

Preliminary Analysis of Embark Dog DNA Tests Performed on 19 AKC Registered Belgian Shepherds

Presented by a group of FB Belgian Shepherd Enthusiasts in 'Embark Belgian Shepherds'
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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 5/15/2018
- ▶ Based on a completed sample of 19 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
- ▶ 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 | 19 |
| Belgian Sheepdog | 15 |
| Belgian Tervueren | 3 |
| Belgian Malinois | 1 |
| Belgian Laekenois | 0 |
| | |
| Male | 8 |
| Female | 11 |
| | |
| *Show lines | 13 |
| *Working lines | 5 |
| *Combo lines | 1 |

*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 identification 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

The background features abstract, overlapping geometric shapes in various shades of green, ranging from light lime to dark forest green. These shapes are primarily located on the right side of the slide, creating a modern, layered effect. The text is positioned on the left side of the slide, set against a plain white background.

Ancestry - Maternal & Paternal Haplotypes of Belgian Shepherds

Dogs have multiple types of DNA, some of which tells us a great deal about the history of their ancestors - Mitochondrial DNA tells us specifically about the female line and the Y chromosome tells us specifically about the male line behind our dogs

- ▶ Mitochondrial DNA (mtDNA) contains about 16,500 base pairs of DNA material ¹
 - ▶ It encodes 37 proteins that provide key instructions and mechanisms for providing for all the energy requirements of the dog¹
 - ▶ mtDNA is normally passed, in an unchanged manner, from the female to all of her offspring, regardless of their sex ¹
 - ▶ The process of establishing relatedness through the use of mtDNA is frequently referred to as “Maternal Haplotype”
 - ▶ 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 characteristics ¹
 - ▶ The Y chromosome is normally passed from the male to his male offspring in an unchanged manner ¹
 - ▶ The process of establishing relatedness through the use of Y chromosome DNA is frequently referred to as “Paternal Haplotype”
- **Embark has been able to identify and associate different characteristics of mtDNA and the Y chromosome with different breeds and track their history and region of origins**

1. Genetics Home Reference, National Institutes of Health: US National Library of Medicine, <https://ghr.nlm.nih.gov/>

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 | 8 |
| Belgian Sheepdog | 5 |
| Belgian Tervueren | 2 |
| Belgian Malinois | 1 |
| Belgian Laekenois | 0 |
| | |
| *Show lines | 6 |
| *Working lines | 2 |
| *Combo lines | 0 |

*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 7 different Maternal Haplotypes identified in the 19 dogs tested with insufficient data to determine any clustering or differences based on coat, color or pedigree

- ▶ At this time, while provocative and very interesting, there is insufficient data to draw any conclusions based on maternal haplotypes
- ▶ Embark classifies the haplotypes into larger haplogroups. The groups containing these haplotypes are:
 - ▶ Haplogroup A1d contains: A11a¹
 - ▶ Haplogroup A1a contains: A16/17/99/100¹
 - ▶ Haplogroup A1e contains: A22, A226¹
 - ▶ Haplogroup B1 contains: B6/8/67, B42 and B57¹
- ▶ The larger Haplogroups will be discussed in the next couple of slides. Further focus on the Haplotypes will wait for the clarification that comes with more data.

| Participants | Maternal Haplotypes | | | | | | |
|--------------------------|---------------------|---------------|-----|------|---------|-----|-----|
| | A11a | A16/17/99/100 | A22 | A226 | B6/8/67 | B42 | B57 |
| Total (N=19) | 2 | 8 | 1 | 3 | 1 | 1 | 3 |
| Belgian Sheepdog | 1 | 8 | 1 | 3 | 0 | 0 | 2 |
| Belgian Tervueren | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Belgian Malinois | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Belgian Laekenois | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Show lines | 1 | 7 | 0 | 3 | 0 | 0 | 2 |
| *Working lines | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| *Combo lines | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

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

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 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 and A226¹

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 B6/8/67, B42 and B57¹

Ancestry - Conjecture, Speculation, and Questions

Maternal Haplotypes

- ▶ It seems like there are multiple, potential interpretations for 7, or more as more data comes in, maternal haplotypes
 - ▶ 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
- ▶ Questions:
 - ▶ How many maternal haplotypes are there, across all breeds?
 - ▶ Is there a 'normal' distribution of maternal haplotypes in purebred dogs?

