Unraveling the Mysteries of Merle

All information from the book "Merle - SINE Insertion from Mc - Mh - The Incredible Story of Merle" 2018 Mary Langevin
www.merle-sine-insertion-from-mc-mh.com

Based on our published research paper – "Merle phenotypes in dogs - SILV SINE insertions from Mc to Mh" - "langevin et al"
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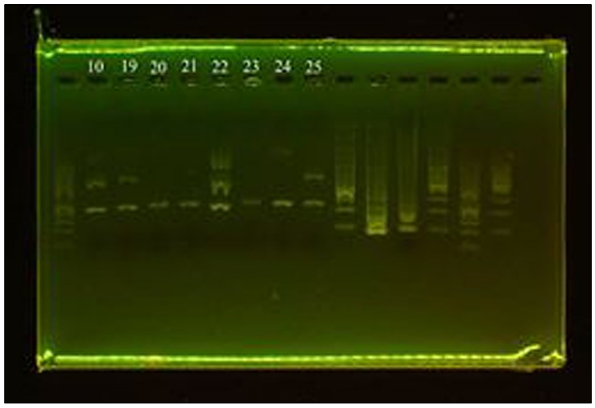
If you have been breeding Aussies for any length of time, it is likely that at some point your dam has whelped a pup which has left you mystified by what you were seeing. Perhaps white body splashes that were not expected and are not to standard; dilute looking pups when d/d is not a possibility; brownish or off-shading on a black offspring; excessive white offspring and yet one parent is phenotypically solid; Merle offspring that were not expected as neither of the parents express a Merle pattern; a solid pup when one parent is M/M and all pups should be Merle; an unusual Tweed Merle pattern unlike that of the Merle parent and other littermates. *The Tweed phenotype is described as a Merle pattern that expresses with random shaded-in or solid areas, usually with two or three distinguishable shades. Once thought to be a modifier of Merle and now shown to be created by several different Merle genotypes.*

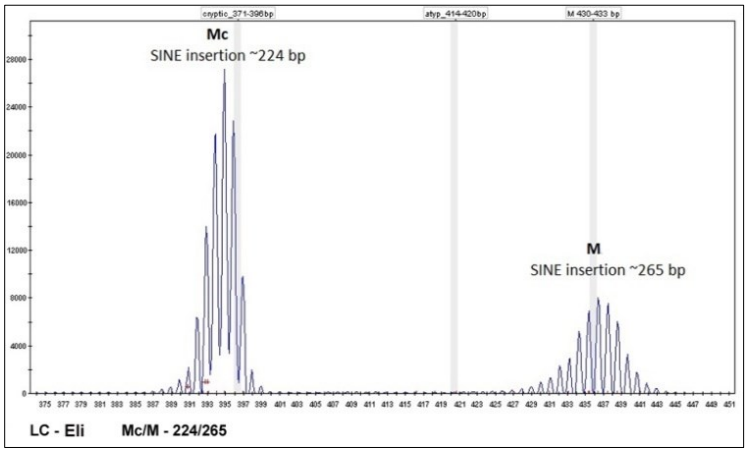
There have always been questions surrounding Merle - a SINE insertion consisting of 3 parts - head, body and tail (poly-A tail) which contains a long string of repeating base pairs. This mutation impairs the ability of cells to produce normal pigment and leaves random areas of the coat which are diluted to a lighter pigment. When Dr. LA Clark identified the Merle mutation in 2006 it was an exciting time! We thought we would get the answers we were searching for that had eluded breeders for generations. What came next was disappointing to say the least - solid dogs testing as M/M that did not produce Merle offspring. How could that be? It left so many questions unanswered that the testing was considered by many to be flawed and in 2009 the only lab testing for Merle under patent removed the test from their color panel.

In 2010 Thermo Fisher introduced the ABI 3500 Genetic Analyzer. We didn't realize it at the time but this advanced analyzer would give us the answers we had been searching for and give breeders the information needed to make educated breeding choices.

The original Merle test could only accurately identify the body of the Merle mutation and the assumption was made that any reasonable length of poly-A tail would produce some type of Merle pattern. Using the new technology available allowed for the "base pairs" of Merle's tail to be counted. This is an extremely challenging process when Merle's long monotonous poly-A tail is involved. The conditions and quality of products used for this process must be of the highest quality to give optimal results.



Original Merle Test Result – reported as Mc/M



Chromatogram High-Definition Test Result Including Base Pair - Mc/M - 224/265

Now the mission would be to assign the length of the base pair numbers (the genotype) to the Merle pattern (the phenotype). The lengths of each allele in the research paper (2018 "langevin et al") were arrived at specifically to address the needs of breeders. Not only how a particular dog would express but how this dog would breed when alleles were combined with those of a mate. In order to achieve these precise results of genotype = phenotype, there must be a working knowledge of dogs in pedigrees; parents, offspring and related individuals through the generations. Although I was responsible for setting the boundary of each of the 6 M* alleles, I did not accomplish this on my own. Many owners and breeders from all breeds worldwide offered testing and pedigree information on hundreds of dogs. This is a prime example of how breeders who are on the frontlines of recognizing the colors and patterns from parent to offspring, have been instrumental in helping labs and researchers to develop new testing.

Note: I am often asked why every lab has not adopted the new testing method. Answer - the ABI 3500 Genetic Analyzer has an approximate cost of $200,000/US, a large sum of money for a lab to invest in if they already have an older analyzer.

