Understanding Merle for Breeders

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Canine color genetics are complicated; with individual genes being interdependent on one another to determine the final outcome of the color and pattern of the dog. The Merle trait is arguably the most complicated in both its independent expression and its interaction with other loci and alleles.
My mission has always been to educate in the most accessible and understandable way for everyone within every breed.

History of the Merle Mutation

Merle is a rare mutation of foreign DNA which impairs the ability of cells to produce normal pigment. This leaves a pattern with random areas of the coat diluted to a lighter pigment combined with areas of full pigmentation. To complicate this expression further, merle is an "incomplete dominant"; one allele does not completely dominate the other and depending on which 2 alleles are inherited in the individual dog this can create an intermediate expression or a completely distinct pattern.

The mutation has occurred only once in history, making every merle dog alive today a descendant from that one unique individual. Based on known breed histories this one dog would have been born in Europe roughly 250 - 500 years ago and was either a hound or herding type, but certainly not a purebred as we think of today.

There are about two dozen documented historically merle breeds having the pattern included in their official standard and now roughly the same number who do not allow for the pattern in their breed standard. This would indicate that the trait has been introduced into the gene pool by way of a non listed outside breed after the stud book was closed. Many breeds falling into this latter group have had the added trait present for perhaps 20 years or more. Through back crossing to the original breed population merle individuals exhibit the standard physical appearance, movement and temperament of the breed in all ways except for the addition of a pattern that was not historically ever found in the breed. Typically these breeds consistently show a 100% match with their breed when tested using services like Embark or Wisdom Panel, which specialize in breed identification. This is because the introduction of an outside breed or breeds occurred multiple generations back.
In these cases it is very important to note that "Purebred Status" and "Genetic Ancestry" are completely separate concepts. "Purebred" status is defined by pedigrees at registration bodies whereas "Ancestry" looks at segments of shared DNA.

Documented Historically Merle Breeds
https://merle-sine-insertion-from-mc-mh.webnode.page/documented-historically-merle-breeds/

Historically Non-Merle Breeds that have had the Merle Pattern added to their gene pool
https://merle-sine-insertion-from-mc-mh.webnode.page/historically-non-merle-breeds/

History of Merle Testing

The merle mutation consists of 3 parts - a head, body and tail (poly-A tail). This tail contains a long string of repeating base pairs. The original test developed by Dr. LA Clark in 2006 identified only the body of the merle mutation with the assumption that any reasonable length of tail produced some kind of pattern. No distinction was originally made between the tail length (base pairs) and the resulting merle phenotype. In 2009 and only two years after being made available to consumers merle testing was suspended indefinitely. The results were considered non-credible with many dogs testing as M/M and yet expressing no merle pattern at all. It was very clear that M locus was more complex than just the two alleles of "m" and "M".

In 2017 I was contacted by MUDr. Soňa Peková, PhD from Vemodia Labs (now Tilia Labs) in the Czech Republic, who expressed great interest in developing a newer testing method that would allow for the reporting of the exact base pair numbers.
The length of merle's poly-A-tail is a "continuum" from 200 - 280 bp's. 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. "

I was tasked with assigning the base pair numbers (the genotype) to the resulting merle pattern (the phenotype). When setting the borders (bins) for each allele it was not as simple as considering the phenotype of each allele as heterozygous, each length had to be looked at in all possible homozygous combinations, especially noting the ability of a said combination to delete pigment to differing degrees of white. This was an immense task; it required a working knowledge of dogs in pedigrees; parents, offspring and related individuals through the generations, many owners and breeders from all breeds worldwide offered testing and pedigree information on hundreds of dogs. This resulted in 28 possible combinations from 7 alleles - 14 of these are considered to be “double merle combinations” which can delete pigment to white within the Merle pattern, and therefore come with a risk of possible hearing and/or vision impairments of varying degrees from low, moderate and high risk depending on the two alleles.
This link contains a downloadable PDF with a description and example photos and of all combinations - https://merle-sine-insertion-from-mc-mh.webnode.page/merle-phenotype-combinations/

These 7 alleles not only offer breeders the knowledge of 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 any given mate.
On Sept, 20, 2018 “Merle phenotypes in dogs - SILV SINE insertions from Mc to Mh” - “langevin et al” - Authors - Mary Langevin, Helena Synkova, Sona Pekova and Tereza Jancuskova, was published by PLOS One, a peer-reviewed open access scientific journal published by the Public Library of Science. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0198536

