

Genetic Diversity Testing for Barbet

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers that will measure genetic heterogeneity and diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions for specified dog populations. This test panel will be useful to dog breeders who wish to use DNA-based testing as a supplement to in-depth pedigrees. Using in-depth pedigrees and DNA based diversity data, along with DNA testing results for desired phenotypes and health traits can aid in informing breeding decisions.

DNA-based testing of the Barbet breed is now in the preliminary results phase with the objective of building a snap-shot of individual- and breed-wide genetic heterogeneity and diversity. This data base will be progressively expanded as more dogs are added with the goal of characterizing all the known alleles for the breed at 33 STR loci across the genome as well as all existing DLA class I and II haplotypes identified by seven STRs. We are accepting Barbet from all parts of the world with a goal of 100 individuals tested to complete the initial phase.

Price: \$50. This is the cost for the dogs that will be tested in the research phase. Once the research phase is complete, the test will be offered at the regular rate of \$80.

[ORDER TEST KITS](#)

Allow 5-10 business days for results.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across the genome were used to gauge genetic heterogeneity and diversity within an individual and across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity, and breed-wide allele frequency is provided.

DLA haplotypes: Seven STR loci linked to the DLA class I and II genes were used to identify genetic differences in regions regulating immune responses and self/non-self-recognition. Problems with self/non-self-recognition, along with non-genetic factors in the environment, are responsible for autoimmune disease.

Internal Relatedness: The IR value is a measure of the genetic relatedness of an individual's parents. The value takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog and two individuals from different sources may have identical IR values but a very different genetic makeup.

I. Introduction to the breed

The **Barbet** (*Fr. barbe* or beard) is a medium-sized French dog breed used initially for water retrieving and listed in Group 8 (retrievers, flushing dogs, water dogs) by the *Société Centrale Canine*, (French Kennel Club) [1, 2]. The Barbet is best known outside of hunting circles for its long, wooly and curly coat. Barbets are solid black, brown, fawn, grey, pale fawn, white, or pied. White chest spots and white paws or legs are often found on the more popular black and brown dogs. Contemporary Barbet in France are still competent water retrievers and often pass the *d'aptitudes naturelles* (TAN), a basic water-retrieving test, and participate in *brevet de chasse a l'eau* (BCE), a general hunting-dog test involving field and water trials. Barbets are kept mainly as pets, although a some also participate in conformation, performance sports, hunting, and service and therapy dog work. They are also being increasingly seen in shows.

The ancestral roots of the Barbet date back at least to the 16th century [2]. A general belief is that water loving, intelligent and highly adaptable dogs slowly spread from Central Asia, Russia, and Eastern Europe, into France and the southern tip of Spain, and west to the UK and Ireland. These original dogs were subsequently bred over several centuries with indigenous regional dogs to better meet the local needs of hunters, farmers and fishermen. These regionalized curly coated dogs ultimately spawned the Komondor and Hungarian Puli in Hungary, Barbet in France, Poodle in Germany, Portuguese water dog, Curly coated retriever and Airedale terrier in England, Irish water spaniel, American water spaniel and more. Identical dogs to the French Barbet were known as the *barbone* in Italy and the *Pudel* in Germany in the 17th to early-18th centuries. The writing of the first Barbet breed standard was in 1891 from France.

The breed has remained relatively unpopular until the last decade or so because of its emphasis on performance traits and difficulty maintaining their coats. The dense curly hair grows quickly and requires continually grooming. However, the breed has become more popular in the last several decades because of the increased interest in rarer regional breeds. The first Barbet was brought into the UK in 2001, but he produced no puppies. Two unrelated females were imported from France in 2007, and most Barbet currently in the UK are said to descend from these two dogs. As of 2018, there are approximately 140 Barbets living in the UK, and they were the 220th breed recognized by The Kennel Club of the UK. Additional breeding stock have been more recently imported from Continental Europe and Sweden. Less than 200 Barbet were estimated to be in the United States in 2013, with a small but steady subsequent increase in imported dogs from Canada and Europe. They are currently registered with the American Rare Breed Association (ARBA) or the United Kennel Club (UKC). There has also been a recent acceptance in the [American Kennel Club](#) (AKC) Foundation Stock Service Program. A November 2018 meeting of the AKC Board reviewed a request from the Barbet Club of America seeking acceptance into the AKC Stud Book and eligibility to compete in the Sporting Group. It was recommended that the AKC Board of Directors approve the request for regular status in the Sporting Group effective January 1, 2020 as well as approve the submitted breed standard.

II. Genetic studies of contemporary Barbet

A. Population genetics based on 33 STR loci on 25 chromosomes

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs, those recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes. Each STR locus manifests several different genetic configurations known as alleles. Each dog inherits one of these alleles from the sire and the other from the dam. Table 1a lists the alleles recognized at each STR locus among 49 Barbet tested to date.

Table 1a. Allele designation and frequency at 33 STR loci for Barbet. The allele that occurs at the highest frequency at each locus is highlighted.

[\(Link to Table 1a\)](#)

Table 1b. Known alleles and the % detected in Barbet for 33 STR markers. Shown are the number of known alleles for all dogs and wolves tested to date and the percent of those detected in the Barbet tested.

| Locus | Known Alleles | % in Barbet (n=49) |
|--------------|----------------------|---------------------------|
| AHT121 | 24 | 29% |
| AHT137 | 17 | 29% |
| AHTH130 | 20 | 30% |
| AHTH171-A | 14 | 57% |
| AHTH260 | 28 | 18% |
| AHTk211 | 7 | 57% |
| AHTk253 | 11 | 45% |
| C22.279 | 13 | 31% |
| FH2001 | 17 | 29% |
| FH2054 | 23 | 30% |
| FH2848 | 24 | 21% |
| INRA21 | 15 | 33% |
| INU005 | 14 | 27% |
| INU030 | 15 | 36% |
| INU055 | 11 | 45% |
| LEI004 | 15 | 20% |
| REN105L03 | 22 | 23% |
| REN162C04 | 14 | 29% |
| REN169D01 | 14 | 36% |
| REN169O18 | 14 | 29% |
| REN247M23 | 11 | 27% |
| REN54P11 | 14 | 50% |
| REN64E19 | 12 | 33% |
| VGL0760 | 26 | 31% |
| VGL0910 | 27 | 30% |
| VGL1063 | 17 | 41% |
| VGL1165 | 23 | 39% |
| VGL1828 | 22 | 36% |
| VGL2009 | 12 | 42% |
| VGL2409 | 13 | 38% |
| VGL2918 | 19 | 42% |
| VGL3008 | 18 | 28% |

The number of known alleles per locus for the 33 autosomal STRs for all dogs and wolves tested ranged from 7 to 28 (Table 1b). The number of alleles identified among the 49 Barbets tested ranged from 3 to 9 per locus and the percent of known alleles from 20% to 57% (average 35%). Therefore, about one-third of known genetic diversity for these 33 loci has been retained in the breed (or two-thirds has been lost) during the entire period of breed evolution.