For this article I have specifically used examples of Australian Shepherds; however some cases may require the use of a different breed. ***Merle acts the same in all breeds.***
NOTE - many Aussies pictured in the article are from Europe where there is a ban on tail docking

7 Alleles on the M Locus

Setting the borders for the new Merle alleles was an immense task that came with an immense responsibility. Merle's poly-A tail is a "continuum" from 200 - 280 base pairs. The word continuum is defined as "a whole with no part of which is noticeably different from its adjacent parts, although the ends or extremes of it are very different from each other. It is without stop, without a break or interruption." So how and why have the base pairs been broken down into different bins and named as separate alleles?

This was done in order to address the three most important concerns that many breeders have.

#1 - which combinations of alleles can express with a Merle pattern?

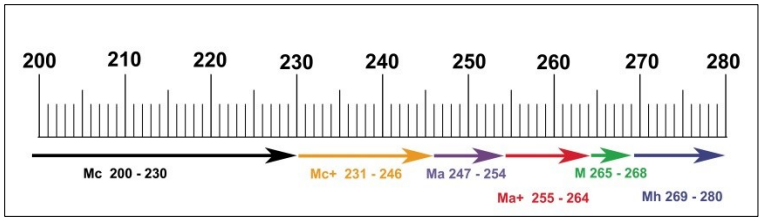
#2 - how crisp and clear will that pattern be?

#3 - which combinations of alleles can delete pigment to white within the Merle pattern, and therefore come with the risk of hearing and/or vision impairments?

These are important distinctions for a breeder who strives to produce Merle patterns that fall within the given guidelines of many breed standards. There is also the issue of not wanting to produce "Double Merle" offspring in a resulting litter. Breeders not only need to know the most typical phenotype expression for a given allele and their combinations; but also how that dog will breed, what patterned offspring this dog can produce when paired with the alleles from the mate. These are the most essential questions and the alleles of m, Mc, Mc+, Ma, Ma+, M and Mh provide those answers.

The Merle trait is an "incomplete dominant", one allele does not completely dominate another. Depending on which 2 alleles are inherited, it can create an intermediate expression or a completely distinct pattern. When the base pair numbers were set for each allele it was not just a matter of looking at the phenotype of each allele as heterozygous, each length had to be looked at as homozygous, as well as when paired with a different length allele. There are 28 possible combinations from 7 alleles. 14 of those combinations can delete pigment to white. In this article I cannot include examples of all combinations so will concentrate on the examples that provide answers to unusual phenotypes.

• **m**	**Non-Merle**	**Wild Type**
• **Mc**	**Cryptic Merle**	**200 - 230 bp**
• **Mc+**	**Cryptic Merle +**	**231 - 246 bp**
• **Ma**	**Atypical Merle**	**247 - 254 bp**
• **Ma+**	**Atypical Merle +**	**255 - 264 bp**
• **M**	**Merle**	**265 - 268 bp**
• **Mh**	**Harlequin Merle**	**269 - 280 bp**



The Scale of Merle - FOR BREEDERS - BY BREEDERS

We can assume that Mh - Harlequin Merle is the original ancestrally allele as it is the longest. So where have all the other allele lengths come from?

Mosaicism

Some of the most exciting test results which explained so many previously unexplained irregularities we often see in some Merle patterns and differences in phenotype from parent to offspring, were the mosaic results. I remember very well my astonishment at seeing that first mosaic result, "WHAT IS THAT?!" And then that moment of profound revelation when suddenly it all came together and made sense.

Mosaicism - "Somatic Mosaicism" or "Somatic Mutation" is the presence of two or more types of cells with different genotypes present in the body of one individual dog. Merle mosaicism results from the shortening of the poly-A tail in one cell in the early stages of embryo development. This mutation is then replicated during cell division. The shortened length/allele will be present in only some of the adult cells and in different parts of the body. From 308 Merle dogs tested, 56 are mosaics - an average of 18% or 1 out of every 5.5 dogs having 3 or more different alleles on the M locus, indicating that mosaic results are not uncommon.

NOTE: Merle's poly-A tail is not unique in this sense of shortening. It is common for the tail of all SINE's to shorten. In this way researchers can estimate the age of the insertion - the longer the tail then the more recent the insertion. For example "at" - Tan Points on the A Locus is surmised to be an older SINE mutation as the poly-A tail length is very short and stable at 99 - 100 bp. However, judging the age of the Merle's poly-A tail in this manner will not apply as the shortening has not been left to nature. As breeders we have artificially kept the longer visible lengths of M and Mh in play by intentionally breeding for the trait. If Merle had been left to nature the visible pattern would likely be gone by now as every dog's length became Mc.

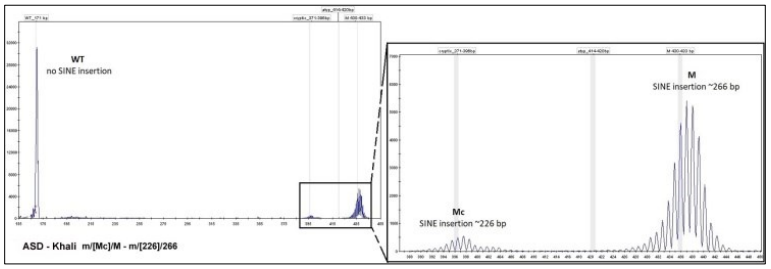
Typically, only a small proportion of cells contain the shortened allele - this is referred to as the "minor" allele/s and represented in test results with the use of [square] brackets. The two alleles with the higher peaks on the chromatogram having the larger fraction of cells are referred to as the "major" alleles. In most cases the major allele/s are the ones with the longest base pair numbers - the alleles inherited from each parent.

