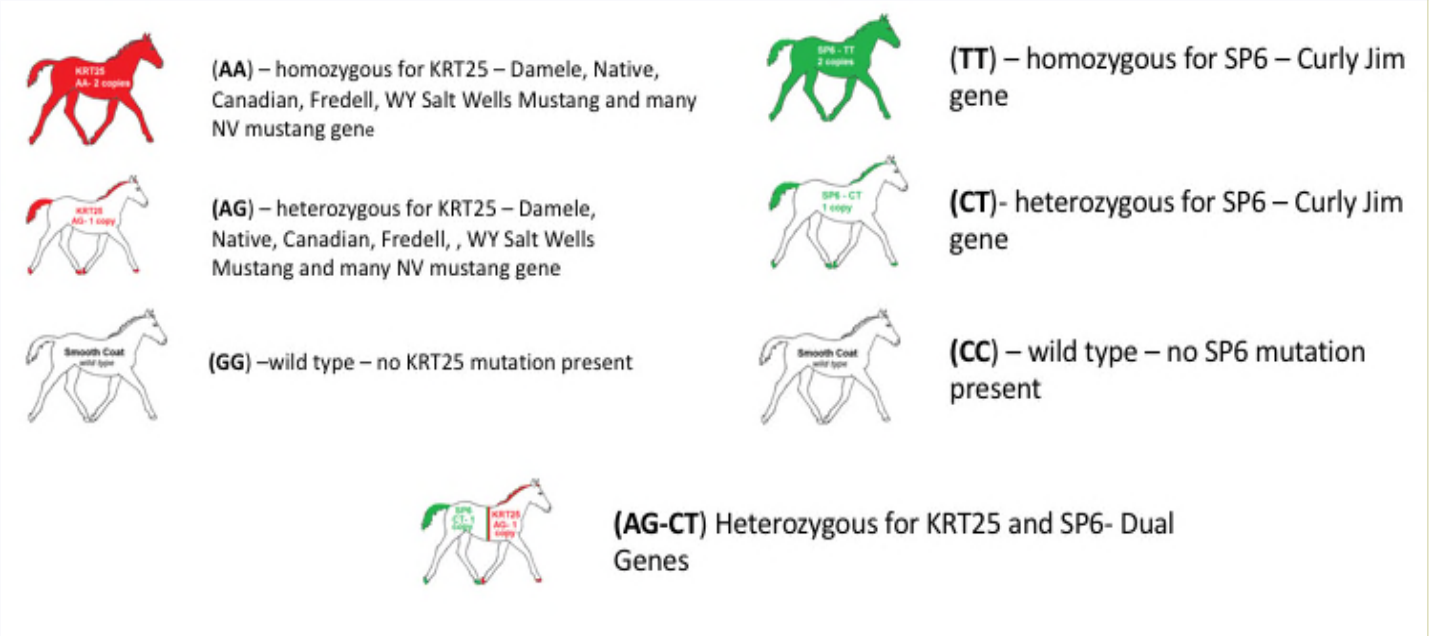
Genetic dominance is an interaction between alleles (two different versions of a gene) at the same gene locus (a specific gene location on a chromosome). Examples: The A allele is dominant over the G allele at the KRT25 locus. Likewise, the T allele is dominant over the C allele at the SP6 locus.

------ so, KRT25 and SP6 are two independent genes at different locations, but the effects of KRT25 mask the effects of SP6 like the gene for albinism masks the effects of a brown hair gene.

In the case of the two curly genes isolated to date, KRT25 is epistatic or masks to some extent the effects of SP6. (6, 15, 19)

Breeding Considerations with Two Independent Genes

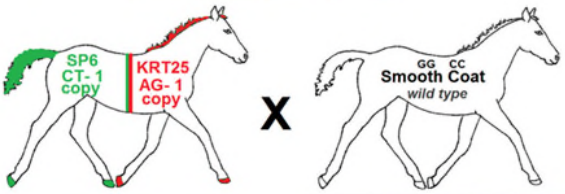
If we look at a horse that carries one copy of the **KRT25** mutation simultaneously with one copy of the **SP6** mutation (**A**GC**T**), and that horse is crossed with a horse that does not have a copy of either the KRT25 nor the SP6 mutation (GGCC), we can see the odds or possibilities for the foal produced. An actual example is shown below with three horses owned by Angie Gaines in Texas.



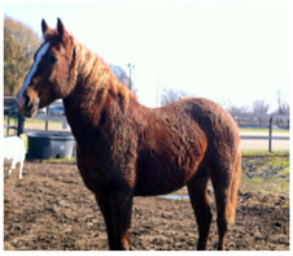
Legend: Combinations of KRT25 and SP6 mutations

Cross:
AGCT × GGCC

	AC	AT	GC	GT
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC
GC	AGCC	AGTC	GGCC	GGTC



Chester



X

Blossom

The cross of a dual gene stallion with a smooth coat mare

Each color represents a different genotype

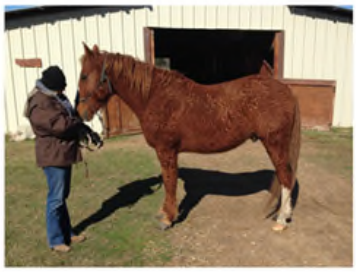
So — — **3/4** chance of having a curly and 1/4 chance of having a straight

1/4 odds of having a foal that is heterozygous for KRT25 but did not inherit SP6 – full mane and tail, but brittle hair

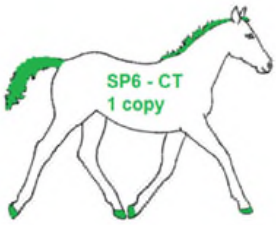
1/4 odds of having a foal that is heterozygous for both SP6 and KRT25 – **dual genes** – full mane and tail, but brittle hair

1/4 odds of having a foal that is heterozygous for SP6 and did not inherit KRT25 – full mane and tail with no brittle hair

0% odds of having a foal with the AA combination which produces sparse mane and tail



Bucky



The foal produced from the mating had one copy of the SP6 mutation

There was a 1/4 chance of this happening

Dr. Distl used the term “hypotrichotic” to describe the scant mane and tail characteristics associated with AA or homozygous horses that inherit the KRT25 mutation. **Hypotrichosis** (scant mane and tail) is a condition characterized by sparse hair or a coat that is not as thick or long as normally expected. It is also used to describe hair coats that have defects in the hair shaft or follicles (dysplastic) that lead to a sparser hair distribution compared to normal hair distribution for the species. (19)

Horses having only one copy of the KRT25 gene mutation or AG are described as having partial hypotrichosis. Dr. D. W. Scott in his 2004 paper on the histology of curly horse hair described the appearance of hair produced by KRT25 mutation as “dysplastic” meaning different from normal horse hair. Both terms can be used to describe the characteristics of hair shafts and coats produced by the KRT25 mutation. (3, 19)

Speculation on the Origins of the SP6 Mutation

In recent years, we have begun to understand that curly coats in horses can be produced by a variety of different and distinct gene mutations. We have also learned that curly coated horses can be found in many places throughout the world. The coats of these horses have distinct phenotypes or properties. As to date, none of the distinct populations have had gene mutations in common. So where did all the curly coated horses originate that we now find in such diverse locations around the world? In the future, we may find that some were imported from different locations and seeded a new population, but we will probably find

the majority resulted from simple and random mutations in local populations of horses.

Somatic Mutations vs Germinal Mutations

Somatic cells produce all non-reproductive tissues in living animals. Mutations in somatic cells are called **somatic mutations**.

Somatic mutations are not passed along to the next generation by sexual reproduction; they die with the animal. Cancer tumors are a unique class of somatic mutations.

In contrast, reproductive cells produce mutations which will be passed on to future generations. These mutations are termed **germinal mutations**. Germinal mutations are not expressed in the parent but are expressed in the offspring and the offspring's descendants. In most cases, only a single offspring will be born with the mutation and other siblings will not have the germinal mutation. (6, 15)

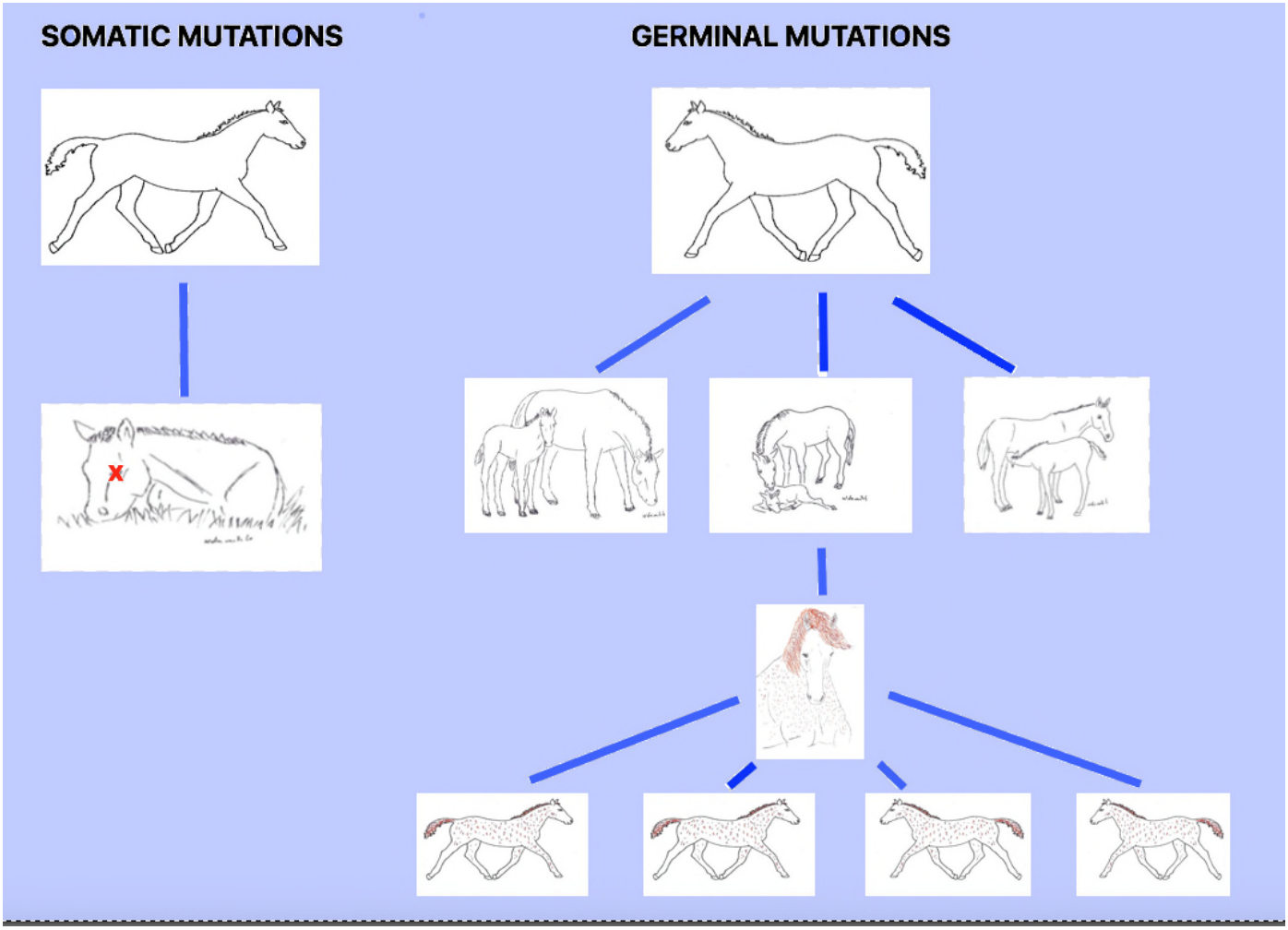


Chart illustrating the difference between somatic and germinal mutations

In the case of Curly Jim, a simple, single DNA base misspelling probably occurred during formation of a reproductive cell which became egg or sperm in a process termed **meiosis**. These reproductive cells or **gametes** have one-half the number of chromosomes found in somatic cells. Gametes are said to be **haploid,** meaning one-half of the animal's genetic material is present. Because there were originally two horses with this curly coat phenotype, the SP6 mutation probably occurred at least two generations before the birth of Curly Jim either in the stallion or mare that were his grandparents.

The SNP that resulted in the SP6 mutation did not affect Curly Jim's grandparents, but was passed as a dominant genetic trait to an offspring that would become one of Curly Jim's parents.

When such things happen, such as two straight haired parents producing an unexpected curly offspring, it is common to deduce that a recessive gene was involved and that both parents were carrying the recessive gene. That can be true, but not always. In this case, it was not.

An unexpected breeding outcome can also happen due to a mutation occurring in either one of the parents' reproductive cells during meiosis. The resulting mutation can be either dominant or recessive. If recessive, it will be passed on and not expressed until a future mating allows the combination of two recessive genes for the same trait to occur in the same individual revealing the dangers of "line breeding". (4, 5)

But if the resulting mutation is dominant, the mutation will increase within the population if it is favorable for survival. In the case of domestic horses like Curly Jim, the selection is due to human favoritism.

Thus, there is a good chance that Curly Jim's coat and the SP6 mutation happened due to a random mutation of a domestic horse in a nearby state. It should be remembered that the SP6 mutation has **not been found** in wild curly horse populations. (20)

Much time and energy have been spent wondering where curly horses originated, and some may have come from other locations, but most will be germinal, dominant mutations that happen spontaneously in local horse populations around the world. No distant curly horse populations to date have matched either KRT25 or Sp6. There is at least one other undiscovered curly producing gene mutation in the domestic curly horse population in the U.S. called the **Cook gene**. A second curly coat gene mutation has been found in **Sulphur Mustangs**. Its outward manifestations are very much like the Curly Jim SP6 mutation. Like the SP6 mutation that has not entered the feral horse

population, the Sulphur Mustang mutation has not yet entered the domestic curly horse population.

