



Tootie is a red-and-white with the genotype  $a^1/a^1, B/B, e/e, K^R/k^r$ .



Ruby, a red-and-white, is  $a^1/a^1, B/B, e/e, K^R/k^r$ .



Ya-Ya is a red-and-white with the genotype  $a^1/a^1, B/B, e/e, K^R/K^R$ .



The sable-and-white Lilly tested  $a^1/a^1, B/B, E/e, k^r/k^r$ .



This sable from Russia has the genotype  $a^1/a^1, B/b^s, E/e, k^r/k^r$ .



Elmo, is genetically a red-and-white:  $a^1/a^1, B/B, e/e, K^R/K^R$ .



This brown-and-white puppy from Russia tested  $a^1/a^1, b^s/b^s, E/e, K^R/k^r$ .



Tink tested  $a^1/a^1, B/B, E/e, k^r/k^r$ , and so has the tan points pattern.



Note the tan point at the top of Meego's front leg.



Rudy, a black-and-white import from Japan, tested  $a^1/a^1, B/B, E/E, K^R/K^R$ .



Tommy, another import from Japan, is also  $a^1/a^1, B/B, E/E, K^R/K^R$ .



Baby, a black-and-white, is  $a^1/a^1, B/B, E/e, K^R/k^r$ .

# JAPANESE CHIN COAT COLOR STUDY 2007

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Last year, the Japanese Chin Club of America Health Committee embarked on a study of coat color. We collected cheek swab samples from Japanese Chin of various colors. The samples were sent to Dr. Sheila Schmutz of the University of Saskatchewan for DNA testing at the A, B, E, and K loci. We are finally ready to share what we learned!

## BACKGROUND & TERMINOLOGY

In order to understand the study results, it may be helpful to review a bit of basic terminology. We describe dogs by genotype and by phenotype. *Genotype* is the inherited information in the genes; *phenotype* denotes the observable characteristics.

*Locus* is simply the location of a gene; the place it lives on a particular chromosome – its street address if you will. *Loci* is the plural. An *allele* is a form of a gene at any given locus. At a locus that determines color, each dog has two alleles, one from each parent. If the two copies of the allele are the same, the dog is said to be *homozygous* for the allele. If the alleles are different from each other, the dog is *heterozygous* at that location. A *dominant* allele may change the dog's color when the dog has just one copy, while a *recessive* allele generally requires two copies to definitively change a trait. A dominant allele is depicted by a capital letter; a recessive allele is depicted by a lower case letter.

*Melanocytes* are the pigment-producing cells of the body. Where there are no melanocytes, there can be no color, so the absence of the melanocytes causes white spotting. Pigment in dogs comes in two basic forms. *Eumelanin* is the dark pigment that is black, brown, or (in other breeds) blue. *Phaeomelanin* is the bright pigment that produces red, yellow, and cream colors in canine coats. Each type of pigment is affected by different genes. Some genes function to affect the intensity of the pigment; other genes are “pattern” genes that may alter the distribution of the pigment. Genes that produce white coats actually disable the body's ability to produce pigment in the hair.

Many of us learned canine color genetics from the old but classic book by Clarence Little, *The Inheritance of Coat Color in Dogs* (Howell Book House 1957). While Little was remarkably accurate in many of his theories, modern molecular studies have demonstrated that there are more or fewer alleles at some genes than originally postulated.

Let us look at some of the genes that affect coat color in the Japanese Chin.

## The S Locus – Spotting (MITF)

Clarence Little suggested that there were four alleles of the Spotting (S) Locus, but recent research suggests that there may be several

genes involved in spotting, each with two or three alleles. One gene that causes a spotting pattern in some breeds was identified last year as MITF (microphthalmia associated transcription factor), a critical gene in the pigmentation pathway; but there is still much to be learned about the genes involved in white spotting.