However in some very interesting cases the mutated cell has replicated at a higher than normal rate or even at such a great rate that there is a larger fraction of the shortened allele then that of the original allele inherited from the parent. This original allele from the parent then becomes the "minor" allele and the mutated/shortened allele is the "major" allele. The expression of the mosaic dog's phenotype will depend on how the mutated cells replicated and the fraction of each allele present in the dog. In most cases it is impossible to know that the dog is a Mosaic Merle based on phenotype alone, however in cases where the cell containing the shortened allele has replicated at a higher rate the phenotype of the dog can be dramatically altered from what would be expected from the 2 original alleles inherited from the parents.

Further, when a mutation occurs very early in development, it may be present in both somatic and germline cells. Somatic cells occur in the body only; they include all cells other than reproductive. Germline cells are found only in the gonads - the ovaries of a female where eggs/ova are produced and testes of the male where sperm are produced. A germline mutation alters the genetic make-up of the reproductive cells, meaning that the cells containing the mutated/shortened allele may be present in either the male's semen or the female's eggs. In this way a germline mutation can affect the progeny of the mosaic Merle dog and subsequent generations of that offspring.

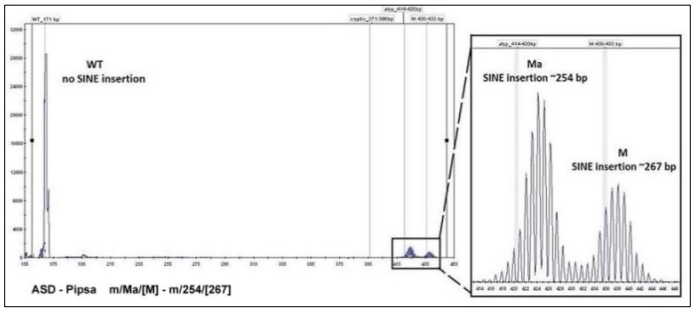
The shortened allele can be passed along to further generations.

Khali is an example of a typical mosaic result. She has both m/M - m/266 cells, the original alleles inherited from her parents and m/Mc – m/226 cells containing the shortened/mutated allele. Note the high peak of the original M allele and the much lower peak of the Mc allele indicating that there are far fewer m/Mc cells in the sample material then m/M cells. Khali's phenotype has not been altered and there would be no way to guess that she is a mosaic.



Khali - m/[Mc]/M - m/[226]/266

In **Pipsa's** mosaic result note the height of the peaks. The cells containing the shortened Ma - 254 allele have replicated at a greater than normal rate and have become the major allele. The original M - 267 allele inherited from a parent has become the minor allele. Pipsa has both m/M - m/267 cells and m/Ma - m/254 cells. The percentage of the different cells in the body have interfered with the expression of Pipsa's Merle pattern and given her an unusual phenotype.

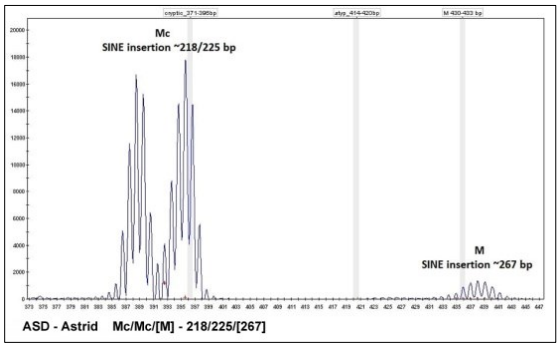


Pipsa - m/Ma/[M] – m/254/[267]

Pippa is an example of the only offspring in a litter with an unexpected Tweed pattern that is not a typical m/M pattern like her dam and other Merle littermates. This has been explained by her mosaic test results. Pippa has some cells that are m/M - m/266 containing the original alleles inherited from her parents and m/Mc+ - m/240 cells containing the shortened Mc+ allele.

m/[Mc+]/M - m/[240]/266

Astrid is an example of a "Minimal Merle" pattern caused by mosaicism. Note the height of her original M - 267 allele inherited from her dam and the height of the original Mc - 225 allele inherited from her sire. *(Both Astrid's parents were tested for this example.)* The cells containing the shortened Mc - 218 allele have replicated at a very high rate leaving Astrid with a small percentage of m/M cells and restricted Merle patterned areas on the body. At this time there is no known reason for the different rates of replication.



Astrid – Mc/Mc/[M] – 218/226/[267]

These four examples demonstrate how easily a new shorter allele can be introduced into a breeding program and future generations without the knowledge of the breeder.