The very popular Curly Jim line of horses owes its popularity to mutations in two transcription factor-producing genes, DMRT3 and SP6. The combination of full and non-shedding curly mane and tail with comfortable gait will continue to make this line of curly horse desirable for many owners.

As a group, curly horse enthusiasts should embrace the fact that the curly horses found in the U.S. and Canada may be part of our unique equestrian heritage and that most will surly prove to be horses that were created by nature in North America.

About the Author:

Dr. Mitch Wilkinson has been a lifelong horse enthusiast. After receiving a bachelor's degree in chemistry and professional dental degrees, he earned a post-doctoral master's degree from Baylor University in biology. Currently, Dr. Wilkinson is Chairman of the Curly Mustang Association and Vice- Chair of the ICHO Research Department. His articles have been published in the United States, New Zealand/Australia, Russia, and Austria.

A special thanks to friends who helped edit and provide helpful suggestions for this article: Dr. Karen Zierler, Dr. Gus Cothran, Earlene" Bunny" Reveglia, and Beverly Arrendell

Dr. Wilkinson and Lucky

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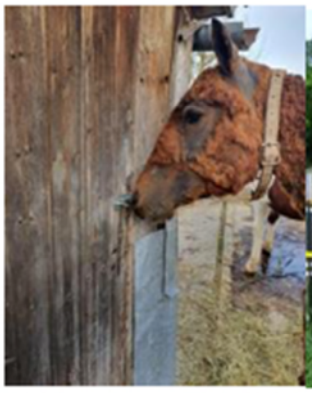
Baldness In Curly Coated Horses

By Dr. Mitch Wilkinson

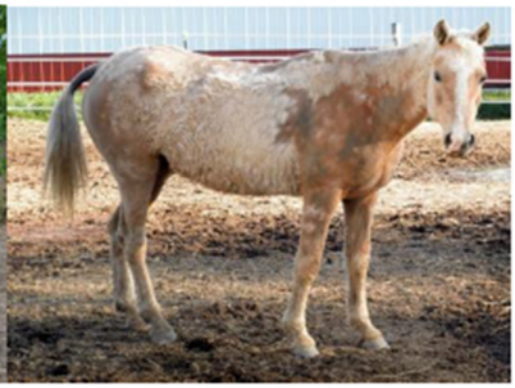
Siberian–Zabaykalskaya – unknown curly causative gene with alopecia

Patagonian – Criollo – unknown curly causative gene with alopecia



N. American KRT25 - Partial alopecia

N. American – KRT25 – Alopecia Totalis



N. American – unknown curly causative coat gene with alopecia

One of the mysteries of curly coated horses around the world is the problem of alopecia (abnormal absence of hair). Alopecia in curly coated horses can range from thin or absent mane and tail hair, to bald patches on body coats, and can include almost complete absence of hair. To make matters even more complicated, we now know that various populations of curly coated horses have multiple causative genes. In North American horse populations alone, we know of

at least five causative mutations which produce a curly winter coat. The five North American genetic mutations are KRT25, SP6, Cook, Unknown Curly Gene “a”, and the Sulphur curly gene. Of these five, only two, KRT25 and SP6, have been located in the horse genome and have corresponding tests to determine their presence. Other North American curly winter coat causative genes may be found in the future.

Worldwide, from Patagonia to Mongolia and from the shores of Lake Baikal in Southern Siberia to Manipur India, horses with curly winter coats can be found. The number and DNA locations (loci) of these different curly winter coat producing gene mutations in these horses is unknown, but if North America is any indication, there may be dozens of different causative mutations in Asia and at least two or more associated with curly coated horses in South America. Most of the Asian and South American curly coated horse populations have been tested for the presence of the two causative gene mutations which have been isolated from North American horses, KRT25 and SP6.(1) Horses from these distant populations have all tested negative for the presence of the two known North American gene mutations, thus leading to the conclusion that their curly winter coats are caused by different genes.(2) The perplexing question concerning curly coated horses is why alopecia is present in widely dispersed populations, and why alopecia is present in unrelated populations with different causative gene mutations? What could possibly be happening to produce such a pathologic condition? It must be stated that alopecia is fairly rare in all the different curly populations, but when it happens, it is devastating both for the animal and the owner. Alopecia in a horse not only affects the horse’s appearance, but has complications such a sun burn and insect induced irritations. These horses require special attention from owners to assure their survival. Many owners do not have the time or resources for such horses.

For a more in-depth explanation of the various types of curly winter coats in horses around the world refer to these two previous articles:

1. https://www.horsetalk.co.nz/2017/12/01/curious-twists-genetics-horses-curly-coats/
2. https://www.horsetalk.co.nz/2018/09/22/curly-jim-domestic-curly-horses-genetic-tale/

Many years ago, one of my mentors, C.E. Jackson, MD who was a physician and a geneticist gave me a wonderful insight into searching for unknown genetic abnormalities. Dr. Jackson said, " Mitch, when you are looking for a genetic abnormality, start every time with family trees. The family tree will tell you what trail to follow". Among many accomplishments in Dr. Jackson's career, he identified a rare abnormality called "Amish Brittle Hair Brain Syndrome" in 1974.

The first curly winter coat mutation to be identified, KRT25, was isolated in 2016 by a joint French and American research team headed by Dr. Gus Cothran , Dr. Rytis Juras, and Dr. Laurent Scribner. (1) Soon after this gene was found, it became evident through further testing of the curly horse population that more than one gene mutation was present in North American curly horses. Many curly horses were tested over the next year in a coordinated effort between Texas A&M University and the International Curly Horse Organization. A genetic data base was created by Earlene "Bunny" Reveglia to catalog the results for future research.(2) When enough information was available, I began looking at family trees. It wasn't long before I stumbled upon something miraculous.

I was looking at the family tree of a horse named Chester who had tested positive for one copy of KRT25 (heterozygous or KRT25 +/-). Chester was bred to a non-curly mare and produced a curly foal that did not have KRT25.

In checking the background of the sire, Chester, it was found that his bloodlines included the Curly Jim line of curly-coated horses. The Curly Jim line was found to have curly coats not produced by KRT25. Because the colt sired by Chester did not carry the KRT25 mutation and his dam did not have a curly coat, the foal's curly coat must be due to the Curly Jim mutation which must also be present in the sire.(3) Although the Curly Jim mutation had not been isolated at this time, the conclusion was that Chester carried two independently inherited mutations for curly coat, the KRT25 mutation and the Curly Jim mutation.

In 2018, a German research team isolated the Curly Jim mutation which was located within the SP6 gene that is found on the 11th equine chromosome (the same chromosome as the KRT25 mutation). (4) Chester and his foal were tested for KRT25 and SP6 in 2018. Chester indeed had KRT25 +/- and SP6 +/- ; his foal only had SP6 +/-.

The stallion Chester is heterozygous for the KRT25 gene, as well as the Curly Jim mutation, SP6. He is owned by Angie Gaines.

After 2018 and more subsequent testing, it was found that there were quite a few horses like Chester that carried two independent curly causing gene mutations. We decided to call these horses "dual gene" horses. The definition of a dual gene horse is a horse that carries two curly winter coat causative gene mutations simultaneously. Back in 2016, I realized what a profound influence my discovery about the inheritance of two or possibly more curly causative genes in the same individual was to play in the future of curly horses, but only a few others did at the time. Is it possible for a horse to be dual gene and be homozygous (two copies) for one or both of the gene mutations involved?

According to the laws of inheritance established by Gregory Mendel in the 19th century, there is only one way a homozygous, dual gene horse could be conceived. To create a "dual gene" horse that has two copies of one or both mutations which produce curly coats, at least one or both parents must be a dual gene genotype. Dual gene horses are not common, and represent a small percentage of the overall curly horse population. Surely, such a breeding has happened, so why do we not have an example of such a horse?

Even with the rare occurrence of two dual gene horses being mated, there is only a 1 in 16 chance of the foal being homozygous for both gene mutations. However, there is a 1 in 4 probability of the foal being dual gene with a double copy of one of the curly coat causative mutations. The mating of two heterozygous "dual gene" horses can be seen in the Punnett Square below. A close examination of other Punnett Square probabilities show that other "dual

gene", homozygous combinations are quite possible with different mating scenarios, but all require at least one parent to be dual gene.

In the Punnett Square below, the red square (upper left) represents a double homozygous, dual gene horse. The substitution of Arginine (A) for guanine (g) creates the KRT25 mutation and a substitution of Thymine (T) for cytosine (c) creates the SP6 mutation. (1) (4) Homozygous KRT25 is shown as AA and homozygous SP6 is represented as TT. The lower case letters represent the wild type genes.

Due to the presence of dual gene horses in the North American domestic population, there seems to be a curious absence of such horses with a homozygous component of KRT25 or SP6. None of the possible homozygous combinations of these two genes have been found in tested, dual gene horses. This could be due to a lack of testing, and eventually an individual with these genotypes will be found and confirmed. However, it has been proposed that the homozygous component in a dual gene foal that includes KRT25 and SP6 may precipitate a spontaneous abortion of the fetus. Several horses with KRT25 +/+ and balding are suspected dual gene, but this involves another gene not SP6. The spontaneous abortion hypothesis is purely speculation at this time.

AgTc × AgTc

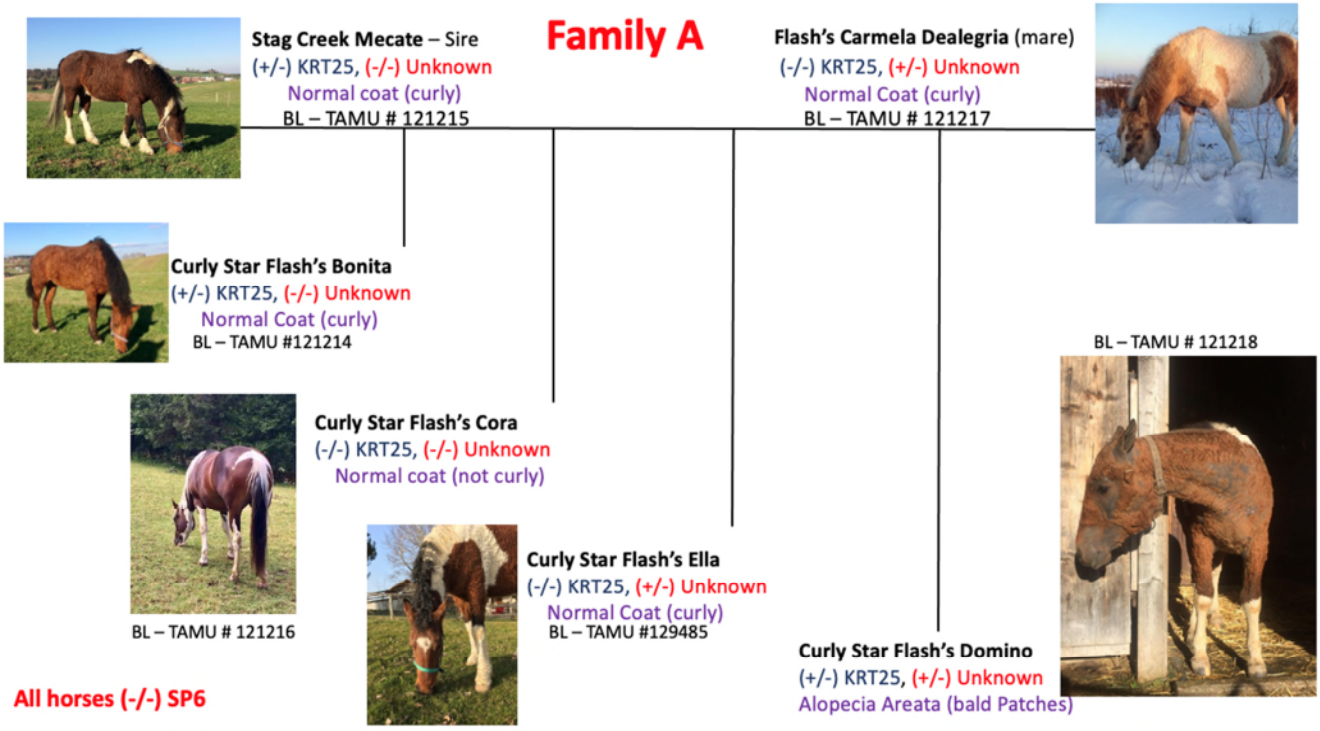
	AT	Ac	gT	gc
AT	AATT	AAcT	gATT	gAcT
Ac	AATc	AAcc	gATc	gAcc
gT	AgTT	AgcT	ggTT	ggcT
gc	AgTc	Agcc	ggTc	ggcc

The coats of curly horses seem to follow certain patterns of appearance called phenotypes. The phenotypes are different enough to imply different genetic causes. The summer and winter coats of North American horses that carry one of

the five North American curly coat causative genes all are distinctive. Superficially, these coats may look similar, but on closer examination, they have distinctive hair growth patterns and microscopic distinctions.(4)

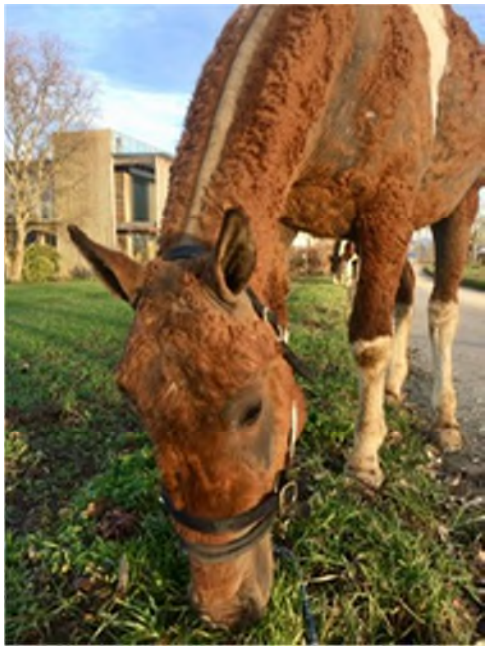
In February of 2020, I was contacted by a horse breeder in Switzerland who was concerned about balding problems in her breeding program. Her name was Veronika Schmid. This wonderful lady was willing to help in any way necessary to help solve the mystery of baldness in her horses, so we started testing samples with Dr. Rytis Juras and Dr. Gus Cothran both from Texas A&M University Animal Genetics Laboratory with the added guidance and help from Earlene "Bunny" Reveglia of the International Curly Horse Organization.

