



BELGIAN SHEPHERD
HEALTH & FITNESS PROJECT

Preliminary Analysis of Embark Dog DNA Tests Performed on 101 Registered Belgian Shepherds or Mixes

- ▶ Presented by a group of FB Belgian Shepherd Enthusiasts in “Embark Belgian Shepherds”
- ▶ Analysis by Kate Hogan, Ph.D.
- ▶ August 2018

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Interested owners of Belgian Shepherds have formed a group to use the available tools to evaluate the health & fitness of the breed

- ▶ The availability and popularity of genetic tools, like the Embark DNA test, make it possible to evaluate the health of the breed in ways not previously available
- ▶ The spread of social media expands the reach of the individual, and groups of individuals, in a way that enables studies of this sort to aspire to be truly global
- ▶ The power of these sorts of studies lies in numbers - Facebook (FB) allows for the creation of networks between people and organizations that would not normally collaborate and allows studies like this to exist outside of a more normal organizational framework and provides a platform to encourage participation
- ▶ The goal is to develop a fuller understanding of the genetic health and diversity of the Belgian Shepherd breed, as a whole, without artificial barriers created by geography or other constraints

Participation is purely voluntary and all data is based on a relatively small number of completed Embark profiles uploaded to the FB group Embark Belgian Shepherds by enthusiasts of the Belgian Shepherds

- ▶ This analysis was completed on 8/15/2018
- ▶ Based on a completed sample of 101 dogs
- ▶ All dogs are identified as their registered breed, regardless of their genetic characteristics
 - ▶ 1 genetically long-coated, AKC-registered Malinois is included in the current data set
 - ▶ 1 mixed breed dog, with some Belgian Shepherd in the mix, is included in the data set
- ▶ Participation is purely voluntary and is NOT sponsored by Embark, breed clubs, or any commercial entity
- ▶ Analysis is to the best of our abilities
- ▶ Interpretation is just that - interpretation - NOT answers
- ▶ The objective is to promote discussion and thoughtfulness
- ▶ Regular updates will be performed as more data becomes available

Participants	Number
Total	101
Belgian Sheepdog	57
Belgian Tervueren	29
Belgian Malinois	14
Belgian Laekenois	0
Mixed Breed	1
Male	40
Female	61
*Show lines	78
*Working lines	14
*Combo lines	8

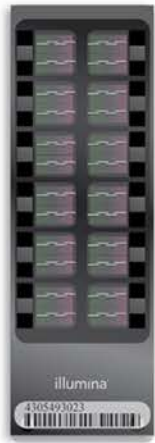
*For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

In addition to separation by coat type and color, Belgian Shepherds have long been bred for different purposes, leading to a division on the basis of pedigrees - generally referred to as show and working bloodlines

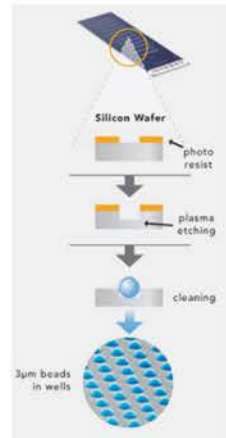
- ▶ These types of selective breeding programs have been in place since at least the 1970s, more than long enough to impact any number of genetic attributes that might be traceable using the Embark panel
- ▶ For the purposes of this investigation, dogs are determined to be either show lines, working lines or combo (a combination of show and working lines)
 - ▶ The owner may volunteer what they believe their dog to be
 - ▶ A discussion may be had by members of the Embark Belgian Shepherd FB group to evaluate a dog's pedigree when they are entering the data for individual dogs into the spreadsheet used for the analysis
 - ▶ Working line dogs are identified by the presence of working Malinois bloodlines in their pedigree
 - ▶ No effort is made to quantify the amount of show or working bloodlines in any individual animal; it is based on perception - if it seems to be primarily working lines - it is so classified; if it seems to be primarily show lines - it is so classified; if it seems to be a combination - it is so classified
- ▶ This analysis is simply exploratory, with an eye to evaluating the entire breed of Belgian Shepherds in as thorough a way as possible

Embark Testing - Basic Methodology of SNP Testing

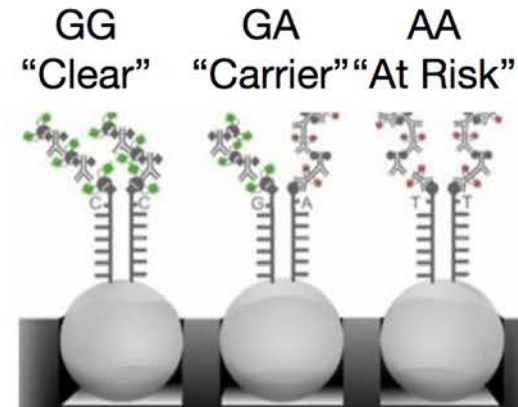
The Embark SNP chip is a custom DNA microarray that queries hundreds of thousands of unique genetic markers across the dog genome.



A SNP chip fits in the palm of your hand and can host millions of DNA sequences. How?



The etched surface of the chip has tiny beads with tethered DNA sequences (probes). Probes are designed to query unique genomic locations with known variants.



We then query whether a dog has one, two, or no copies of a variant. This process is repeated several times per variant, leading to greater than 99.99% accuracy for most tests.



Ancestry - Maternal & Paternal Haplotypes of Belgian Shepherds

Dogs have multiple forms of DNA: Mitochondrial DNA tells us specifically about the maternal line of our dogs

- ▶ Mitochondrial DNA (mtDNA) contains about 16,500 base pairs of DNA material all contained within the mitochondria NOT the nucleus^{1,2}
- ▶ Unlike nuclear DNA, mtDNA is found only in the mitochondria and multiple copies (100s to 1000s) may be found in each cell. There is normally only 1 to 2 copies of nuclear DNA in each cell.²
- ▶ mtDNA encodes 37 proteins, all from a single chromosome, that provide key instructions and mechanisms for providing for all the energy requirements of the dog^{1,2}
- ▶ mtDNA is normally passed, in an unchanged manner, from the female to all of her offspring, regardless of their sex. Males do NOT contribute to mtDNA of their offspring.^{1,2,3}
- ▶ Mutations occur at a higher frequency in mtDNA than in nuclear DNA making it very valuable for studying ancestry³
- ▶ The process of establishing relatedness through the use of mtDNA is frequently referred to as “Maternal Haplotype”
- ▶ 4 to 7 maternal haplotypes per breed is considered normal⁴

1. Embark Dog DNA Testing www.embarkvet.com

2. J-W Taanman, “The mitochondrial genome: structure, transcription, translation and replication”, *Biochim Biophys Acta* 1999 Feb 9;1410(2): 103-23.

3. W.M. Brown, *et al.* “Rapid evolution of animal mitochondrial DNA”, *Proc Natl Acad Sci* 1979 Apr;76(4):1967-71.

4. N. Pedersen, *et al.* “The effects of dog breed development on genetic diversity and the relative influences of performance and conformation breeding”, *J Anim Breed Genet*, 2013 130: 236-248.

The Y-chromosome DNA tells us specifically about the paternal line of our dogs

- ▶ The Y chromosome determines that a dog will be male and contains about 59 million base pairs of DNA material, which is about 2% of all the genetic material in any cell ¹
- ▶ Although, all the genes located on the Y chromosome have not been identified, most are likely to be involved in development of the male and secondary male characteristics¹
- ▶ The Y chromosome is normally passed from the male to his male offspring in an unchanged manner¹
- ▶ The process of establishing relatedness through the use of Y chromosome DNA is frequently referred to as “Paternal Haplotype”
- ▶ 1 to 2 paternal haplotypes are typically found per breed, with one dominating²

1. Embark Dog DNA Testing www.embarkvet.com

2. N. Pedersen, *et al.* “The effects of dog breed development on genetic diversity and the relative influences of performance and conformation breeding”, *J Anim Breed Genet*, 2013 130: 236-248.

So far, the story behind the male lines in the Belgian Shepherds tested is pretty simple - all have the same Paternal Haplotype, suggesting there may be a common ancestral male or small group of related male ancestors

- ▶ All males dogs tested had the same Paternal Haplotype Ha.4, regardless of variety
- ▶ Embark describes the Paternal Haplotype Ha.4 as being a member of A1b group, which it describes this way: *“For most of dog history, this haplogroup was probably quite rare. However, a couple hundred years ago it seems to have found its way into a prized male guard dog in Europe who had many offspring, including the ancestors of many European guard breeds such as Doberman Pinchers, St. Bernards, and Great Danes. Despite being rare, many of the most imposing dogs on Earth have it; strangely, so do many Pomeranians! Perhaps this explains why some Poms are so tough, acting like they're ten times their actual size! This lineage is most commonly found in working dogs, in particular guard dogs. With origins in Europe, it spread widely across other regions as Europeans took their dogs across the world.”*¹
- ▶ Of the Ha.4 haplotype specifically, Embark writes: *“Part of the A1b haplogroup, this haplotype is found in village dogs in North America and Africa. As for breeds, it occurs most frequently in Miniature Pinscher, Great Dane, and Poodle.”*¹

Participants	Paternal Ha.4
Total	40
Belgian Sheepdog	24
Belgian Tervueren	10
Belgian Malinois	6
Belgian Laekenois	0
*Show lines	32
*Working lines	6
*Combo lines	2

*For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

The story behind the female lines in the Belgian Shepherds tested, is not as simple; so far there have been 10 different Maternal Haplotypes identified in the 100 registered dogs tested