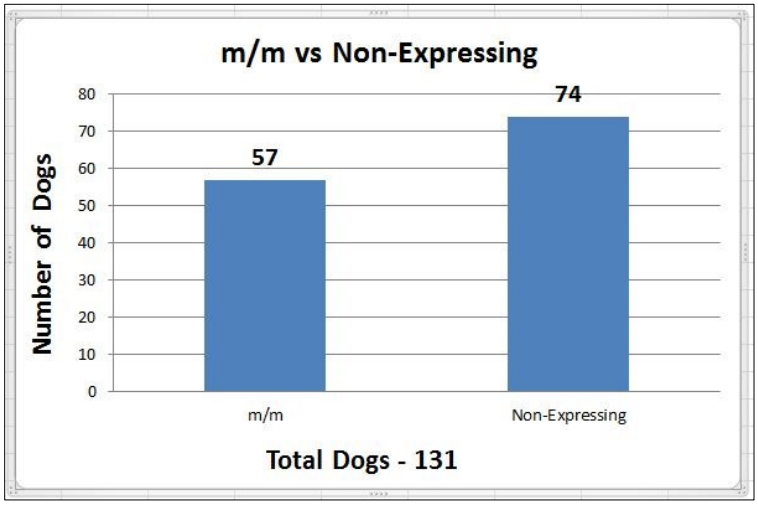
Khali's Mc - 226 pb; Pipsa's Ma - 254 bp; Pippa's Mc+ - 240 bp; Astrid's Mc - 218 bp could potentially all be passed along to future progeny.

These shorter alleles of Mc, Mc+ and Ma are all "Non-Expressing" as heterozygous.

Any resulting solid offspring would be assumed to be non-Merle when in actuality could be m/Mc, m/Mc+ or m/Ma. The Mc allele will not impact future breeding or pattern however Mc+ and Ma certainly have the potential to add white when combined with M. *Breeding with the Mc+ allele will be addressed further in the article.*

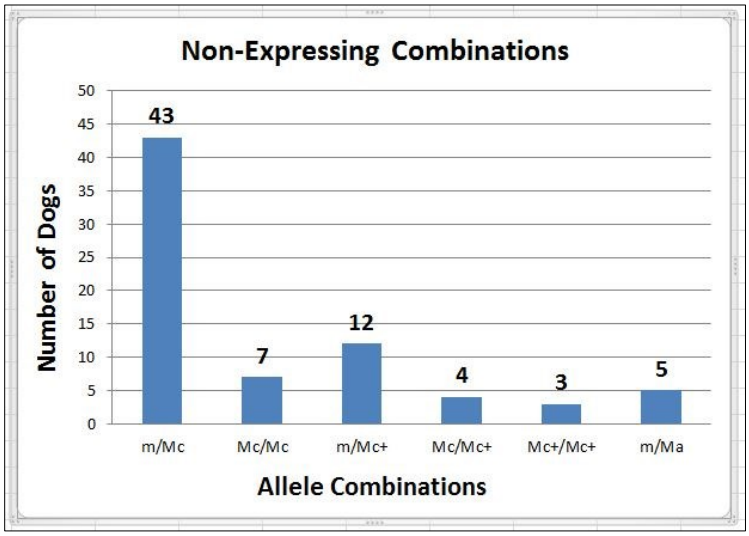
Mc - 200 - 230 bp will not be noticed when added to a breeding program. The poly-A tail has been so shortened (truncated) that it will breed the same as non-merle. In order to give readers a clear understanding of just how common these shorter alleles are, especially Mc, I will provide results of an online survey of Aussie breeders/owners as to the percentage of phenotypically solid dogs having a shorter non-expressing Merle allele of Mc, Mc+ or Ma.

Total dogs - 131



Percentage of m/m - Non-Merle - 43%
Percentage of dogs having a shorter Non-Expressing allele of Mc, Mc+ or Ma - 57%

Breakdown of Non-Expressing Alleles Total 74



38% of phenotypically solids dogs are either m/Mc or Mc/Mc. These dogs will breed the same as non-Merle. When bred to an M - Merle mate no pigment will be deleted to white.

19% of phenotypically solids dogs have either an Mc+ or Ma allele which can delete pigment to white when paired with M.

This small survey is an indicator of just how common the shorter non-expressing alleles are and how often we assume a solid dog to be m/m. With the Mc allele being the most common shorter allele this demonstrates just how often all through history we have unknowingly been including it in our breeding programs with no ill effects.

This percentage would likely hold true for any breed who has traditionally included Merle in breed standards and most often breed for the trait. *In breeds where the trait has been introduced more recently by crossing in a Merle dog from another breed, the percentage of the shorter alleles is likely not as high yet.*

These numbers are not overly surprising. Tracking all the mosaic results to date the most common shortened allele is Mc. There are even three results of Mc+ shortening to Mc. I had a chuckle from these results thinking to myself "I guess Mc+ was not short enough!"

The next section will cover in detail our past usage of the term "cryptic Merle" and now the correct genetic meaning. I consider this information to be the most important that I will cover in this article.

In order to protect the genetic diversity of all Merle breeds it is imperative for breeders to have a full understanding of the Mc allele 200 - 230 bp.

Defining Mc - Cryptic Merle 200 - 230 bp

The origins of the word cryptic come from "crypticus", a Latin word meaning "hidden" or "to hide." The terminology of "cryptic Merle" has been used for decades to mean exactly that - a dog who is a "hidden Merle". You'll most often find the word cryptic also used in conjunction with words such as "hidden", "masked", "ghost" and "phantom". All the same descriptive words with the same meaning - a dog who was assumed by phenotype to be non-Merle and then bred as a Merle producing visibly Merle patterned offspring.