- **Why such a significant difference between the number of maternal and paternal haplotypes?**
- **This is one of the most interesting observations at this stage - it will definitely provide fuel for many interesting conversations, especially over a drink with friends and fellow enthusiasts of Belgian Shepherds!**

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 paternal haplotypes are there, across all breeds?
 - ▶ Do other breeds show such specificity in terms of paternal haplotypes or is this unique to Belgian Shepherds?
 - ▶ Is it easier to lose a paternal haplotype than maternal haplotype, since the paternal haplotype only exists in male dogs?

Coefficient of Inbreeding (COI)

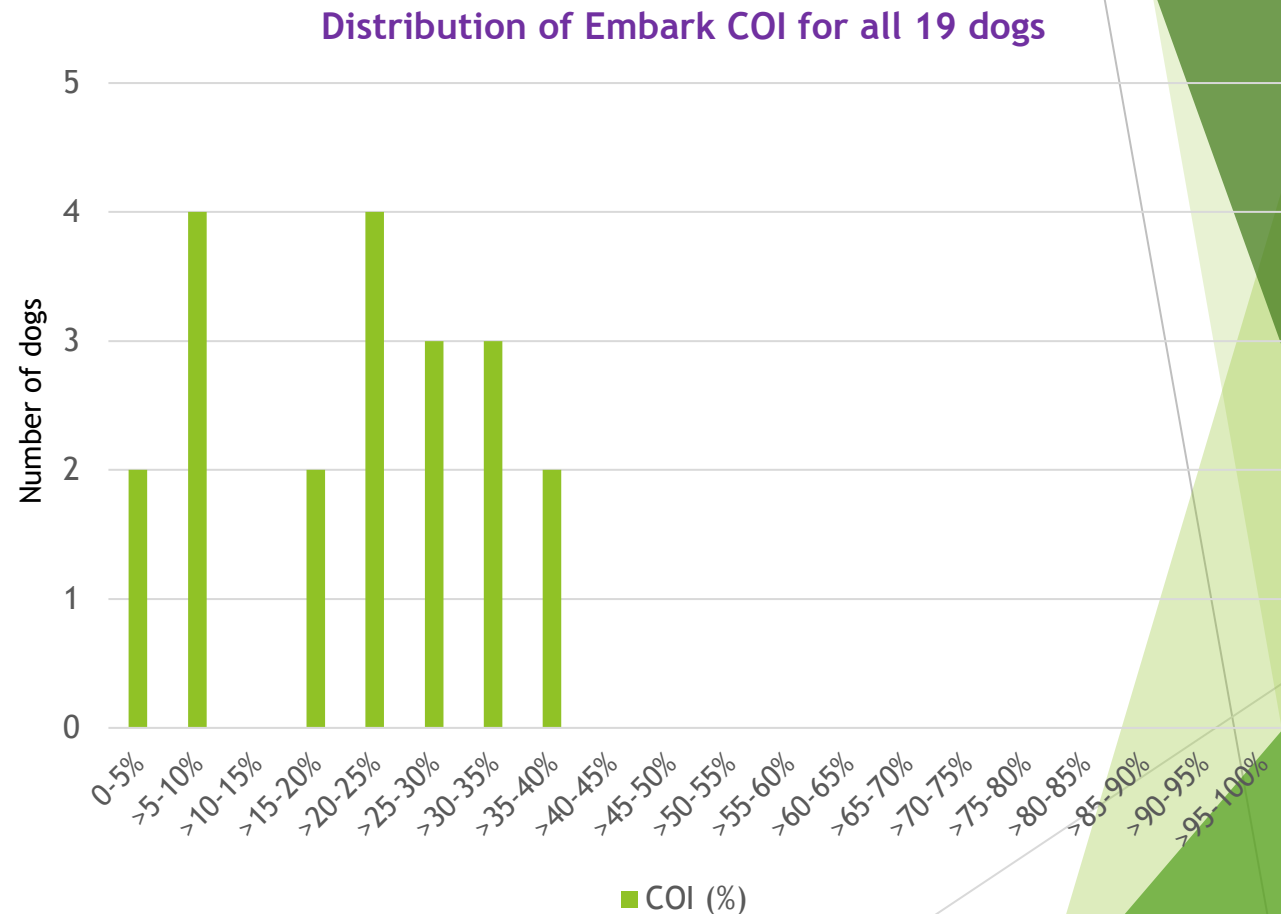
COI is one of the standard approaches to pedigree analysis and breeding decisions by many breeders - the ability to evaluate COI on the basis of an individual's specific DNA represents a paradigm shift