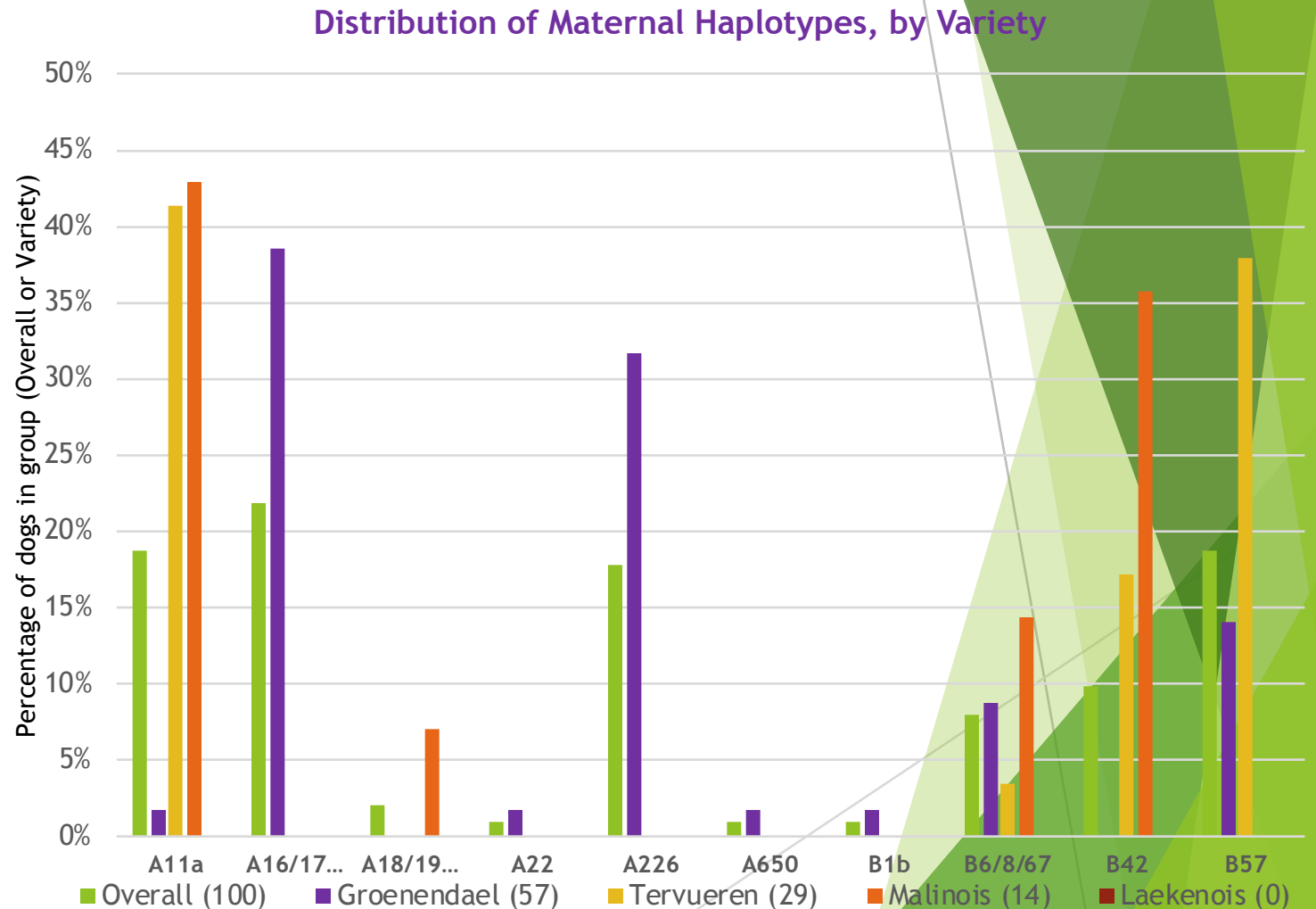
- ▶ Embark classifies the haplotypes into larger haplogroups. The larger haplogroups are likely to be branches from a common maternal line that has evolved over time. The groups containing the haplotypes identified so far are:
 - ▶ Haplogroup A1d contains: A11a¹ - 19 dogs in this group
 - ▶ Haplogroup A1a contains: A16/17/99/100¹ - 22 dogs in this group
 - ▶ Haplogroup A1b contains: A18/19/20/21/27/36/94/109¹ - 2 dogs in this group
 - ▶ Haplogroup A1e contains: A22, A226, A650¹ - a total of 20 dogs in this group
 - ▶ Haplogroup B1 contains: B1b, B6/8/67, B42 and B57¹ - a total of 37 dogs in this group
- ▶ All of these haplogroups have strong connections to central Europe and make sense for Belgian Shepherds
- ▶ The larger Haplogroups will be discussed in the appendix slides. Further focus on the Haplotypes is shown in the next few slides.

Sharon Lafuse, another member of the Embark Belgian Shepherds group, devoted some time trying to identify the most distal female that could be associated with each haplotype through a paper pedigree analysis

- ▶ The results are quite fascinating, and potentially, revealing:
 - ▶ A11a: 19 dogs, all dogs except 1 traced back to **Lionne1 c. 1916**
 - ▶ A16/17/99/100: 22 dogs, traced back to 2 different females - **Folette (Ledoux) c. 1917 (probably incorrect)**, and **Sarmen 1939**
 - ▶ A18/19/20/21/27/36/94/109: 2 dogs, untraced for now
 - ▶ A22: 1 dog, traces back to Bobine or Cora c. 1895, depending on reference
 - ▶ A226: 18 dogs, all traced back to **Mirette c. 1912**
 - ▶ A650: 1 dog, traced back to Diane, 1925
 - ▶ B1b: 1 dog, untraced for now
 - ▶ B6/8/67: 8 dogs, most inconsistencies - multiple dogs traced to **Poes**, 1 each to Sarah c. 1928, Idarina 1934, and Dora (Devray) c. 1920
 - ▶ B42: 10 dogs, all dogs except 1 traced back to **Sarah c. 1928**
 - ▶ B57: 19 dogs, all dogs except 1 traced back to **Folette (Ledoux) c. 1917**, 1 dog traces to Dora (Devray) c. 1920
- ▶ Clearly, all these results can not be correct, as a single female can NOT contribute multiple haplotypes, nor can unrelated females contribute the same haplotype
- ▶ However, the inconsistencies are almost certainly related to the history of the breed and in no way reflect the quality of the research done by Sharon

With 10 Maternal Haplotypes identified so far, we are just beginning to observe some trends that might be meaningful

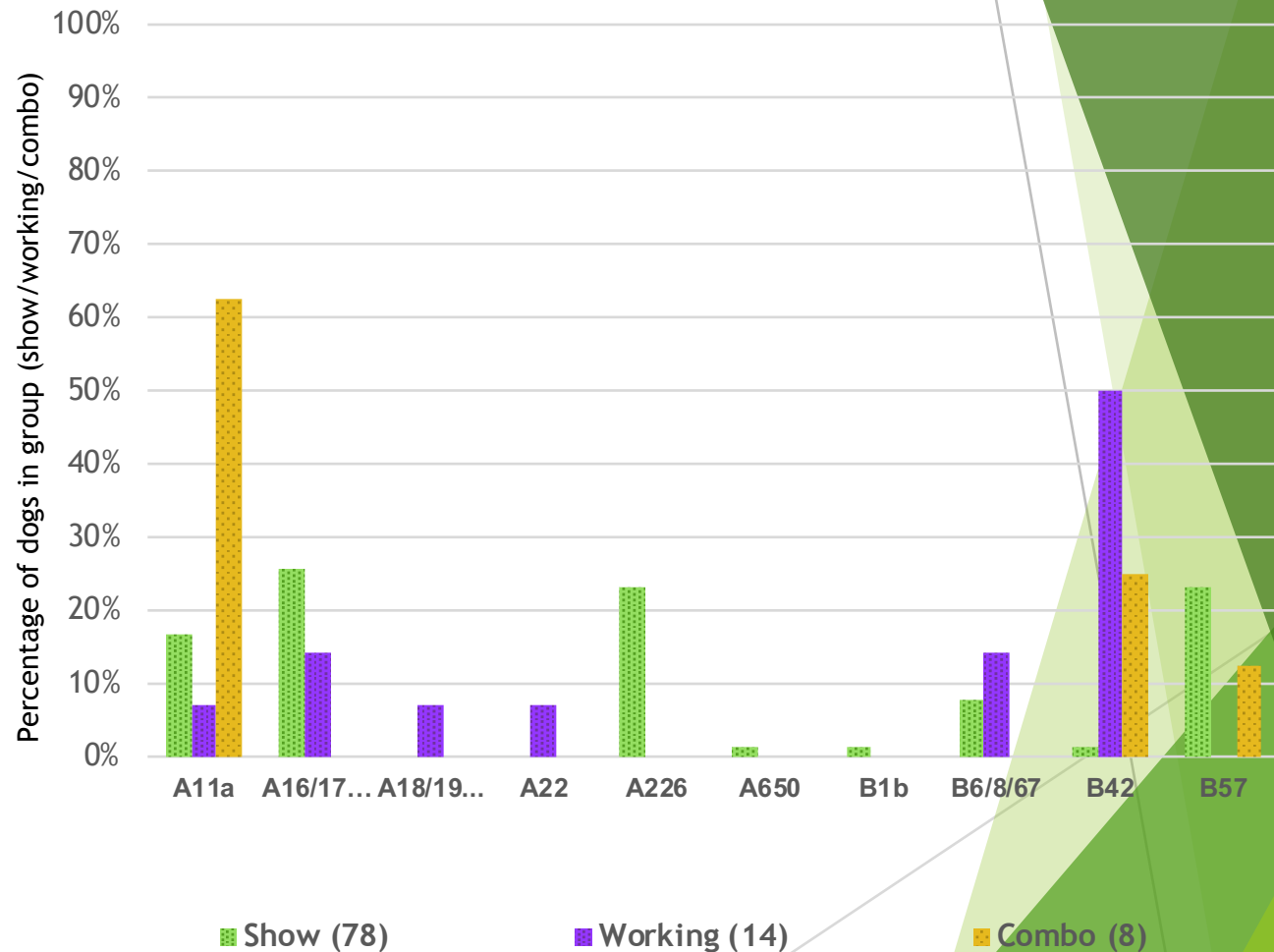
- ▶ At this time, while provocative and very interesting, there is insufficient data to draw any solid conclusions based on maternal haplotypes
- ▶ Observations:
 - ▶ About 70% of BSDs/Groenendaels occupy unique maternal haplotypes without other varieties of Belgian Shepherds, that's a bit surprising
 - ▶ A16/17/99/100 and A226 seem to be specific to Groenendaels
- ▶ It will be interesting to see how this evolves as more dogs from different countries are added



As with the primary analysis, there are some hints that might be interesting to watch as more dogs are added, particularly of working line descent

- ▶ It is still too early to draw any conclusions, but some trends to watch, based on this and the previous slide:
 - ▶ A16/17/99/100 and A226 seem to be specific to Groenendaels; A226 perhaps more specifically to show line Groenendaels, while A16/17/99/100 includes both show and working lines
 - ▶ B42 may be specific to dogs with at least some working line heritage
 - ▶ B57 may be specific to dogs with at least some show line heritage
- ▶ It will be very interesting to see how this plays out as more dogs are added, particularly dogs from other parts of the world

