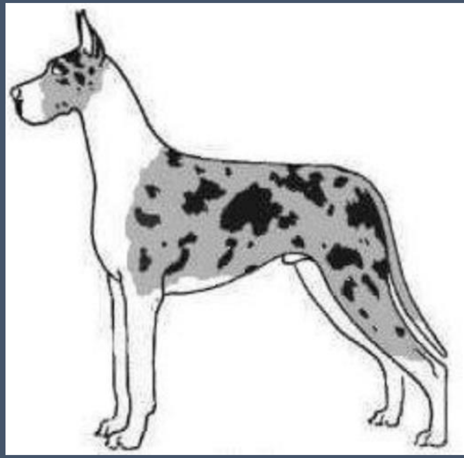
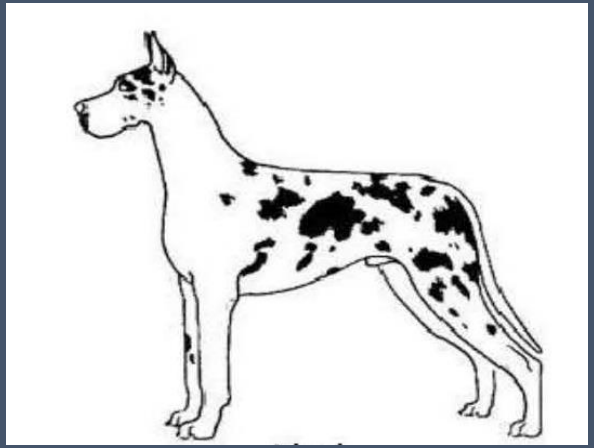
Dawn of Genetic Testing, How do we do it, and What does it Mean?



Neil O’Sullivan PhD



A Presentation for

Midland & West of England Great Dane Club

Genetic Testing

"Genetic tests are powerful tools, whose use can have a significant positive or negative impact on a breed's gene pool. As with all power tools, they should come with an instruction manual on safety and their proper use."

Jerold S Bell DVM

Content

- Basics of genetic testing.
- A History of colour in our breed.
- Which color tests are available, and the gene being tested, in each case. Colour testing allows us to venture safely, but with an abundance of caution, to broaden the gene pool.
- Health testing with genetic tests and the genes being tested.
- COI Coefficient of Inbreeding, calculate by pedigree, calculated by genetic testing.
- Summary

Basics of Genetic Testing

- A sample of cheek cells, a blood sample, a semen sample etc. are collected from an individual, identified and sent to a genetic testing lab.

- The lab extracts the DNA, using a method specific to the nature of the sample. Then increase the quantity of the DNA, placing tiny sample of the DNA with reagents on a multi-well plate.

- Each cell on the plate runs a separate test. Big multiplex testing SNP chip plates, can run thousands of tests on each plate. The testing is universal, all traits, currently known in dogs. Embark and Optimal Selection are examples of these testing methodology.

- Some labs you order a few tests for your dog, then the lab will use a section of a SNP chip plate. This custom method drives up the costs of the individual test. But it does allow special tests which involve measuring base pair difference.

- The entire process is fully automated using robotics and barcodes at all stages, unless you ordered customized testing.

Colour History

- While our breed has lots of genes interacting to give us our beautiful breed colours, for our safety, we have mostly interbred in three colour families. Namely, Fawns and Brindles, Blues and Blacks and the Harlequins, Mantles, and Merles.

- These families groupings insured the colours bred true for the most part.

- However it did somewhat restrict gene flow across the colour families. Which can have a negative impact of inbreeding, increasing inbreeding in less popular colours.

