“The Coat of Many Colors"

"Life is a celebration of passionate colors." **(L. Cator)**

Introduction:

Much is being discussed of late - actually for the past few decades - about sable merle collies, the breed standard, and color/pattern genetics. Thanks to the "leaps & bounds" growth of DNA research and the work of a few dedicated veterinary geneticists, we have far more solid science available than ever before to teach us about color and pattern in our wonderful breed.

This article is about the dedicated work of Dr. Leigh Anne Clark and her colleagues at Texas A&M and that of Dr. Phil Sponenberg of Virginia Tech and his colleagues. Their studies and research with various dog breeds have brought color/pattern genetics into focus and answered important questions for breeders.

In 1984, Dr. Sponenberg published a study in **The Journal of Heredity** involving 66 puppies from a double merle Australian Shepherd from which he concluded that the merle gene was due to a mutation by what is called “transposable DNA .”

In 2005 Dr. Clark and her colleagues successfully mapped the merle gene in all breeds, sequenced the merle gene of Shetland Sheepdogs, and published their findings in **The Proceedings of the National Academy of Sciences**, in January 2006, under the catchy title, ***Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog***. Dr. Clark’s research was based on Dr. Sponenberg’s earlier work with Aussies. I am the conduit for their expertise and that of my good friend, molecular biologist Dr. Mike Vaughan. Mike is a fellow collie fancier and CCA member who helped me "translate" the science so that I could relay it accurately to you.

Collie Color:

The two base colors in collies are sable and black & tan. Most of us know, that in collies, sable is dominant and tri-color recessive. A puppy inherits one color allele from each parent. **An allele is an alternate form of a gene.** Therefore, if a collie inherits a dominant sable allele from each parent, he/she is a "pure for sable" (homozygous for sable). On the other hand, if a recessive black & tan color allele is contributed by each parent, the collie will be a tri-color (homozygous for black & tan). Some collies inherit a sable allele from one parent and a black & tan one from the other making them "tri-factored" sables (heterozygous genetically although still sable in appearance).

Dr. Mike explains the process:

> Specialized skin cells in Collies produce **pigments called melanins**; these pigments come in black or tan. These are not free dyes, dissolved in the cell water but instead are **solid pigment particles built up in tiny bodies made of proteins called melanosomes.** The skin pigment cells pass these melanosomes into hair follicle cells which produce the hair strand.

If a hair follicle does not receive pigmented melanosomes, the hair strand it forms is white, as in the majority coat of a white Collie or the neck region of colored collies (from the Irish pattern gene). If all the melanosomes that a growing hair strand receives contain black melanin, the hair strand itself will be black, as in the black areas of a tri-color Collie. And if the melanosomes the follicle receives from the pigment cells are all tan, the hair strand will be sable, as in the coat of a pure sable or tan points on a tri-color.

All cells of a Collie have the same basic genes, but no single cell expresses all those genes; instead, in each cell, some genes control the expression of others, turning some on and some off. This complex and miraculous control process not only produces beautiful puppies; it continues throughout the life of the dog.

An example is the mahogany sable coat of tri-factored sables. At birth these puppies are usually dark tan. But as older puppies, many look much like pure sables, and almost all the developing hair in the first coat is sable. However, as the tri-factored collie ages, some black melanosomes are put into the growing hair strands, so the coat develops a deepening dark appearance.

Sable Merle & Blue Merle Genetics – Separating Fact from Fiction:

Dr. Mike tells us, "The merle allele is not a color allele; in fact, it produces no pigment at all." A misconception that is apparently still alive and well is that sable merles are a mysterious mishmash of colors in conflict. This is fiction. A sable merle is not a combination of sable, tri and blue. Here's why. **There is no color gene in collies for blue coat color, only sable and black.**

The merle allele "dilutes" color on the body. In genetically black dogs, it turns the black coat grey but allows some melanosomes to be fully loaded with black pigment so that black color comes through completely on some parts of the coat. The merle gene has the same affect on a genetically sable collie, turning most such collies a lighter sable.

Of course, when a collie has one sable color allele and one black & tan color allele, it is still a sable, just a darker one. This phenomenon is called incomplete dominance (an interaction between alleles/genes). Incomplete dominance means that the black and tan recessive allele in a tri-factored sable is only partially suppressed by the dominant sable color allele. The sable phenotype (appearance) is expressed, so it is dominant (or, more accurately, incompletely dominant) while the suppressed black & tan allele is incompletely recessive because it does darken the sable by adding in more black. The resulting collie is, of course, still a sable. So when a merle allele with no pigment acts upon the coat, various shades of sable come through.

Sable merles, whether pure for sable or tri-factored sable, are still sable collies; one is simply a darker sable than the other. There is no conflict among the genes; only a normal, expected and predictable interaction!