B. Assessment of population heterozygosity using standard genetic parameters

Allele frequencies across all 33 STR loci taken from Table 1 were used to calculate a mean (average) observed heterozygosity (Ho) and expected heterozygosity (He) for the Barbet (Table 2). The population of 49 Barbet which were initially tested had a mean number of alleles (Na) of 5.58 across all 33 genomic STR loci. The average number of alleles per locus was low compared to many larger breeds such as the Italian greyhound (Na=7.12), Labrador retriever (Na=7.27) and Golden Retriever (Na=8.23), but higher compared to breeds such as the Swedish Vallhund (Na=4.67). The Flat coated retriever is similar with an Na=5.70 alleles/locus. The mean effective alleles (Ne) (i.e., alleles contributing most to heterogeneity) per locus were 3.57. The fact that a few alleles contribute to most of the heterogeneity of the breed is a typical finding for most pure breeds of dogs.

Table 2. Summary of Standard Genetic Assessment for Barbet using 33 STR loci. (Updated October 10, 2019)

| | N | Na | Ne | Ho | He | F |
|-------------|----------|-----------|-----------|-----------|-----------|----------|
| Mean | 63 | 5.879 | 3.615 | 0.735 | 0.690 | -0.065 |
| SE | | 0.331 | 0.203 | 0.021 | 0.019 | 0.012 |

The mean observed heterozygosity (Ho) was 0.74, which was higher than the expected heterozygosity (He) of 0.69. He is the heterozygosity that would be found in this group of dogs if the parents of these dogs had been chosen in an entirely random manner (i.e. Hardy-Weinberg equilibrium or HWE). The fact that Ho was higher than He indicates that these particular dogs were products of parents purposefully selected for un-relatedness.

Ho and He can be used to calculate an inbreeding coefficient F. An F value of -1.0 would occur if the parents of all test dogs were totally unrelated to each other, while a value of +1.0 would indicate that the parents were genetically identical. The F value for these 49 Barbet was -0.07, indicating a 7% excess of heterozygosity over what would have been expected if mate selection from within the population was entirely random (i.e., at HWE). These findings for 49 individuals are encouraging, if they accurately reflect the breed, as they indicate an active attempt to seek out and use the most unrelated sires and dams.

Although the Ho, He and F values look very good for this group of dogs, these scores are averages for the group and thus these do not represent the genetic heterogeneity of individual

dogs. The genetic relatedness of a given dog's sire and dam are better reflected by internal relatedness (IR) scores (see below).

C. Standard genetic assessment values for individual STR loci

The allele frequencies (Table 1) can be used to do a standard genetic assessment of heterozygosity at each STR locus (Table 3). The N_a values for individual STR loci for this population of 49 Barbets ranged from a low of 3 to a high of 9 alleles/locus, while the N_e ranged from 1.81 to 5.66. The numbers of alleles that were identified at each of the 33 STR loci was only a fraction of the alleles known to exist in all dogs and was relatively low compared to large breeds, as was shown by the mean N_a values for the entire group. The F value was negative for 27/33 loci, with 11 of those loci having $F \leq -0.10$ (loci highlighted). This indicates that either this group of dogs was pre-screened for increased heterozygosity or that there is an active attempt by breeders at this time to find and mate the most unrelated sires and dams.

Table 3. Standard Genetic Assessment for Barbet using 33 STR loci. Loci with F values < -0.10 are highlighted. (Updated October 10, 2019)

| # | Locus | N | N_a | N_e | H_o | H_e | F |
|----|------------------|----|-------|-------|-------|-------|--------|
| 1 | AHT121 | 63 | 7 | 3.751 | 0.714 | 0.733 | 0.026 |
| 2 | AHT137 | 63 | 5 | 3.930 | 0.810 | 0.746 | -0.086 |
| 3 | AHTH130 | 63 | 6 | 4.765 | 0.825 | 0.790 | -0.045 |
| 4 | AHTH171-A | 63 | 8 | 5.694 | 0.857 | 0.824 | -0.040 |
| 5 | AHTH260 | 63 | 6 | 3.793 | 0.810 | 0.736 | -0.099 |
| 6 | AHTk211 | 63 | 4 | 2.129 | 0.571 | 0.530 | -0.078 |
| 7 | AHTk253 | 63 | 5 | 3.192 | 0.730 | 0.687 | -0.063 |
| 8 | C22.279 | 63 | 5 | 3.272 | 0.778 | 0.694 | -0.120 |
| 9 | FH2001 | 63 | 5 | 2.766 | 0.667 | 0.638 | -0.044 |
| 10 | FH2054 | 63 | 7 | 5.198 | 0.794 | 0.808 | 0.017 |
| 11 | FH2848 | 63 | 5 | 2.363 | 0.587 | 0.577 | -0.018 |
| 12 | INRA21 | 63 | 7 | 3.472 | 0.873 | 0.712 | -0.226 |
| 13 | INU005 | 63 | 3 | 1.858 | 0.508 | 0.462 | -0.100 |
| 14 | INU030 | 63 | 6 | 3.138 | 0.778 | 0.681 | -0.142 |
| 15 | INU055 | 63 | 5 | 3.332 | 0.683 | 0.700 | 0.025 |
| 16 | LEI004 | 63 | 3 | 2.308 | 0.603 | 0.567 | -0.064 |
| 17 | REN105L03 | 63 | 5 | 3.995 | 0.857 | 0.750 | -0.143 |
| 18 | REN162C04 | 63 | 4 | 2.182 | 0.603 | 0.542 | -0.113 |
| 19 | REN169D01 | 63 | 5 | 3.214 | 0.714 | 0.689 | -0.037 |