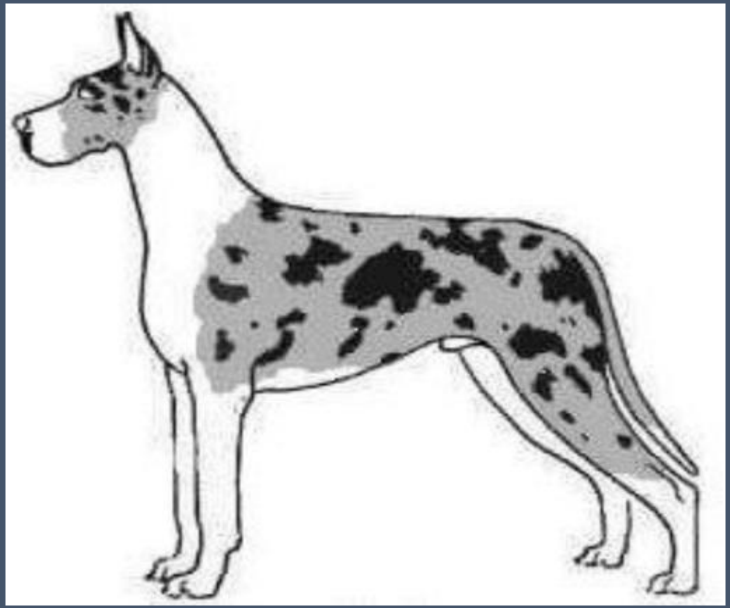
Colour History

- There are colour genes segregating in Great Danes which are undesirable and untypical of our breed. Tan points, Chocolate (Brown), mask-less Fawns and Brindles, Piebald, and Ticking.
- All but Ticking are recessive. Ticking is expressed on the Harlequin, Merles, but not in the other colors. Blacks, Blues, Brindles and Fawns often have dominant ticking but it isn't expressed on their coats.
- The mantle (Irish marking) is desired for it's specular affect on all members of the Harlequin, Mantle, and Merle family. However outcrossing to solid colors Blacks, Blues, Brindles and Fawns, results in this recessive Irish Marking being covered over by dominant solid markings patten.

Colour History: Compare a Mantle Merle to a Solid Merle

Genotype Mm s^i s^i

Genotype Mm S s^i or SS



Colour Gene Testing. Agouti or A Locus

- Technically called Agouti Signal peptide (ASIP).
- Located on canine chromosome 24
- Two forms of the Agouti gene segregate in Great Danes, a^y and a^t. The a^y gives us fawns and brindles in Great Danes without a copy of dominant black (K^B allele at another gene), a^t is our bad actor, giving us tan points, as seen in Dobermans or Rottweilers.
- Almost all Great Danes carry two copies of a^y so they are a^ya^y genotype.
- Colour testing Agouti is therefore recommended so you avoid mating two a^t carriers together.

Colour Gene Testing. Brown or B Locus

- Technically called Tyrosinase Related Protein 1 (TYRP1).
- Located on canine chromosome 11
- Two forms of gene TYRP1 segregate in Great Danes. B which has full black pigmentation and recessive b which only expresses brown (chocolate) pigmentation.
- Almost all Great Danes carry two copies of B so they are BB genotype.
- Colour testing TYRP1 (B locus) is therefore recommended so you avoid mating two b carriers together.

Colour Gene Testing. Dilution or D Locus

- Technically called Melanophilin (MLPH) gene.
- Located on canine chromosome 25
- Two forms of gene MLPH segregate in Great Danes. D which has full pigmentation and recessive d which dilutes black to blue, and in fawn and brindles the fawn to a lighter color.
- Great Danes widely segregate for D and d alleles. All blues are dominant black dogs (K^B) with dd genotype.
- Colour testing MLPH (D locus) is therefore recommended to ensure that blue dogs are produced where desired. Avoiding blue brindles and blue fawns.

Colour Gene Testing. Extension or E Locus

- Technically called Melanocortin 1 Receptor gene (MC1R).
- Located on canine chromosome 5
- Two forms of gene MLPH segregate in Great Danes. E^m which is dominant and causes masking expressed on Fawns and Brindles. e a recessive allele results in no masks in Brindle and fawn with ee genotype.
- Great Danes widely segregate for E^m and e alleles. Many Great Danes from the Black, Blue, Harlequin, Mantle and Merle families are ee or E^me genotypes.
- Colour testing MC1R (E locus) is therefore recommended in Brindles and Fawn to prevent mask-less progeny.

Colour Gene Testing. Harlequin or H Locus

- Technically called Proteasome B7 (PSMB7).
- Located on canine chromosome 9
- Two forms of gene PSMB7 segregate in Great Danes. H which is dominant and in Merle (Mm) causes Harlequin, in homozygous Merle (MM) causes an almost all White. h is a recessive allele with no effects on coat color.
- Great Danes widely segregate for H and h alleles. HH genotypes are in utero lethal.
- Colour testing PSMB7 (H locus) is recommended in Mantles.