It is very important to note that the word "cryptic" in this sense has been used only as a general "descriptive" word and not in a true "genetic" manner. Used to describe something we could not understand as we did not yet have the technology available to us in order to discern the precise genetics of Merle's poly-A tail. *NOTE: in some breeds cryptic is also used to describe a dog who is Minimal Merle. This expression has 2 distinct Merle genotypes, one which has been covered in the previous section on Mosaicism using Astrid as an example. The second genotype will be discussed in the Mh - Harlequin Merle section.*

In 2015 a paper was published officially naming the "Cryptic Merle" allele - Mc. However this research was done still using the old testing method and based only on phenotype, not on breeding outcomes. This resulted in an Mc allele that was much too long in length. This length encompasses the "langevin et al" alleles of Mc, Mc+ and Ma which are all "Non-Expressing" as heterozygous but do not breed the same.

It was unfortunate that this paper named the allele Mc - Cryptic Merle as the term "cryptic" has become so convoluted over the years; used in such general form to mean so many different things to different people and in different breeds.

My choice for the allele would have been Mt - Truncated Merle. "Truncated" meaning "shortened", "curtailed", "cut short" which describes the Mc allele perfectly.

I mentioned earlier that setting the boundaries for each allele was an immense task that came with an immense responsibility. The base pair numbers for the Mc allele were by far the most important. We need to ensure for every breeder that when a dog testing as Mc 200 - 230 bp is bred to M, there is no deletion of pigment to white due to the combination of both alleles and therefore no risk of vision and/or hearing impairments caused due to Merle.

Any dog tested as m/Mc or Mc/Mc can safely be bred to M with the Mc allele acting the same as non-Merle.
A dog who is Mc/M will have no pigment deleted to white due the allele combination.

So what does this have to do with protecting the genetic diversity of all Merle breeds?

The Mc allele is the most common non-Expressing allele. As already mentioned the SINE insertion of Merle's poly-A tail shortens more often to Mc then to any other length and continues to shorten even from a length of Mc+. This has been happening by way of mosaicism since the Merle mutation first occurred. We can presume that all breeds who consistently breed for the Merle trait have on average 38% of their phenotypically solid dogs having at least one Mc allele and a percentage having two. We have always been breeding with Mc involved; we just did not know it. The basic rule has been to breed "Merle x Solid" in order to avoid any resulting "Double Merle" offspring, which for the most part has served breeders fairly well over the years. *There is however exceptions when a Merle x Solid breeding does not turn out as expected that I will provide examples of later.*

Before moving on I'd like to address the terminology of "Double Merle". This is technically not a genetic term. We would never say "Double Tan Points" or "Double Black". The terminology in the past has not only been used to describe a dog who is M/M - homozygous for M, but more so refers to the white that can be created by the combination of two Merle alleles and the possibilities of hearing and/or vision impairments. With the new understanding of the alleles that are dependent upon the length of the poly-A tail of the SINE insertion, questions have arisen as to what constitutes a "Double Merle". Or more so, which combinations of two Merle alleles can delete pigment to white and therefore come with the risk of hearing and/or vision impairments? Out of the 28 possible Merle allele combinations there are 14 that have the ability to delete pigment to white. I cannot provide example photos of all the combinations but will list all 14 grouped by the longest allele.

Mc+/Ma+, Ma/Ma+, Ma+/Ma+

Mc+/M, Ma/M, Ma+/M, M/M

m/Mh, Mc/Mh, Mc+/Mh, Ma/Mh, Ma+/Mh, M/Mh, Mh/Mh
Note - the Mh allele can delete pigment even as heterozygous.

We need to start redefining what we refer to as a Double Merle. Mc/Mc+ is not a DM dog; Mc+/Ma is not a DM; Ma/Ma is not DM.

And most importantly Mc/M is not Double Merle as no pigment will be deleted to white.

ZZ - m/Mc - m/210

Kai - Mc/Mc - 225/225

Harper - Mc/M - 221/268

Kenzie - Mc/M - 200/267

Now back to the issue of genetic diversity. There are currently 16 labs offering Merle testing. A full list is available at this link - www.merle-sine-insertion-from-mc-mh.com/labs-offering-merle-testing. Five of these labs are offering the new high resolution testing method but only two of these are offering base pair numbers, mosaic results and up-to-date information in regard to the Mc allele. Eleven labs are still using the old method of testing and only two are offering correct genetic information in regard to Mc. That leaves us with 12 labs that have old outdated and inaccurate non genetic information on their websites. Along with literally hundreds of websites who repeat this information over and over again, as well as old information cited from old Merle studies, some that were based only on phenotype and not on genetic testing.

Any Google search for "Cryptic Merle" will result in pages and pages of websites using the old catch-all, convoluted and non-genetic meaning of the term. I have seen very limited progress over the past year from most labs and breed websites to update their Merle information. The damage being done could be disastrous to many breeds as dogs testing as Mc - 230 bp and under are neutered/spayed and removed from breeding programs due to the fear that Mc means "hidden", "ghost" and "phantom" Merle. That their Mc dog could possibly breed the same as a Merle and may produce impaired Double Merle offspring when bred to another visible Merle dog.

The following information is taken from Google search - *"Many solid dogs are actually cryptic or phantom merles and can produce both Merle and double merles. A cryptic ghost or phantom Merle is a dog which phenotypically appears to be a non-merle or very faint patches of Merle that can go unnoticed. Animals that do not present the Merle phenotype may possess the Merle genotype and subsequently produce Merle offspring. These dogs are known as cryptic Merles."*

Scary stuff indeed!!