| | | | | | | | |
|-----------|------------------|----|----|-------|-------|-------|--------|
| 20 | REN169O18 | 63 | 4 | 2.479 | 0.667 | 0.597 | -0.117 |
| 21 | REN247M23 | 63 | 3 | 2.353 | 0.651 | 0.575 | -0.132 |
| 22 | REN54P11 | 63 | 7 | 5.247 | 0.841 | 0.809 | -0.039 |
| 23 | REN64E19 | 63 | 4 | 2.296 | 0.556 | 0.564 | 0.016 |
| 24 | VGL0760 | 63 | 9 | 5.404 | 0.825 | 0.815 | -0.013 |
| 25 | VGL0910 | 63 | 9 | 4.079 | 0.857 | 0.755 | -0.136 |
| 26 | VGL1063 | 63 | 7 | 1.767 | 0.429 | 0.434 | 0.012 |
| 27 | VGL1165 | 63 | 11 | 5.008 | 0.889 | 0.800 | -0.111 |
| 28 | VGL1828 | 63 | 8 | 5.192 | 0.841 | 0.807 | -0.042 |
| 29 | VGL2009 | 63 | 5 | 4.477 | 0.873 | 0.777 | -0.124 |
| 30 | VGL2409 | 63 | 5 | 3.701 | 0.778 | 0.730 | -0.066 |
| 31 | VGL2918 | 63 | 9 | 5.555 | 0.889 | 0.820 | -0.084 |
| 32 | VGL3008 | 63 | 5 | 2.941 | 0.730 | 0.660 | -0.106 |
| 33 | VGL3235 | 63 | 7 | 4.435 | 0.667 | 0.775 | 0.139 |

D. Differences in population structure as determined by principal coordinate analysis (PCoA)

Principal coordinate analysis (PCoA) uses genetic distance based on allele sharing to demonstrate genetic differentiation between individuals in related or unrelated populations. The resulting data is multi-dimensional (spherical) but can be accurately portrayed in a two-dimensional graph by selecting values from the two coordinates that represent the greatest proportions of individuals (coordinate 1 and 2 in this case). Figure 1 is a PCoA plot of Barbets that shows them clustering as a single breed, as would be expected. However, individuals are not tightly clustered around the central X/Y axis but are spread at some distance from each other across the graph. This is another indication that these 49 dogs were genetically diverse.

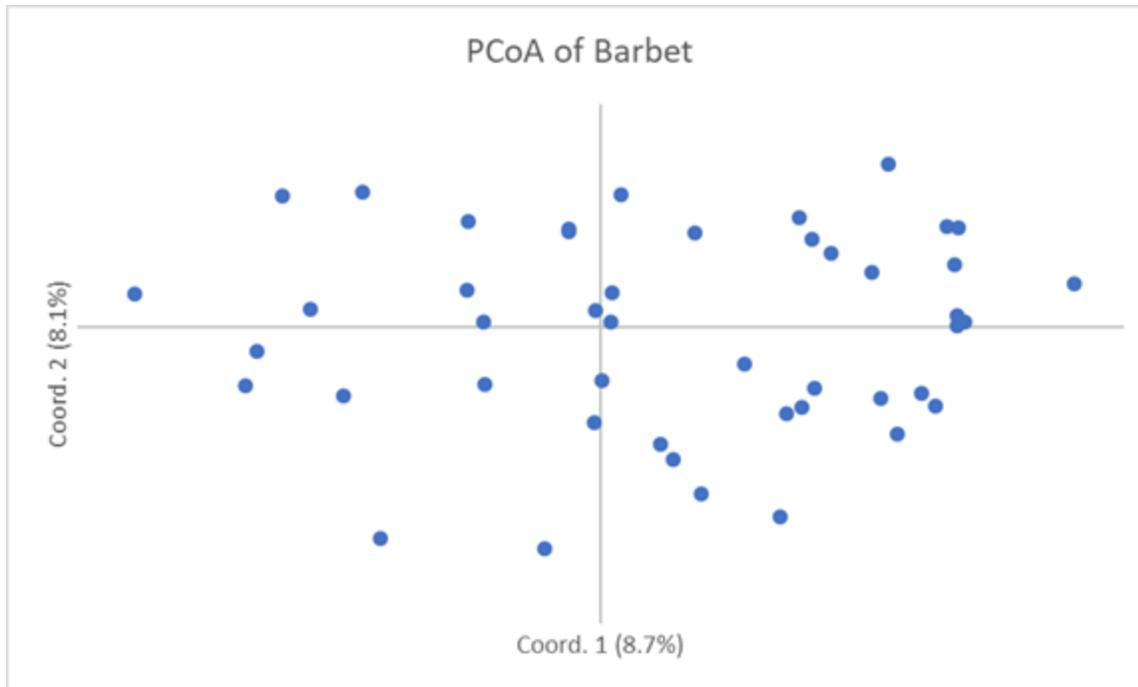


Figure 1. PCoA plot showing the genetic relationship of 49 Barbet dogs

Comparing Barbets to both distantly related and less distantly related breeds will force related individuals and breeds to become more aligned. Figure 2 displays a PCoA plot comparing Barbet, Standard Poodle and Italian Greyhound. The Barbet and Standard Poodle are more closely aligned with each other than either is to the Italian Greyhound, as might be expected given a recent co-evolution of Standard Poodle and Barbet and an unrelated evolution of either breed with Italian Greyhound. It is noteworthy that individual Barbets are still somewhat scattered in the plot and not forming the tight clustering seen in the Standard Poodle and Italian Greyhound. This is another confirmation that the tested Barbet were more genetically diverse than many other pure dog breeds.