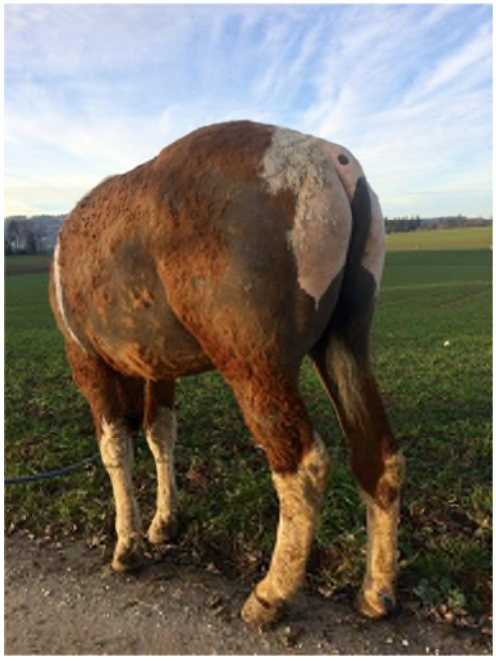
Again, I went back to Dr. Jackson's advice and started with family trees. One branch of the Schmid's horse breeding program included alopecia problems and is shown below.



In Family A show above, the dam having one copy of an unknown gene is the only scenario in which the observed inheritance pattern can work. If she had two copies of the unknown gene, all the foals would have one copy of that gene and be affected. The fact that one foal is not curly shows that both the dam and sire were heterozygous. The sire's genotype (KRT25 +/-) was confirmed by testing. Also, the mare had a daughter that was negative for KRT25, but was curly and had the same coat phenotype as the dam. The only offspring with symptoms was Domino who has KRT25 +/- and was speculated to have inherited an additional copy of the unknown gene from the dam. The forth foal was KRT25 +/- like the sire and did not have symptoms.

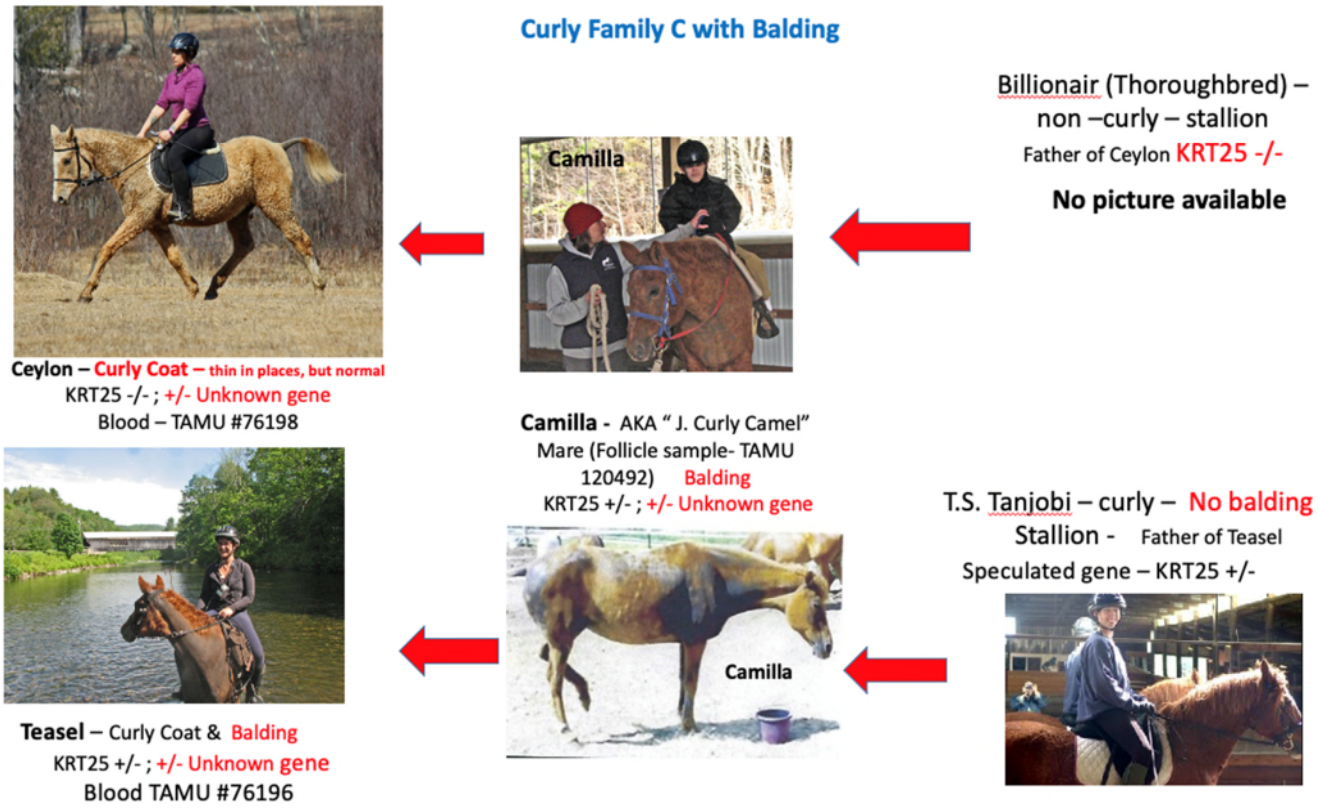
From the family A tree, it became evident that the form of baldness in this family of horses was due to an unknown gene that was hidden in "plain sight" and could have dated back to the beginning of North American domestic curly breeding in the 1940s.(6) Various forms of alopecia in North American Curly horses have plagued breeders from the beginning. In the family tree above, the suspected unknown gene is in red letters. I termed the gene, Unknown Curly Gene "a" with the expectation there were other unknown genes yet to be found. This form of alopecia had a very distinctive phenotype as seen in a close-up picture of Curly Star Flash's Domino. Domino fits the definition of a dual gene horse. His genotype is KRT25 +/- and Curly Unknown Gene "a" +/- .





Domino: One copy of KRT25 and one copy of Unknown Curly Gene "a"

Unknown Curly Gene "a" when mixed with KRT25 in a dual gene horse produces a phenotypical coat which has bald areas surrounded by patches of curly coat. Mane and tail hair is almost absent. With the help of Earlene Reveglia and the wonderful genetic records kept at the International Curly Horse Organization, I began to construct other family trees which might involve the suspected Unknown Curly Gene "a".



Appearance or phenotype of Curly Unknown Gene "a"

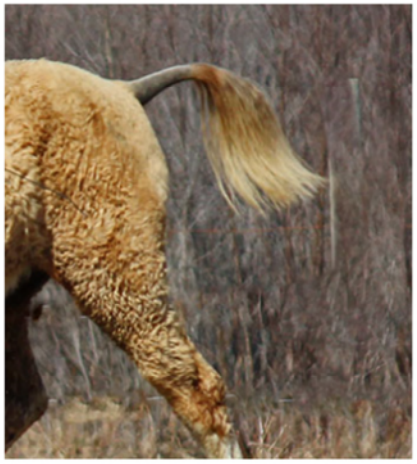
Horses like Flash's Carmela Dealegria and Curly Star Flash's Bonita from "Family A" which only have one copy the Unknown Curly Gene "a" with no other curly genes present have a very distinctive coat pattern. This is characterized by a short mane, acceptable winter coat, very thin summer coat with some bald spots, and a very unusual tail hair morphology. These horses will test negative for KRT25 and SP6, but can usually be identified by the unusual pattern of tail hair.

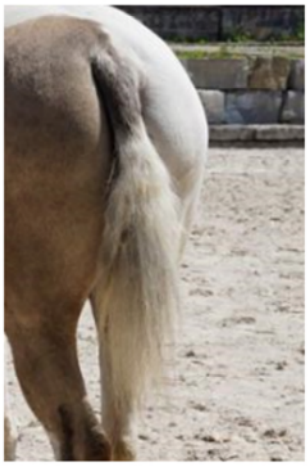
Is the Unknown Curly Gene "a" an acceptable curly gene for future breeding with the associated alopecia problems? That decision will be up to curly horse breeders. We must also explore the possibility of a homozygous Unknown Curly Gene "a' horse. What would that look like? Regardless, the combination of Unknown Curly Gene "a" with KRT25 gives an unacceptable coat.

Horses like Domino are also "dual gene" horses, but the combination in his case is heterozygous KRT25 and heterozygous Unknown Curly Gene "a". If a dual gene horse like Domino is bred, dual gene homozygous combinations are

possible. These combinations may produce complete baldness or other phenotypes.

Spring Coat of Curly Unknown Gene "a" before shedding





Two examples of Curly Unknown Gene "a": Winter coat and tail (left) Summer coat and tail (middle and right)

The Confusion Over the Term "Extreme"

The phenotype (appearance) of homozygous (two copies) KRT25 mutation is easily confused with other balding phenotypes due to sparce or little mane and tail, but by the accepted definition of "extreme" there should not be bald areas. Certain fungus infections can produce loss of coat in affected areas and this is common with curly coated horses kept in a moist warm climate. The question of whether a curly horse is an extreme or has some genetic induced balding pattern can easily be answered by testing. If the results of the test show KRT25 +/+, the horse is an extreme curly. If the results show KRT25 +/- or KRT25 -/- then something else is affecting the horse, and the possibility of another curly gene must be considered. A horse that tests KRT25 +/+, but has bald patches must also be considered as having another gene present.

An extreme curly from Salt Wells Creek HMA – photo by Pat Doak

The winter coat of a horse with alopecia that tested KRT25 +/+. This is not the normal homozygous KRT25 phenotype. Another gene probably is present and Unknown Curly Gene "a" is suspected.

Besides KRT25, SP6 and Curly Unknown Gene "a" in the North American curly horse population there is a rare gene mutation called the Cook Gene. This gene is so rare we have no idea how it combines with other curly coat producing genes. Due to the rarity of this type of curly horse, it would be a good idea not to mix the Cook Gene with other curly genes.

With at least 5 different curly causative mutations present in the North American horse population, there is the possibility of dual gene horses being created from other combinations besides KRT25, SP6, and Unknown Curly Gene "a". No suspected dual gene combinations have been found involving the Cook or Sulphur causative gene mutations. Subsequently, the phenotypes are unknown.

Karma – Maiden mare with one copy of the Cook Gene - KRT25 -/-, SP6 -/-

Other Combinations

As more horses are tested in the future, final judgements about these other combinations will be made. Could something like this be happening in curly coated horse populations in other parts of the world? If there are two different causative coat producing genes in the population, it is certainly a possibility.

It is now known that Curly Unknown gene "a" when combined with any form of KRT25 produces an unacceptable coat. The combination of Unknown Curly Gene "a" with SP6 is unknown.

With so many horses needing homes, it is probably not wise to produce extreme curly horses (KRT25 +/+) except for breeding purposes. The lack of mane and tail makes these horses difficult to place.

The Future

Money has been raised for the isolation of Curly Unknown Gene "a", and work has begun at the Texas A&M Animal Genetics Laboratory by Dr. Rytis Juras and colleagues, but so far the gene mutation has not been found in the millions of possible DNA bases which make up the DNA chain of any animal.

The search for a causative gene mutation is slow painstaking work. The search for a "curly gene" was begin in the early 1990s. The first gene, KRT25, was not found till 2016. However, the incidence of unacceptable curly coat mutation combinations seems to be rising, and this at a time when finding homes for any horse is becoming increasingly challenging. For North American Curly horses to continue to exist for future generations, it is critical for the curly coat producing genes to be found, isolated, and tested in breeding horses. Very few people want horses without manes and tails and even fewer people want horses that have alopecia. So the future of these horses rests with responsible breeding and scientific research to unravel the complicated genetics of curly horses.

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4. Annika Thomer, Maren Gottschalk, Anna Christmann, Fanny Naccache, Klaus Jung, Marion Hewicker-Trautwein, Ottmar Distl & Julia Metzger. "An epistatic effect of KRT25 on SP6 is involved in curly coat in horses," *Scientific Reports* ,volume 8, Article number: 6374 (2018).
5. Curly Horse Pedigree Database. www.curlyhorses.info.
6. Thomas, Shan. *Myth and Mystery: The Curly Horse in America*, New York: C.S. Fund Inc.,1989.

