

# Genetic Diversity Testing for Biewer

(includes the Biewer, Biewer Terrier, and Biewer Yorkshire Terrier)

## 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 determine genetic diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions. This test panel will be useful to Biewer breeders who wish to track and increase genetic diversity of their breed as a long term goal.

Genetic testing of Biewer is now in the preliminary results-phase please see [Enrolling a Breed](#). During this phase, we will continue to test Biewer dogs and send results. Testing 50 or more Biewer of each variety will further converge the baseline genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their variety of the breed. We encourage breeders to submit samples from active dogs to further build the database. The goal is to test enough dogs so that no new alleles or DLA haplotypes are recognized.

[Free tests are available for selected dogs!](#) [BetterBred](#), in collaboration with a generous donor promoting diversity testing for the health of the breed, are offering a free diversity test for select dogs. Please see this [questionnaire page](#) for more information.

## Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across the genome were used to gauge genetic 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: 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 genetic diversity within an individual that 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 cannot be compared between dogs. Two dogs may have identical IR values but with very different genetic makeups.

## I. History of Biewer dogs

The Biewer (Beeva, BeeVare) is a new and rare “breed.” It is known by several other names, including *Biewer Terrier*, *Biewer Yorkshire à la Pom Pon*, *Biewer Yorkie*, *Biewer Yorkshire*, *Biewer Yorkie Terrier*, and *Biewer Yorkshire Terrier*. It evolved in Germany in the early 1980s in the Friedheck Yorkshire Terrier kennel of Werner and Gertrud Biewer. They chose UK Yorkshire terriers as the foundation stock of the von Friedheck Yorkshire terrier. The heart of the breeding program (for the Biewer) most likely began with the arrival of *Streamglen Richard*, one of many UK Yorkshire terriers imported from Streamglen Kennels in the United Kingdom.

*Streamglen Richard* is present on both sides of the Master Parents, two outstanding blue and tan Yorkshire Terriers, *Darling von Friedheck* and *Fru Fru von Friedheck*, introduced to the kennel in 1980 (see pedigree below). A recessive white spotting (piebald) gene appeared among the Friedheck line with the birth in 1984 of a blue, white and gold female puppy named *Schneeflöckchen von Friedheck*. Whether this was due to a mutation that had been latent in Yorkshire Terriers for some time, a spontaneous mutation, or an introgression from another similar breed of small dogs has long been a source of debate (since the Biewer's arrival in the US). Herr and Frau Biewer claimed that white spotting was not the result of an outcross with a similar breed (which would have carried the piebald mutation). If this is true the most logical conclusion is that the parents of *Schneeflöckchen* carried the recessive piebald mutation in a latent manner, which is known to occur in Yorkshire terriers.

Margot Eskens, (a famous German singer) undoubtedly helped to bring this unique phenotype to the public's eye by posing for pictures with *Schneeflöckchen* and *Schneemann* which were used in an advertisement/flyer. Margot was also a proud owner of one of the first Biewer Yorkshire Terriers produced in the von Friedheck kennels. Herr and Frau Biewer directed their subsequent breeding efforts to develop dogs of *Schneeflöckchen*'s bearing and appearance. This was done purportedly within their kennels without outside introductions for five years. The breedings resulted in a line of distinctive “Yorkshire terriers” with white patches of varying size, on the underside and muzzle, including four white legs starting with the paws and extending up the leg. The Biewer's were highly discerning in the dogs they bred. The demand for their dogs was great and breeding was very selective. The last of the championed von *Friedheck Yorkshire* terriers were placed in pet homes when Herr Biewer became ill in 1992.

The uniqueness of the Biewer Yorkshire Terriers led their breeders to seek recognition as a distinct “variety” to be called *Biewer Yorkshire Terriers*. The Biewer Yorkshire Terriers were ultimately recognized as a distinct breed in 1989 by the **Allgemeiner Club der Hundefreunde Deutschland** (ACH) as Biewer Yorkshire a la Pom Pon. This long and cumbersome name was ultimately shortened by many in different ways but always preceded by the name “Biewer.” Because of the many names for this breed currently in use by various breeders and breed clubs, the varieties of Biewer tested in this study will be referred to simply as “*Biewer*” or “*Biewers*” for purposes of uniformity and simplicity.

In the early 2000's, there were only a handful of breeders in America. Controversy soon surrounded the breed as it took off in popularity in America in the mid-2000's. To keep up with demand for Biewers, German breeders continued to introduce German Yorkshire terriers into

their breeding programs. They accepted this practice since Herr Biewer used traditional Yorkies to produce his tricolor dogs. Some American breeders also engaged in the practice of breeding to German Yorkshire terriers. Biewers imported into the US were frequently bundled with a German Yorkshire terrier to start breeding programs. The **Biewer Terrier Club of American (BTCA)** accepted the practice of breeding with Yorkshire terriers until Dec 2007 when the club stated, "[The BTCA, Inc. only allows Biewer to Biewer breeding...](#)"

Biewers are accepted by the **Biewer Breeders International Registry (BBIR)**, although some US breeders still use German registries. Biewer Terriers are also accepted by the **Biewer Terrier Registry of America (BTRA)** based having their "purity" confirmed by the MARS wisdom test, version 3.0, which evaluates a panel of DNA markers against dogs of accepted Biewer ancestry.