Maternal Haplotypes, by Bloodlines



*For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

- ▶ **Maternal Haplotypes:** It seems like there are multiple, potential interpretations for 10, or more as more data comes in
 - ▶ There may have always been more diversity in terms of the female founders of the breed, which are relatively less well known than the males
 - ▶ Efforts to restore the breed after WWI & WWII may have relied upon different types of females than had been available before the war
 - ▶ Cross-breeding at any time during the history of the breed
- ▶ **Paternal Haplotypes:** **FASCINATING** that all male dogs share the same haplotype, so far
 - ▶ Supports the history of the breed going back to a limited number of male dogs from a specific geographic region
- ▶ **Questions:**
 - ▶ How many maternal/paternal haplotypes are there, across all breeds?
 - ▶ Is it easier to lose a paternal haplotype than maternal haplotype, since the paternal haplotype only exists in male dogs?
 - ▶ Do other herding breeds from this region of the world, Bouvier, Dutch Shepherds, Picards share the same maternal and/or paternal haplotypes?
- ▶ **These are among the most thought-provoking findings and will definitely provide fuel for many interesting conversations, especially over a drink with friends and fellow enthusiasts of Belgian Shepherds**

Ancestry - Maternal & Paternal Haplotypes: Conjecture, Speculation, & Questions



Coefficient of Inbreeding (COI)

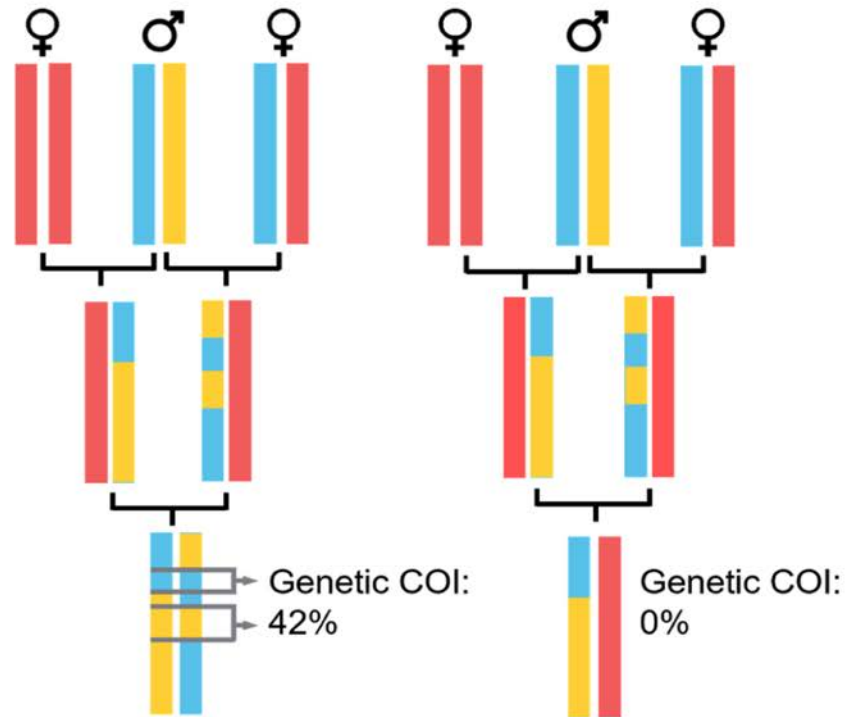
COI is one of the standard approaches to pedigree analysis and breeding decisions by many breeders - the ability to evaluate COI on the basis of an individual's specific DNA represents a significant paradigm shift

- ▶ Until recently COI analysis was a calculation performed on a paper pedigree; as such it has some drawbacks
 - ▶ Requires complete pedigree information for as many generations as possible
 - ▶ Is only as good as the information it is based upon - incomplete or falsified pedigrees invalidate the calculation
 - ▶ Assumes that all littermates are equivalent - does not allow for normal variation known to occur in mammalian reproduction, particularly those species that produce multiple offspring in a litter
- ▶ We will, inevitably, come to appreciate the drawbacks associated with a whole DNA scan for COI, but for now it seems to be primarily beneficial - addresses the 3 issues above
- ▶ Embark utilizes the genome-wide COI method; their markers are distributed across all 38 chromosomes and cover more than 1 million base pairs¹
- ▶ For a general primer on COI and the consequences of inbreeding, see Carol Beuchat's article²

1. Oedipus Rex: Dog Inbreeding, its consequences and its quantification, Embark, <https://embarkvet.com/oedipus-rex-inbreeding-its-consequences-and-its-quantification/>
2. COI FAQs: Understanding the Coefficient of Inbreeding, The Institute of Canine Biology, <http://www.instituteofcaninebiology.org/blog/coi-faqs-understanding-the-coefficient-of-inbreeding>

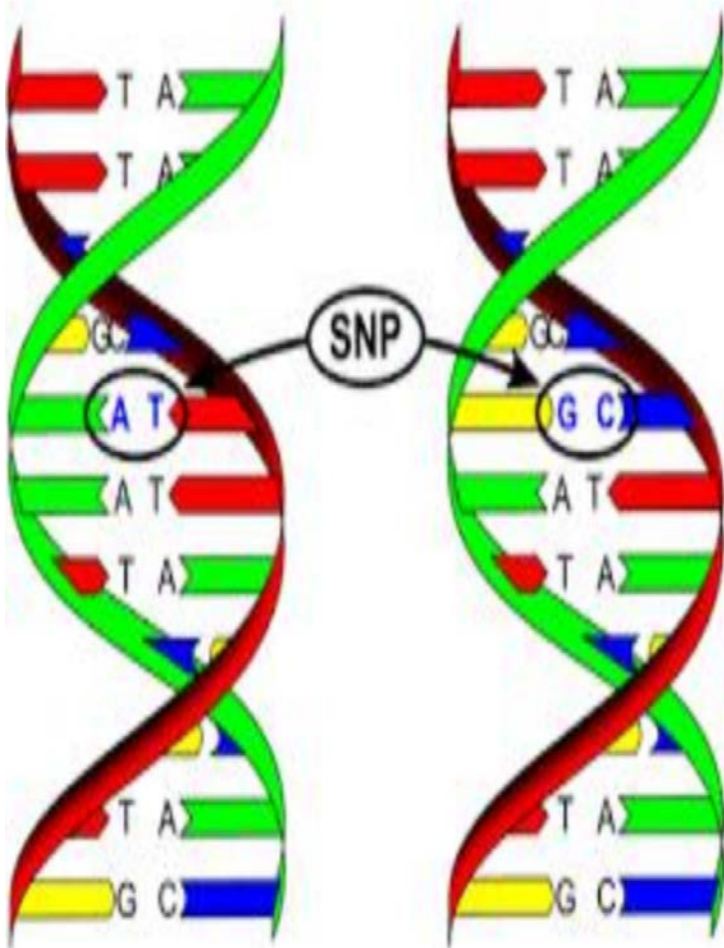
Below is an example showing how 2 dogs from the same parents could have significantly different DNA-based COI

Inbreeding | Pedigree vs. Genetic COI



Let us consider instead using the DNA of an individual to assay genetic COI. Here, we simply assay how related a dog's parents are based on how similar their chromosomes are. If a pedigree is somewhat outbred (which we've delineated here by coloring the chromosomes different colors), we can see that **just by sheer luck, two individuals might have extremely different COIs, but have identical pedigrees.**

Genome-wide association scans using SNP probes, like Embark's, determine COI based on a metric called "runs of homozygosity"



- ▶ SNPs (single nucleotide polymorphisms) occur with some degree of frequency throughout the dog genome and have been used to generate a map of the entire dog genome^{1,2,3}
- ▶ Probes have been developed to interact with the SNPs that may or may not be present within an individual's DNA in a manner that can be measured allowing the individual's genotype to be mapped without reading each individual base pair of DNA^{1,2,3}
- ▶ The Embark panel uses over 200,000 markers which interact with about 8% of any individual dog's DNA⁴
- ▶ Runs of homozygosity are stretches of DNA that show the same pattern of interaction with SNP markers for each of the parental chromosomes (or other stretch of DNA) - suggesting that the individual has inherited identical haplotypes from each parent in that region^{1,2,3}
- ▶ The longer these runs are, the greater the degree of genetic relatedness, or inbreeding, the individual has^{1,2,3}

1. A.K. Wong, *et al.*; "A Comprehensive Linkage Map of the Dog Genome", *Genetics*, February 1, 2010 vol. 184(2):595-605

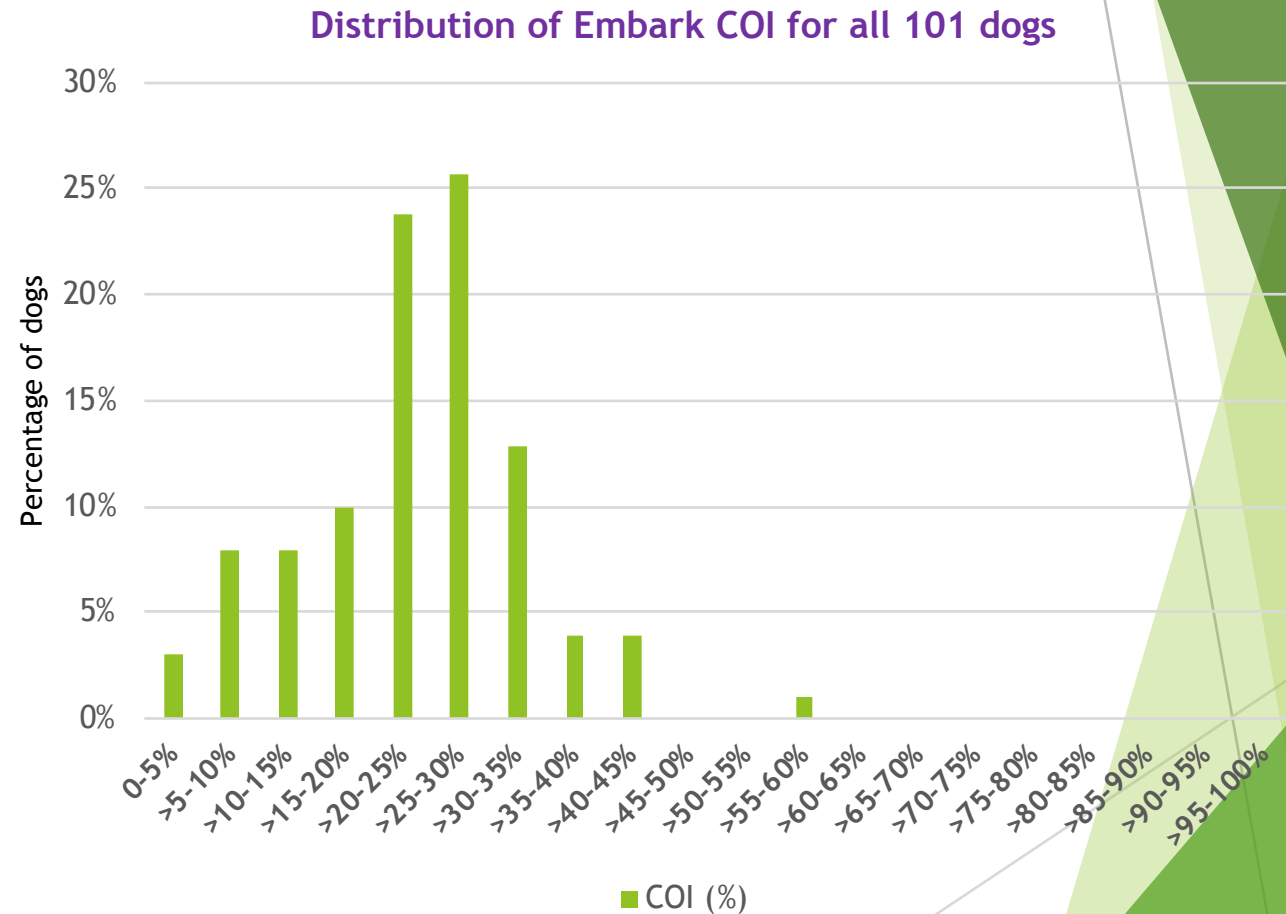
2. D.P. Howrigan, *et al.*; "Detecting autozygosity through runs of homozygosity: A comparison of three autozygosity detection algorithms", *BMC Genomics* 2011, 12:460