We did not test any of our study subjects for spotting genes, so for now, we can only say that the white areas on the dog represent an absence of melanocytes and therefore, an absence of color. These areas may vary in size and location, but the Japanese Chin breed is usually white on the chest, lower legs, and tip of the tail. Breeders tend to select for a white muzzle, a white blaze between the eyes, and patches of color surrounding the eyes and ears. The preference for these color patches on the head may be partially protective against a type of deafness that occurs in piebald dogs. Patches are distributed somewhat randomly elsewhere on the body, with the color of these patches largely determined by the loci A, B, E, and K.

## The A Locus – Agouti Signal Protein (ASIP)

- a<sup>v</sup>* sable/fawn – In the Japanese Chin, produces sable: solid black hairs intermingled among red hairs. In some other breeds, produces fawn (red/yellow/cream hairs with black tips).<sup>2</sup>
- a<sup>w</sup>* wolf sable – Agouti “wild-type” allele; wolf-grey coloration or banded black/red hairs.
- a<sup>t</sup>* tan point allele – Produces the tan points pattern.
- a* recessive black – Produces recessive black in German Shepherds, Shetland Sheepdogs, and Belgians.

At the Agouti Locus, we believe the Japanese Chin to have either *a<sup>v</sup>* (sable) or *a<sup>t</sup>* (tan points) alleles. We do not believe that the breed carries the recessive black or wild-type alleles possible at this locus in other breeds. We know that *a<sup>v</sup>* is dominant to *a<sup>t</sup>*, so a Japanese Chin with even one copy of *a<sup>v</sup>* will be sable unless it is masked by homozygous recessive *e* or a dominant *K<sup>B</sup>*. A Japanese Chin with two copies of *a<sup>t</sup>* will have tan points (again, unless they are masked by *e/e* or a dominant *K<sup>B</sup>*).

One of the most important realizations we made during the study was that it is very likely that many of our Japanese Chin carry alleles for tan points (*a<sup>t</sup>*). Although we cannot directly test for *a<sup>t</sup>* yet, several Chin were determined by the process of elimination to be homozygous for *a<sup>t</sup>*, since they tested negative for *a<sup>v</sup>* and negative for recessive black and obviously are not *a<sup>w</sup>* (wild grey). The tan points are only observed in a Japanese Chin that has at least one dominant *E* and that is homozygous for recessive *k<sup>v</sup>* and *a<sup>t</sup>*, such as the pictured tricolor puppy, Tink.

Japanese Chin that are black-and-white-with-tan-points (often colloquially called “tricolors” by breeders) are historically controversial, because most major kennel clubs outside the United

States do not include tan points as an allowed color pattern in their standards for Japanese Chin. The American Kennel Club (AKC) standard for the Japanese Chin was changed to allow the tan point pattern effective 1993.

Since most Japanese Chin in the United States in the early 1900s were either black-and-white or red-and-white, it is likely that most of the Japanese Chin in the United States were homozygous for dominant  $K^B$  prior to about 1950, which is around the time when Kitsu of Yevot was imported by Catherine Cross. The alleles for tan points and sable can only be expressed if a Chin is homozygous for the recessive allele  $k^v$  and carries at least one copy of a dominant  $E$ . One hypothesis is that an imported dog that caused a sudden appearance of sable or black-and-white-with-tan-points possibly introduced copies of recessive  $k^v$  into the gene pool, allowing the  $a^v$  and  $a'$  alleles that were there all along to be expressed.

Several of the study subjects tested positive for  $a^v$ . As would be expected, the sables, like Lilly, carry at least one copy of  $a^v$ . Tootie, a red-and-white, carries a copy of  $a^v$ , but the sable allele is masked by  $e/e$ . The two black-and-white imports from Japan, Rudy and Tommy, were homozygous for  $a^v$ ; but their sable alleles were masked by their dominant  $K^B$  alleles. Rudy and Tommy will be unable to sire sable offspring, because they are homozygous for  $K^B$ . Tootie carries a recessive  $k^v$ , so she would be able to produce sable offspring if bred to a dog with a dominant  $E$  and a recessive  $k^v$ .