CURLY HORSE GENETICS VIDEOS

by Dr Mitch Wilkinson with guest speaker Dr Gus Cothran

Sponsored by Curly Horse Advocates

PART I Curly Horse Genetics

https://youtu.be/RsHmZAu0jxE

PART II Curly Horse Genetics

https://youtu.be/bXRx3salOFM

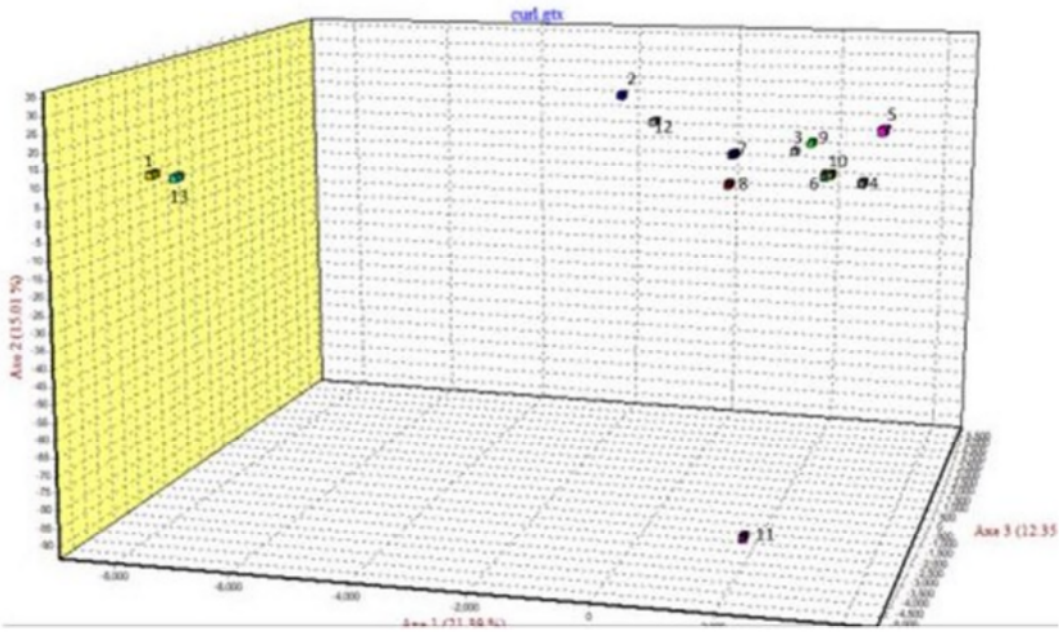


Fig.? Principle Component Analysis graph of alleles from 15 microsatellite markers showing relationships between different groups of curly coated horses Populations: (1) Curly Argentines (20015 samples), (2) Simpson Park & Rocky Hills Nevada, (3) Curly Ponies, (4) Zabaikalskaya Siberia, (5) Salt Wells Creek Wyoming, (6) Mongolian, (7) Damele Lines, (8) Warrior Lines - native, (9) Curly Jim, (10) Cook lines, (11) Sulphur Mustangs Utah, (12) Fish Creek Nevada, (13) Curly Argentines (2018 samples).

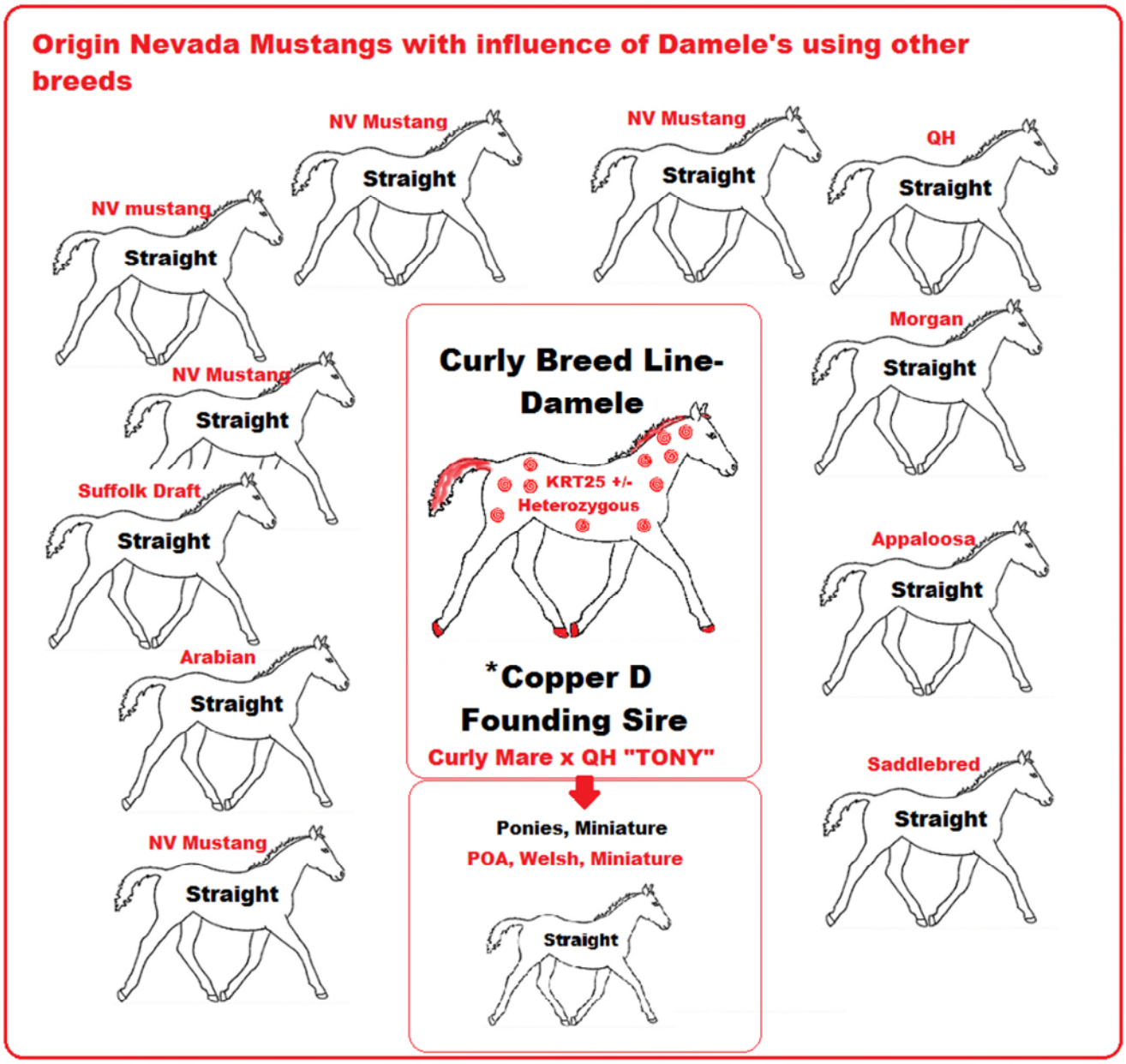
From the research findings by Dr Mitch Wilkinson & Dr Gus Cothran of the PCoA Graphing and grouping of original Curly Horse Herd groups/lines, ICHO has founded the ICHO Preservation Association. This allows Curly owners and breeders to know and value the type of origins the Curly horse carries. The goal is to preserve the original Curly lines without mixing and identify them as their own Curly breed group/line.

<u>The ICHO Curly Horse Preservation Association Mission Statement</u>:

Identify and preserve unique original Curly Horse types/lines in the North American Curly Horse Population so as not to lose origin genetics and coat genes. Curly horses that qualify will receive a certificate stamp at the time of ICHO registration or a card with stamp for those already ICHO registered.

<u>The known Curly Breed Lines</u> eligible for Preservation Full Lineage status that are of one Curly Breed Line without being mixed with other Curly lines are:

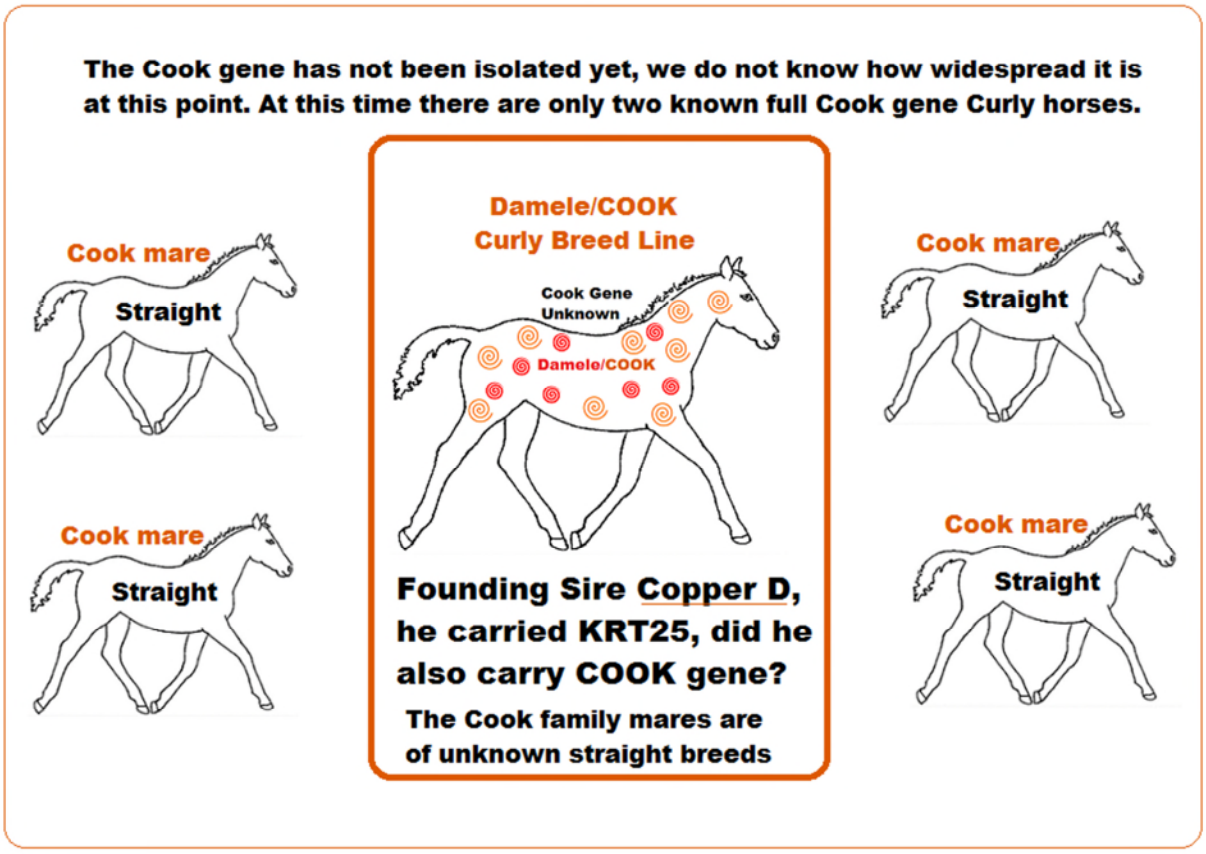
<u>Damele</u> -Coat gene KRT25 and unknown Curly gene (a) Foundation sire Copper D, foundation breeds used by the Damele's were~ QH, Morgan, Arabian, Saddlebred, Appaloosa, Suffolk Draft. Damele horses are almost identically related to the Fish Creek NV Mustang herd. Founding sire Copper D (Damele Curly mare x QH Tony)



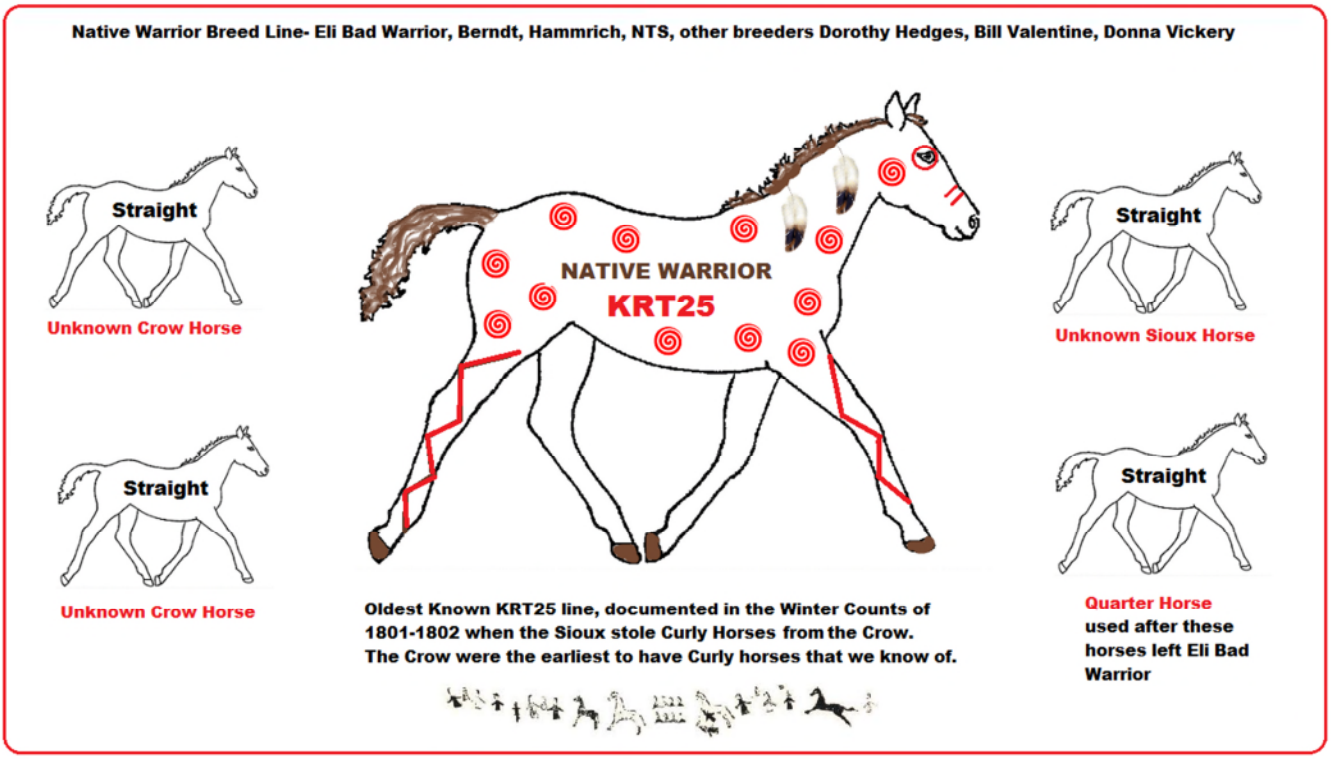
<u>Damele Curly Pony/Miniature-</u> KRT25 (Bancroft) The Ebony Twist line of Damele horses crossed with Miniature horses.