The **Biewer Terrier** was provisionally accepted by the AKC as a new breed in 2014, but they are only recorded by the Foundation Stock Service (FSS). FSS breeds are not eligible for AKC registration but are allowed in "Companion and Performance Events." They are considered a breed in development.

Several other clubs such as the **Biewer Breed Club of America (BBCA)** have also been active in trying to get the breed accepted by the AKC. All of these individuals, clubs and registries insist on only dogs that can trace themselves directly to the Biewer's original kennel with no introgressions from other Yorkshire Terriers or phenotypically similar small breeds.

Pedigree - SCHNEEFLOECKCHEN VON FRIEDHECK			
		CHAMPION <b>MUCCU VON FRIEDHECK</b> Blue & Tan Germany 1975	CHAMPION <b>STREAMGLEN RICHARD</b> Blue & Tan England 1972
			<b>STREAMGLEN FLORA</b> Blue & Tan England
		<b>IWY VON FRIEDHECK</b> Blue & Tan Germany 1974	CHAMPION <b>STREAMGLEN RICHARD</b> Blue & Tan England 1972
			<b>BONNY VON FRIEDHECK</b> Blue & Tan Germany 1972
<b>SCHNEEFLOECKCHEN VON FRIEDHECK</b> White-Blue-Gold ** Germany 1984	World Junior Champion 1981 <b>DARLING VON FRIEDHECK</b> Blue & Tan Germany 1980	<b>QUARDI VON FRIEDHECK</b> Blue & Tan Germany 1977	CHAMPION <b>STREAMGLEN RICHARD</b> Blue & Tan England 1972
			World Champion 1976 + 1977 <b>GRAZIELLA VON FRIEDHECK</b> Blue & Tan Germany 1974
			CHAMPION <b>STREAMGLEN RICHARD</b> Blue & Tan England 1972
			<b>ANJA VON FRIEDHECK</b> Blue & Tan Germany 1970
	World Junior Champion 1981 <b>FRU FRU (*1980) VON FRIEDHECK</b> Blue & Tan Germany 1980	<b>TSCHI TSCHI VON FRIEDHECK</b> Blue & Tan Germany 1976	

World Pedigree DataBase Biewer Yorkshire Terrier - <http://ingrus.net/biewer/en>

\*\*Note that *Schneeflöckchen* registration indicates "blue and tan," see images below.

**KLUB FÜR TERRIER e.V. von 1894**  
 Die Freiheit der Rasse  
 ein Zeichen für die Verantwortung  
 für die Gesundheit der Tiere

**AHNTAFEL**  
 Nach Prüfung der Urkunde  
 erhebt sich die Verantwortung  
 für die Vererbung der  
 Mängel der Rasse

Name: **BRUNNEN - BIERER** Nummer: **33**  
 Farbe: **blau + weiß / gelb und blau** Haltungsart: **ausgew. 1984**  
 Farbe des Halses: **schwarz / schwarz** Geschlecht: **weiblich**  
 Züchtername: **von Fritschke**  
 Züchter: **Stratmann, Kist, Straßburg, Fritschke**

Datum: **20.08.1984** Platz: **283**

nr.	Elter	nr.	Elter	nr.	Elter
1	Walt - Jg. - Sieger 91	2	KFT - Jg. - Champion Kob. - Jg. - Sieger Wespa - Jg. - Sieger Dr. Jg. - Jg. - Champion Wespa - Jg. - Sieger - 3144	3	Dr. - Champion Friedrich - Richard - VEGOT 454
4	Walt - Jg. - Sieger 91	5	Dr. - Jg. - Champion Kob. - Jg. - Sieger Wespa - Jg. - Sieger Dr. Jg. - Jg. - Champion Wespa - Jg. - Sieger - 3144	6	Dr. - Champion Friedrich - Richard - VEGOT 454
7	Walt - Jg. - Sieger 91	8	Dr. - Jg. - Champion Kob. - Jg. - Sieger Wespa - Jg. - Sieger Dr. Jg. - Jg. - Champion Wespa - Jg. - Sieger - 3144	9	Dr. - Champion Friedrich - Richard - VEGOT 454
10	Walt - Jg. - Sieger 91	11	Dr. - Jg. - Champion Kob. - Jg. - Sieger Wespa - Jg. - Sieger Dr. Jg. - Jg. - Champion Wespa - Jg. - Sieger - 3144	12	Dr. - Champion Friedrich - Richard - VEGOT 454
13	Walt - Jg. - Sieger 91	14	Dr. - Jg. - Champion Kob. - Jg. - Sieger Wespa - Jg. - Sieger Dr. Jg. - Jg. - Champion Wespa - Jg. - Sieger - 3144	15	Dr. - Champion Friedrich - Richard - VEGOT 454