- ▶ 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 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, Carol Beuchat has a good article online²

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>

Embark measures the Coefficient of Inbreeding (COI) by comparing the individual strands of each chromosome to each other, providing a, potentially, different result than that based on pedigree analysis alone

- ▶ This represents a 1st attempt to look at DNA-based COI of all Belgian Shepherds, regardless of official breed, in the US
- ▶ 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 20% range
 - ▶ It may also represent a bi-modal distribution, again with insufficient data, with averages somewhere in the 6% and 25% range
- ▶ Hopefully, we can continue to collect data to further this type of analysis
- ▶ Question: not sure how much of the DNA is being surveyed for COI calculations in the Embark analysis?



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 (previous slide) and in a segmented manner to evaluate any differences within the breed

| Participants | COI (%) | | |
|-------------------------|---------|---------|---------|
| | Minimum | Maximum | Average |
| Total (N=19) | 4% | 37% | 20% |
| Belgian Sheepdog (N=15) | 4% | 37% | 21% |
| Belgian Tervueren (N=3) | 9% | 32% | 19% |
| Belgian Malinois (N=1) | 6% | 6% | 6% |
| Belgian Laekenois (N=0) | N/A | N/A | N/A |
| *Show lines (N=13) | 16% | 37% | 26% |
| *Working lines (N=5) | 4% | 9% | 6% |
| *Combo lines (N=1) | 10% | 10% | 10% |

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

Coefficient of Inbreeding - Conjecture, Speculation, and Questions

- ▶ 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?

Belgian Shepherd 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 studied in the Embark panel

- ▶ 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
- ▶ K-locus: Gives rise to the dominant black coat that is desired by many breeders of Belgian Sheepdogs¹
- ▶ 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

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 options reported than dogs tested
- ▶ Observations:
 - ▶ The majority of dogs tested so far (14/19) are homozygous for a melanistic mask
 - ▶ Nearly all dogs tested so far (18/19) carry at least 1 allele for a melanistic mask
 - ▶ Only 1 dog is homozygous for the E/E genotype, associated with extension masking
- ▶ Fascinating data, but we need more dogs with such a wide variety of available genetic options

| Participants | Em [Melanistic mask] > Eg [Grizzle] > E [Black] > e [Red] ¹ | | | | | | | | | |
|--------------------------|--|-------|------|------|-------|------|------|-----|-----|-----|
| | Em/Em | Em/Eg | Em/E | Em/e | Eg/Eg | Eg/E | Eg/e | E/E | E/e | e/e |
| Total (N=19) | 14 | 0 | 4 | 3 | 0 | 0 | 0 | 1 | 0 | 0 |
| Belgian Sheepdog | 10 | 0 | 4 | 3 | 0 | 0 | 0 | 1 | 0 | 0 |
| Belgian Tervueren | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Belgian Malinois | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Belgian Laekenois | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Show lines | 9 | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Working lines | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Combo lines | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

¹For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

One of the most recognizable traits of Belgian Shepherds, particularly of the Malinois and Tervueren, is the color pattern of their 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:
 - ▶ There is a significant group of dogs that are *ay/ay*, sable (8/19)
 - ▶ There is a significant group of dogs (12/19) that carry at least 1 copy of *a*, the recessive black allele
 - ▶ There is a significant group of dogs (10/19) that carry at least 1 copy of *at*, the black & tan allele
 - ▶ 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

| Participants | A Locus - <i>ay</i> [Sable] > <i>aw</i> [Wolf sable] > <i>at</i> [Black & tan] > <i>a</i> [Recessive black] ¹ | | | | | | | | | |
|-------------------|--|--------------|--------------|-------------|--------------|--------------|-------------|--------------|-------------|------------|
| | <i>ay/ay</i> | <i>ay/aw</i> | <i>ay/at</i> | <i>ay/a</i> | <i>aw/aw</i> | <i>aw/at</i> | <i>aw/a</i> | <i>at/at</i> | <i>at/a</i> | <i>a/a</i> |
| Total (N=19) | 8 | 0 | 2 | 5 | 0 | 0 | 0 | 3 | 5 | 2 |
| Belgian Sheepdog | 4 | 0 | 2 | 5 | 0 | 0 | 0 | 3 | 5 | 2 |
| Belgian Tervueren | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Belgian Malinois | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Belgian Laekenois | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Show lines | 3 | 0 | 0 | 4 | 0 | 0 | 0 | 3 | 5 | 2 |
| *Working lines | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Combo lines | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