For the Japanese Chin, we defined “sable” as red hairs with intermingled black hairs. At birth, a sable may appear to be black-and-white, but sables tend to lighten with age. While a few adult sable Japanese Chin may have abundant intermingled black hairs in their red coat, even a single black hair may be hard to find in some adults. Black hairs on the sable are often most prominent in the ears or on the back. If the black hairs are hard to find, a clue to the genotype is that the nose pigment on a sable tends to be darker than the nose pigment of a pure red-and-white ( $e/e$ ) Japanese Chin.

Since the allele for tan points is recessive to the allele for sable, tan points will only be expressed if there are two copies of  $a'$  in the absence of masking alleles. Consequently, tan point is the least common pattern seen in Japanese Chin. In a black-and-tan breed such as the Rottweiler, the pattern would cause tan points to appear on the eyebrows, muzzle, cheeks, throat, chest, legs, feet, and under the tail. The tan point can only be seen where there is melanin, so except for an occasional tiny fleck, tan will usually only be visible in the homozygous  $a'$  dog where there is adjacent colored coat. Because of the typical pattern of melanin distribution in the Japanese Chin, tan is likely to be on the face (eyebrows, cheeks, and a little under the ears) but much less likely where the coat is usually white, such as the muzzle, throat, chest, legs, and feet. If the rear end of the tan-point-patterned dog is black, there will be some tan at the anal vent and under the proximal part of the tail. Our standard does not mention it, but if melanocytes are present on the leg of a tan-point-patterned Japanese Chin, there may be a little tan on the leg (see the top of Meego’s leg).

### **The B Locus - Brown (TYRP1)**

- $B$  Dominant allele that allows for black eumelanin.
- $b^s$  Recessive permitting expression of brown eumelanin when

- there are two copies of the allele.
- $b^d$  Recessive permitting expression of brown eumelanin when there are two copies of the allele.
- $b^c$  Recessive permitting expression of brown eumelanin when there are two copies of the allele.

This is a relatively easy one. The TYRP1 gene has four known alleles, one dominant and three recessive, and determines whether the dark pigment expressed will be black or brown.<sup>3</sup> Dominant  $B$  allows the production of black pigment, provided it is not masked by  $e/e$ . Two copies of a recessive  $b$  will allow brown where black would normally be. A brown-and-white ( $b^s/b^s$ ) Japanese Chin will have a brown nose; it will not produce black nose pigment.

It is likely that most, if not all, of the Japanese Chin in the United States are homozygous for dominant  $B$ , since brown-and-white Chin are not observed in America. Brown-and-white Japanese Chin have been photographed in Russia and the Netherlands and were reported to be exhibited at a show in Poland, so we tested DNA from a brown-and-white puppy from Russia and his sable sire. The brown-and-white puppy was homozygous for  $b^s$ ; the sable sire carried one copy of  $b^s$ . The dam was not tested but would have to carry at least one copy of  $b^s$  in order to produce the brown-and-white puppy. All tested Chin from America, including imports from Japan, were negative for recessive  $b$  and therefore are unable to produce puppies that are genetically brown-and-white.

### **The E Locus – extension (MC1R)**

- $E^M$  Melanistic mask – Darkened face seen in Pug, Boxer, Tervuren, etc.
- $E$  Eumelanin – Produces black, brown, or in some breeds, blue.
- $e$  Pheomelanin – Red, yellow, or cream produced.

This locus, which many of us knew as the Extension Locus, is now referred to by geneticists as the Melanocyte Stimulating Hormone Receptor Gene or Melanocortin Receptor 1 (MC1R).<sup>4</sup> When dominant  $E$  is present in a dog, it has some dark pigment in its coat because of the production of eumelanin. Dominant  $E$  may also allow expression of the Agouti Locus alleles ( $a^v$  or  $a'$ ), so a Chin with a dominant  $E$  may be sable, black-and-white, or have tan points, depending on what alleles are at the Agouti and K loci.