3. F.C. Ceballos, *et al.*; "Assessing runs of Homozygosity: a comparison of SNP Array and whole genome sequence low coverage data", *BMC Genomics* 2018; 19:106

4. Embark Dog DNA Testing www.embarkvet.com

Embark measures the Coefficient of Inbreeding (COI) by measuring runs of homozygosity, providing a different result than that based on pedigree analysis

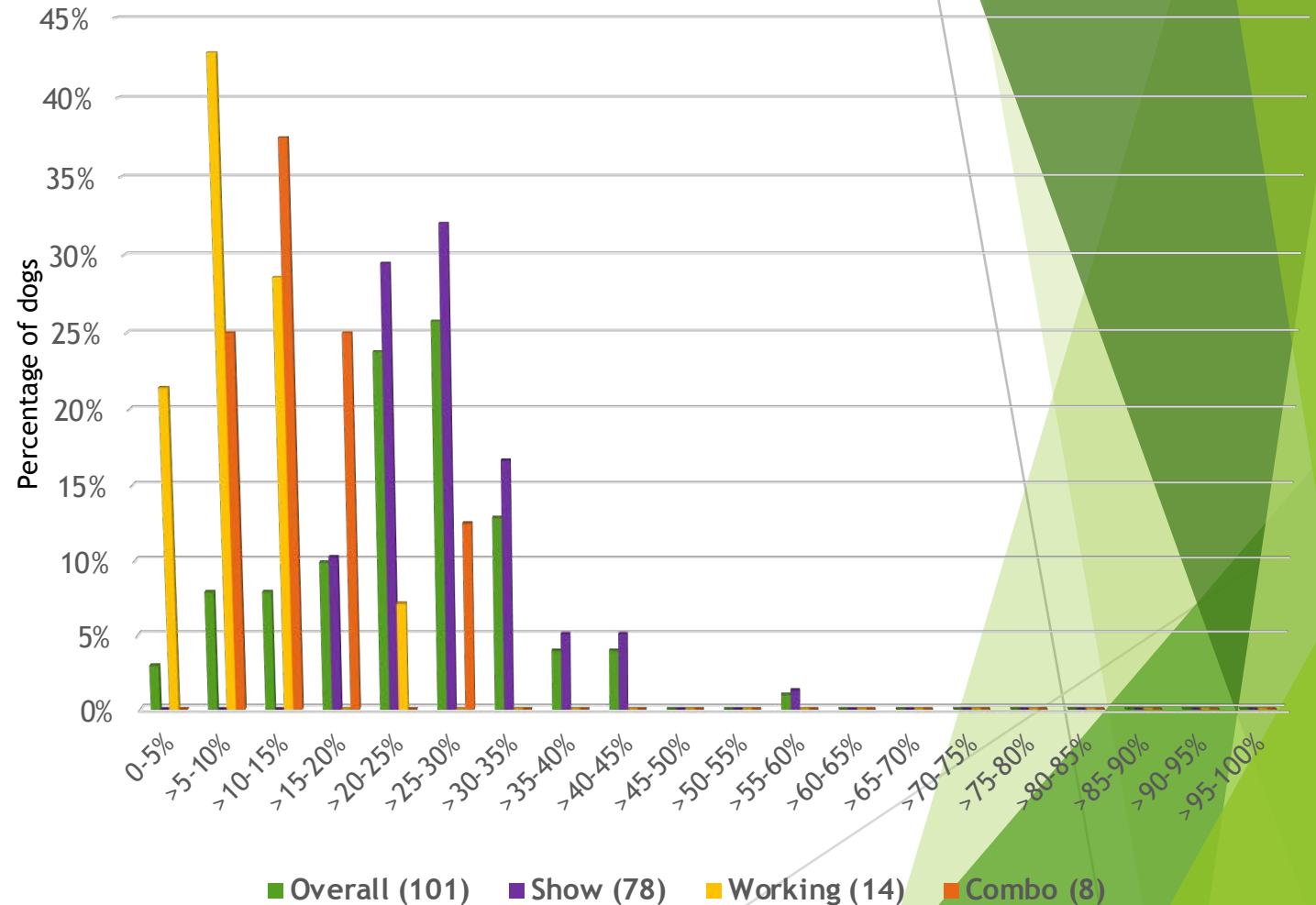
- ▶ This represents a 1st attempt to look at DNA-based COI of all Belgian Shepherds, regardless of official breed, in the US
- ▶ Includes 1 mixed breed dog with Belgian Shepherd in Embark breed results
- ▶ The limited number of data points make it unclear how to interpret the distribution at this time
 - ▶ It may represent a single, normal distribution with insufficient data, with an average somewhere in the 25% range
 - ▶ It may also represent a bi-modal distribution, again with insufficient data, with averages somewhere in the 10% and 25% range
- ▶ Hopefully, we can continue to collect data to further this type of analysis



Dividing the data into groups based on bloodlines shows some very interesting trends - the most obvious separation, when viewing the raw data, seems to fall along bloodlines

- ▶ Though there is not yet enough data to draw any conclusions, the separation by bloodlines produces a good fit to the data
- ▶ Currently:
 - ▶ There is not a single show line dog with a DNA-based COI of less than 15%
 - ▶ Greater than 90% of working line dogs have a COI of 15% or less
 - ▶ The dogs with a combination of both show and working bloodlines fall between the distribution of show and working lines, some skewed more toward the show line distribution and others more toward the working line distribution
- ▶ There is an additional view of this slide, that does not include the combo group in the Appendix
- ▶ With only 14 working line dogs and 8 combination line dogs in the data set, I expect this situation to change, but it will be interesting to watch ...

Distribution of Embark COI by Bloodlines



*For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

Note: Mixed breed dog included in overall sample, but NOT in sub-groups

While there are insufficient data to draw any conclusions at this time, analysis of this type will help to determine whether there are any significant differences in COI across the Belgian Shepherds

- ▶ At this time, while provocative and very interesting, there is insufficient data to draw any conclusions based on COI
- ▶ Data will continue to be analyzed in total and in a segmented manner to evaluate any differences within the breed

Participants	COI (%)		
	Minimum	Maximum	Average
Total (N=101)	4%	58%	24.4%
Belgian Sheepdog (N=57)	4%	58%	27.1%
Belgian Tervueren (N=29)	9%	40%	23.8%
Belgian Malinois (N=14)	5%	26%	13.7%
Belgian Laekenois (N=0)	N/A	N/A	N/A
Mixed Breed (N=1)	11%	11%	11%
*Show lines (N=78)	16%	58%	28.0%
*Working lines (N=14)	4%	21%	9.8%
*Combo lines (N=8)	9%	26%	15.0%

*For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

- ▶ The basic hypothesis going into this is that all the varieties will be inbred to a similar degree, though, perhaps, on different dogs/lines
 - ▶ Any variations from that may ultimately be reflected in differences in the overall health and fitness of that sub-population of Belgian Shepherds, see Beuchat, COI FAQs¹
 - ▶ Such observations might prompt breeders to make different breeding decisions
- ▶ Every breeder has their own criteria for breeding; the issue is whether enough are breeding for similar traits to in any way skew statistics like COI in a sub-population of dogs
- ▶ **Questions:**
 - ▶ Do we know whether COI numbers tend to be higher or lower when done by DNA analysis as compared to traditional pedigree analysis?
 - ▶ Can we get 'normal' numbers and distributions for DNA-based COI, using the Embark system, for other breeds as a comparison?

Coefficient of Inbreeding: Conjecture, Speculation, and Questions



Belgian Shepherd Breed Traits Genetics - Coat, Color, Size, etc.