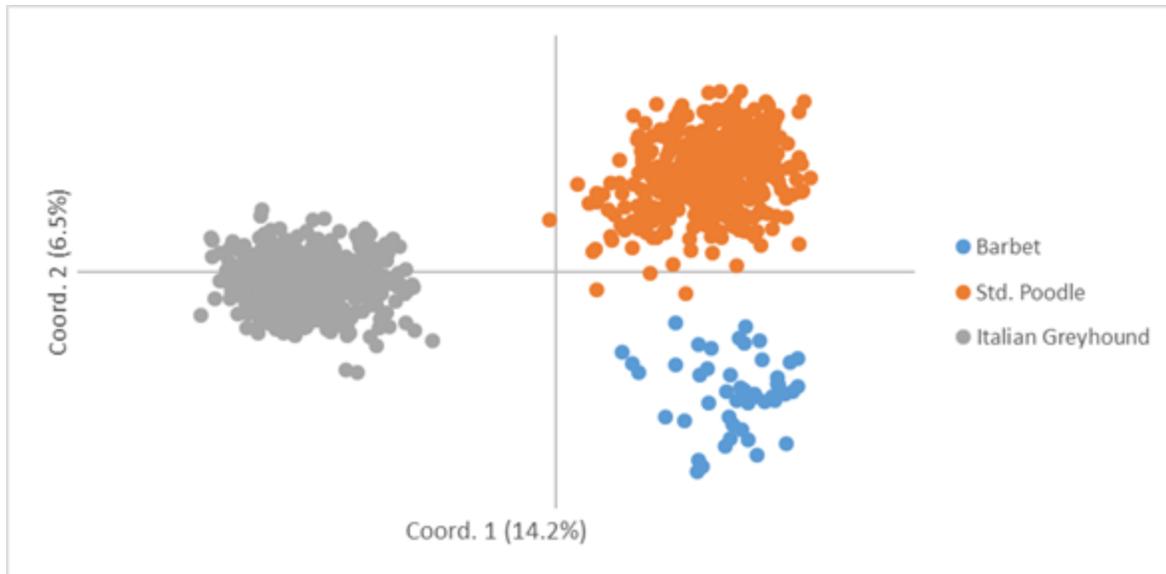


Figure 2. PCoA plot showing the relatedness of Barbet with Standard Poodle and Italian greyhound

Figure 3 is a PCoA plot comparing the relatedness of Barbet to Standard Poodles, Italian Greyhounds, and to the Samoyed. Samoyeds were included in the comparison because they share no class I and only one class II haplotypes with Barbet, and only one class I and no class II haplotypes with Italian Greyhound (see Table 8). However, Italian Greyhounds do share several DLA class I and II haplotypes with Standard Poodles (see Table 8). This suggests a greater degree of relatedness between Italian Greyhounds and Standard Poodles than indicated by the 33 STR loci and may explain why Barbets and Standard Poodles did not cluster closer together in the three breed comparison.

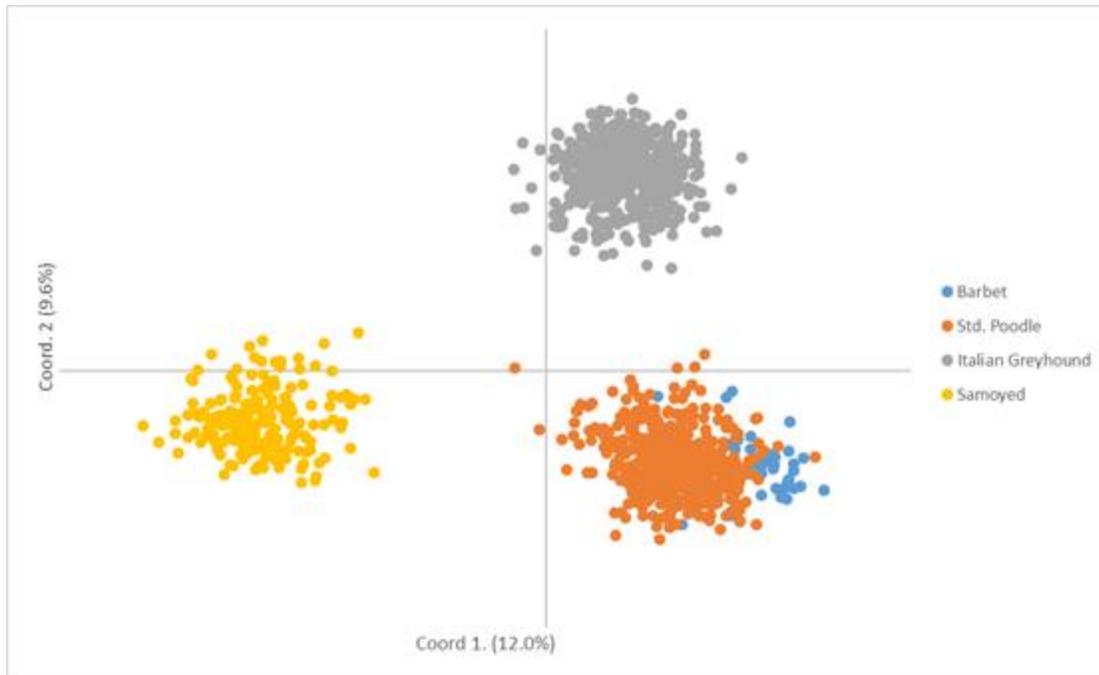


Figure 3. PCoA plot showing the genetic relatedness of Barbet, Standard Poodle, Italian Greyhound and Samoyed.

When PCoA was done on four breeds, including one breed (i.e., Samoyed) that was strongly unrelated to the others, the shared ancestry of Barbet and Standard Poodle becomes apparent (Fig. 3). Not only were Barbets and Standard Poodles much more closely aligned to each other than to Samoyed and Italian Greyhound, but at least four Barbets among the 49 dogs tested were more closely related to Standard Poodles than to other members of their breed. These dogs appear as outliers in Fig. 3. Also note that individual Barbets were still not as tightly clustered as Standard Poodle, Italian Greyhound or Samoyed populations, indicating that individuals were either intentionally selected for the least possible relatedness or that the population they came from was under intense negative selection.

E. Internal relatedness (IR) of individuals and the population as a whole

1. IR testing

Genetic assessments such as those presented in Table 2 are indicators of population-wide heterozygosity and do not reflect the genetic diversity of individuals within the population. The genetic diversity of an individual dog is largely determined by the diversity inherited from each of its parents. Internal Relatedness (IR) is a calculation that has been used to determine the relative genetic contributions of both parents to an individual. The IR calculation evaluates homozygosity and assigns greater importance to rare and uncommon alleles (Table 4). IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0 (Fig. 4). A dog with a value of -1.0 has parents that are totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were genetically identical at all loci. An IR value of

+0.25 would be equivalent to offspring of full sibling parents from a random breeding population. IR values >0.25 occur when the parents of the full sibling parents were themselves highly inbred.