Collie Merle Pattern:

Again, keep in mind as you continue to read that the merle allele is NOT a color allele. I know we've said this before, but it's worth repeating!

We'll need a few definitions to understand how merle works. For example, we briefly mentioned the meaning of **allele** earlier. Here's an expanded definition: An allele is one of two or more alternative forms of a gene at the same site on a chromosome that determine alternative characteristics in inheritance.

SILV, a term in the title of Dr. Clark's paper excerpted later in this article, is a gene that is important in pigmentation because it produces a protein that forms the matrix (background) on which melanin is deposited. When *SILV* is used to refer to a gene, it is italicized in scientific writing. When SILV refers to proteins in such writing it is not italicized. I have tried to adhere to that practice in this article.

Transposable DNA, also called **SINE** or **retrotransposon**, is another term we need. These three terms are interchangeable. These refer to sections of DNA able to copy themselves and inset themselves at random into other areas of DNA. All merles have a SINE inserted in the *SILV* gene. A SINE is organized into 3 parts: a head, a body and a tail. The tail is a fragile string of **"A" nucleotides**. Nucleotides are chemical letters in the DNA code. The "A" tail may shorten or lengthen each time it is copied.

The length of the "A" tail controls the degree of merling seen in a collie. A collie with little or no "A" will present as a cryptic. A cryptic is a merle who is not physically distinguishable as such or who mainly looks tri-color or sable with only small areas of merling. Collies with longer "A" tails in SINE present as merles, but the tail length controls the degree of merling and is responsible for the random degree of mottling as well as the randomness of eye color.

Dr. Clark summarizes the merle allele's affects this way:

> Merle is a coat pattern caused by a dominant mutation in a pigmentation gene called *SILV*. The mutation (an insertion of repetitive, mobile DNA) causes dilution of the base fur color and often blue eye color. Merle affects all coat colors, but is more apparent in dogs with darker-colored coats (e.g., black) than those with lighter-colored coats (e.g., sable). Coat color is determined by other genes. Because of the type of mutation that causes merle, merling is random and characteristics such as the size and number of colored patches and eye color cannot be predicted.

So how does the mobile, inserted "transposable DNA" (remember that synonyms are SINE & retransposon) in merle interfere with the production of SILV protein needed to form pigmented melanosomes? Dr. Mike explains with an analogy that the insertion works on pigment in the following way: "It is as if a nonsense string of letters is inserted at random into a meaningful sentence, making it impossible for us to read it."

A blue merle is a tri-color (homozygous black & tan) with the merle allele of the *SILV* gene. A sable merle is a sable that can be either homozygous sable or heterozygous sable with the addition of a non-color producing merle allele. Merle actually has the affect of preventing some of the color from fully appearing on the collie.

Dr. Mike explains further:

> With the addition of merle, things get interesting! The "transposable DNA" inserted into the merle gene contains a long string of "A's" that "turn off" the *SILV* gene so that it cannot produce the SILV protein helping to form melanosomes. It turns out that mutations occur in this string of "A's"quite often as cells divide, decreasing the string's length. The result is that the body of a tri-color (with merle) develops, first as an embryo and later after birth, with clones of skin cells with new mutations in the "A" string. The shortened "A" prevents the "transposable DNA" from inactivating the *SILV* gene, allowing normal melanosomes to be made again. In other words, now there are two mutations present, with one reversing the other. So this clone of skin cells, with normal melanosomes loaded with black pigment, will produce a spot of hair that is black or dark grey. This spot on the puppy now has two mutations, one reversing the other. Since there are a lot of these spots, some dark gray and some quite black, scattered randomly over the dog's body, the puppy ends up with the classic "blue merle" appearance.
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> This is amazing! When you next look at a blue merle Collie, look at the spots of black and grey on its body, giving it its beautiful coat and think that you are literally looking at mutations, creating each unique spot. The SINE gene is still there in the SILV in those spots; it has just been inactivated in them, by the second mutation decreasing the length of the string of "A's." One mutation is canceling the effect of another. You always knew Collies were clever!
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> I should add that the merle mutation also often affects the eye color of dogs inheriting it; the effect is to produce one eye or both eyes that are completely or partially blue. This does not always happen; some merle dogs have two dark eyes, though close examination may show a fleck of blue coloration.

Merle Gene Mapping:

The 2005 mapping of the merle gene using 50 Shetland Sheepdogs not only tells us what genetic mechanisms produce merle genetics but also that merle is the same in all breeds.