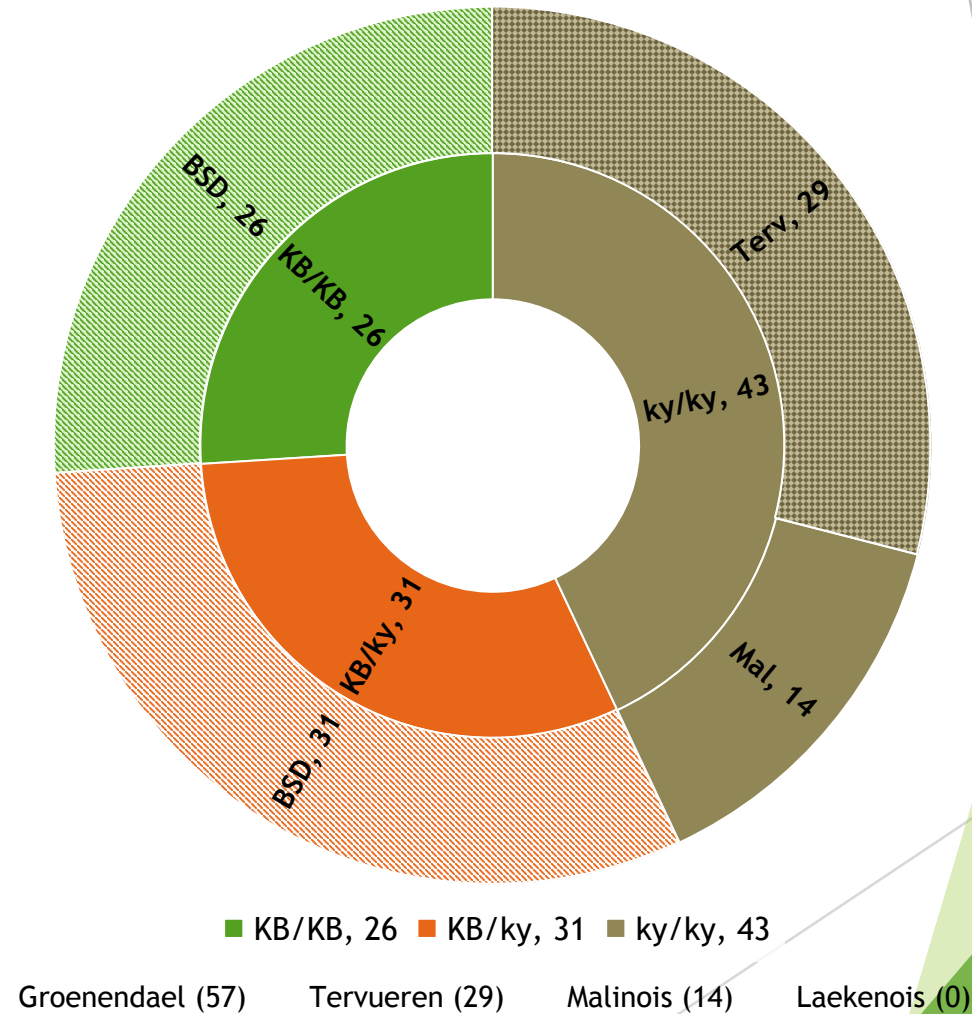
Like all purebred breeds of dogs, Belgian Shepherds have a characteristic ‘look’ that is the result of numerous genetic traits, several of which are included in the Embark panel

- ▶ K-locus: Gives rise to the dominant black coat that is desired by breeders of Groenendaels/Belgian Sheepdogs¹
- ▶ E-locus: Controls the melanistic mask characteristic of the Belgian Shepherds¹
- ▶ A-locus: Determines whether the color of the hair shaft is banded or solid¹
- ▶ Other color genes¹:
 - ▶ Dilute
 - ▶ Brown
- ▶ Coat type¹:
 - ▶ Long vs. short coat
 - ▶ Curly coat
 - ▶ Furnishings
 - ▶ Shedding
- ▶ Several genes contributing to size are included in the Embark panel¹

The K-Locus is particularly important within the Belgian Sheepdog population - the Belgian Sheepdogs included so far are relatively evenly split between heterozygous and homozygous at the dominant black locus

- ▶ There is insufficient data to draw any conclusions based on K-locus genetics
- ▶ Observations:
 - ▶ Among the Belgian Sheepdogs, there are 5 more dogs that are KB/ky than KB/KB, at this time
 - ▶ The mixed breed dog included in the sample group is ky/ky
- ▶ Fascinating data, but we need more dogs

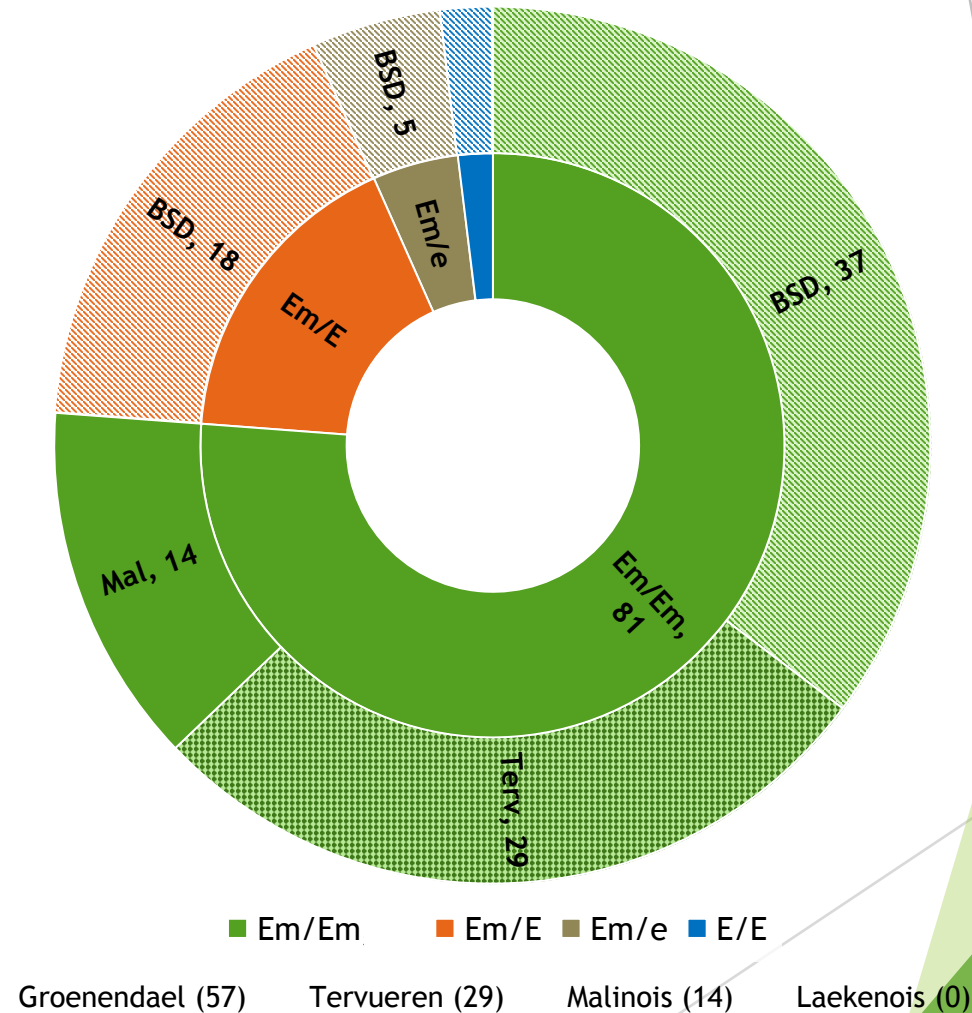
Distribution of Alleles at the K Locus, by Variety



One of the most recognizable traits of Belgian Shepherds is their mask, determined primarily by inheritance at the E locus

- ▶ There is insufficient data to draw any conclusions based on masking genetics
- ▶ In some cases, Embark was unable to determine among the possible genotypes for a specific dog, so there are more results reported than dogs tested
- ▶ Observations:
 - ▶ All Tervueren and Malinois tested, so far, are homozygous for the melanistic mask, all other genotypes were found only in the Groenendael
 - ▶ More than 60% of Groenendaels tested, so far, are homozygous for the melanistic mask
 - ▶ Only 2 dogs, both Groenendael, do not carry an allele for the melanistic mask, both are homozygous E/E genotype
 - ▶ There were no dogs with the *Eg/Eg*, *Eg/E*, *Eg/e*, *E/e*, or *e/e* genotypes detected
- ▶ Fascinating data, but we need more dogs with such a wide variety of available genetic options

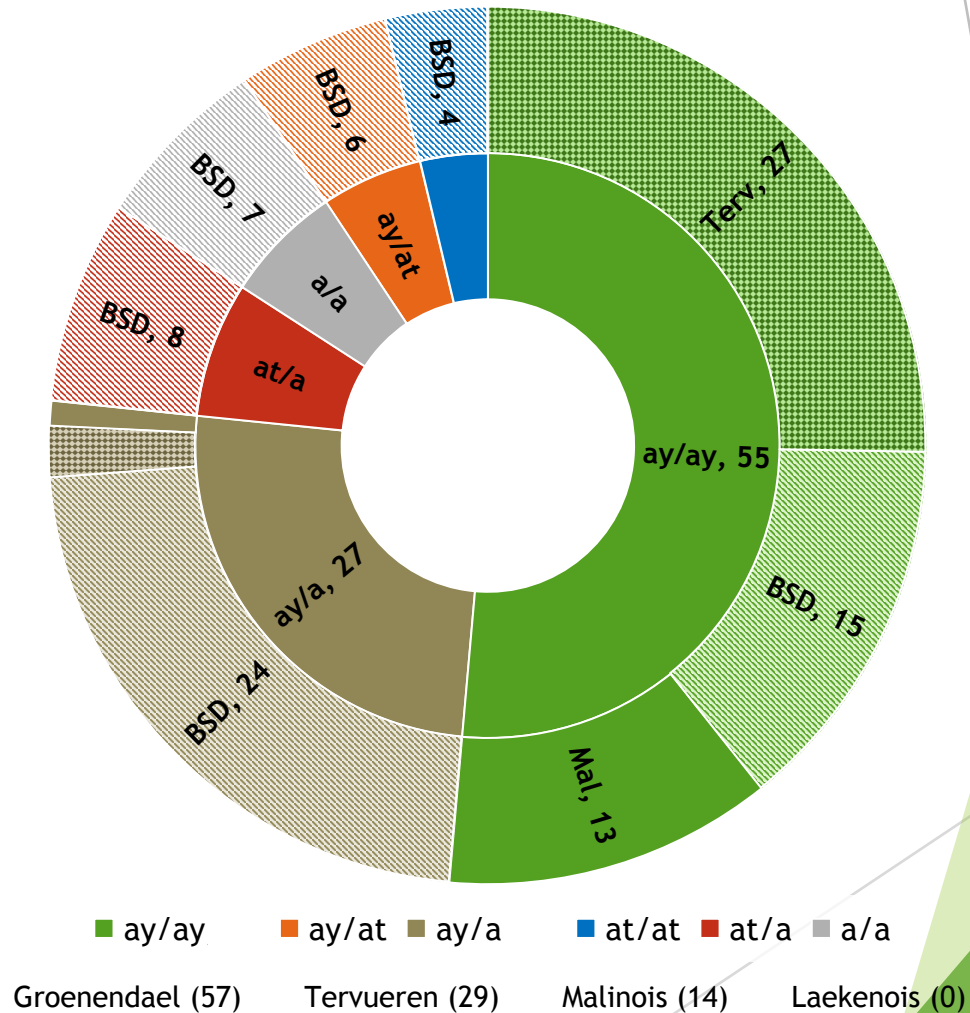
Distribution of Alleles at the E Locus, by Variety