Table 4. IR and IRVD values for Barbet (n=49)

| | IR | IRVD |
|---------------|-----------|-------------|
| Min | -0.3157 | -0.1574 |
| 1st Qu | -0.1207 | 0.0482 |
| Mean | -0.0691 | 0.1140 |
| Median | -0.0813 | 0.1044 |
| 3rd Qu | -0.0249 | 0.1893 |
| Max | 0.2070 | 0.4353 |

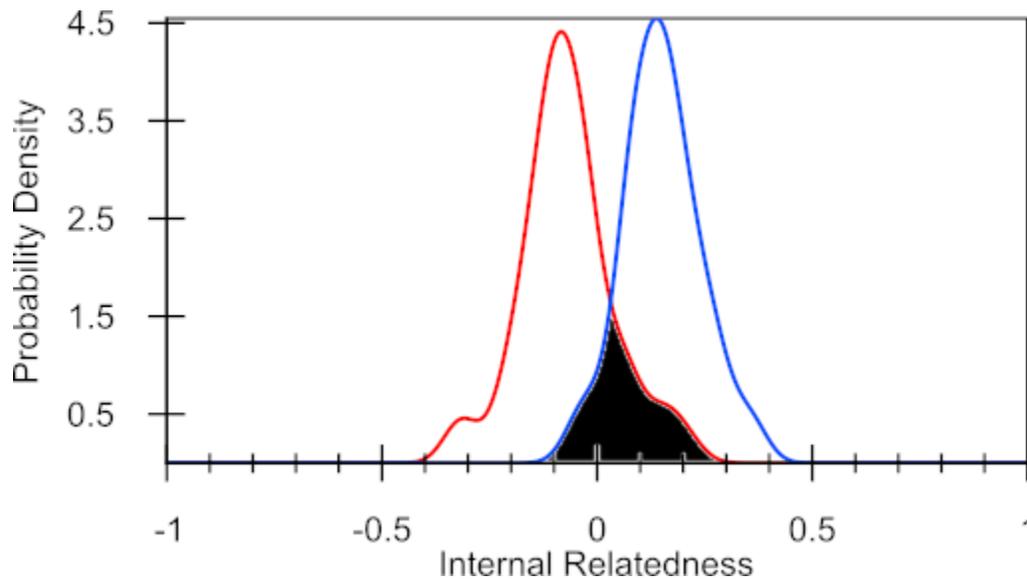


Fig. 4. Graph of IR values (red line) for 49 Barbet dogs. Allele and allele frequencies of these 49 dogs were compared with the frequency of those same alleles in a large village dog population which is ancestral to most modern dog breeds to yield IR village dogs (IRVD) (blue line).

The value of IR over standard genetic assessments is apparent in this group of dogs. The entire population is on average slightly outbred with a mean IR score of -0.070 (Table 4, Fig. 4). Nonetheless, the population contains a small number of dogs that are products of highly related parents. One half of the dogs had IR values from -0.081 to +0.207 and one fourth from -0.025 to +0.207. Therefore, some of these inbred dogs had IR scores as high as +0.207, near the level of offspring of full sibling parents. This group of more inbred dogs is balanced by a similar percentage of the population of outbred dogs with IR scores as low as -0.121 to -0.316. This is one of the most outbred groups of pure breed dogs that VGL has observed. This balancing of

highly outbred and inbred dogs is why the average H_o , H_e and FIS values based on allele frequency data from the 33 genomic STR loci were misleading.

2. Adjusted IR values based on village dogs (IRVD) as a measure of lost or retained genetic diversity

The IR values can be evaluated in such a way as to provide one estimate of the amount of species-wide genetic diversity that still exists in a breed from the time of its creation (closure of registry to outside dogs) to current time. This amount of retained genetic diversity is measured by comparing breed associated alleles and allele frequencies with the frequency of those same alleles among present-day village dogs from the Middle East, SE Asia and Island Pacific nations. Village dogs are the most random bred and genetically diverse population that has been studied to date and are ancestral to most modern breeds such as the Barbet. The IR value adjusted to village dogs is known as IR-village dogs or IRVD. The IRVD curve for Barbets was shifted well to the right, reflecting a 77.8% loss (or 22.2% retention) of potential genetic diversity during breed development (Fig. 4, Table 4). This 78% estimate of lost genetic diversity is not dissimilar to the 65% estimate of lost diversity obtained from known vs. observed alleles and their frequencies presented in Table 1.

F. DLA Class I and II haplotype frequencies and genetic diversity

1. A genetic assessment of allele and allele frequencies of STRs associated with DLA I and II haplotypes

The DLA consists of four gene rich regions (classes I-IV) comprising part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. The class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at STR loci associated with each of the three Class II genes are strongly linked and inherited as a single block or haplotype (Table 5). One haplotype comes from each of the parents. Specific class I and II haplotypes are often linked to each other and inherited as a genetic block with limited recombination over time. Therefore, DLA class I and II haplotypes can be viewed as reasonable surrogate markers for breed founders. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune and autoinflammatory diseases.

Class I and II haplotypes can be determined by direct sequencing or by their association with linked STR loci. Sequencing is time consuming and expensive, while the use of linked STR markers is simpler and much less expensive. There are four STR loci that are linked to the DLA class I region and three STR loci associated with the DLA class II region (Table 5).

Table 5. Standard Genetic Assessment of individual STR loci within the DLA region based on alleles and their frequencies. The first four STR loci are linked to the DLA class I region and the last three STR markers to the DLA class II region. (Updated October 10, 2019)

| # | Locus | N | Na | Ne | Ho | He | F |
|---|-------------------|----|----|-------|-------|-------|-------|
| 1 | DLA I-3CCA | 63 | 8 | 5.567 | 0.778 | 0.820 | 0.052 |
| 2 | DLA I-4ACA | 63 | 6 | 4.378 | 0.730 | 0.772 | 0.054 |
| 3 | DLA I-4BCT | 63 | 6 | 2.424 | 0.524 | 0.587 | 0.108 |
| 4 | DLA1131 | 63 | 6 | 4.894 | 0.778 | 0.796 | 0.022 |
| 5 | 5ACA | 63 | 4 | 2.485 | 0.508 | 0.598 | 0.150 |
| 6 | 5ACT | 63 | 5 | 3.292 | 0.667 | 0.696 | 0.043 |
| 7 | 5BCA | 63 | 6 | 3.947 | 0.746 | 0.747 | 0.001 |

The seven DLA-associated STR loci are very polymorphic and the number and incidence of alleles parallels that of the 33 more genome-wide autosomal STR loci shown in Tables 1 and 2 (Table 5). The Ho values for the DLA region of the 49 Barbet dogs were high, mirroring the higher than usual heterogeneity among this group of dogs (Table 6). The expected heterozygosity for these dogs was near equal to the actual observed heterozygosity yielding near zero F values. This indicated that the DLA region was in equilibrium with the rest of the genome.

