

2017 ICHO GAZETTE

International Curly Horse Organization

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FALL GAZETTE 2017

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Happy Holidays from ICHO!



Greetings ICHO members and friends! This is an **extraordinary** 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 <u>A missense variant in the coil1A domain of the keratin 25 gene is associated with the dominant curly hair coat trait (Crd) in horse.</u> The direct link is https://gsejournal.biomedcentral.com/articles/10.1186/s https://gsejournal.biomedcentral.com/articles/10

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.

Happy Holidays to All, Joan Henning, ICHO President, and Angel Leggs.



ICHO Calendars make great Curly gifts!

Order yours at www.cafepress.com/icho

Use coupon code SHOP20 at checkout

GAZETTE AD DEADLINE

We now have a new Gazette ad deadline schedule for submitting ads as follows:

WINTER: Dec 15 SPRING: Mar 15 SUMMER: June 15

FALL: Sept 15

Ads that are received after the deadline will be run in the next following issue. Payments must also be made before the deadline. All ads and payment must be sent to the office. Thanks!

ARTICLES- We are always looking for interesting articles for the Gazette. The article could be about training, health tips, an interesting story about a Curly or anything of interest horse related. Please send all articles to the office before the deadlines. Awaken the writer within you!



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ICHO now offers DNA testing for:

Ancestral only TX A&M	\$25.00
Parentage Only U of KY	\$30.00
Lp color TX A&M	\$35.00
CA- (Cerebellar Abiotrophy)VetGen	\$35.00
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Visit the website for more info or contact the office.

Gazette Ad Specials!

Stallion Station Ad-only \$10.00 per issue, \$40.00 for the year, color ad.

Business Card Ad-only \$10.00 per issue, \$40.00 for the year, color ad

All color Ads at Black and White Prices! All Gaz Color Ad fees reduced!!

Full Page Color \$40.00, 1/2 Pg Color \$20.00, 1/4 Pg Color \$10.00, Classified \$10.00

Submitting ads: *Pay for ads online on our webpage- http://ichocurlyhorses.org

All ads must be copy ready in word doc format or jpg, png. Since we have reduced our prices we need the ads in ready to print format. For photos png is preferred since it has the best resolution. We can no longer accept pdf format because it does not insert clearly for the Gazette editing process.

All Ads must be sent to the office with payment. Ads also appear on the ICHO webpage.

Bring in the New Year with The 2018 ICHO Calendar! CALENDAR





2018 ICHO CALENDAR IS AVAILABLE!

at- www.cafepress.com/icho
IMPORTANT- coupon code SHOP20 must
be used at checkout. You must choose Jan
2018 when choosing the 2018 calendar!
Be sure to go to the calendar month links at
top of the ICHO CafePress page to get items
with the month page on them. These make
great Xmas gifts!

Thank you to all our participants and Curly friends!



DNA Requirements

NEW DNA REQUIREMENT for ALL newly Registered Curly horses in 2018 and all future years, all horses must be DNA typed. Geldings are excluded if you desire, you may DNA test geldings if you wish. ICHO registered breeding Stallions and Breeding Mares are required to be DNA typed for offspring to be registered. Stallions & Mares- All registered ICHO/NACHR stallions & mares used for breeding are required to be DNA typed. If your horse is already DNA tested thru another Lab or Registry, send a copy of the DNA report and pay the Submit Other Lab/Registry DNA fee of \$10.00.

DNA testing thru ICHO and UKY is \$30.00.

KRT25 Curly Coat Gene Test- ICHO is offering testing for your Curly horse at \$35.00. If your horse is unknown, BLM or from the Damele. Native/Warrior line or Canadian Line, they could be carrying KRT25. Go to our homepage for KRT25 form and payment link- ichocurlyhorses.org



Genetics Fact!

DNA stands for Deoxyribose Nucleic Acid. It is the molecule that carries the genetic instructions used in growth, development, functioning and reproduction of all known living organisms and many viruses. Discovered in the 1950's, the DNA structure was found to be a double helix, resembling a twisted ladder!



Calling all ICHO Stallions!



The winter issue of the ICHO Gazette is the traditional stallion issue, and in that tradition, we again offer the FREE stallion ad for ICHO members and ICHO stallions!

