Norwich Terrier Coat Color: Grizzle
Jane R. Schubart, Chair
NTCA Health Committee
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This report summarizes one of the key results of our recent Norwich terrier color study: the genetic explanation for the grizzle-color.

Background

Coat color is determined by melanin, a substance produced by cells in the skin. There are two forms of melanin: eumelanin and phaeomelanin, and each has a "default" color. For eumelanin, black is the default. The second form of melanin is phaeomelanin (yellow). Unlike eumelanin, phaeomelanin pigment doesn't appear in distinct colors. Rather it occurs in shades ranging from off-white to dark red. In Norwich, "yellow" is our RED color. The placement and pattern of coat pigment can be modified by various genes. The *ASIP* (Agouti Signaling Protein) gene affects color in dogs.

The first A-allele tests for canine coat color had **two** alleles pertinent to our Norwich: *AY* and *at*. A dog that tested *AY/at* might be red or he might be grizzle. For our Norwich terriers, the old A-locus testing identified nearly every Norwich as *AY/AY* (red), *AY/at* (red or grizzle), or *at/at* (black saddle). But this test could not distinguish between the varying amounts of black seen in primarily red dogs, such as we see in grizzle.

New understanding of *ASIP* gene expression

ASIP gene expression was not fully understood until recently. In 2021, Bannasch et al. published the results of their work identifying new allele types that better define many dogs, including Norwich terriers. They found variations in two regions of the *ASIP* gene which determine how black pigment is expressed in the coat and explaining five distinctive dog color patterns.[1]

There were twenty Norwich terriers included in the Bannasch study. In addition, one of the authors, a geneticist (Robert Loechel), separately analyzed 30 cheek swab samples that I collected, along with photos, from Norwich Terrier Club of America members to represent the variety of color variations seen in our breed. Today, thanks to this team of researchers, we have a complete analysis of the A locus and an answer to the "grizzle" puzzle.

Eight *ASIP* alleles were identified of which **three** are relevant to Norwich. These are called DOMINANT YELLOW ($ASIP^{DY)}$, SHADED YELLOW ($ASIP^{SY}$), and BLACK SADDLE ($ASIP^{BS}$). The important new finding is that there are two kinds of "yellow." (This means we have two genetic kinds of red in Norwich.) We also know the order of dominance. Because the expression of *ASIP* promotes pheomelanin synthesis, *ASIP* alleles associated with yellow are dominant to those associated with black. In fact, shaded yellow is far less common than dominant yellow by about ten-fold. Coat color tests are now available (VetGen). It should be mentioned that grizzle in Norwich is different from the "grizzle" that occurs in sighthounds and some other breeds.

So, what about grizzle?

The newly identified allele $ASIP^{SY}$ explains grizzle. A grizzle Norwich is a shaded yellow carrying the black saddle. In the study, all grizzle Norwich tested as *AY/at* by the old tests but were SHADED YELLOW ($ASIP^{SY}$) carrying BLACK SADDLE ($ASIP^{BS}$) in the new test. The table summarizes the old legacy and the new A alleles.

Color Phenotype	A-allele, NEW		A-allele, OLD
Red	$ASIP^{DY}$/ $ASIP^{DY.}$ $ASIP^{DY}$/ $ASIP^{BS}$ $ASIP^{DY}$/ $ASIP^{SY}$	Dominant Yellow (DY) Dominant Yellow (DY) carrying Black Saddle (BS) Dominant Yellow (DY) carrying Shaded Yellow (SY)	AY/AY AY/at
Grizzle	$ASIP^{SY}$/ $ASIP^{BS}$	Shaded Yellow (SY) carrying Black Saddle (BS)	AY/at
Black and Tan (saddle)	$ASIP^{BS}$/ $ASIP^{BS}$	Black Saddle (BS)	at/at

Other genes examined in our color study

ASIP is just one part of the picture for Norwich terriers. Every dog carries several coat color genes, and it is the interplay of the genes that control both color and distribution of pigment to give the complete coat picture. One additional locus was examined. The study found the MFSD12 red dilution mutation in our breed. MFSD12 (Major Facilitator Superfamily Domain Containing 12) is a protein coding gene that plays a role in pheomelanin synthesis, thereby regulating pigmentation. Dogs with two copies of the MFSD12 mutation will have their red

pigment reduced to the point where the dog is buff colored (wheaten). In our color study, some dogs were a distinct red wheaten color, and others were medium red.

Also, the study verified that two color variations are caused by previously reported mutations. A true "pinkie" is *ee* at the MC1R gene (Melanocortin 1 Receptor, which is also known as the Extension locus or E locus.) An uncommon dilute color called "blue" is *d1/d1* at the MLPH gene (Melanophilin, which corresponds to the D locus). Variants in MLPH alter melanosome transport in melanocytes and are associated with inherited forms of coat color dilution in many breeds.

[1]Bannasch, D.L., Kaelin, C.B., Letko, A. et al. Dog colour patterns explained by modular promoters of ancient canid origin. Nat Ecol Evol (2021). https://doi.org/10.1038/s41559-021-01524-x