Allele Lengths

The 7 alleles in order of length

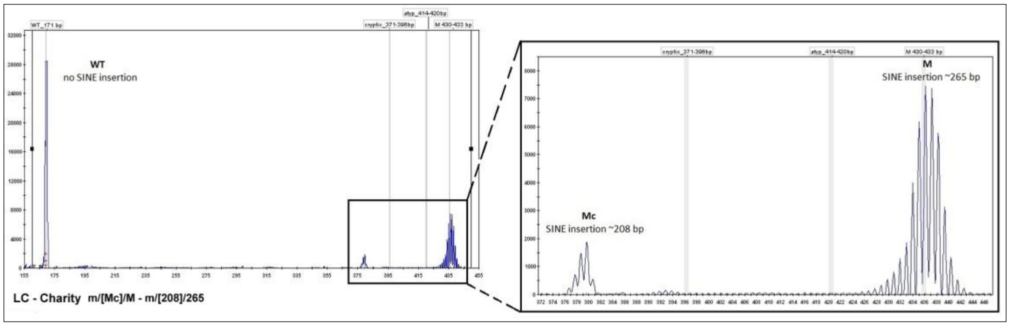
• m	Non-Merle Wild Type
• M^c	Cryptic Merle 200 - 230 bp
• M^{c+}	Cryptic Merle + 231 - 246 bp
• M^a	Atypical Merle 247 - 254 bp
• M^{a+}	Atypical Merle + 255 - 264 bp
• M	Merle 265 - 268 bp (Classic merle)
• M^h	Harlequin Merle 269 - 280 bp

NOTE - The alleles of M^c, M^{c+} and M^a are all “non-expressing” in their heterozygous state, they will all appear the same as non-merle, however ONLY M^c will breed the same as non-merle in that it will not delete pigment to white when paired with an M allele.

Mosaicism

But where do all the different lengths come from? We must presume that the very first merle dog had a visibly expressing pattern from a longer allele of either M or M^h. This is where merle becomes even more complicated.
Mosaicism - “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. On average, 18% or 1 out of every 5 merle dogs will have 3 or more different alleles on M locus, indicating that mosaic results are not uncommon. 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 a^t - 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 bp. Piebald - s^p is also an older mutation with a stable length of 254 bp. However, judging the age of 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 expressing lengths in play by intentionally breeding for the visible trait. If Merle had been left to nature the expressing pattern would likely be gone by now by way of mosaicism/shortening to the non-expressing alleles.
The M^c allele is the most commonly shortened to length in a mosaic dog.

Color mutations can happen on any loci, the most noticeable being an area of black pigment on a recessive red e/e dog. However these color mutations are "somatic" variants in that one particular individual, occurring in the body cells only and are not present in reproductive cells, hence they cannot be passed along to offspring. Merle mosaicism is unique in that it is a "germline mutation". The cells containing the mutated/shortened de novo allele can also be present in the gonads - the ovaries of a female where eggs/ova are produced and testes of the male where sperm is produced. In this way the shortened allele can be inherited by progeny and subsequent generations of that offspring. Most always this de novo allele is one of the three non-expressing shorter alleles and offspring could be assumed to be non-merle based on phenotype. It is strongly advised that any offspring from a merle parent who may potentially be considered as a future breeding prospect be tested.



Test example of a chromatogram showing a mosaic result.
Offspring have a 50% chance of inheriting "m" and a 50% chance of inheriting either M^c or M from this parent.

Now that we understand how all the different lengths are created I want to focus on the shortest allele of M^c - Cryptic Merle.
I mentioned earlier that setting the boundaries for each allele was an immense task. The base pair numbers for the M^c allele were by far the most important that came with an immense responsibility.

Defining M^c - 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".

In 2015 a paper was published officially naming the "Cryptic Merle" allele - M^c. However this research was done still using the old testing method and based only on phenotype, not on breeding outcomes and possible homozygous results. This resulted in an M^c allele that was much too long in length all the way to 252 bp. This length encompasses the "langevin et al" alleles of M^c, M^{c+} and M^a which are all "non-expressing" as heterozygous but do not breed the same nor express the same as homozygous especially when combined with longer alleles. It was unfortunate that this paper named the allele M^c - 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 M^t - Truncated Merle. "Truncated" meaning shortened, curtailed, cut short which describes the M^c allele perfectly.

Moving forward "cryptic merle" genetically refers to M^c, an allele on the M Locus that is so shortened/truncated that it can no longer express a Merle pattern and will breed the same as non-merle. The base pairs for M^c were set at a low number to ensure for every breeder that when a dog testing as M^c 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/M^c or M^c/M^c can safely be bred to M with the M^c allele acting the same as non-merle. A dog who is M^c/M will have no pigment deleted to white due the allele combination. Any merle allele combination that cannot delete pigment to white (a safe combination) is a referred to as a "homozygous combination". Any allele combination that can delete pigment and therefore comes with some level of risk for impairments is referred to as a "double merle" combination.