Please send to the ICHO office, www.ichocurlyhorses.org, your stallion's information and a good photo. The ads are a quarter page, so limit information to details. I will try my best to include as much information as possible without compromising your beautiful photos. If you have more than one stallion, perfect! Send them all for an individual ad for each! The extended deadline is January 15, 2018 for stallion ads. If you have any questions, do not hesitate to contact me, Joan Henning, at jhenning_joanie@yahoo.com or the ICHO office. Thank you!

THANKFULNESS!

At this time of year we reflect on being thankful with our families and friends. And so it is with Curly Horse Rescue being thankful for their "family" of foster homes!

We are thankful for Fred and Annise Finch at Deer Creek Farm in Texas who has been a foster home for many, many years. Currently they have Violet and Dash in their care. Violet and Dash were both very rude and untrained and with the help of a sponsor to pay for training and Holly Jones to help Annise with training, they now have good ground manners and are waiting for their forever homes!



We are thankful for Jane Matlock and her niece and husband, Jenny and Allen Burch at Red Roof Stables for taking on Brulee and Merangue and their future babies! Jane was originally set up to take the 4 Storm mares, but then Brulee and Merangue came up at auction and were closer, so they took them on and will take care of them and their foals until they're ready for adoption. One mare is due in December, as estimated by the vet, so maybe there will be a Christmas baby! The anticipation is high at Red Roof Stables for the first baby to be born. With the training Jane and her family are providing, the mares are becoming easier to handle and will make wonderful companions!

We are thankful for Kirsten Vanpoolen and her family in Colorado for taking the 4 Storm mares in. All 4 are untrained and Kirsten is working diligently to get them halter broke so they will be more adoptable. She has taken on a HUGE job that most could not or would not,

and we so appreciate it!



Earlier this year the Hylers at Still Meadow Farm fostered Chance and Faith. Faith was a rack of bones, literally, and they have brought them both back to beautiful horses. Dt wasn't too long and they fell in love and have adopted them. But they are ready to foster again if needed.

And Darlene Lambert also fostered Cinnaman for us for a couple of months until BABBS adopted him to be their ambassador.

We thank all of these people for the devoted attention and time they are putting into these horses so that they are wonderful companions! We also are thankful for YOU for your financial support of our rescue. Without you, none of this would be possible and these horses wouldn't have the chance for a new home!

Curly Horse Rescue wishes all of you a happy holiday season!

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

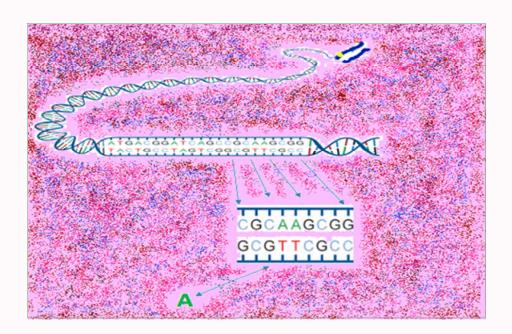
A substitution of a single base pair within the site of a known gene called KRT25 seems to be the causative factor in a large percentage of curly coated horses tested. This mutation has been designated a numerical location on the DNA chain of the 11th chromosome and is mapped as p.R89H. The substitution 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 todes for a gene, it becomes a point mutation.

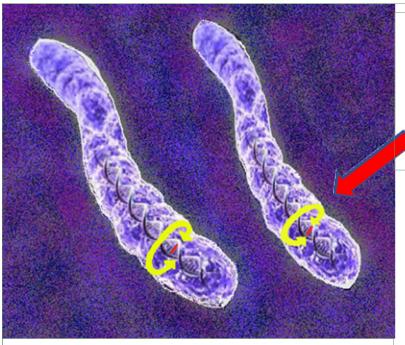
The **missense**, **point mutation** in KRT25 was found to be present in a little more than half of the curly coated horses that have been tested. [1] [10]

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 Mustang**) 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]

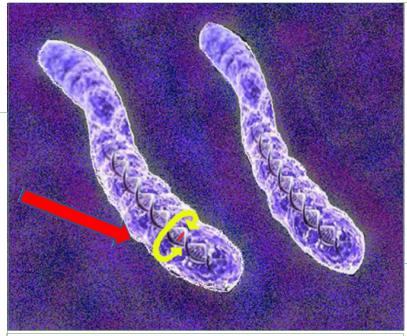
The KRT25 mutation has a dominant mode of inheritance. This means that an individual horse only needs one copy of the mutated gene to show traits. [3] Most of the curly coat traits are thought to have a dominant mode of transmission. [11]



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.

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.

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.

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.