Information such as this has not been overly detrimental in the past when there was really no reliable test for Merle or the Mc allele. Breeders based their information on breeding results of litters produced. Now that we have the technology available for the accurate testing of Merle's poly-A tail we are aware of just how common a result of Mc - 200 - 230 bp is at an average of approximately 38%.

What if 38% of all phenotypically solid dogs were removed from breeding programs based exclusive on a result of Mc?

Genetic diversity would be greatly impacted. I have already seen dogs tested as Mc spayed/neutered and removed from the genetic pool either based on information the owner found through an internet search or following the advice of labs stating not to breed Mc to M for the fear of producing Double Merle offspring and passing this "unsafe" Mc allele to the future generation.

It is even more imperative in Europe that the Mc allele is understood completely as many registries and clubs do not allow the breeding or registering of litters from a Merle x Merle cross. This still includes Mc x M and even Mc x Mc. Imagine the irreversible damage that could be done to a closed gene pool by restricting the breeding of Mc dogs? A gene pool will get smaller when the number of gene variants decrease and are lost due to dogs not reproducing and passing their genetics on to future descendants.

It is a travesty to remove a dog from a breeding program based solely on a result of Mc and totally unnecessary! The base pairs for the Mc allele were set at 230 bp in order to guarantee it will breed the same as Non-Merle, that no pigment will be deleted to white when bred to M. I know that "guarantee" is a strong word here and not scientific in the least. However in this case it is a word I am confident using. The limit that was placed on the Mc allele could likely have been slightly higher, maybe even to 234 bp but I have seen examples of 235 bp x M starting to delete pigment.

NOTE: Even if every Mc dog was removed from the breeding population there would never be an end to the eradication. Mosaicism is always happening. There is no way to stop the natural shortening of any poly-A tail. With 18% of Merle dogs being mosaics and 55% of those dog having a shortened Mc allele there is just no way to "get rid of" it.

The following example shows a dam who is phenotypically solid and producing offspring with "white body splashes" when bred to an m/M - m/266 sire. Testing confirmed that Figgy is not m/m but has a non-expressing Mc+ 240 bp allele. The combination of her Mc+ and the sire's M allele has resulted in 240/266 in the offspring which has deleted to pigment to white. **Pirate is bilaterally deaf and unilaterally vision impaired due to this Merle combination. This is the very important reason that the length for the Mc allele was set at 230 bp.**

Sire - Royce m/M - m/266

Dam - Figgy m/Mc+ - m/240

Offspring - Mc+/M - 240/266, S/S

Offspring - Mc+/M - 240/266

I sincerely hope that all this information will give breeders confidence when they receive an Mc 200 - 230 bp result back on a phenotypically solid dog, knowing this dog can safely be bred to a visible Merle. But I think we need to address one more important fact before moving on.

Mc Does Not Lengthen to M

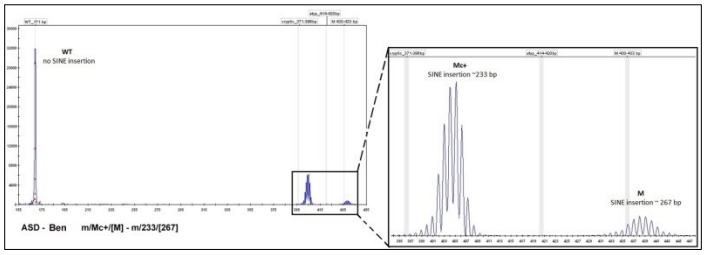
In the past this type of information was based solely on phenotype and the outdated method of testing, which could not provide accurate base pair numbers and most importantly could not provide any possible mosaic results. In the example given of Astrid's mosaic results there is a minor M allele present that was inherited from her dam. Astrid will breed as a Merle. But what if she had been tested using the old method? The result would have been Mc/Mc, there would have been no M allele included in her results. Since she expresses a Minimal Merle pattern and can produce Merle offspring, an assumption would be made that Mc can produce limited Merle areas on the body and will somehow lengthen to M and produce Merle offspring.

Any dog tested as Mc and producing Merle patterned offspring must have an M allele present.

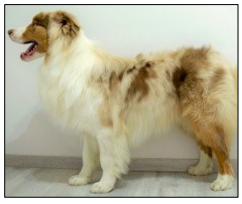
Any case providing an example of Mc lengthening to M (even based on the new method of testing) is really just a matter of due diligence not being done to investigate further. The following is an example of a Minimal Merle sire tested as m/Mc using the new high-definition method of testing and yet he produces Merle offspring. An assumption was then made that m/Mc can produce "small areas of pattern" and can lengthen to M. This is not possible.

I was given the opportunity to investigate this case further and arranged to test Germline cells (a semen sample) from this male. **Simply put - if he can produce Merle patterned offspring then he has an M allele.** As previously mentioned when mosaicism is involved the different cells will be present in only some of the adult cells and in different parts of the body. Obviously Ben's minor M allele was not present in the first sample material provided but must be present in the body and Germline cells in order for Merle offspring to be produced. Of course this was the case and Ben does have a minor M allele.

m/Mc+/[M] - m/233/[267]



Ben's Germline Result Clearly Shows a Minor M Allele.