<u>Nevada Curly Mustangs</u> KRT25 and an Unknown Coat Coat gene, origin of Damele Curly horses from Fish Creek, NV.

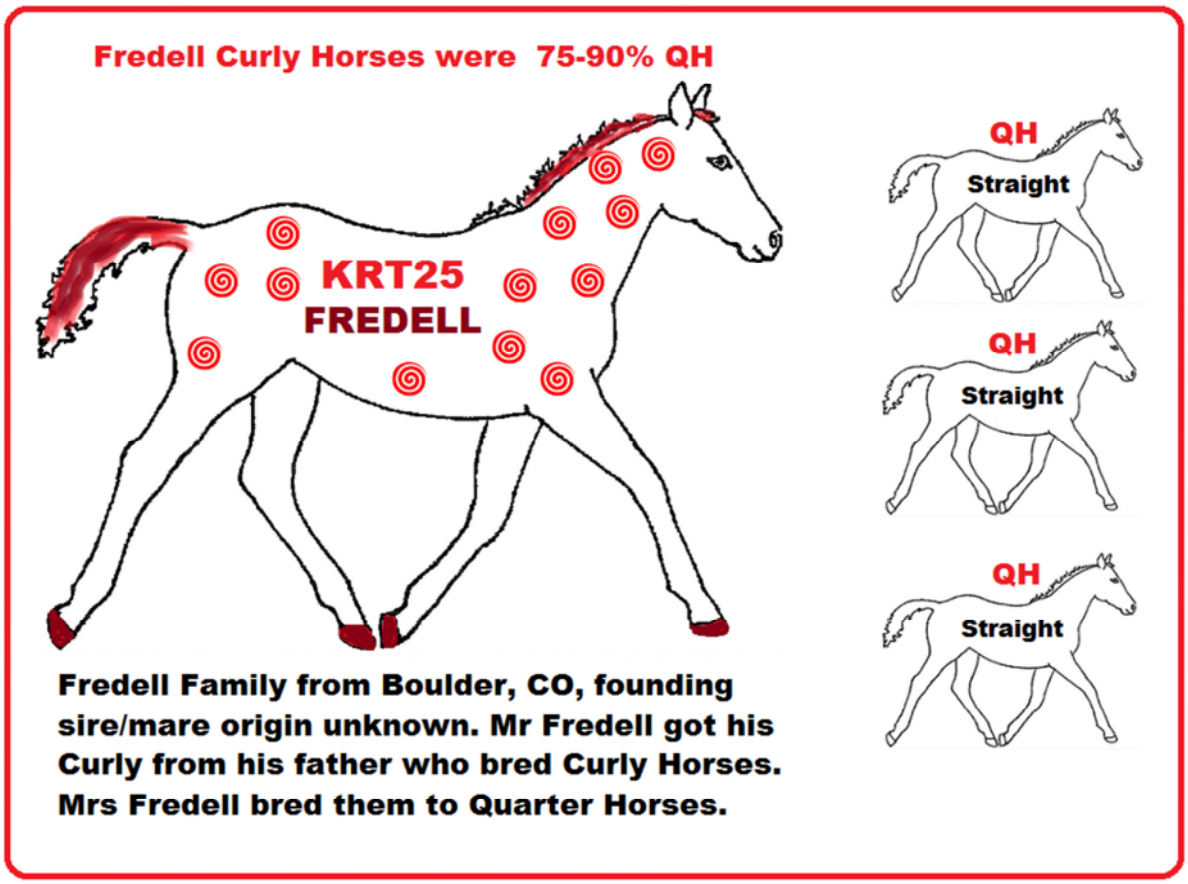
Damele/COOK Unknown Coat Gene, Foundation sire is Copper D bred to Cook mares, Cook mares are unknown.



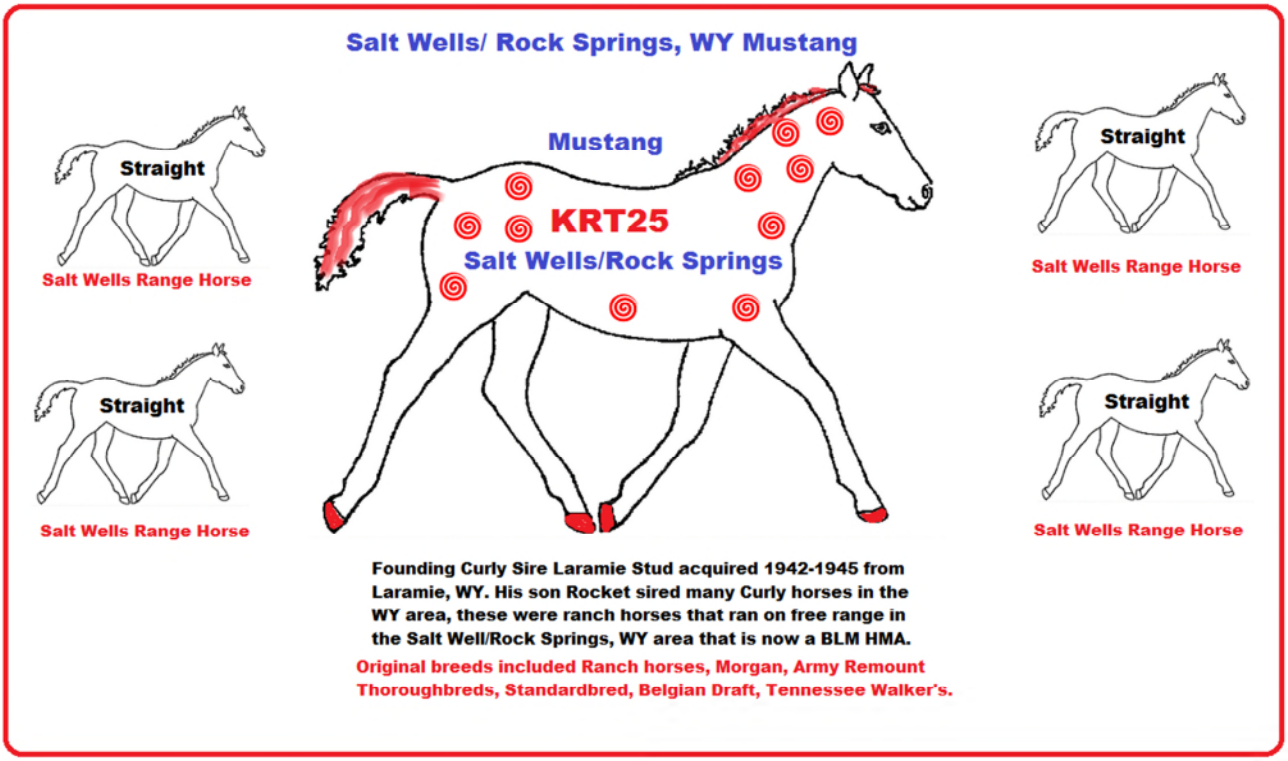
Native/Warrior (Eli Bad Warrior/Berndt/Hammrich/NTS) Coat gene KRT25 from Eli Bad Warrior Native horses of Cheyenne River Sioux Reservation heavily bred to old type QH.



Fredell Coat Gene KRT25 unknown original Curly line heavily bred to QH's. Fredell's said this line is up to 75-90% QH.

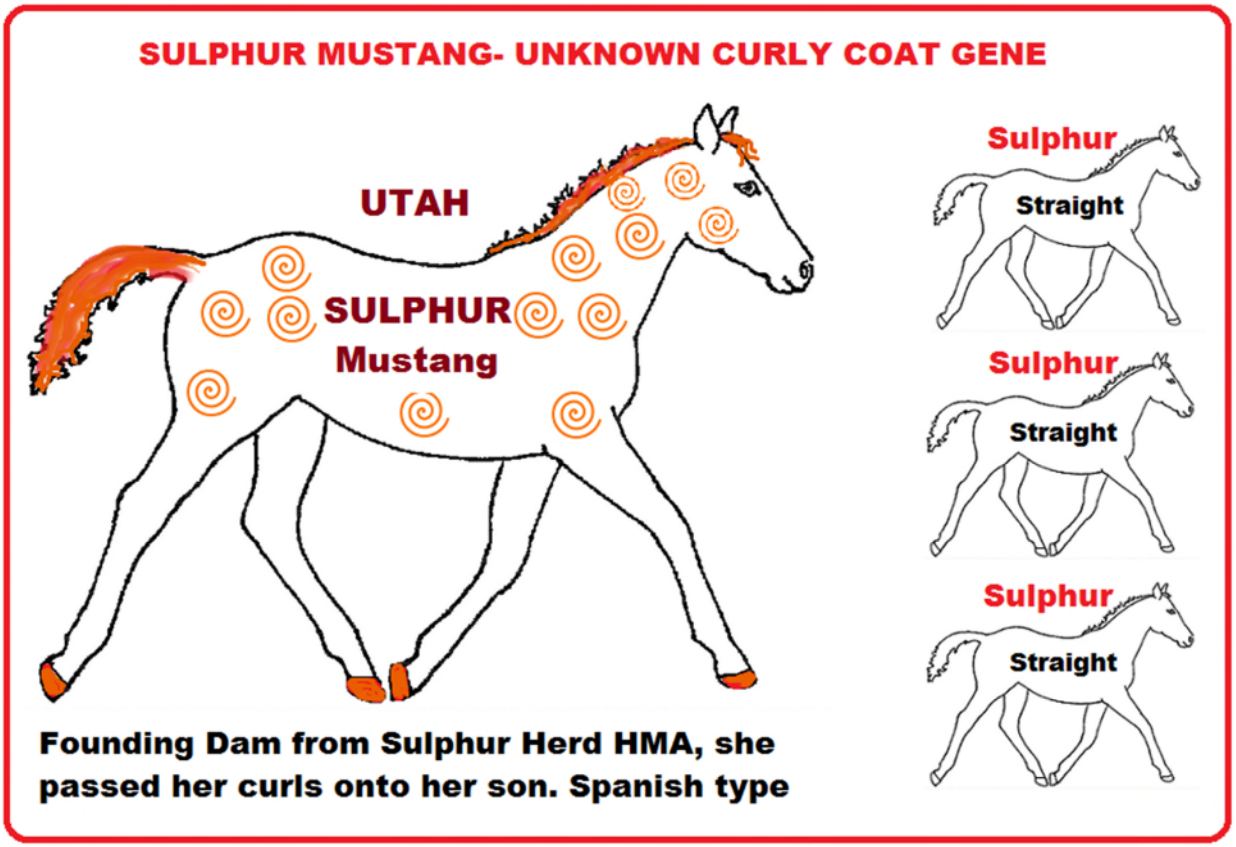


WY Salt Wells/Rock Springs Mustang Coat Gene KRT25, Foundation sire Laramie Stud and son Rocket.

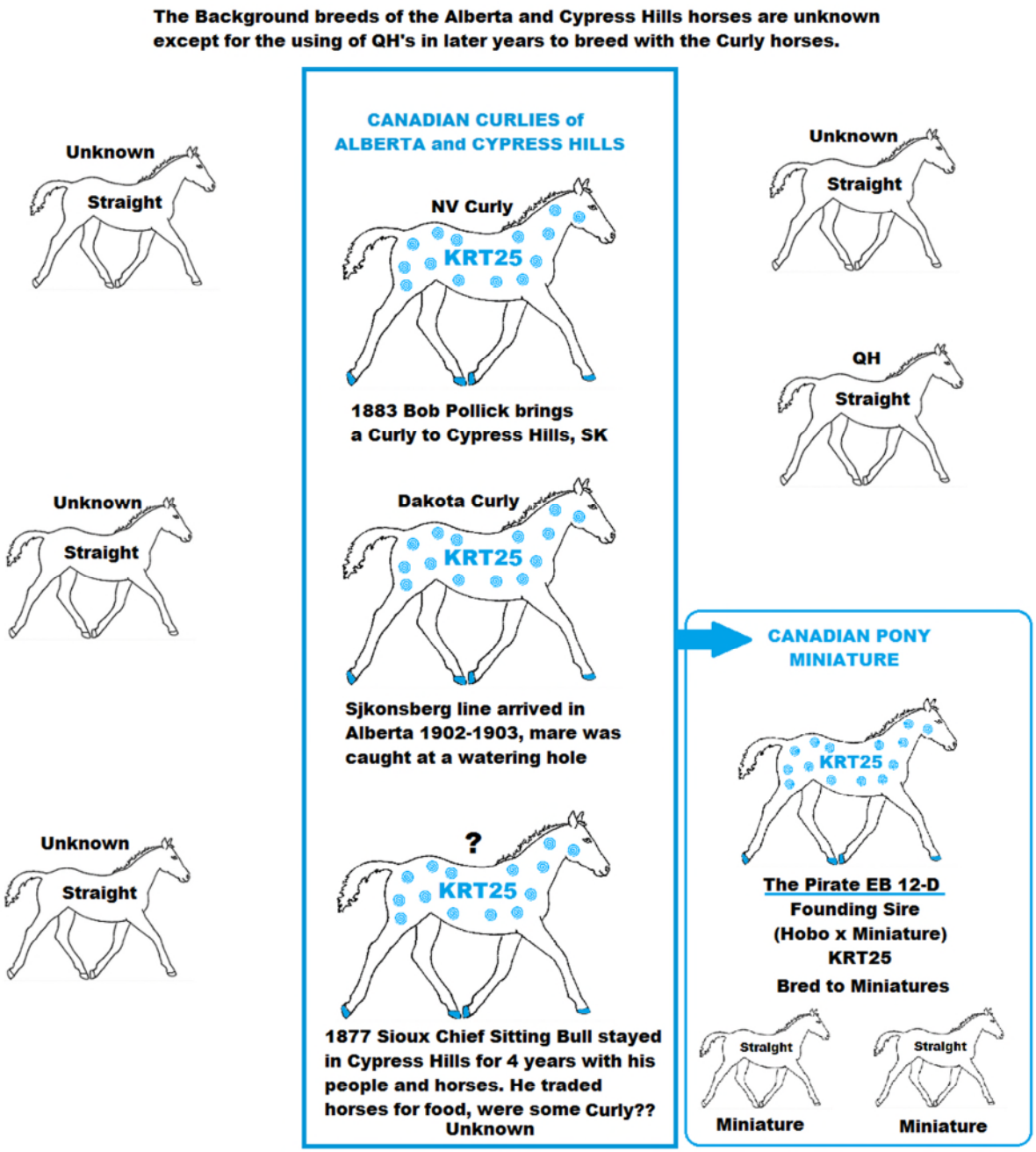


<u>Sulphur Curly Mustangs</u> Unknown Curly Coat gene, very rarely found in the Sulphur Mustangs, possible new mutation.