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"

 $\mathbf{A}\mathbf{A}$

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]

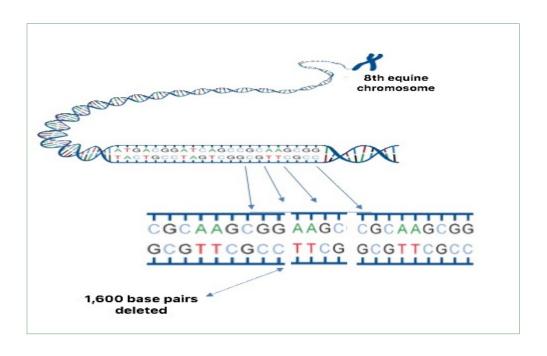
Spanish colonial horses arrived for the first time in numbers to establish a population in 1589. In the decades before this, the southwest was being explored by Spanish expeditions and treasure seekers who were mounted on stallions and geldings, for mares were forbidden to be taken from the Caribbean 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



${\bf Renegade}$

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.





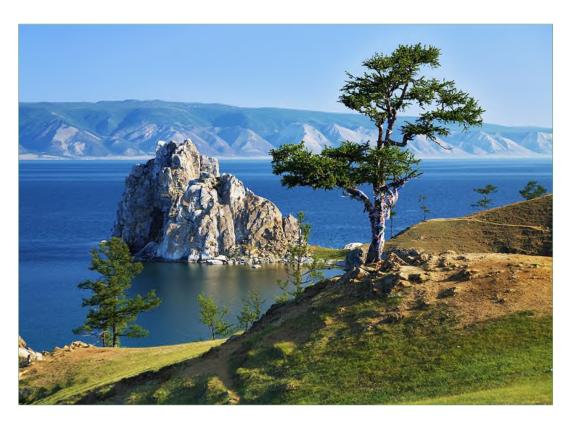
curly coated horses

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]

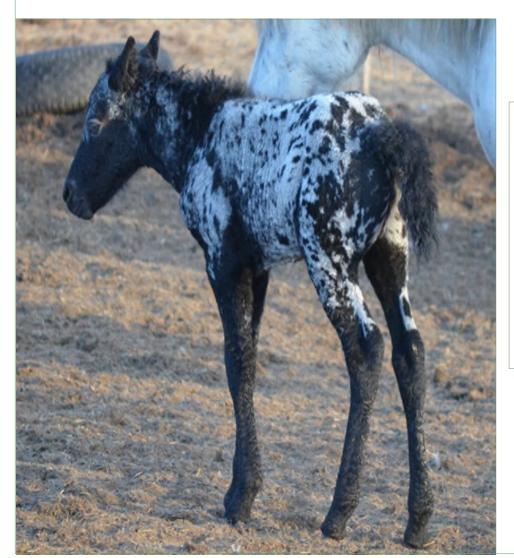
Hair Follicle samples were shipped to Texas A&M lab from Siberia. Little else is known about these horses.



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 $23^{\rm rd}$ 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 non-sense 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 $23^{\rm rd}$ 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 $23^{\rm rd}$ pair has the nonsense mutation where an adenine was substituted for a cytosine. Finally, the symbol, "AA", is used to donate a $23^{\rm rd}$ 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 $23^{\rm rd}$ 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]

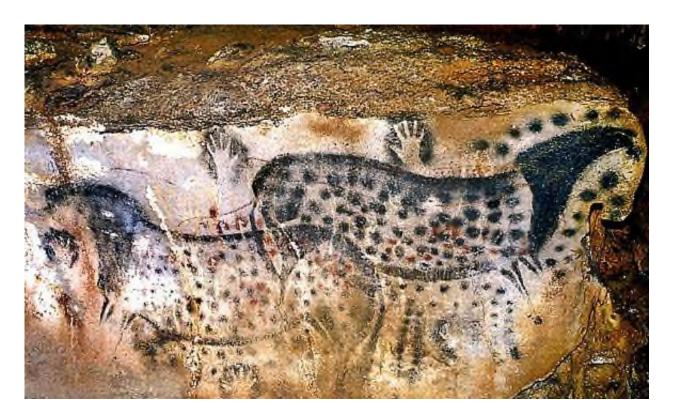
This brings us to another topic which has been a point of much discussion. Where did the KRT25 missense mutation originate? As we can see from the gait-keeper mutation, horses have been exported and traded from ancient times. But the absence of the KRT25 mutation in known curly coated horse populations 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 reintroduction 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

Tests are also available for KRT25 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:

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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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