Dr. Clark and her colleagues explain the merle gene mapping phase of their work in ***Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog*** as follows:

> (We) carried out a whole-genome scan using the Shetland Sheepdog and were able to map the merle locus… To determine whether the *SILV* mutation causing merle patterning in the Shetland Sheepdog population was breed specific, merle

> and nonmerle dogs representing six other breeds (Collie, Border Collie, Australian Shepherd, Cardigan Welsh Corgi, Dachshund, and Great Dane) were analyzed for the insertion. Merle dogs from all six breeds were heterozygous and one double merle Great Dane was homozygous for the insertion.

Merle Gene Influence on Eye Color AND Its Influence on Ear & Eye Abnormalities in Double Merles:

Both blue merles (homozygous, for black & tan) and sable merles (homozygous for sable, as well as heterozygous for sable) have random chances for pigmentation in the eyes. The same genetic information mechanism involving mutations in the length of the "A" tail in the SINE that randomly puts merling spots on these collies also produces the following possibilities on eye color: both eyes brown; both eyes blue; one eye of each color; partial blue & brown in one or both eyes; and blue flecks in brown eyes. So while eye size, shape and set are genetically controllable through selective breeding, the presence of blue in the eyes of merles is not. While dark brown eyes would be the preference of most breeders & fanciers in both blue and sable merles, blue in eyes may or may not detract appreciably from the merle's expression and is an individual matter of proper evaluation.

Below is the explanation for the relationship between pigmentation and its effect on normal eye and ear development from the Clark et al. paper ***Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog*** (bold below is mine):

> **Melanocytes are pigment-producing cells present in many tissues, including the epidermis, hair follicle, inner ear, and choroid of the eye** (33). Melanocyte cell populations differentiate from unpigmented melanoblasts released from the neural crest during embryogenesis (33). The complex process in which melanoblasts migrate and differentiate into melanocytes is not fully understood; however, the study of pigmentary anomalies may accelerate identification of genes important for normal development.

This research and prior work done by Dr. Sponenberg tells us that normal inner ear and eye development is, in part, controlled by presence of pigmentation. One copy of the merle gene causes no harm in the collie; however, two copies are a different matter. **Fanciers and breeders call homozygous merle collies, those that receive a merle allele from each parent, by a variety of names, all of which describe the same collie: double merle, double dilution and white merle. These collies may have varying degrees of vision and auditory abnormalities.**

As Dr. Mike says, "Despite these defects, double merles may live normal lives. In fact, some are of superior conformation and when mated to non-merles have produced splendid Collies that are perfectly normal blue merles or sable merles, successful in the show ring and at performance events."

Again, the predisposition for abnormalities in double merles varies widely with some having both usable vision and hearing, some having one or the other, and some having neither. However, when a homozygous (two copies) merle is bred to a non-merle collie, the resulting offspring receive color/pigmentation from both parents but the merle allele from only the homozygous merle parent. All resulting puppies develop into blue merles or sable merles depending upon the base colors of each parent. These offspring will NOT have or produce any eye or ear abnormalities related to pigmentation since they only have one merle allele from the double merle parent and none from the non-merle parent. Their own merle offspring will also be normal.

Genetic Testing for the Merle Gene:

A genetic test for the merle gene will be available again soon and can give complete confidence that a dog is or is not carrying the gene. Nothing more than a simple cheek swab is needed from your collie – a non-invasive test. For more information, go to: http://www.idexx.com/animalhealth/laboratory/realpcr/index.jsp

Normally, we know whether or not our collies are blue merles or sable merles. However, there are some questionable situations that may arise. Cryptic merles are an example of a collie that may need genetic testing.

In phenotype (appearance) some collies can appear to be tri-colors or sables without merle. However, these individuals may produce as merles because this is their genotype. If that happens, then a genetic test is in order. Furthermore, if there is any doubt at all as to whether a collie you own and plan to breed has a merle genotype, then testing prior to breeding is needed. Because of the probability of producing 25% double merles, merle to merle breedings would only be appropriate in very specific situations.

Conclusion:

We've come full circle in our discussion of the collies' beautiful colors and patterns. Genetics is an amazing and wondrous science with a great deal to teach us as breeders and fanciers. If we take the time to educate ourselves properly and make an effort to keep up with the latest that science has to offer, our beloved collies will be all the better for our knowledge.

Excellent quality sable merles, like excellent quality blue merles, sables, tri-colors and color-marked whites, have a great deal to contribute to the collie gene pool. Fear and misinformation should not stand in the way of what they have to teach us! As Dr. Mike explains, "There is absolutely no fundamental distinction between blue merle collies and sable merle collies other than the fact that blues, having inherited black & tan alleles, have diluted grey bodies with grey and black spots while sable merles have similar spots on sable bodies. The fact that a Collie is a merle, blue or sable, is simply an important characteristic to be noted, not one to be used to stigmatize it."

"Continuity gives us roots; change gives us branches, letting us stretch and grow and reach new heights." (P. Kezer)

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