Table 6. Standard genetic assessment of the DLA region of 49 Barbet dogs based on seven linked STR loci. (Updated October 10, 2019)

| | N | Na | Ne | Ho | He | F |
|-------------|----|-------|-------|-------|-------|-------|
| Mean | 63 | 5.857 | 3.855 | 0.676 | 0.716 | 0.061 |
| SE | | 0.425 | 0.417 | 0.040 | 0.033 | 0.018 |

2. DLA class I and II haplotypes in Barbet

The genes of the DLA region are in strong linkage disequilibrium (LD), which means they are inherited as a block with one block from the sire and one from the dam. Genetic recombination in this region is less than for other regions, and the genetic makeup (genotype) of this block tends to remain the same over very long periods of time. Therefore, these blocks of DLA genes can also be used to trace founders of a pure breed. The actual combination of genes that are inherited is indicated by the seven alleles that are present in the associated STRs. These alleles are linked together to form what are known as haplotypes. These haplotypes are designated according to the combination of alleles that they contain.

DLA class I haplotypes are defined by linked alleles at four different STR loci, and DLA class II by linked alleles at three different loci (Table 7). The STR-based haplotype nomenclature used by the VGL in this breed diversity analysis is based on numerical ranking with the first haplotypes identified in Standard Poodles being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class II haplotypes. It is common for various dog breeds to share common and even rare haplotypes, depending on common ancestry. To date, the VGL has identified 205

unique DLA I and 112 DLA II haplotypes among all dogs. DLA class I and II regions are in looser linkage than each region alone, leading to 355 combinations of DLA class I/II haplotypes.

Table 7. DLA class I and Class II haplotypes and their frequencies

| DLA Class I Haplotype Frequencies (Updated Oct 10, 2019) | | |
|--|------------------|----------------------|
| DLA1 # | STR types | Barbet (n=62) |
| 1001 | 380 373 281 182 | 0.040 |
| 1002 | 380 365 281 181 | 0.056 |
| 1003 | 387 375 277 186 | 0.121 |
| 1020 | 388 369 289 184 | 0.129 |
| 1030 | 380 373 293 178 | 0.024 |
| 1033 | 382 379 277 181 | 0.016 |
| 1035 | 386 373 277 184 | 0.153 |
| 1046 | 376 379 291 180 | 0.032 |
| 1092 | 376 379 277 181 | 0.032 |
| 1093 | 386 379 277 180 | 0.169 |
| 1159 | 395 379 277 181 | 0.113 |
| 1200 | 394 367 273 178 | 0.113 |
| DLA Class II Haplotype Frequencies (Updated Oct 10, 2019) | | |
| DLA2 # | STR types | Barbet (n=62) |
| 2001 | 343 324 284 | 0.185 |
| 2003 | 343 324 282 | 0.129 |
| 2006 | 339 325 280 | 0.008 |
| 2009 | 351 324 280 | 0.032 |
| 2017 | 343 322 280 | 0.032 |
| 2023 | 341 323 282 | 0.024 |
| 2032 | 339 323 280 | 0.169 |
| 2035 | 341 323 280 | 0.048 |
| 2043 | 343 324 296 | 0.105 |
| 2047 | 339 331 280 | 0.113 |
| 2082 | 339 325 268 | 0.145 |
| 2113 | 343 324 292 | 0.008 |

The 49 Barbets in this study possessed 12 DLA class I and 11 DLA class II haplotypes (Table 6). Four DLA class I (1003, 1020, 1035, 1093) and four DLA class II (2001, 2003, 2032, 2082) are found in 60-70% of individuals and are likely to remain dominant Barbet haplotypes even as

more dogs are tested. However, several lower incidence haplotypes will undoubtedly be identified as more dogs are tested.

The current number of DLA class I and II haplotypes make-up 12/205=5.8% and 11/112=9.8%, respectively, of all haplotypes known to exist in all dogs tested at the VGL to date. These percentages may increase somewhat as more haplotypes are identified. However, they provide an estimate of canine genetic diversity that has been retained by Barbet during proto-breed creation, closure to further introgressions, and up to present time. The percentage of known DLA class I and II haplotypes retained by the Barbet (approximately 10%) is about one-third of the retained genetic diversity determined for all known alleles vs Barbet alleles (Table 1) and that were identified in the IR/IRVD comparison of Barbet and indigenous village dogs (Figure 4). This difference is due to a higher number of haplotypes in the DLA class I and II regions than alleles in any of the 33 autosomal STR loci. It has been proposed that genetic polymorphisms in the DLA region are subject to more positive selection than any other region of the genome, due to continuous exposure to new pathogens that have appeared throughout canid evolution.

3. DLA class I and II haplotype sharing with other breeds

DLA class I and II haplotypes found in the 49 Barbets tested were extensively shared with other breeds, however one DLA class I haplotype; namely 1200, has not been identified in other breeds to date (Table 8). Strong DLA class I and II haplotype sharing occurs with the Standard Poodle (8/12 DLA class I and 6/11 class II haplotypes). Somewhat less extensive class I and II haplotype sharing was also observed between the Barbet and Miniature Poodle, Golden Retriever, Havanese, and Italian Greyhound, all European breeds. Little haplotype sharing was observed with more Asian breeds such as Akita and Shiba Inu.