¹For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

The K-Locus is particularly important within the Belgian Sheepdog population and is included in the Embark panel - the Belgian Sheepdogs included in this evaluation are evenly split between heterozygous and homozygous dominant black

- ▶ There is insufficient data to draw any conclusions based on K-locus genetics
- ▶ Observations:
 - ▶ Among the Belgian Sheepdogs, there was an equal proportion of dogs that were homozygous for dominant black as heterozygous
- ▶ Fascinating data, but we need more dogs

| Participants | K Locus - <i>KB</i> [Dominant black] > <i>ky</i> [Sable] (coat color for <i>ky/ky</i> dogs is determined by A locus ¹) | | |
|--------------------------|--|--------------|--------------|
| | <i>KB/KB</i> | <i>KB/ky</i> | <i>ky/ky</i> |
| Total (N=19) | 7 | 8 | 4 |
| Belgian Sheepdog | 7 | 8 | 0 |
| Belgian Tervueren | 0 | 0 | 3 |
| Belgian Malinois | 0 | 0 | 1 |
| Belgian Laekenois | 0 | 0 | 0 |
| *Show lines | 6 | 5 | 2 |
| *Working lines | 0 | 3 | 2 |
| *Combo lines | 1 | 0 | 0 |

¹For purposes of additional analysis, dogs were determined to be of show, working or a combination of bloodlines, by pedigree review

Other coat and color genes were tested in all 19 dogs, very little variation was observed among these dogs

- ▶ Additional tests color traits indicated¹:
 - ▶ None of the dogs tested carry the dilute allele at the D-locus
 - ▶ None of the dogs tested carry the brown allele at the B-locus
- ▶ Additional tests for coat characteristics revealed¹:
 - ▶ All 19 dogs tested are homozygous for the FGF5, long-coat gene
 - ▶ None of the dogs tested carry the KRT71, curl-coat gene
 - ▶ 1 dog is 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
 - ▶ 4 of the dogs are heterozygous for the LMBR1, hind dew claws gene
 - ▶ 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 - None of the dogs tested carry the small allele
 - ▶ IGF1R gene - None of the dogs tested carry the small allele
 - ▶ STC2 gene - 2 of the dogs tested are heterozygous for the small allele
 - ▶ GHR (E195K) - 1 of the dogs tested is heterozygous for the small allele
 - ▶ GHR (P177L) - None of the dogs tested carry the small allele

Belgian Shepherd Traits - Conjecture, Speculation, and Questions

- ▶ 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?

Belgian Shepherd Health & Disease Genetics

The background of the slide is white with abstract, overlapping green geometric shapes on the right side. These shapes include triangles and polygons in various shades of green, from light to dark, creating a modern, layered effect.

Embark evaluates the Alanine Aminotransferase enzyme, as a measure of liver health in individual, it might also be relevant to the breed, as a whole

- ▶ Alanine Aminotransferase is an important liver enzyme normally measured in complete blood panels. Different genetic forms of the enzyme are known to be associated with different activity levels¹. Knowing that your dog is low or low normal may be clinically relevant for an individual dog, and has potential implications for the breed as a whole.
- ▶ Observations:
 - ▶ About half of the dogs tested, regardless of variety or bloodlines, tested for the heterozygous form of ALT, which would result in low normal clinical activity levels
- ▶ Fascinating data, but we need more dogs

| Participants | Alanine Aminotransferase Activity | | |
|-------------------|-----------------------------------|-------------------|---------------|
| | <i>Low</i> | <i>Low Normal</i> | <i>Normal</i> |
| Total (N=19) | 0 | 11 | 8 |
| Belgian Sheepdog | 0 | 10 | 5 |
| Belgian Tervueren | 0 | 1 | 2 |
| Belgian Malinois | 0 | 0 | 1 |
| Belgian Laekenois | 0 | 0 | 0 |
| | | | |
| *Show lines | 0 | 9 | 4 |
| *Working lines | 0 | 2 | 3 |
| *Combo lines | 0 | 0 | 1 |