Ram - Mc/M - 220/268


Drum - Mc/Mc+ - 220/234


Tuna - m/Mc - m/219

These three resulting offspring of Ben's have each inherited one of his 3 different Merle alleles - the first pup inheriting his M, the second pup his Mc+ allele and the third pup his m allele. *Note - +/- 1 bp is the margin of accuracy. The Mc - 220 allele has been inherited from the dam.*

When I started this article I did not intend to spend so much time discussing Mc but given that it is such an important issue for breeders and genetic diversity I want to ensure that I cover it in as much detail as possible so that any existing doubts will be addressed and answered.

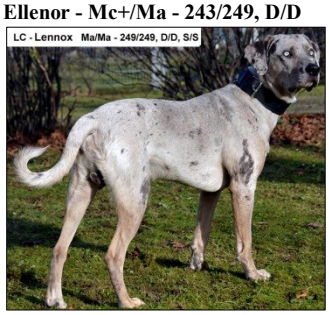
Unique Phenotypes

I mentioned at the beginning of the article numerous oddities that a breeder may unexpectedly see in their whelping box. Pippa is an example of *"an unusual Tweed Merle pattern unlike that of the Merle parent and other littermates."* This was due to mosaicism. Figgy and Royce's litter is an example *"White body splashes that were not expected and are not to standard."* This was due to the combination of Mc+ and M.

The following are examples of a *"dilute expression when d/d is not a possibility."* The shorter alleles and their combinations very often result in a diluted look to the coat.

Ellenor - Mc+/Ma - 243/249, D/D

Indie - Mc/Ma - 222/247, D/D



Catahoula- Ma/Ma - 249/249

Catahoula - Mc+/Ma, 245/249, D/D

The following are examples of *"brownish or off-shading on a black offspring."* Combinations of Mc and Mc+ may often result in unusual shading.

Maverick - Mc/Mc+ - 224/235

Sky Mc+/Mc+ 234/246

Mayla - Mc/Mc+ - 211/233

Rabbit - Mc/Mc - 225/225

The following is an example of *"Merle offspring that were not expected as neither parent expresses a Merle pattern."* In cases like this it is a matter of both parents having a shorter non-expressing Merle allele as heterozygous and the combination expressing in the offspring as homozygous.

Sire - Rico m/Ma - m/247

Dam - Mac m/Ma+ - m/258

Merle Patterned Offspring Ma/Ma+ - 247/258

These pups could very well be mistaken for a typical m/M - Merle but will not breed as such. If crossed to an m/m mate all resulting offspring would be non-expressing m/Ma - m/247 or m/Ma+ - m/258.

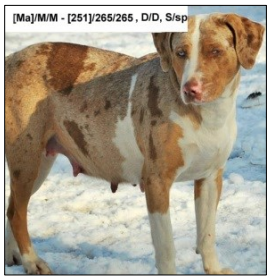
The following example demonstrates this quite well.

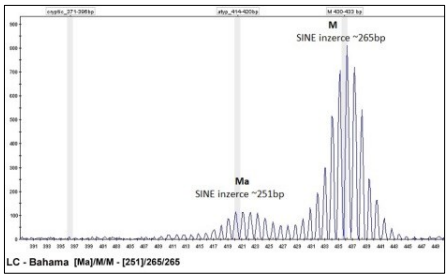
Sire - Boaz - m/m

Dam - Selah - Ma/Ma - 250/250

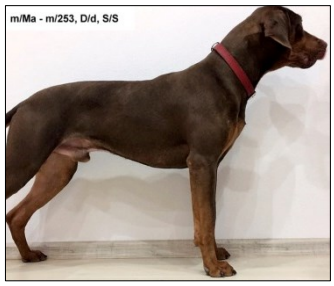
All resulting offspring are m/Ma - m/250 non-expressing Breed - Catahoula

The following is an example of *"a solid pup when one parent is M/M and all pups should be Merle."* This is a result of mosaicism and an offspring inheriting a minor non-expressing allele. In the past a case like this would have been extremely confusing for sure!





Bahama - [Ma]/M/M - 251/265/265



One offspring has inherited Bahama's minor Ma allele.

There is still one very important issue to cover. "Excessive white offspring and yet one parent is phenotypically solid".

Occasionally this is a case of one parent expressing a Merle pattern and another parent having a "masked" or "hidden" Merle pattern due to other coat color genetics. Phaeomelanin or the "red pigment" of e/e - Recessive Red/Yellow and Ay - Clear Sable will not allow for the expression of M - Merle. This is the reason why many breed standards do not allow for these colors due to their ability to mask a Merle pattern. In genetic terms this is referred to as "epistasis" when one locus or allele suppresses the expression of another.

Border Collie - m/M - m/267, e/e Merle pattern masked by Recessive Red

Welsh Shepherd - m/M m - 268, Ay Merle pattern masked by Clear Sable

Note: The Mh allele may express a slight pattern on Clear Sable and Recessive Red

Another reason for a Merle pattern possibly not expressing is due to mosaicism. As previously mentioned mosaic Merle dogs having a higher percentage of cells containing the shortened allele may often express an unusual pattern and in these cases no pattern at all.

Catahoula - m/[Ma]/M - m[250]/266

Catahoula - [Mc]/Ma/M - [225]/251/165

The third reason for a Merle pattern possibly not expressing is the Mh allele. Harlequin Merle has proven to be very common in the Aussie breed.

Mh - Harlequin Merle

The Mh allele has a broad range of phenotypes with three different expressions, two of which are very recognizable.