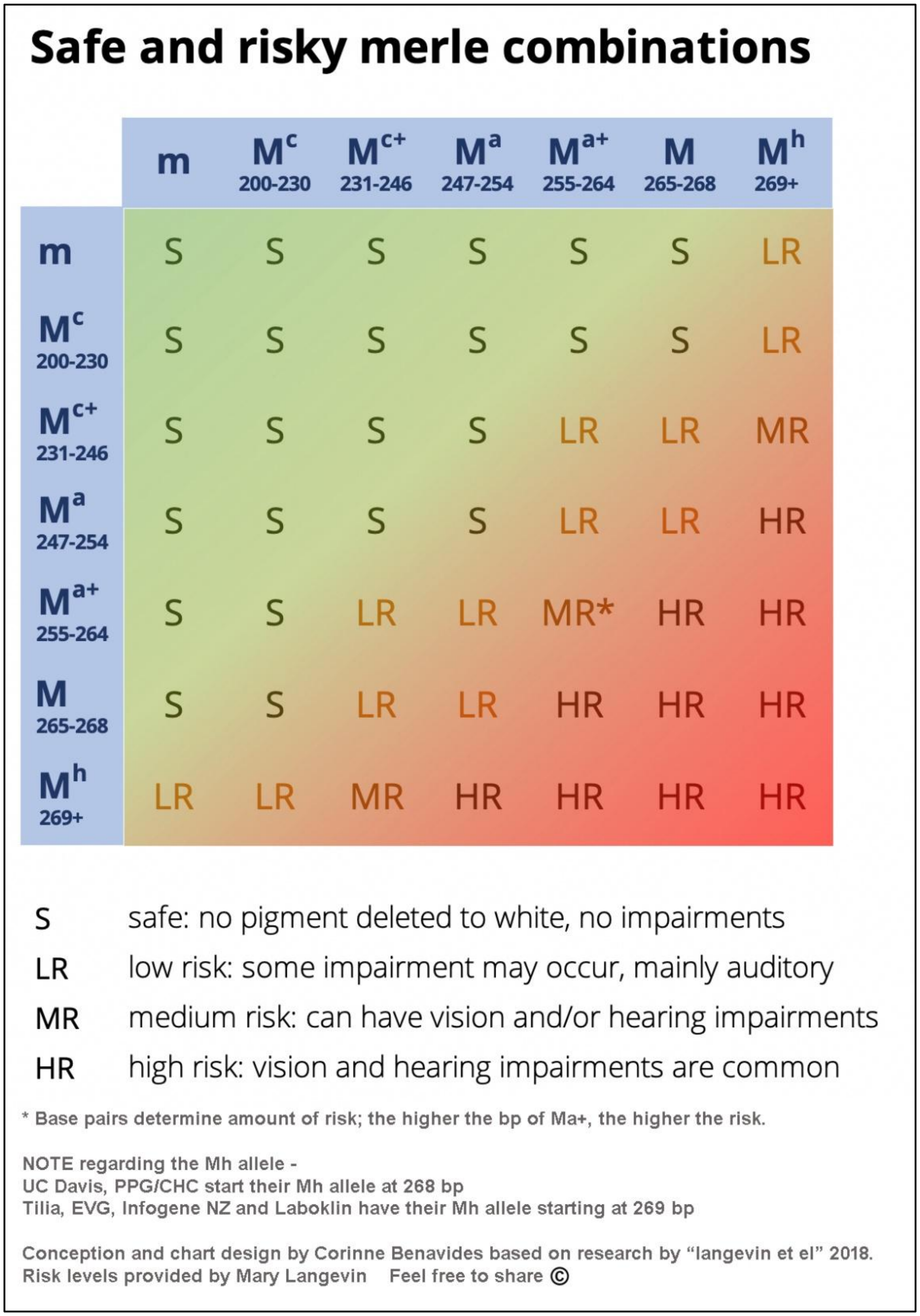
“Homozygous Merle” vs “Double Merle”

These two terms differentiate between safe homozygous merle combinations and combinations that come with risk.

"Homozygous Merle" is the proper genetic term when an individual has inherited the same versions (alleles) of a genomic marker from each parent. Thus, an individual who is homozygous for a genomic marker has two versions of that marker - M*/M*.
By contrast, an individual who is heterozygous for a marker has two different versions of that marker - m/M*
(**M*** indicates a merle allele of unknown length.)

"Double Merle" is not a genetic term but rather is a “descriptive term” used to indicate that the dog has a homozygous merle allele combination that has the ability to delete pigment to white and therefore comes with low, moderate or high risk for impairments depending on the two alleles.

The following chart shows the safe homozygous allele combinations and the double merle combinations that come with varying degrees of risk for vision and hearing impairments.