Clear red animals (MC1R homozygous recessive or  $e/e$  dogs) are unable to express eumelanin pigment in their hair. The homozygous recessive gene is so restrictive of black hair pigment that dogs can “carry” for black and it will not be visible. An  $e/e$  Chin may only produce pheomelanin (red, yellow, cream) and should not have any black hairs, even as a puppy. No matter what  $K$  and  $A$  alleles are present, if a dog is  $e/e$  (homozygous for recessive  $e$ ), other colors and patterns are going to be suppressed. This phenomenon is called *epistasis* but is often referred to as a *masking* effect. Unlike sables which typically lighten with age, clear red-and-white ( $e/e$ ) dogs usually darken with age. At birth, the  $e/e$  dog may appear so pale as to be almost white, but the color patches on the adult may vary from a cream or lemon to a deep red.

### **The K Locus – Dominant Black**

- $K^B$  = Eumelanin dominant (black, brown, or in some breeds, blue)

$k^{br}$  = Brindle

$k^y$  = Recessive that allows patterns/colors of agouti to express

The discovery of the K Locus location is the focus of brand new research<sup>5</sup> announced October 18, 2007. Once thought to be at the Agouti Locus, dominant black has been found to be at the K Locus – recently identified as  $\beta$ -defensin 103 (CBD103). The test for K was not commercially available at the time we began our study, so we were fortunate to form a relationship with a research laboratory able to test for  $K^B$ .

All black-and-white Japanese Chin without tan points must have at least one copy of dominant  $K^B$ . All tricolors (tan point patterned) and sables must have two copies of recessive  $k$ , most likely  $k^y$ , since we know of no true brindle Chin. Clear red-and-whites (never any black hairs) can be any combination of dominant  $K^B$  or recessive  $k^y$ , because a homozygous  $e/e$  “trumps” all the other alleles when it comes to color – any black hair pigment the  $e/e$  Chin may “carry” genetically cannot be seen in the coat. In the study, the sables and the black-and-white-with-tan-points were all  $k^y/k^y$ , homozygous for recessive  $k$ , as would be predicted.

### Examples of allele combinations that might be found in Japanese Chin in the United States

1. These puppies will all be pure red-and-white, because they are  $e/e$ , which is epistatic to all.

$a^t/a^t$  B/B  $e/e$   $K^B/k^y$   
 $a^t/a^t$  B/B  $e/e$   $K^B/K^B$   
 $a^t/a^t$  B/B  $e/e$   $k^y/k^y$   
 $a^y/a^t$  B/B  $e/e$   $k^y/k^y$   
 $a^y/a^t$  B/B  $e/e$   $K^B/k^y$   
 $a^y/a^t$  B/B  $e/e$   $K^B/K^B$   
 $a^y/a^y$  B/B  $e/e$   $K^B/k^y$   
 $a^y/a^y$  B/B  $e/e$   $K^B/K^B$   
 $a^y/a^y$  B/B  $e/e$   $k^y/k^y$

2. These puppies will all be black-and-white. They have at least one dominant  $E$  and one dominant  $K$ .

$a^t/a^t$  B/B  $E/e$   $K^B/k^y$   
 $a^t/a^t$  B/B  $E/E$   $K^B/k^y$   
 $a^t/a^t$  B/B  $E/e$   $K^B/K^B$   
 $a^t/a^t$  B/B  $E/E$   $K^B/k^y$   
 $a^t/a^y$  B/B  $E/e$   $K^B/k^y$   
 $a^t/a^y$  B/B  $E/E$   $K^B/k^y$   
 $a^t/a^y$  B/B  $E/E$   $K^B/K^B$   
 $a^y/a^y$  B/B  $E/e$   $K^B/k^y$   
 $a^y/a^y$  B/B  $E/E$   $K^B/k^y$   
 $a^y/a^y$  B/B  $E/e$   $K^B/K^B$   
 $a^y/a^y$  B/B  $E/E$   $K^B/k^y$

3. These puppies will be sable because they are homozygous for  $k^y$  and have at least one copy of  $a^y$  and dominant  $E$ .