#1 - "Minimal Merle" - a large percentage of the body features solid colored pigment with only small random areas of Merle patterning. These areas are normally on the outer extremities of the body - head/muzzle, legs and shoulders. Individuals may also express extended white out of the normal area of the typical Irish Spotting pattern – this may include a large white collar, white up legs past the elbow, white past shoulders extending onto withers and white on the belly extending up the side. **Occasionally the Merle pattern is so "minimal" or has been deleted by white that no Merle is visible at all or could very be easily missed.**

#2 - The more classically thought of pattern that has been referred to as "herding harlequin" in the past. Random diluted areas of pigment are deleted to white, leaving solid patched areas that may be Tweed patterned including different shades mixed with a Merle pattern. The extended white patterning mentioned in description #1 may be present but is less noticeable due to the deleted white areas on the body.

#3 - Some dogs may express more as m/M, yet are still able to produce offspring with a phenotype as described above in example #1 and #2 - these offspring have inherited the same length of base pairs as the parent and yet express in either of the 3 ways presented here.

m/Mh, Mc/Mh, Mc+/Mh, Ma/Mh, Ma+/Mh, M/Mh and Mh/Mh combinations can be phenotypically indistinguishable.

Note - M/Mh and Mh/Mh may express with a greater percentage of white over the body.

Expression #1 - Minimal Merle

Peetu - m/Mh - m/272


Peetu - Only Merle Area on Body

Ellie - Mc+/Mh - 234/271

Border Collie - m/Mh -m/269

Baxter - m/[Mc+]/Mh - m/[246]/271

Zoya - m/Mh - m/269

Expression #2 - Classic Pattern

In the past referred to "herding harlequin"

Ehaw - m/Mh - m/273

Winnie - m/Mh - m/273

Harley - m/Mh - m/272

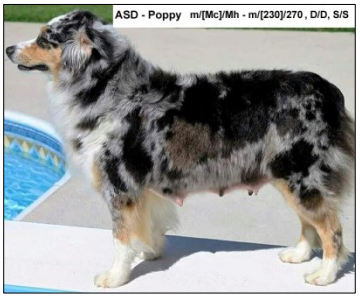
Quinn - m/Mh - m/274

Expression #3

The following dogs have a phenotype that could easily pass for a typical m/M pattern.

Stella - Mc+/Mh - 235/269

Baxter - m/Mh - m/270



Poppy - m/[Mc]/Mh - m/[23]/270

Winne - Mc/Mh - 217/269

Mh offspring will not necessarily express the same type of Harlequin Merle pattern as a parent even though they have inherited the same length of base pairs. Any of the three Mh phenotypes may be expressed in a single litter.

This example shows 3 generations all with the same Mh - 271 bp allele.

Flutter - Minimal Merle


Lucchese - "herding harlequin"

Goose - Sire - "herding harlequin"

Ellie - Grandam - Minimal Merle

This example of a Minimal Merle phenotype could easily be missed by the breeder and future owner.

Willow - m/[Mc+]/Mh -m/[240]/271

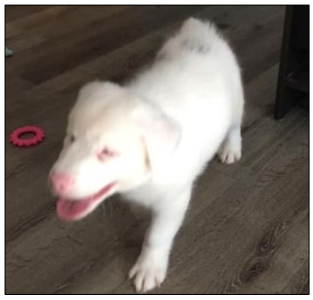
An example of an Mh a Catahoula with no Merle pattern at all.

Catahoula - mMh - m/272

And now an example of **"Excessive white offspring and yet one parent is phenotypically solid".** This is due to an Mh male who expresses no visible Merle pattern at all and was assumed to be non-Merle and his resulting offspring when bred to a Merle female.

Tripp - m/[Ma]/Mh - m/[250]/277

Merle Dam With Litter



Excessive White Offspring Bilaterally Deaf - Bilaterally Vision Impaired.

Conclusion

Over the past two years new technology has propelled us forward towards a new understanding of Merle's many expressions. From knowing very little to having most phenotype questions answered. So many breeding and test results that did not make sense in the past can now be explained. I have heard from long time breeders worldwide that they finally have answers to questions that have perplexed them for decades. The reporting of mosaic results and the inheritance of minor alleles has been one of the most important additions to genotype test results. These de novo Merle alleles from a parent are easily passed along to further generations, changing genotype in offspring and in a line of dogs.

We can now count the exact base pairs of Merle's poly-A tail and preciously allocate the 6 lengths of the Merle SINEs so that genotype = phenotype. This is especially crucial for the Mc allele. For the future health and genetic diversity of all our breeds it is imperative for breeders to have a full understanding of the Mc allele and how it will breed. We must take it upon ourselves for this further education and pass this knowledge along to our fellow breeders. It is time for us all to unlearn what we have learned and rethink what we have come to believe, in regards to "cryptic Merle" and no longer rely on a Google search ☺. This must be an unequivocal refutation of the old belief and acceptance of the science of genetic testing; there is no in between here if we want to preserve the welfare of our future generations.

"It ain't what you know that gets you into trouble. It's what you know for sure that just ain't so."
~ Mark Twain ~

Acknowledgements

Karen Smiley Combs - For tirelessly educating so many in the Australian Shepherd community. For guiding owners through the process of Merle testing, for explaining results and helping breeders to understand the oddities they see in their whelping boxes. For all her encouragement and continued support. There would not have been the great number of Aussies tested for the published paper or my book if it had not been for Karen. My deepest thanks!!

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