Another recognizable trait of Belgian Shepherds, particularly of the Malinois and Tervueren, is the color pattern determined primarily by inheritance at the A (Agouti) locus

- ▶ There is insufficient data to draw any conclusions based on A-locus genetics
- ▶ In some cases, Embark was unable to determine among the possible genotypes for a specific dog, so there are more options reported than dogs tested
- ▶ Observations:
 - ▶ The majority of dogs are homozygous *ay/ay*, sable
 - ▶ There is a significant group of dogs (12/19) that carry at least 1 copy of *a*, the recessive black allele, including 2 Tervuerens and 1 Malinois that are *ay/a*
 - ▶ The *aw*, wolf sable, allele was not identified in any of the dogs tested
- ▶ Fascinating data, but we need more dogs with such a wide variety of available genetic options

Distribution of Alleles at the Agouti Locus, by Variety



Other coat and color genes were tested in all 101 dogs

- ▶ Additional tests color traits indicated¹:
 - ▶ Only 5 of the dogs tested carry the dilute allele at the D-locus, one Tervueren, 3 Malinois, and the mixed breed dog
 - ▶ None of the dogs tested carry the brown allele at the B-locus
- ▶ Additional tests for coat characteristics revealed¹:
 - ▶ 87 of 101 dogs tested are homozygous for the FGF5, long-coat gene, 3 were heterozygous, and 6 were homozygous for the short coat allele
 - ▶ 3 of the dogs tested carry the KRT71, curly-coat gene - 1 BSD/Groenendael and 2 Malinois
 - ▶ 10 dogs are heterozygous for the MC5R gene, associated with reduced shedding
 - ▶ None of the dogs tested carry the RSPO2, furnishings gene
- ▶ Other traits tested, related to miscellaneous characteristics¹
 - ▶ 26 of the dogs are heterozygous and 2 are homozygous at LMBR1, the gene for hind dew claws
 - ▶ None of the dogs tested carry the EPAS1, altitude adaptation gene
- ▶ The Embark panel also tests for 5 different genes associated with smaller size¹
 - ▶ IGF1 gene - 2 of the dogs tested are heterozygous, 1 is homozygous for the small allele
 - ▶ IGF1R gene - None of the dogs tested carry the small allele
 - ▶ STC2 gene - 12 of the dogs tested are heterozygous for the small allele, and 1 dog is homozygous for the small allele
 - ▶ GHR (E195K) - 12 of the dogs tested are heterozygous for the small allele, and 3 dogs are homozygous for the small allele
 - ▶ GHR (P177L) - None of the dogs tested carry the small allele

- ▶ This is just interesting, basic genetics; the more data we can get, the better
- ▶ **Questions:**
 - ▶ Why do some dogs come up with multiple possibilities on the A-locus (Agouti) and E-locus genotyping?
 - ▶ Wouldn't it be fun to actually measure the heights of the dogs to see which of the "small" genes really do play a significant role in Belgian Shepherds?
 - ▶ Would love to be able to do all sorts of subgroup analysis on this, by
 - ▶ Geography
 - ▶ Bloodlines
 - ▶ Inter-variety breedings
 - ▶ Multi-variety heritage
 - ▶ Etc.
- ▶ Just need more data to add to all of this interesting stuff ...

Belgian Shepherd Traits: Conjecture, Speculation, & Questions



Belgian Shepherd Health & Disease Genetics

Alanine Aminotransferase (ALT) enzyme activity levels are used as an indicator of overall liver health; Embark can identify 3 different genotypes

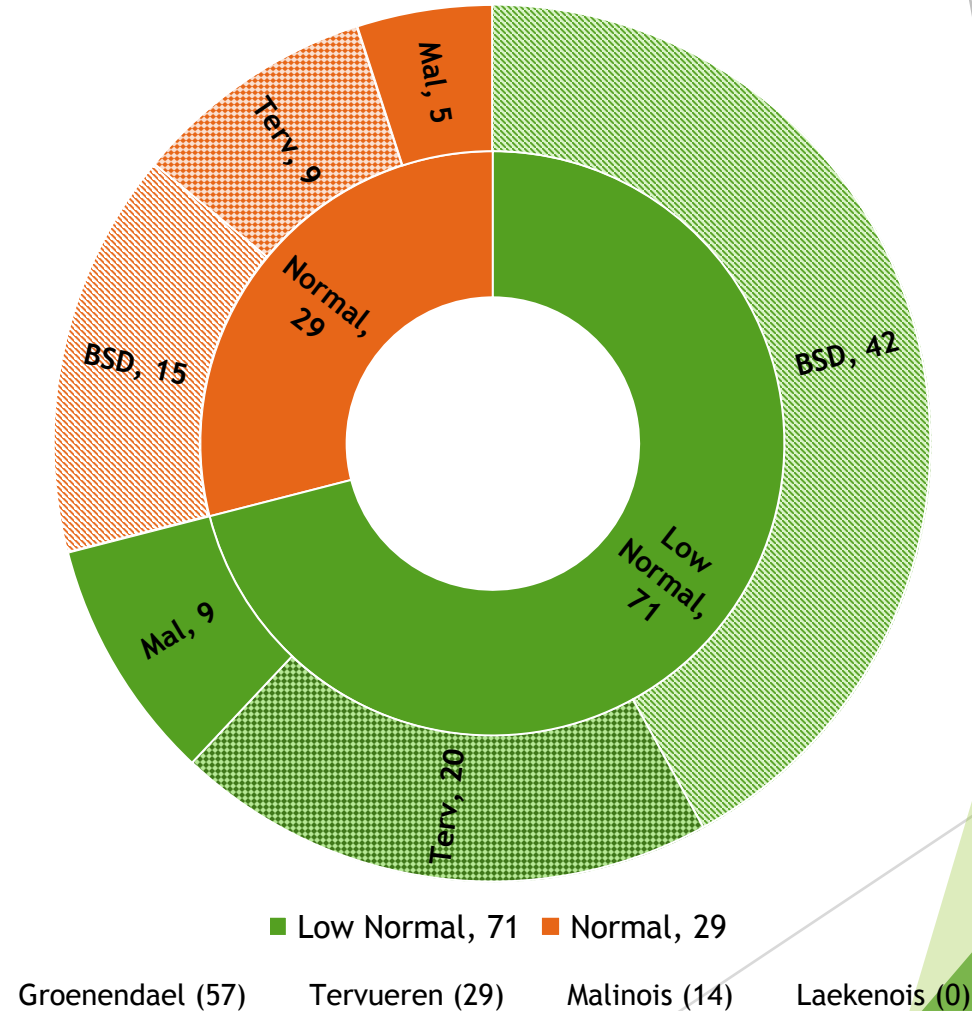
- ▶ Known to be highly expressed in liver cells, activity levels of alanine aminotransferase, or ALT, is a common value on most blood chemistry panels and is known to be a sensitive measure of liver health.
- ▶ Genotypic variation is associated with different clinical activity levels
 - ▶ Dogs with two ancestral G alleles show "normal" activity
 - ▶ Dogs that have one or two copies of the derived A allele may have lower resting levels of ALT activity, known as "low normal"
 - ▶ These dogs may show low levels of ALT activity even during when the dog is suffering from liver disease
- ▶ Dogs with "low normal" activity levels should be brought to the attention of your veterinarian. Then when a blood chemistry panel is being interpreted the values that you and your veterinarian consider "normal" may need to be adjusted.
- ▶ Neither a "normal" nor a "low normal" result for this predicts a disease state or increased risk for liver disease. This mutation does not associate with increased levels of ALT: If your dog has high ALT levels, please consult your veterinarian.

Over 70% of reported Belgians show the 'Low Normal' genotypes for Alanine Aminotransferase - this could be an important finding for the breed, as well as for the individual dog

► Observations:

- Over 70% of the dogs tested, regardless of variety or bloodlines, carry at least 1 allele of the form of ALT, which results in low normal clinical activity levels
 - Dogs that have 1 or both alleles showing the A allele may show a "Low Normal" phenotype
 - This does NOT represent a risk to the dog but may encourage your veterinarian to evaluate your dog's liver enzyme levels on a different scale
- At present, the sub-group combination lines, consisting of 8 dogs, does show greater than 50% of its members in the Normal range
- Equally interesting, at this time, the majority of both show and working lines exhibit this genotype, but combination lines are more likely to have 2 alleles coding for normal ALT activity levels; again likely to be a result of relatively small numbers for some sub-groups of dogs
- This could be an important finding for the breed, as a whole, if the trend continues

Distribution of ALT Activity, by Variety



The Major Histocompatibility Complex (MHC), aka Dog Leukocyte Antigen (DLA) in dogs, plays a critical role in the regulation of immune responses

- ▶ Comprised of a tightly linked cluster of genes primarily on chromosome 12 that are highly polymorphic. Normally, when we think of genes, we tend to think of genes with 2 alleles, 1 dominant and 1 recessive. The DLA genes have multiple alleles.
 - ▶ There have been 4 Class I genes identified with over 75 alleles between them
 - ▶ There have been 4 Class II genes identified with over 200 alleles between them
- ▶ Though there are a huge number of possible combinations of the DLA complexes, in reality, most breeds have 4 or 5 frequent haplotypes (combination of specific alleles) with perhaps a few others that are less frequent. The specific haplotypes vary by breeds, though some are more common across breeds.
- ▶ Certain DLA haplotypes have been associated with a number of different conditions, including:
 - ▶ Diabetes
 - ▶ Hypothyroid disease
 - ▶ Immune-mediated hemolytic anemia
 - ▶ Vaccine reactions, particularly to rabies

1. J.L. Wagner; "Molecular Organization of the Canine Major Histocompatibility Complex", *Journal of Heredity*, Volume 94, Issue 1, 1 January 2003, Pages 23-26
2. L. Kennedy; "Major Histocompatibility Complex Diversity in Dogs & Disease Associations", Tufts' Canine & Feline Breeding & Genetics Conference, 2009
3. Janeway CA Jr, Travers P, Walport M, et al. "The major histocompatibility complex and its functions", in *Immunobiology: The Immune System in Health and Disease*. 5th edition. New York: Garland Science; 2001.