| DLA Class I Haplotype Frequencies (Updated Dec 11, 2018) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|-----------------|-------------------------|-----------------------|---------------|------------------------|-----------------------|---------------------------------|-------------------------------|--------------------|---------------------------|-------------------------|------------------------|-------------------------------|-------------------------|--------------------------|------------------|-----------------------------------|---------------------------|------------------------|----------------------------|-------------------------|--------------------|--------------------------|--------------------------------|-----------------|-----------------|------------------|---------------------|------------------|--------------------|--------------------------|------|
| DLA1 # | STR Types | Alaskan Klee Kai (n=83) | American Akita (n=91) | Barbet (n=48) | Biewer Terrier (n=110) | Biewer Terrier (n=92) | Biewer Yorkshire Terrier (n=53) | Black Russian Terrier (n=124) | Blend Akita (n=53) | Doberman Pinscher (n=490) | English Bulldog (n=163) | English Mastiff (n=36) | Flat Coated Retriever (n=419) | Giant Schnauzer (n=186) | Golden Retriever (n=695) | Havanese (n=392) | Irish Red and White Setter (n=41) | Italian Greyhound (n=764) | Japanese Akita (n=330) | Labrador Retriever (n=150) | Lakeland Terrier (n=45) | Magyar Agar (n=44) | Miniature Poodle (n=254) | Polish Lowland Sheepdog (n=16) | Poodle (n=2376) | Samoyed (n=187) | Shiba Inu (n=90) | Shiloh ISSA (n=146) | Shepherd (n=121) | Toy Poodle (n=121) | Yorkshire Terrier (n=16) | |
| 1001 | 380 373 281 182 | -- | -- | 0.04 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.014 | -- | 0.2652 | -- | -- | 0.007 | 0.017 | -- | |
| 1002 | 380 365 281 181 | -- | -- | 0.06 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.0007 | -- | -- | -- | -- | -- | -- | -- | -- | 0.002 | -- | 0.1791 | -- | -- | -- | 0.008 | -- | |
| 1003 | 387 375 277 186 | -- | -- | 0.14 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.1403 | 0.034 | -- | -- | -- | -- | 0.003 | -- | 0.1 | 0.01 | -- | 0.1677 | -- | -- | -- | -- | -- | |
| 1030 | 380 369 289 184 | -- | -- | 0.15 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.002 | -- | 0.0057 | -- | -- | -- | 0.017 | -- |
| 1030 | 380 373 293 178 | -- | -- | 0.01 | 0.477 | 0.446 | 0.349 | -- | -- | 0.101 | -- | 0.16 | -- | -- | 0.0007 | 0.001 | -- | 0.0236 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.31 |
| 1033 | 382 379 277 181 | -- | -- | 0.02 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.14 | 0.006 | -- | 0.0025 | -- | -- | -- | -- | -- | |
| 1035 | 386 375 277 184 | -- | -- | 0.14 | -- | -- | 0.009 | -- | -- | -- | -- | 0.007 | -- | -- | 0.006 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.007 | 0.004 | -- | -- |
| 1040 | 376 379 291 180 | -- | -- | 0.03 | 0.014 | 0.054 | -- | -- | -- | 0.003 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.0002 | -- | -- | -- | -- | -- | -- |
| 1092 | 376 379 277 181 | -- | -- | 0.01 | -- | -- | 0.038 | 0.258 | 0.009 | -- | 0.006 | -- | -- | 0.237 | -- | 0.087 | -- | -- | 0.295 | -- | -- | -- | -- | -- | -- | 0.0002 | -- | -- | -- | -- | -- | 0.06 |
| 1093 | 386 379 277 180 | -- | -- | 0.21 | -- | -- | -- | 0.081 | -- | -- | -- | -- | -- | -- | -- | 0.028 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 1159 | 395 379 277 181 | -- | -- | 0.1 | -- | -- | -- | -- | -- | 0.001 | -- | -- | -- | -- | 0.169 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 1200 | 394 367 273 178 | -- | -- | 0.09 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

| DLA Class II Haplotype Frequencies (Updated Dec 11, 2018) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|-------------|-------------------------|-----------------------|---------------|------------------------|-----------------------|---------------------------------|-------------------------------|--------------------|---------------------------|-------------------------|------------------------|-------------------------------|-------------------------|--------------------------|------------------|-----------------------------------|---------------------------|------------------------|----------------------------|-------------------------|--------------------|--------------------------|--------------------------------|-----------------|-----------------|------------------|---------------------|------------------|--------------------|--------------------------|-------|-------|
| DLA2 # | STR Types | Alaskan Klee Kai (n=83) | American Akita (n=91) | Barbet (n=48) | Biewer Terrier (n=110) | Biewer Terrier (n=92) | Biewer Yorkshire Terrier (n=53) | Black Russian Terrier (n=124) | Blend Akita (n=53) | Doberman Pinscher (n=490) | English Bulldog (n=163) | English Mastiff (n=36) | Flat Coated Retriever (n=419) | Giant Schnauzer (n=186) | Golden Retriever (n=695) | Havanese (n=392) | Irish Red and White Setter (n=41) | Italian Greyhound (n=764) | Japanese Akita (n=330) | Labrador Retriever (n=150) | Lakeland Terrier (n=45) | Magyar Agar (n=44) | Miniature Poodle (n=254) | Polish Lowland Sheepdog (n=16) | Poodle (n=2376) | Samoyed (n=187) | Shiba Inu (n=90) | Shiloh ISSA (n=146) | Shepherd (n=121) | Toy Poodle (n=121) | Yorkshire Terrier (n=16) | | |
| 2001 | 343 324 284 | -- | -- | 0.21 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.008 | 0.141 | 0.04 | -- | -- | -- | -- | -- | -- | -- | -- | 0.016 | -- | 0.6065 | -- | 0.006 | 0.007 | 0.008 | -- |
| 2003 | 343 324 282 | -- | -- | 0.15 | 0.223 | 0.255 | 0.208 | -- | -- | -- | 0.598 | -- | 0.134 | 0.038 | 0.0223 | 0.223 | 0.004 | 0.0072 | -- | 0.0072 | 0.02 | 0.72 | -- | 0.13 | 0.016 | -- | 0.304 | -- | 0.0886 | 0.013 | -- | 0.01 | 0.438 |
| 2006 | 339 324 280 | -- | -- | 0.01 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.156 | -- | 0.004 | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.0322 | -- | -- | -- | -- | -- | -- |
| 2009 | 351 324 280 | -- | -- | 0.03 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.25 | -- | -- | -- | 0.0322 | -- | -- | 0.006 | -- | -- | -- |
| 2017 | 343 322 280 | -- | 0.016 | 0.03 | -- | -- | -- | -- | 0.019 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.01 | -- | -- | 0.0109 | -- | -- | -- | 0.006 | -- | 0.037 |
| 2023 | 341 323 282 | -- | -- | 0.01 | 0.477 | 0.446 | 0.349 | 0.004 | -- | 0.101 | -- | 0.215 | 0.09 | 0.001 | -- | 0.0417 | 0.009 | -- | 0.2199 | 0.006 | -- | 0.06 | 0.35 | -- | 0.38 | 0.0029 | -- | -- | 0.39 | 0.004 | -- | 0.31 | |
| 2012 | 339 323 280 | -- | -- | 0.21 | -- | -- | -- | 0.081 | -- | -- | -- | -- | 0.16 | -- | 0.0007 | 0.0236 | 0.001 | -- | -- | -- | -- | -- | -- | -- | 0.014 | -- | -- | -- | 0.011 | -- | -- | -- | |
| 2035 | 341 323 280 | -- | -- | 0.03 | -- | -- | -- | -- | 0.009 | -- | -- | -- | -- | -- | -- | -- | 0.029 | -- | 0.0465 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 2043 | 341 324 296 | -- | 0.229 | 0.09 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.07 | 0.0963 | 0.295 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 2047 | 339 331 280 | -- | -- | 0.1 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.0108 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 2082 | 339 325 268 | -- | -- | 0.13 | -- | -- | 0.009 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.09 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 8. DLA class I and II haplotype sharing between Barbets and other breeds