Breed to other Sulphur mustangs or Spanish breeds.

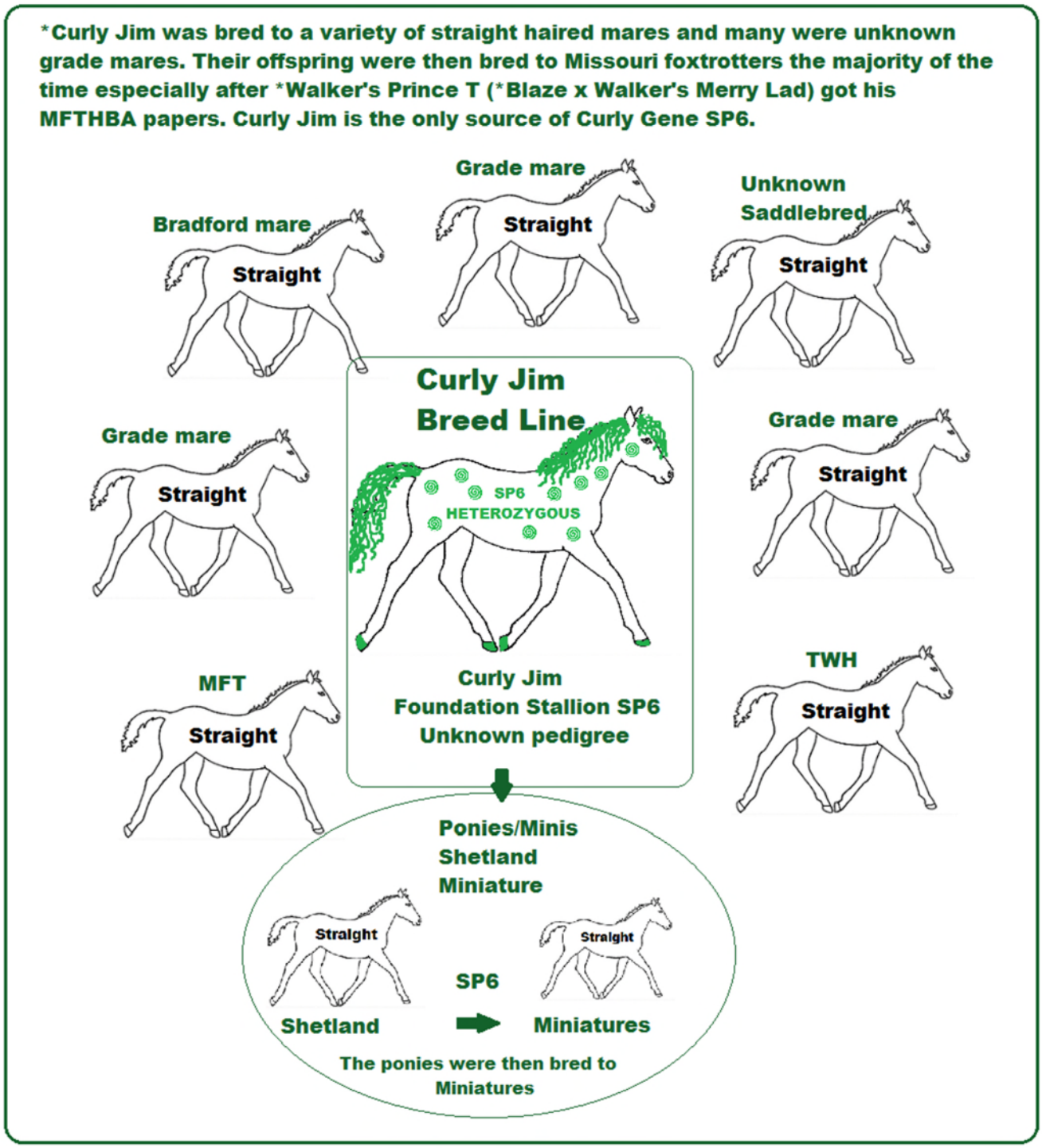


Canadian Curly Coat Gene KRT25, origin could be a captured Curly from NV 1880's and a captured Curly from SD 1900's taken to Canada, and could be wild Curly horses found in Alberta, Cypress Springs region where Sitting Bull (Sioux) and White Bird of Nez Perce camped during late 1800'S as well. This group is being genetic tested and PCoA graphed to see if they are their own type or a sub type.



Canadian Curly Pony/Mini (Bancroft) KRT25 Founding sire Hobo and son The Pirate who goes back to Roy Olepeter and full line Canadian Curly crossed with American Miniature Horses.

Curly Jim MFT Coat gene SP6, Founding sire Curly Jim (unknown) the majority were bred to Missouri Foxtrotter's.

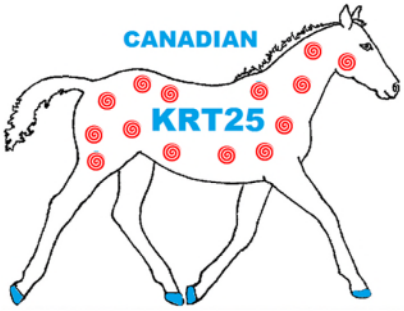


Curly Jim Pony/Mini (McKay/Circle B) Coat gene SP6 Curly Jim and Walker's Prince T line of ponies bred to Shetlands, Miniatures. Some bred to Welsh and POA for a larger sized pony.

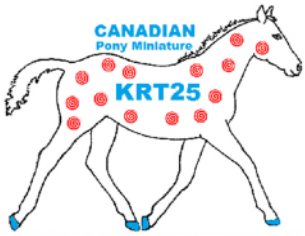
**Under investigation:* Aishihik- Joe Mead line of Curlies. This line is said to be from a Curly Yukon horse Joe acquired while in Alaska. Joe bred this line to original Appaloosa horses that trace back to Sam Fisher, a Palouse Native American who bred quality Palouse horses. Genetic tests and PCoA graphs will be done to see how this line compares to the other Curly lines. They are also mixed with Damele, so it is unknown how they will test and if they are their own type or sub type of Damele. The most common sire is Yellow Hornet (Damele x Aishihik/Appaloosa) in this line. There is no Aishihik only line anymore, but the one line we are testing is the linebred Yellow Hornet horses. Coat Gene is KRT25.

To Preserve a Full Lineage Curly line, breeding's should be done to keep the Curly line the same and not mixed with other Curly lines. Other breeds unique to that line should also be used for breeding. Example- Curly Jim MFT should breed to other Curly or Smooth Coat Curly Jim MFT and other straight MFT's to keep this line true to breed type and Coat gene type SP6.

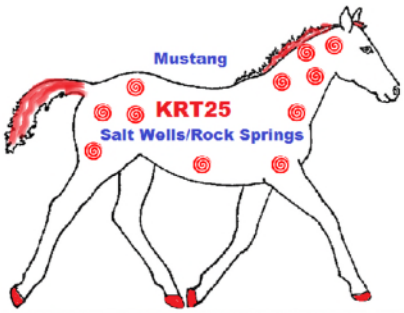
CURLY BREED LINES- Known Coat Genes KRT25 and SP6



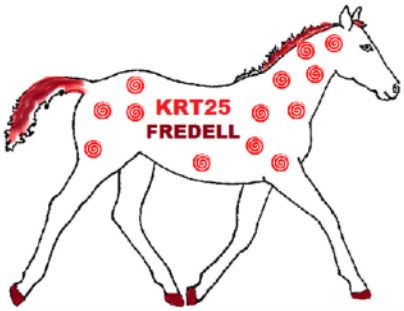
AB/Cypress Hills/SK



North/South Dakota



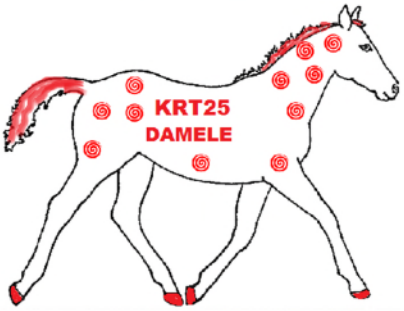
Wyoming Mustang



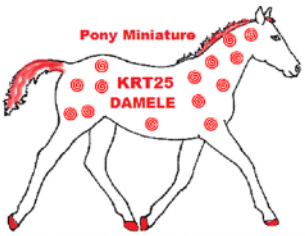
Colorado

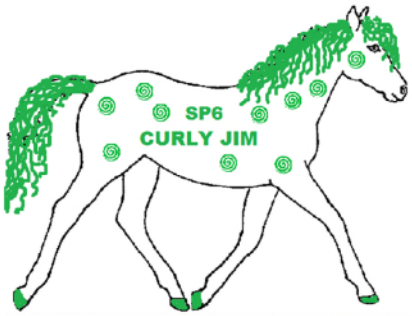
KRT25 Curly coat mutation found in Western USA

Historical Curly Lines that carry KRT25 mutation:

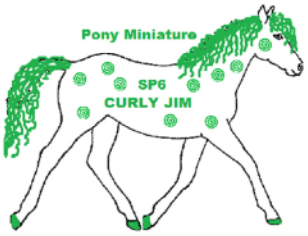


Nevada
Fish Creek Mustang





Tennessee



Eastern USA
SP6 Curly Jim

Tennessee

A Curly Breed Line has an Origin/ Pedigree and a Coat Gene that matches the Origin/Pedigree

Why should we preserve the original Curly types?

With genetic testing research by Dr Mitch Wilkinson (ICHO Research Dept) and Dr Gus Cothran of TX A&M, the grouping of these horses genetically has shown that each Curly Line is distinct from each other. A Curly from Fish Creek NV HMA (Damele) is a distinct breed Line compared to a Salt Wells/Rock Springs WY HMA Curly, who is also their own Breed Line. The Curly Jim line Curly is also distinct from other lines of Curly horses. It is showing us that yes the Curly horse does have Breed Lines that are not related to each other and should be bred separately. In nature only 7-10 % of the horse herd is Curly coated if there is a Curly coat gene in the group and is a trait that is passed on. These Breed Lines contain Curly Coats and Smooth Coats, they are of the same herd and Breed Line.

The Preservation Association keeps these Curly Breed Lines in their own Preservation Curly breed division with a Preservation Seal attached to the Registration certificate. This gives breeders and owners the opportunity to know who their Curly horse actually is and the best breeding choices to make for the Curly Breed Line they are. This is our goal to preserve these original Curly horses to their breed line status as unique Curly Breed Lines. Sadly many types are in danger of being lost forever. The Fredell line is about extinct as is the Cook line with its own unique coat gene. The Native/Warrior line horses are almost extinct as well. The Native/Warrior horses have been mixed with Damele and other lines and are now almost gone.

Curly Blends- The Curly Horse population around the world has been mixed and bred to each other for many years. The result is that the majority of Curly horses are now of mixed breed lines. The thought that Curly Coat x Curly Coat was making a breed was not valid. We now know this is false because our original lines were already a Curly breed with their own coat gene for curls and unique ancestry. By mixing them all together, the breed ended up with a mixed Curly who lost its original breed line. These mixed Curly groups will not be breeds but Breed Blends. The coat genes have mixed as well and since we have an unknown balding gene in the mix, it is making breeding a guessing game especially Curly x Curly. When the balding gene (Unknown Gene (a) is identified, it will help us all make better breeding choices. The fact that most all of us have Curly Blends, gives rise to the question, what do we do now? For owning a Curly, Curly blends are wonderful horses and make fine companions and athletes. They are part of the Curly population now. You can breed Curly Blends to other Curly Blends. But we can work toward preserving the original lines of Curly while we still can.

If you are a breeder, look at your breeding horses and find out if you have any Curly horses that are of one breed line and pick breeding partners to keep that line true to type without mixing other Curly lines with it. If you have a full line Curly Jim horse, breed only to Curly Jim line Curly or Smooth Coat Curly Jim horses or MFT's. If you have a Curly Jim line Miniature bred, breed that line only to Curly Jim Miniature line or Miniatures horses. This will preserve the line as its own Curly Breed Line. We also have divisions for Pony and Miniature sized Curly by height.

The whole population of Curly horses today is a mixed bag, but with good future breeding choices we can preserve original Breed lines and have several Curly Preservation Breed Line Divisions.