Safe and risky merle combinations

	m	M^c 200-230	M^{c+} 231-246	M^a 247-254	M^{a+} 255-264	M 265-268	M^h 269+
m	S	S	S	S	S	S	LR
M^c 200-230	S	S	S	S	S	S	LR
M^{c+} 231-246	S	S	S	S	LR	LR	MR
M^a 247-254	S	S	S	S	LR	LR	HR
M^{a+} 255-264	S	S	LR	LR	MR*	HR	HR
M 265-268	S	S	LR	LR	HR	HR	HR
M^h 269+	LR	LR	MR	HR	HR	HR	HR

S	safe: no pigment deleted to white, no impairments
LR	low risk: some impairment may occur, mainly auditory
MR	medium risk: can have vision and/or hearing impairments
HR	high risk: vision and hearing impairments are common

* Base pairs determine amount of risk; the higher the bp of Ma+, the higher the risk.

NOTE regarding the Mh allele -
UC Davis, PPG/CHC start their Mh allele at 268 bp
Tilia, EVG, Infogene NZ and Laboklin have their Mh allele starting at 269 bp

Conception and chart design by Corinne Benavides based on research by “langevin et el” 2018.
Risk levels provided by Mary Langevin Feel free to share ©

Hidden or Masked Merle

Merle only expresses on black pigment (eumelanin). Using the wording of “hidden or masked merle” is the appropriate term for red pigment (pheomelanin) not allowing for the expression of a merle pattern.

This would include e/e - Recessive Red, A^y - Clear Sable and a^w - Agouti (merle may fainting express on any black pigment on the latter two alleles). The true genetic word for this is "epistasis" but the definition also means "masked" or "hidden" - both these words have long been used in the language of Merle.

a^t - Tan Points is also red pigment that merle will not express on.

NOTE - M^h and any double merle combinations are “strong enough” to express on pheomelanin.

“Hidden” vs “Non-Expressing”

Hidden refers to an otherwise expressing merle allele that is masked by red pigment. These are the longer alleles of M^{a+}, M and M^h. When these alleles are inherited by offspring who are not e/e, A^y or a^w they will express a visible merle pattern.

This resulting Mudi litter demonstrates this inheritance quite well.

Sire - e/e, m/M - recessive red hidden/masked merle

Dam- E/E, m/m

Offsprings' E locus of E/e does not mask a merle pattern as the sire's e/e does.

"Non-expressing" refers to the alleles of M^c, Mc^+ and M^a. They will not express a pattern no matter what the base color is.

Note - Ma+ (<260 bp) can also be non-expressing.*

** 260 bp is the most typical length for the Ma+ allele to start expressing a visible pattern.*

** 255 - 259 can sometimes cause a faux dilute or brownish shading to the coat.*

Non-Expressing m/Mc 210

Non-Expressing m/Mc+ 235

Non-Expressing m/Mc+ 241

Non-Expressing m/Ma 251

Non-Expressing m/Ma+ 258

Non-Expressing m/Ma+ 258

Example of a non-Merle in appearance sire assumed to be m/m and the resulting offspring when bred to a visible Merle dam.
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NOTE - the M^c allele is the only non-expressing allele that will breed the same as non-merle.

M^{c+}, M^a and M^{a+} come with a low, moderate or high risk respectively when combined with the M allele.

It is extremely important for breeders to understand and make use of the terms **"Non-Expressing", "Hidden or Masked" and "Cryptic Merle"** in the proper genetic reference.

Merle + Piebald

There seems to be a notion that there is risk when breeding merle and piebald together because this somehow could create impairments due to “extra white”, this is an invalid assumption with no genetic basis.
The loci of M - Merle and S - Piebald - s^p do not interact with each other. They do not "create white" when combined together.
Piebald - s^p/s^p can create white and in some cases an “extreme” amount of white which can result in hearing impairments when the pigment deletion is close to the ear area.
Double merle allele combinations can also create white which can cause vision and/or hearing impairments.
However, the two loci do not somehow multiply to produce more white when combined.
This breeding is a good example.

Sire - M/M - 265/265, S/S

Dam - m/m - sp/sp

All Offspring - m/M - m/265, S/sp

Example of resulting offspring from two extreme white parents.
Sire - “Double Merle”, Non-Piebald - M/M, 265/265
Dam - Piebald, Non-Merle - sp/sp

White created from a Merle combination - M Locus and white created from Piebald - S Locus do not interact.
M and S locus are 2 separate loci, they do not combine to create white.
All offspring are well colored Merles and Piebald carriers with no white - m/M, S/sp

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Conclusion

I hope article has helped to answer questions surrounding the mysteries of merle; it’s history and testing, myths and speculations, descriptive terms vs true genetic terminology. Moving forward this is information that every breeder should have a full understanding of and share with others in order to educate.

This PDF may be shared in its original version.
Link for full 145 page PDF **“Merle - SINE Insertion from Mc - Mh - The Incredible Story of Merle”** can be order here - https://merle-sine-insertion-from-mc-mh.webnode.page/book-order/

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