$a^y/a^t$  B/B  $E/e$   $k^y/k^y$   
 $a^y/a^t$  B/B  $E/E$   $k^y/k^y$   
 $a^y/a^y$  B/B  $E/e$   $k^y/k^y$   
 $a^y/a^y$  B/B  $E/E$   $k^y/k^y$

4. These puppies will be tricolor (black-and-white with tan

points). To have tan points, a puppy has to be homozygous for  $a^t$  and carry at least one copy of dominant  $E$ .

$a^t/a^t$  B/B  $E/e$   $k^y/k^y$   
 $a^t/a^t$  B/B  $E/E$   $k^y/k^y$

### A Few Things We Still Want to Know

The gene that makes some  $e/e$  dogs darker red than others is not presently known. Some  $e/e$  red-and-white Chin are so light as to be informally called lemon-and-white; some even look to be cream-and-white. Clarence Little hypothesized that the C Locus alleles were responsible for diluting red and yellow to cream color and assigned dilution of pheomelanin to alleles he designated as chinchilla, complete albinism, extreme dilution, and full pigmentation. In several species the C Locus is considered to be tyrosinase (TYR) because albinism is caused by mutations at this locus in mice. However, a 2007 DNA study of TYR in red/yellow/cream dogs found no polymorphism consistently associated with color dilution.<sup>6</sup> So, the jury is still out on why Elmo is phenotypically so much lighter than YaYa, even though both are genotypically  $a^t/a^t$  B/B  $e/e$   $K^B/K^B$ . A dilution gene for dark eumelanin pigment (melanophilin gene, MLPH) was found in 1995 and relegated to the D Locus; the discovery of a dilution gene for the brighter pheomelanin pigments cannot be far behind.

There is also much yet to be understood about the white spotting genes. We do not know what genes determine that the muzzle, chest, feet, and tail tip of the Japanese Chin are usually almost free of melanocytes, just as we do not know why some Japanese Chin have more melanocytes and therefore, more color than others.

We are not certain what causes the flecks of color sometimes appearing in places that are traditionally white on the Japanese Chin breed, particularly the feet. While these flecks of color are often referred to as “ticking,” they are usually not nearly as large or pervasive as the ticking seen in breeds like the English setter. Although the belton markings seen on the English setter may be due to the dominant ticking allele described by Little, the color flecks that sometimes appear on the legs and feet of the Japanese Chin may not be due to a ticking allele. Instead, they may simply be little areas where a white spotting gene failed to stop melanocyte development. As we continue to learn about the involved genes, hopefully, time will tell.

As mentioned earlier, the actual DNA test for  $a^t$  (the tan point allele) has yet to be discovered. Determining the presence of tan point alleles is presently a process of elimination, since we are able to test for recessive black and for sable at the A Locus. Determining the presence of  $k^y$  is also presently a process of elimination, since we are able to test for  $K^B$ . Work on canine color genetics continues at some major universities and laboratories; DNA from this study is being retained for further testing as more discoveries are made. We will keep you posted.

### CONCLUSION

The dates on some of the articles in the bibliography bear testimony as to how fortunate we were to take advantage of cutting edge research to gain useful insights into the color of the Japanese Chin. Many thanks to Sheila Schmutz, PhD, our liaison with the

HealthGene Molecular Diagnostic and Research Center, for her invaluable guidance. We also extend our appreciation to Annette Sonja Jesse, Larissa Plotnikova, Dr. Michael Billow, Susan Lawrence, Peg Schnarrenberger, Adrienne Wilder, and Rae Hunt, who were instrumental in acquiring DNA samples. Questions may be addressed to Dr. Leanne Bertani, bertani@rrohio.com.

## ENDNOTES

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