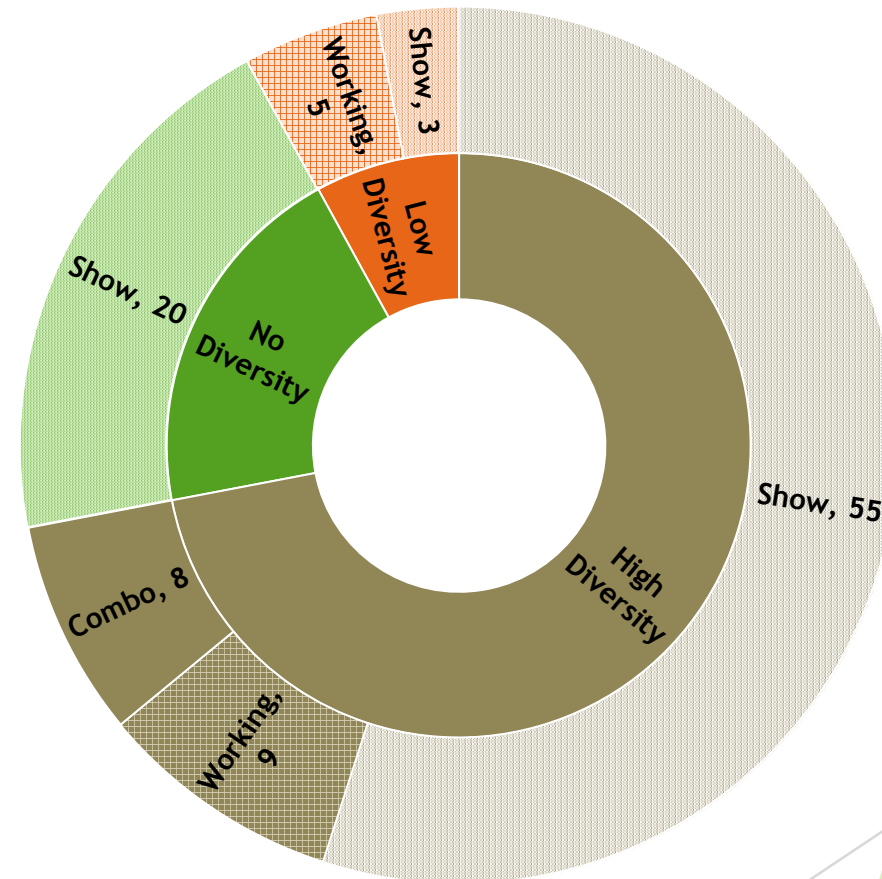
The DLA haplotypes previously identified, and in some cases associated with disease, tend to make use of a technology that is inconsistent with Embark's SNP panel

- ▶ Embark has resolved this by evaluating the diversity of the DLA regions associated with 3 specific gene products: DLA-DRB complex and DLA-DQA1 and DQB1
- ▶ Definitions¹:
 - ▶ High Diversity - the dog has inherited highly dissimilar DLA haplotypes from its parents
 - ▶ Low Diversity - the dog has inherited similar DLA haplotypes from its parents
 - ▶ No Diversity - the dog has inherited identical DLA haplotypes from its parents
- ▶ While this is somewhat less useful than actual haplotype identification would be, it still provides useable information to help guide breeding decisions
 - ▶ For example, if a bitch had No Diversity in either of her DLA regions breeding her to a male with High Diversity provides at least a 50% probability that the offspring would have higher diversity than their mother
- ▶ In the ideal world, Embark will continue to do the research necessary to be able to identify the major DLA haplotypes using their SNP panel

Nearly 30% of all Belgians tested showed No to Low Diversity in their DLA-DRB1 complex - this could be an important finding for potential autoimmune conditions like hypothyroiditis

- ▶ There are at least 61 different alleles of the DLA-DRB complex²
- ▶ A lack of diversity in the MHC DLA-DRB1 has been associated with Cushing's Disease in some studies¹
- ▶ Observations:
 - ▶ About 1/3 of all the dogs tested had No or Low Diversity in their DLA-DRB1 haplotype
 - ▶ All the dogs from combination bloodlines had high diversity at this DLA region
- ▶ Could this lack of diversity, in a region specifically designed to be diverse, be related to some of the immune conditions reported in Belgian Shepherds?

Distribution of Diversity level at DLA-DRB1 Complex, by Bloodlines



■ Diversity ■ Low Diversity ■ High Diversity

Groenendael (57) Tervueren (29) Malinois (14) Laekenois (0)

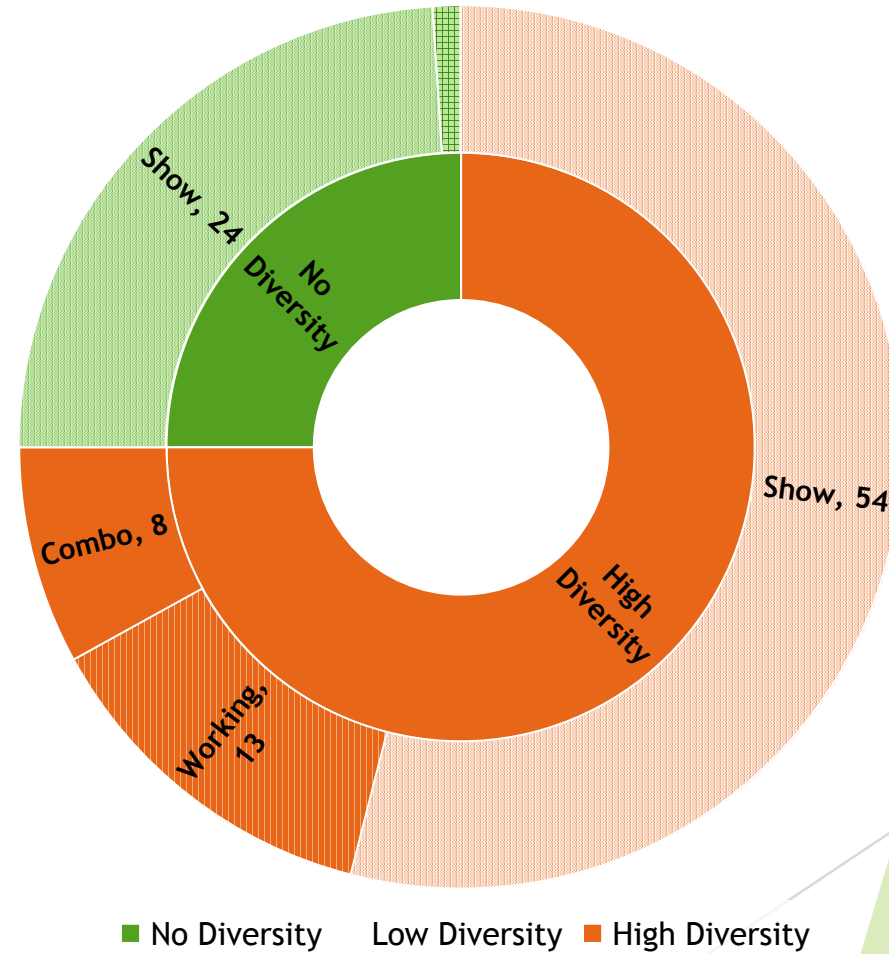
1. Embark Dog DNA Testing www.embarkvet.com

2. J.L. Wagner; "Molecular Organization of the Canine Major Histocompatibility Complex", *Journal of Heredity*, Volume 94, Issue 1, 1 January 2003, Pages 23-26

Reduced diversity in the DLA-DQA1 & DQB1 region may be tied to some autoimmune diseases

- ▶ There are at least 65 different alleles of the DLA-DQA1 & DQB1 complex²
- ▶ A lack of diversity in the MHC DLA-DRB1 has been associated with autoimmune conditions in some studies¹
- ▶ Observations:
 - ▶ 24 of all the show line and 1 of the working line dogs tested had No Diversity in their DLA-DQA1 & DQB1 haplotype
 - ▶ No dogs had low diversity in this region - it was either high or no - does that mean anything?
 - ▶ The combination line dogs all had High Diversity in this DLA
- ▶ Could this lack of diversity, in a region specifically designed to be diverse, be related to some of the immune conditions reported in Belgian Shepherds?

Distribution of Diversity level at DLA-DQA1 & DQB1 Complex, by Bloodlines



Groenendael (57) Tervueren (29) Malinois (14) Laekenois (0)

1. Embark Dog DNA Testing www.embarkvet.com

2. J.L. Wagner; "Molecular Organization of the Canine Major Histocompatibility Complex", *Journal of Heredity*, Volume 94, Issue 1, 1 January 2003, Pages 23-26

Mutations associated with 3 different conditions have been identified in a small number of the Belgian Shepherds tested, so far

- ▶ 7 dogs have been identified that carry the PDK4¹ mutation associated with dilated cardiomyopathy (DCM) in Doberman Pinschers - 5 Groenendael and 2 Tervueren
- ▶ Several of the dogs are related
- ▶ The PDK4 mutation is found in breeds other than Dobermans and is not associated with DCM in those breeds, making its finding in Belgian Shepherds somewhat unclear as to meaning
- ▶ 1 dog, a Malinois, was identified as a carrier of the mutation in gene SLC2A9¹ that has been associated with hyperuricosuria and hyperuricemia or urolithiasis
- ▶ In the homozygous form, this mutation is associated with bladder and kidney stones

- ▶ Though none has yet been reported by owners of dogs in the FB group, Embark reports that a mutation associated with degenerative myelopathy, SOD1A¹, has been detected in Belgian Shepherds
- ▶ Like the PDK4 mutation, the SOD1A mutation has been identified in a variety of breeds in which it does not seem to be associated with degenerative myelopathy, making its finding in Belgian Shepherds somewhat unclear as to meaning

PDK4 Mutation



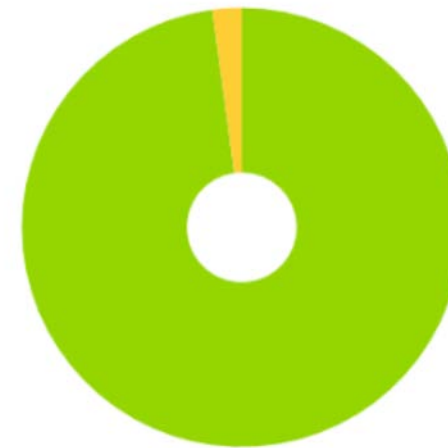
■ Clear ■ Carrier ■ Affected

SLC2A9 Mutation



■ Clear ■ Carrier ■ Affected

SOD1A Mutation



● Clear ● Carrier

Other than the PDK4, SLC2A9, and SOD1A mutations, none of the Belgian Shepherds included in this survey have tested positive, as either affected or a carrier, for any of the other 160+ mutations associated with genetic diseases detected by the Embark panel