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

Embark tests the diversity of the Major Histocompatibility Complex (MHC) as a way of evaluating immune health in individuals, and potentially, more broadly, in a larger population of animals; Reduced diversity in the DLA-DRB1 may be tied specifically to Cushing's Disease

- ▶ Though the science in this area is NOT uniform, it is likely that increasing diversity in the MHC and Dog Leukocyte Antigen (DLA) is associated with a stronger immune system. When the DLAs inherited from the parents are more similar, there is less immunological diversity.¹
- ▶ A lack of diversity in the MHC DLA-DRB1 has been associated with Cushing's Disease in some studies¹
- ▶ 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
- ▶ Observations:
 - ▶ About 1/3 of all the dogs tested had No or Low Diversity in their DLA-DRB1 haplotype

| Participants | Major Histocompatibility Complex II - DLA-DRB1 | | |
|-------------------|--|---------------|----------------|
| | No Diversity | Low Diversity | High Diversity |
| Total (N=19) | 5 | 2 | 11 |
| Belgian Sheepdog | 4 | 1 | 9 |
| Belgian Tervueren | 1 | 1 | 1 |
| Belgian Malinois | 0 | 0 | 1 |
| Belgian Laekenois | 0 | 0 | 0 |
| *Show lines | 5 | 1 | 7 |
| *Working lines | 0 | 1 | 3 |
| *Combo lines | 0 | 0 | 1 |

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

Embark tests the diversity of the MHC as a way of evaluating immune health in individuals, and potentially, more broadly, in a larger population of animals; Reduced diversity in the DLA-DQA1 & DQB1 may be tied to some autoimmune diseases

- ▶ The basic science is the same as for the MHC DLA-DRB1, on the previous slide.¹
- ▶ A lack of diversity in the MHC DLA-DRB1 has been associated with autoimmune conditions in some studies¹
- ▶ 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
- ▶ Observations:
 - ▶ About 1/2 of all the show line dogs tested had No Diversity in their DLA-DQA1 & DQB1 haplotype
 - ▶ The working line dogs all had High Diversity in this MHC

| Participants | Major Histocompatibility Complex II - DLA-DQA1 & DQB1 | | |
|--------------------------|---|----------------------|-----------------------|
| | <i>No Diversity</i> | <i>Low Diversity</i> | <i>High Diversity</i> |
| Total (N=19) | 6 | 0 | 13 |
| Belgian Sheepdog | 5 | 0 | 10 |
| Belgian Tervueren | 1 | 0 | 2 |
| Belgian Malinois | 0 | 0 | 1 |
| Belgian Laekenois | 0 | 0 | 0 |
| *Show lines | 6 | 0 | 7 |
| *Working lines | 0 | 0 | 5 |
| *Combo lines | 0 | 0 | 1 |

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

None of the 19 Belgian Shepherds included in this survey have tested positive, as either affected or a carrier, for any of the 160 genetic diseases detected by the Embark panel

- ▶ Certainly, some of these 160 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
 - ▶ Degenerative Myelopathy (SOD1A)
 - ▶ Dilated Cardiomyopathy (PDK4)
- ▶ Despite all of this, it is likely that Belgian Shepherds will test positive for genetic diseases at some point
 - ▶ Embark is working on incorporating the Spongy Degeneration with Cerebella Ataxia, Forms 1 & 2, tests into the next version of its chip - this condition may be specific to working line Belgian Shepherds
 - ▶ It is inevitable that other things will show up, eventually

Belgian Shepherd Health & Disease Genetics - Conjecture, Speculation, and 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 to show 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'. This is not an uncommon phenomenon, as there are many things that result in damage to the liver.
 - ▶ 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:
 - ▶ Will Embark change their reporting of specific genetic conditions if any Belgian Shepherd tests positive for that condition?
 - ▶ As scientific advancements are made and incorporated into the Embark panel, how will people access those tests and that information?
- **Is there anything more important than the health of these spectacular dogs?**

Thank You

All the members of the FB Group, Embark Belgian Shepherds

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