Breeding for a Curly Breed Line- FULL LINEAGE

Damele- Fish Creek mustangs, original horse breeds like QH, Appaloosa, Morgan, Arabian, are good choices. The goal is to keep the line true to type, not just breed Curly x Curly.

Damele/COOK- To keep the Cook gene separate, choose Smooth Coat Damele horses or Fish Creek NV mustangs.

Damele Pony/Miniature- , breed to other Miniature horses for miniature size (Bancroft Twist line), or Shetland for larger size, some Damele may already be POA or Welsh bred ponies. This will give size A & B sized ponies & miniatures.

Native/Warrior- breeding to other Native/Warrior horses, Curly or Smooth Coat or QH's is a good choice to keep this line true to type. Appaloosa may be another choice since they carry quite a bit of QH.

Fredell- It maybe too late to save this line, I only know of one Fredell mare, but if you have a Fredell only line horse, breed to a QH to preserve it and let us know about your horse.

WY Salt Wells/Rock Springs- breed to other Salt Wells horses, Smooth Coat or Curly to keep this line true to breed type.

NV Curly Mustangs- breed to other mustangs from the NV HMA or breeds found in the ancestry of the herd.

Sulfur Curly Mustangs- breed to other Sulfur mustangs or Spanish type breeds to preserve that unique Curly coat gene.

Canadian Curly- breed to other Canadian Curly horses (Smooth coat or Curly), possibly others after we know more about this Curly type through research. Look for future recommendations.

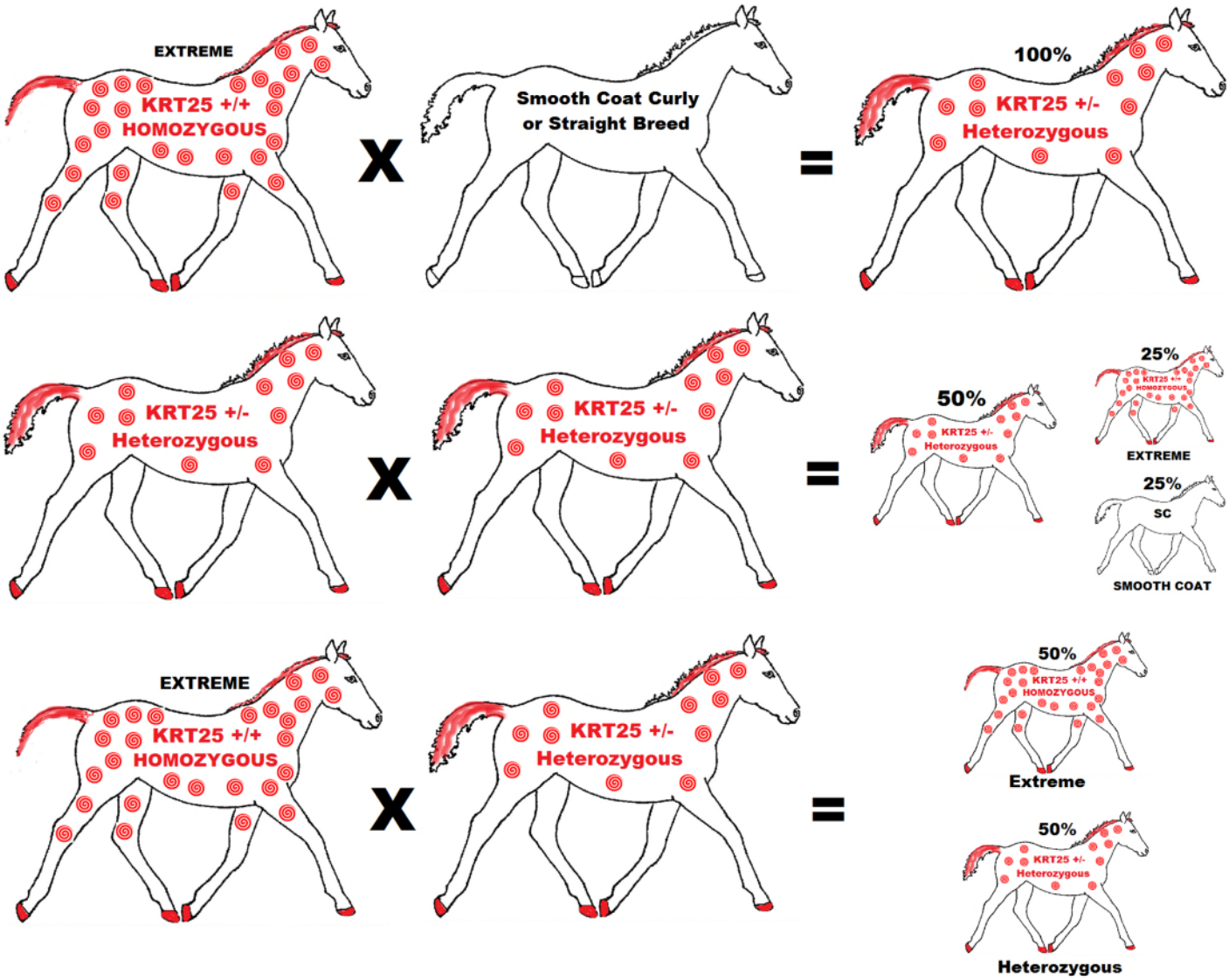
Canadian Pony/Miniature- Breed this line (founding Sire- The Pirate) to other Smooth Coat or Curly Pirate line or to Miniature horses.

Curly Jim MFT- bred this line to other Curly Jim line horses (Smooth Coat or Curly) or to Missouri Foxtrotters if they are also registered with MFTHBA. If not an MFTHBA registered Curly horse, Tennessee Walking horses are another possibility, since some of the founding horses of the Missouri Foxtrotter Association. Tennessee Walking horses were also used in one line of Curly Jim founding horses (Sir Patrick), so there is historical and breed history/tradition for breeding Missouri Foxtrotters and Tennessee Walking horses to Curly Jim horses.

Curly Jim Pony/Miniature- McKay used Shetlands for their pony line and many have used Miniatures. There are sized A and B ponies and miniatures in this line. The Miniature horse for smaller sizes and the Shetland for ponies are good choices. We offer Miniature and Pony classification by height measurement in A and B categories

Breeding for a Full Curly Breed Line with a Curly Coat KRT25:

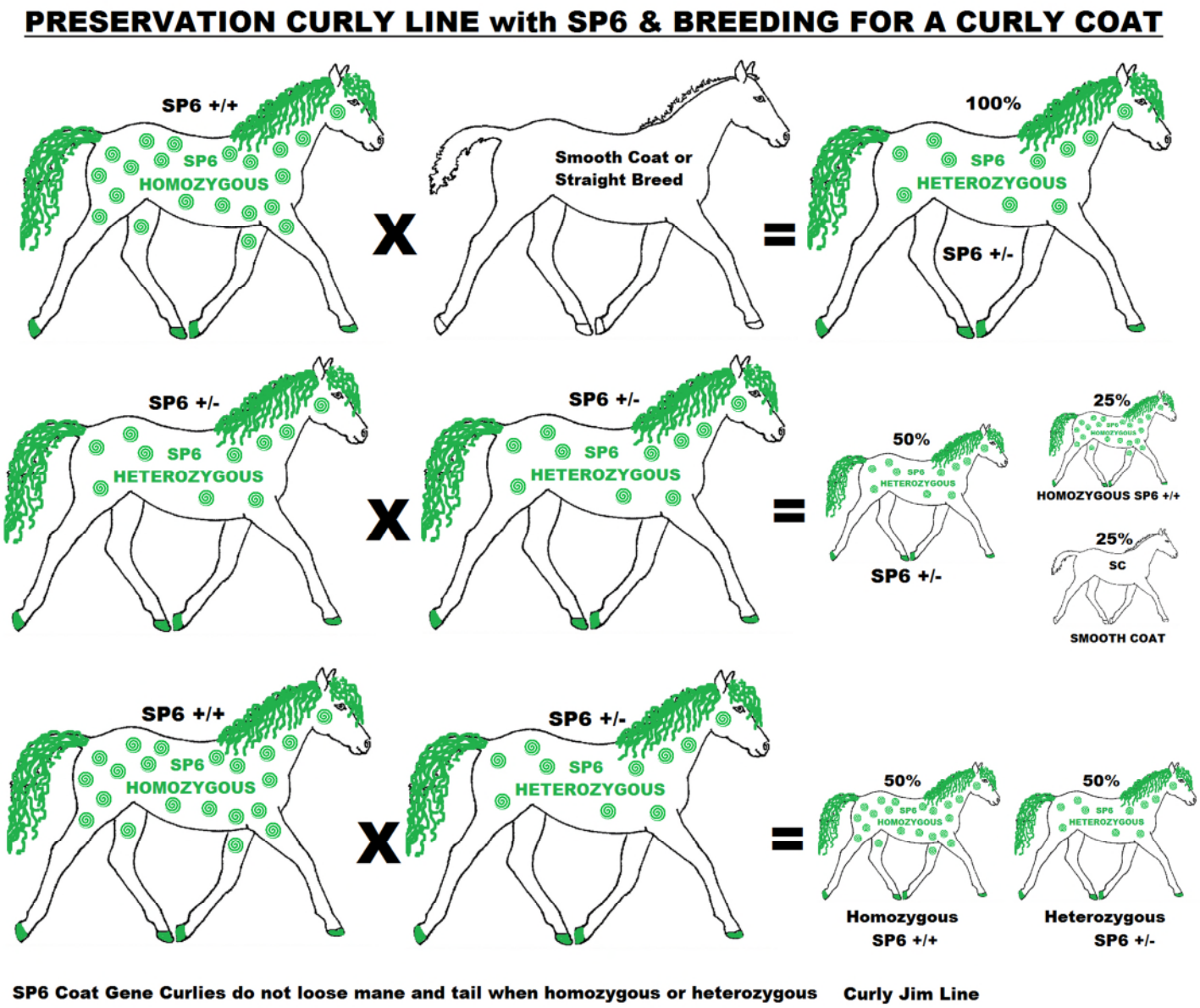
PRESERVATION CURLY LINE with KRT25 & BREEDING FOR A CURLY COAT



It must be taken into account that Homozygous extreme KRT25 +/+ horses are not as marketable as heterozygous KRT25 +/- horses are to the general buying public. KRT25 Lines- Damele, Native/Warrior, most NV mustangs, Salt Wells, WY mustangs, Canadian, Fredell

***Please note this chart is only accurate if Unknown Gene (a) and Cook gene are NOT present.**

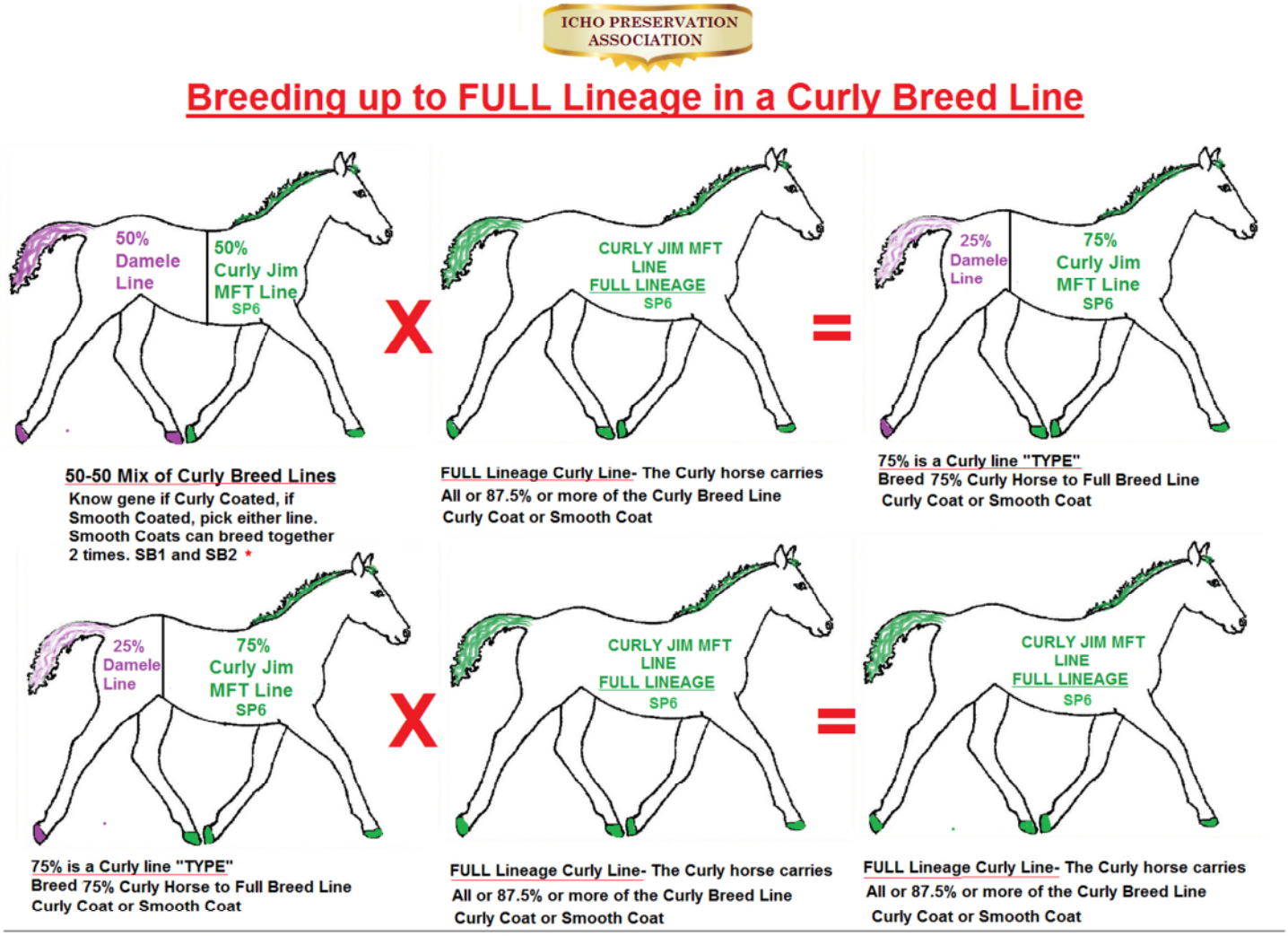
Breeding for a Full Curly Breed Line with Curly Coat SP6:



SP6 Coat Gene Curlies do not loose mane and tail when homozygous or heterozygous **Curly Jim Line**

<u>Preservation Breeding for "Type" without full lineage-</u> Since we know our original Curly breed lines with full lineage are getting very hard to find, we will group Curly horses that have <u>2 two different Curly pedigree lines</u> to one Curly breed line even though they may have one other pedigree line to another Curly breed line. This will help save our lines that are very scarce.