- ▶ Certainly, many of these conditions are highly specific to other breeds of dogs
- ▶ Some genetics conditions are considered to be more broadly expressed in a variety of breeds, so it is good to know that no Belgian Shepherds have tested positive for conditions like
 - ▶ MDR1-mediated Drug Sensitivity
 - ▶ PRA-prcd
- ▶ Despite all of this, it is likely that Belgian Shepherds will test positive for serious genetic diseases at some point
 - ▶ Embark is working on incorporating the Spongy Degeneration with Cerebella Ataxia (SDCA), Forms 1 & 2, tests into the next version of its chip - this condition may be specific to working line Belgian Shepherds; the validation tests are currently ongoing
 - ▶ It is inevitable that other things will show up, eventually

Belgian Shepherd Health & Disease Genetics: Conjecture, Speculation, & Questions

- ▶ Though Belgian Shepherds are a healthy breed, overall, with few identified genetic diseases, it is easy to see how data generated by Embark can contribute to improved health of the individual dog and the breed, as a whole:
 - ▶ Knowing that your dog has the potential for Low Normal ALT levels has the potential to have an immediate impact on any individual, by changing the amount of elevation required to be considered 'high'.
 - ▶ Information on the MHC DLA complexes diversity could be used immediately when making breeding decisions
- ▶ Identifying carriers for any genetic conditions should certainly be considered in breeding decisions, though there are few relevant tests that are available for conditions more frequently seen in Belgian Shepherds
- ▶ **Questions:**
 - ▶ We really need to put together a health survey to try to tease out any relationships with some of the autoimmune conditions seen in Belgians. Which conditions should we focus on?
 - ▶ Is Embark planning to continue their work in the DLA region to be able to correlate their findings with previous work done using older methods showing relationships between specific haplotypes and autoimmune conditions?

Thank You

- ▶ All the members of the FB Group, Embark Belgian Shepherds
- ▶ Kate Hogan, Ph.D., Analysis
- ▶ August 2018





Appendix

Descriptions of Maternal Haplogroups from the Embark profiles

Haplogroup A1d

- ▶ *“This female lineage can be traced back about 15,000 years to some of the original Central Asian wolves that were domesticated into modern dogs. The early females that represent this lineage were likely taken into Eurasia, where they spread rapidly. As a result, many modern breed and village dogs from the Americas, Africa, through Asia and down into Oceania belong to this group! This widespread lineage is not limited to a select few breeds, but the majority of Rottweilers, Afghan Hounds and Wirehaired Pointing Griffons belong to it. It is also the most common female lineage among Papillons, Samoyeds and Jack Russell Terriers. Considering its occurrence in breeds as diverse as Afghan Hounds and Samoyeds, some of this is likely ancient variation. But because of its presence in many modern European breeds, much of its diversity likely can be attributed to much more recent breeding.”¹*
- ▶ Contains Haplotype A11a¹

Haplogroup A1a

- ▶ *“A1a is the most common maternal lineage among Western dogs. This lineage traveled from the site of dog domestication in Central Asia to Europe along with an early dog expansion perhaps 10,000 years ago. It hung around in European village dogs for many millennia. Then, about 300 years ago, some of the prized females in the line were chosen as the founding dogs for several dog breeds. That set in motion a huge expansion of this lineage. It's now the maternal lineage of the overwhelming majority of Mastiffs, Labrador Retrievers and Gordon Setters. About half of Boxers and less than half of SharPei dogs descend from the A1a line. It is also common across the world among village dogs, a legacy of European colonialism.”¹*
- ▶ Contains Haplotype A16/17/99/100¹

Descriptions of Maternal Haplogroups from the Embark profiles, continued

Haplogroup A1b

- ▶ *“This female lineage was very likely one of the original lineages in the wolves that were first domesticated into dogs in Central Asia about 15,000 years ago. Since then, the lineage has been very successful and travelled the globe! Dogs from this group are found in ancient Bronze Age fossils in the Middle East and southern Europe. By the end of the Bronze Age, it became exceedingly common in Europe. These dogs later became many of the dogs that started some of today's most popular breeds, like German Shepherds, Pugs, Whippets, English Sheepdogs and Miniature Schnauzers. During the period of European colonization, the lineage became even more widespread as European dogs followed their owners to far-flung places like South America and Oceania. It's now found in many popular breeds as well as village dogs across the world!”¹*
- ▶ Contains Haplotypes A18/19/20/21/27/36/94/109¹

Haplogroup A1e

- ▶ *“This female lineage likely stems from some of the original Central Asian wolves that were domesticated into modern dogs starting about 15,000 years ago. It seemed to be a fairly rare dog line for most of dog history until the past 300 years, when the lineage seemed to “explode” out and spread quickly. What really separates this group from the pack is its presence in Alaskan village dogs and Samoyeds. It is possible that this was an indigenous lineage brought to the Americas from Siberia when people were first starting to make that trip themselves! We see this lineage pop up in overwhelming numbers of Irish Wolfhounds, and it also occurs frequently in popular large breeds like Bernese Mountain Dogs, Saint Bernards and Great Danes. Shetland Sheepdogs are also common members of this maternal line, and we see it a lot in Boxers, too. Though it may be all mixed up with European dogs thanks to recent breeding events, its origins in the Americas makes it a very exciting lineage for sure!”¹*
- ▶ Contains Haplotypes A22, A226 and A650¹

Descriptions of Maternal Haplogroups from the Embark profiles, continued

Haplogroup B1

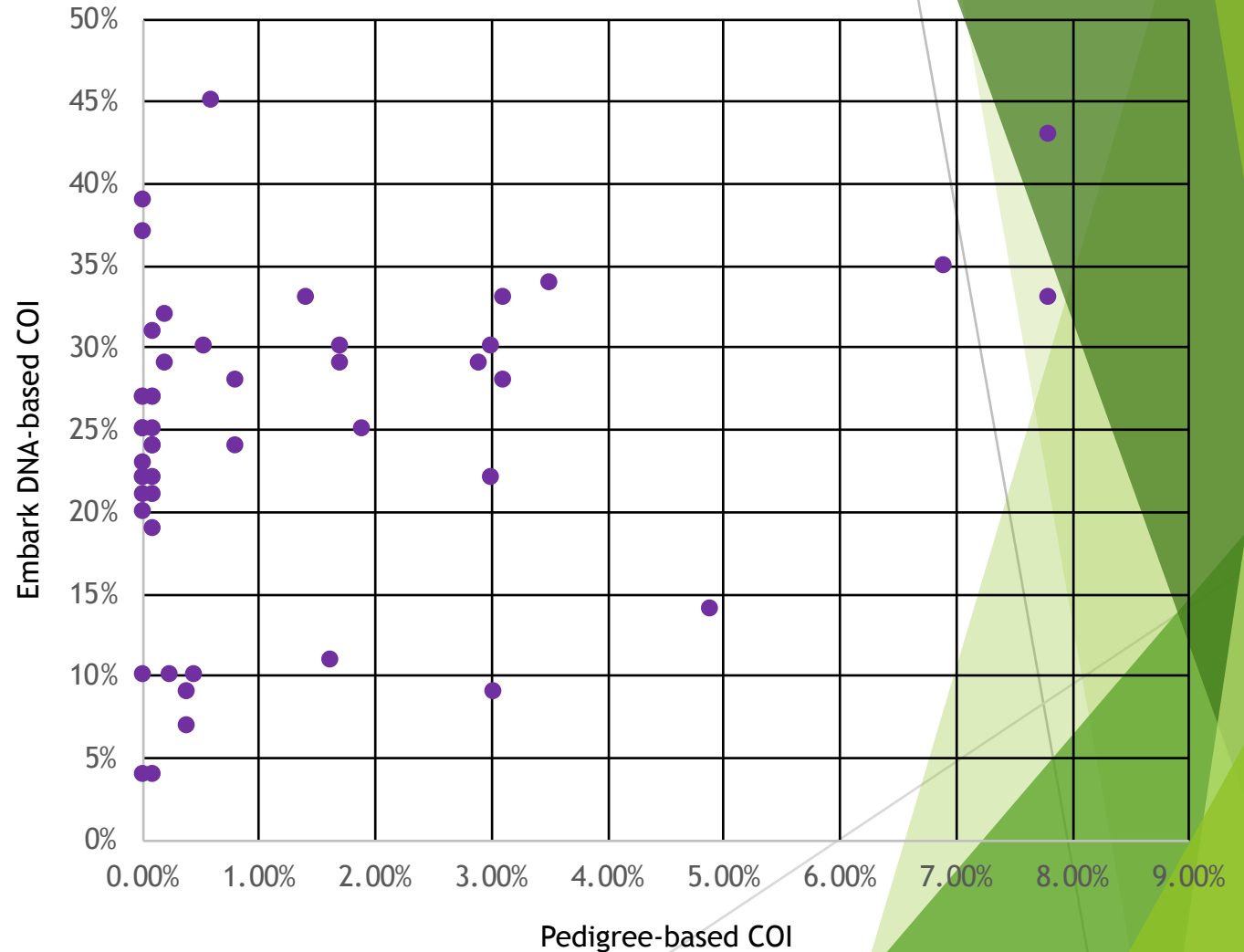
- ▶ *“B1 is the second most common maternal lineage in breeds of European or American origin. It is the female line of the majority of Golden Retrievers, Basset Hounds, and Shih Tzus, and about half of Beagles, Pekingese and Toy Poodles. This lineage is also somewhat common among village dogs that carry distinct ancestry from these breeds. We know this is a result of B1 dogs being common amongst the European dogs that their conquering owners brought around the world, because nowhere on earth is it a very common lineage in village dogs. It even enables us to trace the path of (human) colonization: Because most Bichons are B1 and Bichons are popular in Spanish culture, B1 is now fairly common among village dogs in Latin America.”¹*
- ▶ Contains Haplotypes B1b, B6/8/67, B42 and B57¹



SLIDES IN PROGRESS/WORK IN PROGRESS

There appears to be little correlation between paper pedigree-based COI and DNA-based COI in the dogs tested so far

- ▶ At this time, while provocative and very interesting, there is insufficient data to draw any conclusions based on COI
- ▶ Data will continue to be analyzed in total (previous slide) and in a segmented manner to evaluate any differences within the breed



Thank You

- ▶ All the members of the FB Group, Embark Belgian Shepherds
- ▶ Kate Hogan, Ph.D., Analysis
- ▶ August 2018