III. What does DNA-based genetic testing tell us about contemporary Barbet

It is not possible based on 49 individual dogs to make final conclusions, but the results of initial testing do provide important insights. The 49 dogs selected for testing appear to exhibit surprisingly more heterozygosity than expected. Assuming these dogs are representative of the breed as it currently exists, it appears that breeders have done an excellent job of finding and using the least related parents from within the existing pool of breeders.

Although the initial 49 dogs that were tested were heterogeneous (i.e., not inbred), the breed appears to have below average genetic diversity. The breed has retained an estimated 22% of the genetic diversity known to exist in village dogs from the ancestral area of the breed founders. A lack of genetic diversity was also seen with the low percentages of known canine DLA class I (6%) and class II (10%) haplotypes that were represented in the Barbet that were tested.

It will be important to test 50-100 additional dogs from a wide geographic area, including North America, Finland, Switzerland, The Netherlands, Sweden, Germany and Poland, in order to obtain a complete and more accurate genetic blueprint for the breed. A low genetic diversity, if confirmed, is not in itself serious. If, as it appears, founder dogs were relatively free of lifespan shortening deleterious genetic polymorphisms and random breeding was strictly adhered to during the breed's post-registration history, the health of the breed can be sustained at the present course. However, a low degree of genetic diversity makes it much more difficult to avoid the problems that might occur as a breed changes from performance to conformation and from relatively unknown to very popular. The problems that arise when breeds go from performance to conformation have been well documented [4]. Performance breeds vary more in appearance (phenotypes). Variation in phenotypes is due to variations in the DNA, which indicates more genetic diversity. Performance traits are also less heritable than conformation traits, which helps resist popular sire and popular bloodline effects. Booms and busts in breed popularity can also encourage poor breeding practices and bouts of inbreeding [4].

IV. Heritable disorders of Barbet

A. Overview and complex genetic disorders

The average lifespan of the Barbet is said to be 13–15 years, which is typical for medium-sized dogs. This long average life span indicates that health problems in Barbets, when they occur, are more apt to be of a chronic nature and amenable to symptomatic care. The health problems described for the breed also tend to be common to all dogs and amplified to varying degrees from breed-to-breed by founder selection and genetic descent. Ear infections are a problem in Barbets, as they are in other curly coated breeds. Several disorders common to pure breeds and of complex genetic origin occur in the breed. Undescended testicles and undershot/overshot bites occur in the breed and are often associated with pure-breeding and inherited by descent from certain ancestors. Problems such as epilepsy, hernias, hip dysplasia, elbow dysplasia and entropion, have been traced back 4–6 generations or more. There is no specific history of autoimmune disorders such as hypothyroidism, Addison's disease or sebaceous adenitis in Barbet. These are serious problems in Standard Poodles.

homozygosity at any genomic loci or DLA class I and II haplotype and encourage the use of dogs with less common genomic alleles or DLA haplotypes. Maintaining existing genomic diversity will require using IR values of potential mates based on the 33 STR loci to assure puppies of equal or greater overall diversity, like what is being done by many Standard Poodle breeders. However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

The next step is to compare the DLA class I and II haplotypes. You want to avoid breeding pairs that will produce puppies that will be homozygous for the same haplotypes, and once again, less common haplotypes may offer more diversity than common ones.

Breeders who do not have access to computer programs to predict the outcome of matings based on IR values of sire and dam can also compare values by manual screening. Potential sires and dams should be first screened for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Some extra weight should be given to rare vs common alleles. This information is included on all certificates and on the breed-wide data on the VGL website.

Puppies, once born, should be tested for their actual IR values, which will reflect the actual genetic impact of each parent on internal diversity. Considerations of mate choices for genetic diversity should be balanced with other breeding goals but maintaining and/or improving genetic diversity in puppies should be paramount.

Barbets still retain their close relationship to Standard Poodles and 4/49 of the Barbets tested were more related to Standard Poodles than to other members of their breed. Unfortunately, no DNA data was available to test for relationships to other "water dog" breeds (e.g., Portuguese and Spanish water dogs, Curly coated retriever, Logotto Romagnolo, American and Irish water spaniels). The close genetic relationship of Barbets to Standard Poodles is almost to the level of varieties, such as seen between Japanese and American Akita or North American and European Italian Greyhounds. Varieties (i.e., distinct bloodlines) would provide an opportunity for outcrossing between breeds to improve genetic diversity. However, Barbets have no reported problems with autoimmune disorders, while disorders such as chronic thyroiditis, Addison's disease and sebaceous adenitis are serious problems in Standard Poodles [7, 8]. If Barbets are indeed at low risk for autoimmune diseases, the risk of introducing autoimmune disorders into Barbets by outcrossing to Poodles would be too great. The close relationship of Barbets and Standard Poodles could also be exploited for determining the origins and complex heritability of autoimmune diseases in Standard Poodles doing genome wide association studies (GWAS) [7, 8] or by comparing whole genome sequences of disease-free Barbets and diseased Standard Poodles.

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