Breeding up to Full Chart:

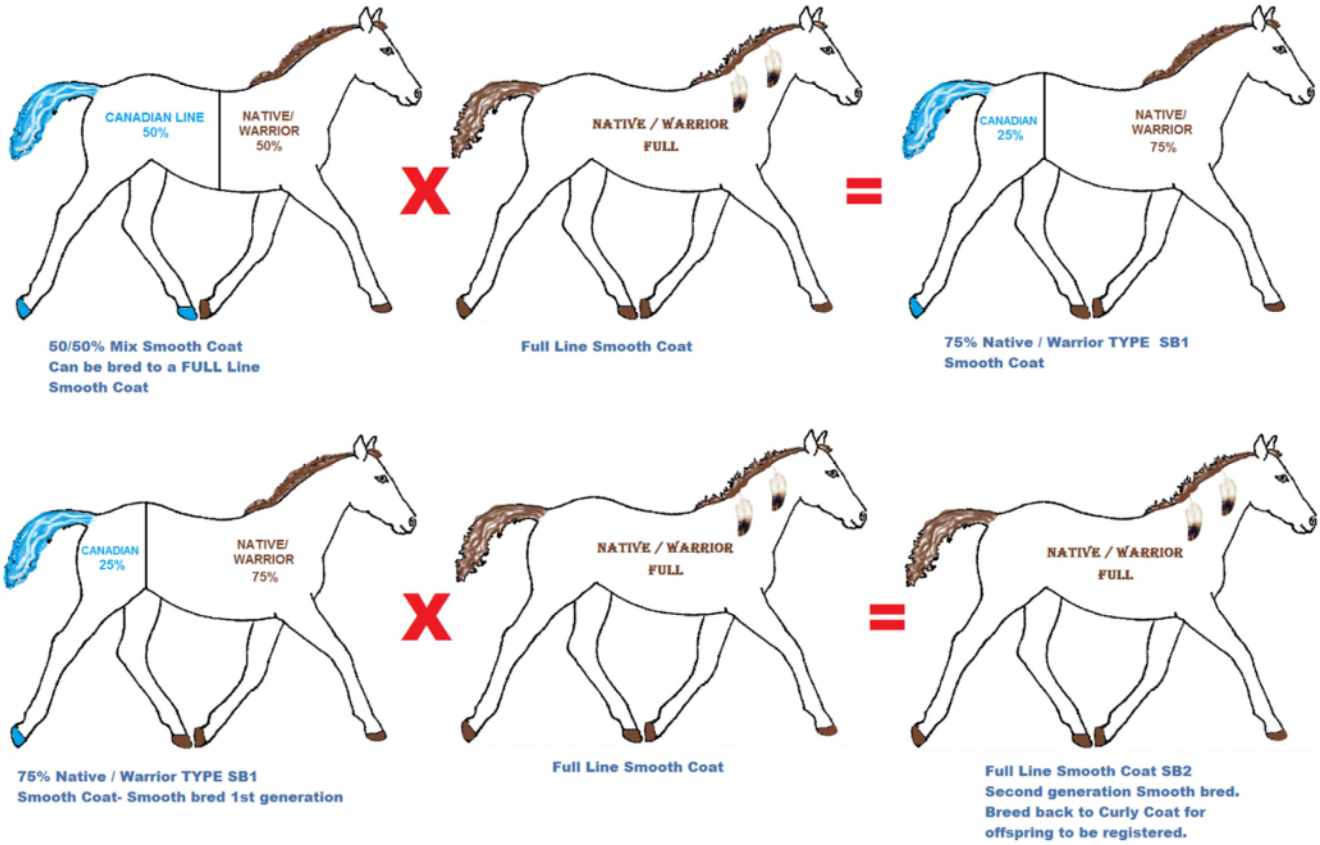


* Smooth Coats (SC) are an important part of the Curly Breed Line, especially to breed to homozygous Curly Coats. We allow SC X SC = SB1 Smooth Bred 1 generation. SB1 x SB1 = SB2, second generation Smooth Bred. An SB2 must be bred back to a Curly Coated Breed Line horse. This makes saving a Preservation Line easier especially for those breed lines of low numbers. The Smooth Coats could save these almost extinct lines from disapearing. Smooth Breds SB1 and SB2 have all registration rights in the Smooth Coat Division and Preservation Line Status.

Using Curly Smooth Coats to Persevere a Curly Breed Line:

ICHO PRESERVATION ASSOCIATION

Breeding up to FULL Lineage using Smooth Coats in a Curly Breed Line



In some instances Smooth Coats could help save an entire endangered Curly Breed Line if only a few can be found with Curly coats and the rest are smooth coats

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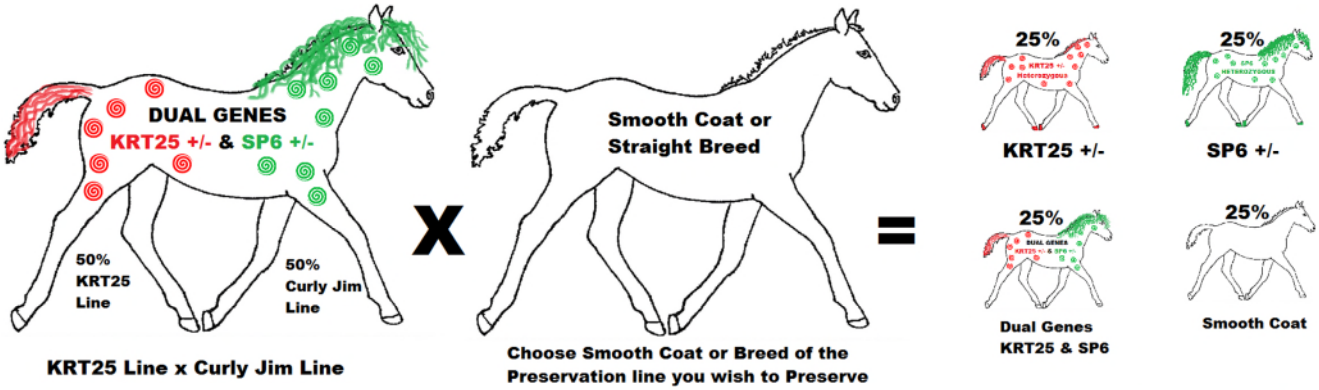
Preservation Breeding using a Dual gened horse to breed up



This is most likely the most difficult way to breed up and save a preservation line since you only have a 25% chance of getting the Curly gene you desire and a 25% chance of getting a smooth coat.

It would be easier to do on a Curly pony miniature line becasue either SP6 or KRT25 is found in the Curly Miniatures and the other breed used could be a Miniature straight coat. There is a 75% chance of a Curly Coated Foal.

Offspring would be 50% Preservation line if Curly coated and one coat gene inherited, would be typed to Preservation line the coat gene is related to. Example- SP6 inherited, dam was a Miniature- Foal 50% Curly Jim Type

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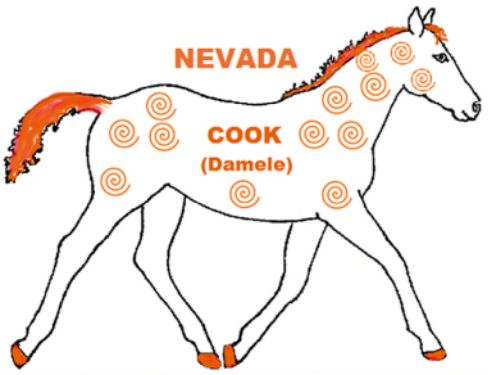
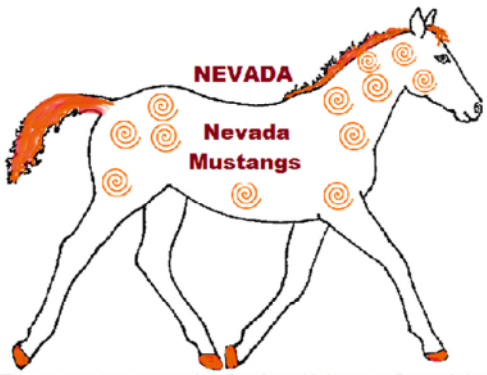
Unknown Curly Coat Genes and their Breed Line Origins (Only breed to that one line or straight breeds, do mix with other Curly Horse Lines because these Coat genes are unknown and we do not know the effect of mixing unknown genes:

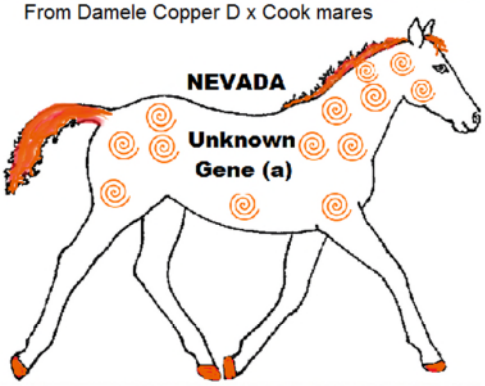
UNKNOWN CURLY COAT GENES- All tested negative for KRT25 & SP6



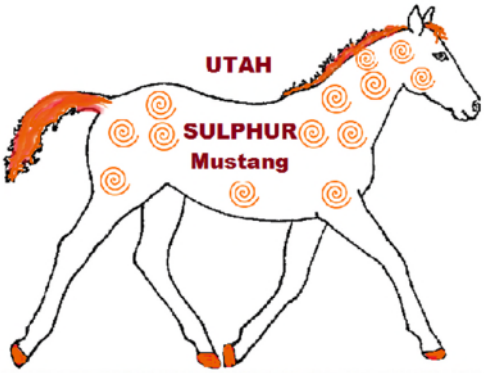
From Damele Copper D x Cook mares



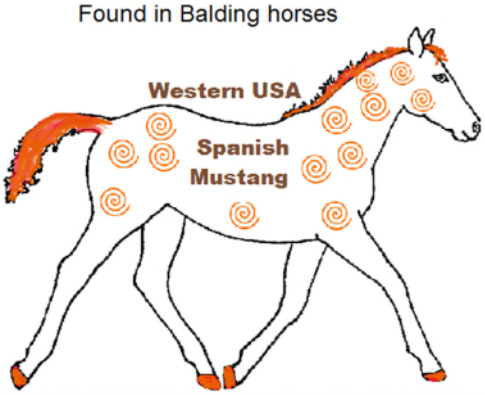
These mustangs maybe Cook or Unknown Gene (a)



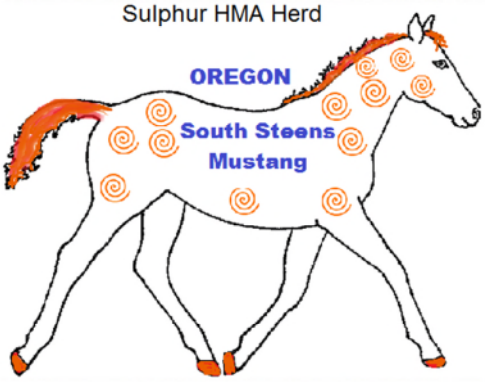
Found in Balding horses



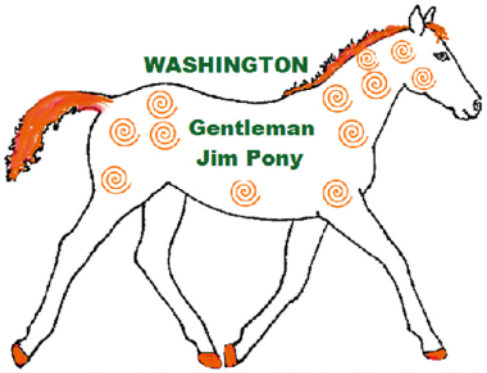
Sulphur HMA Herd



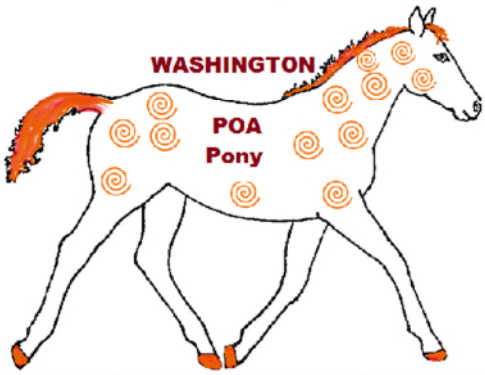
Native American horses taken from Spanish Stock



Rare for a Curly Horse to be found here



Curly Jim Pony x straight mini



Actual bloodlines unknown

**Please note, this article will be updated as new Research information becomes available.*

Bunny Reveglia/ Dr Mitch Wilkinson