Colour Gene Testing. K Locus

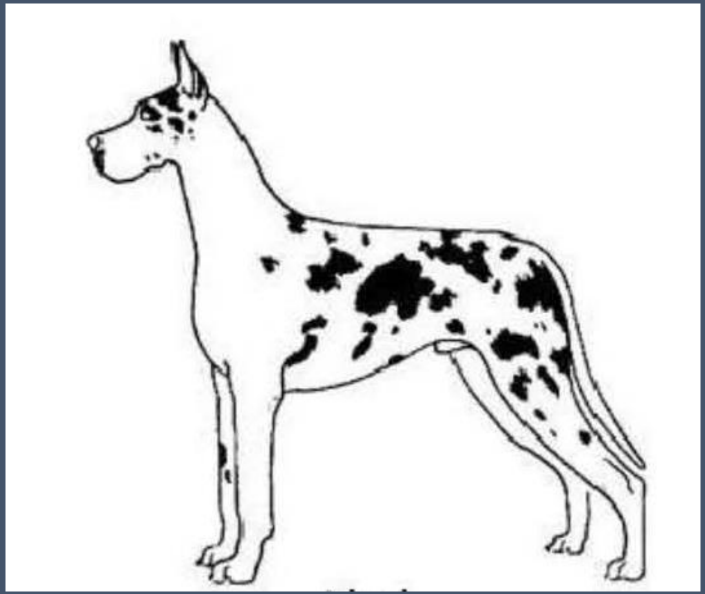
- Technically called Canine Beta Defensin 103 (CBD103).
- Located on canine chromosome 16
- Three forms of CBD103 gene segregate in Great Danes. K^{B} which is dominant black, K^{br} is brindle and it is dominant to fawn k^{y} , both K^{br} and k^{y} are recessive to black .
- Great Danes widely segregate for all three alleles. So a black can carry fawn or brindle but not both. K^{B} K^{br} is a black that carries brindle and K^{B} k^{y} is a black that carries fawn.
- Colour testing CBD103 (K locus) commercially only offer results in K^{B} and k^{y} alleles.

Colour Gene Testing. Merle or M Locus

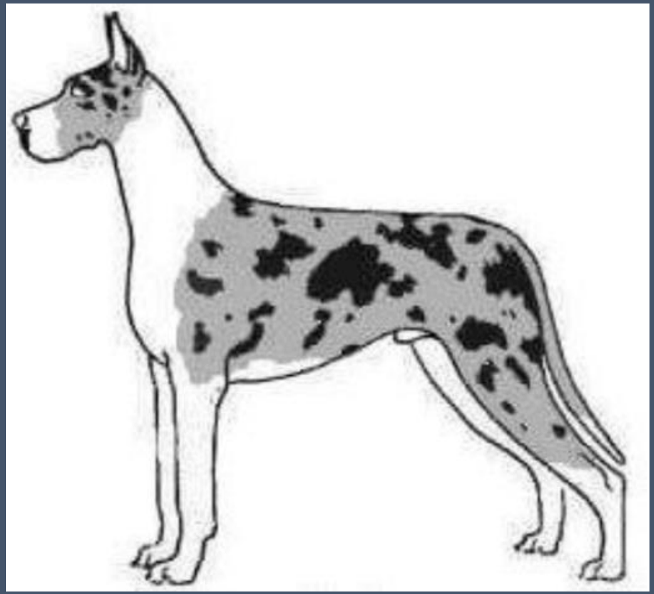
- Technically called Premelanosome protein (PMEL). Used to be (SILV)
- Located on canine chromosome 10
- Two forms of gene PMEL segregate in Great Danes. M which is dominant and results in Merle when heterozygous(Mm). Merlequin is a homozygous merle (MM) which does not carry H. m is a recessive allele with no effects on coat color.
- Great Danes widely segregate for M and m alleles. Variation in the A tail of merle gene length causes differences in expression.
- Colour testing PMEL is recommended in Harlequins, Mantles and Merles at Labs where they report the base pair numbers to indicate gene expression.

Harlequins cannot be Bred without the Merle

Genotype: Hh Mm



Genotype: hh Mm



Colour Gene Testing. Solid or S Locus

- Technically called Microphthalmia Associated Transcription Factor (MIFT).
- Located on canine chromosome 20
- Three forms of the MIFT gene segregate in Great Danes. S which is dominant solid, s^i which is mantle (Irish Spotting), and recessive to solid and dominant over piebald s^p. s^is^i and s^is^p are both Mantle genotypes. s^ps^p is the piebald genotype.
- Great Danes widely segregate for S, s^i , and s^p alleles.
- Colour testing MIFT S and s^p alleles are commercially available. The GDCA is currently funding a study to validate an s^i test with UC Davis Vet Med Genetics Lab.

Colour Gene Testing. Ticking or T Locus

- Gene is not sequenced.
- Located on canine chromosome is unknown
- Two forms of the Ticking gene segregate in Great Danes. T which is dominant ticking, t is recessive and results in clean white areas in harlequins. .
- Great Danes widely segregate for T, and t alleles. Most Harlequin, Mantle, and Merle have been cleared of ticking.
- Colour testing not commercially developed. Outcrossing Harlequin, Mantle, and Merle to Solid colors, Black, Blue, Brindle and Fawn risk reintroduction of the Ticking allele.

Genetic Health Testing.

Known Health Disorder of Great Danes with a Genetic Test.

- Centronuclear Myopathy (CNM) is caused by a mutation in the Bridging Integrator 1 (BIN1) gene, and leads to a rapidly progressing muscle myopathy, less than 20% of affected Great Danes reach adulthood. Affected individuals are homozygous for the recessive mutation, which was discovered in Great Danes on Chromosome 2.

- Ichthyosis is caused by a mutation in Splice Acceptor Site (SLC27A4) and leads to epidermal (skin) and follicular (hair growth sites inside the skin) orthokeratosis and hyperkeratosis. “Cornification” concentrated in the skin of head and extremities like feet. Affected individuals are homozygous for the recessive mutation, which was discovered in Great Danes on Chromosome 9.

Genetic Health Testing.

Health Disorder with a Genetic Test which may help Great Danes.

- Von Willebrand's Disease type 1 (vWD) is caused by a mutation in the von Willebrand's factor (vWF) gene, which makes a blood clotting protein. Affected individuals are homozygous for the recessive mutation, which is on Chromosome 27.

- Multi-Drug Resistance 1 (MDR1) this is a mutation in the Transporter P-glycoprotein. Affected individuals are homozygous for this recessive mutation, located on canine chromosome 14, and this affects the metabolization of common drugs like Ivermectin, Loperamide, among other common drugs.

Coefficient of inbreeding (COI), calculate by pedigree, calculated by genetic testing.

Calculation by pedigree:

This method was developed by Sewell Wright. This is a probability calculation that two alleles are identical by decent from a common ancestor. So the more ancestors shared by the sire and dam and the more recently these shared ancestor(s) appear in the pedigree the higher the COI is.

This method while easily calculated has some limitation. In order to make the calculations manageable people usually looks at a 10 generation pedigree.

This method doesn’t allow for mutations. While most mutations are of no significance what so ever a small handful are either negative or positive to the animals chance of being selected for breeding.

Coefficient of inbreeding (COI), calculate by pedigree, calculated by genetic testing.

Calculation by pedigree:

Wright COI assumes all ancestors are of equal merit. They are not, some are really superior in terms of their ability to pass along genes which come closer to the breed ideal than others do.

Each individual has a unique “genetic load”. Genetic load is the number of deleterious traits carried by that individual. Looking only at recessive traits, an average individual carries three to six different, deleterious recessive alleles.

So an ancestor who was not only genetically superior for desirable traits but is also desirable because of low genetic load will be measured the same as an individual with an extremely high genetic load.

Who do you want to line breed to?

Coefficient of inbreeding (COI), calculate by pedigree, calculated by genetic testing.

Calculation by genetic testing:

This measures how much of the genome tested is homozygous in an individual. That is at each site tested what percentage are homozygous for the same allele.

From a recent ancestor in common long areas of the chromosomes can be identical.

So an ancestor who is in common, and was itself highly inbred will result in much higher percentage which are homozygous for the same allele.

Again we still don’t know if this high COI was to individual with high or low genetic load?

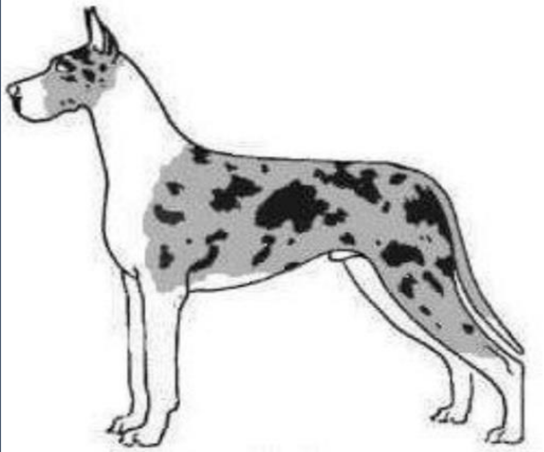
Summary.

- Genetic Testing has given us almost the complete picture of coat colour genetics in Great Danes. Let's be sensible and cross colour families only with great care and due planning for future generation.
- Remember Nellie Ennals about saved Blue Great Dane from extinction post WWII. If you doubt me run back any pedigree of blues today to their 1940's ancestors. She did this by clever mixed color breeding.
- While we have a handful of health conditions we can genetically test for today. All these conditions are rare in our breed, genetic testing for more common challenges, DCM, Wobblers, Megaesophagus are all in various stages of research.
- We have a very large healthy gene pool across the Globe. Cooperate with breeders who do the tradition health testing and publish their results, as well as the new tools of genetic testing.

Summary.

- Carriers of recessive traits, even undesirable traits, if otherwise worthy of reproduction must be used to maintain a broad healthy gene pool.
- Breeders with a non carrier for trait X, but still only want to breed to other non carriers are rapidly narrowing the gene pool. *Dangerous*
- Once you get comfortable with all the new scientific mumbo jumbo around genetic testing. You will find it a brilliant tool to help you not sweat the small stuff.
- A well balanced breeding program gathers all the information possible but one must prioritize. Make sure you go from big picture to smaller and smaller details.

Thank You



Questions?

Please.