## II. The Canine Genetic Diversity Test and What It Tells Us about Biewers

### A. Standard genetic assessments based on 33 STR loci on 25 chromosomes and allele frequencies

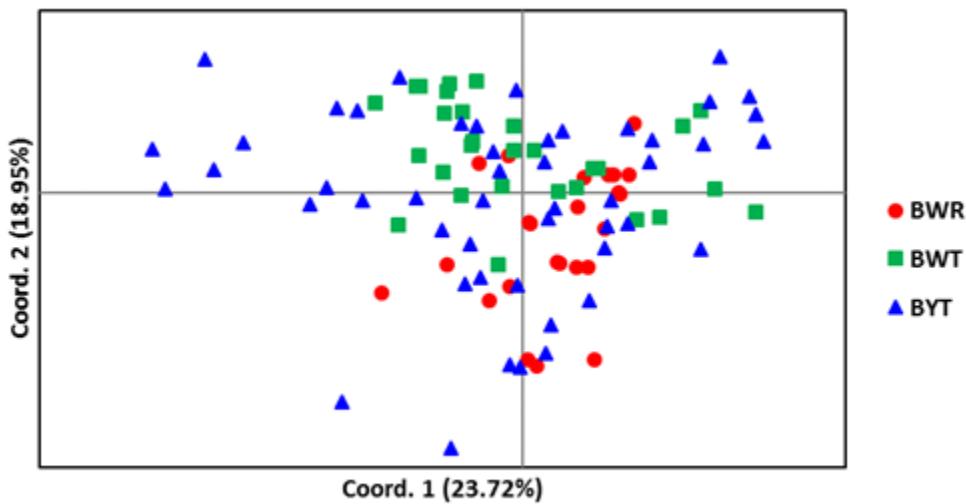
STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) with additional markers developed by the VGL for forensic purposes. Each STR locus manifests a number of different genetic configurations known as alleles. Each individual inherits one of these alleles from the sire and the other from the dam. Allele designations, allele size and allele frequencies at each of the 33 STR loci for 104 Biewer dogs that were tested is listed in Table 1 ([link to table 1](#)). The 104 dogs included 50 dogs described as Biewer Yorkshire Terriers (BYT), 25 dogs listed as Biewer (BWR) and 29 dogs called Biewer terriers (BWT). Results for each of the “varieties” are presented separately as well as for the total population. With the exception of a few uncommon alleles, the same alleles are seen in all three varieties and the most common alleles in one variety are often the most common in the other varieties. This intense sharing of alleles at the same approximate frequencies indicates that BWR, BWT and BYT are highly related.

**Table 1.** The frequency of known alleles at each of the 33 genomic STR loci in three different Biewer populations. Individual alleles are designated by numbers representing their size in gels. N = number each variety of Biewer tested. The most common allele (shaded) tends to be shared between all three varieties, and this also is true for alleles of lessening frequency.

[\(link to table 1\)](#)

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

The first objective was to determine how the three “varieties” of Biewer were related to each other. This is best done by comparing alleles and allele frequencies at each of the 33 STR loci using a technique called principal coordinate analysis (PCoA). PCoA is comparable to the multidimensional scaling (MDS) plots used by others. A PCoA graph is actually multi-dimensional (ball-like), but the results are displayed in two dimensions based on the two coordinates that best portray the genetic relationship of individuals within the total population (Fig. 1). The more distant points (i.e., dogs) are from each other the greater the genetic differences and *vice versa*. This plot indicates that BYT, BWR and BWT are members of the same population (i.e., they belong to a single breed) with the bulk of the three subpopulations clustering in the middle. Genetic outliers exist for all three varieties with BYT having more outliers than BWR and BWT. However, the differences are not significant, and clusters are expected to “tighten up” as more samples are tested.



**Fig. 1.** PCoA of three varieties of Biewer (n=104) using comparative allele frequencies at 33 STR loci.

## 2. The use of allele frequencies at the 33 STR loci to measure fixation indices as indicators of breed-wide heterozygosity

The fixation indices, which are genetic assessments of heterozygosity, for the three “varieties” of Biewer, are shown in Table 2.  $N_a$  is the mean number of alleles identified at each of the 33 STR loci,  $N_e$  is the mean effective number of alleles at each locus,  $H_o$  is the observed heterozygosity,  $H_e$  is the expected heterozygosity for a random breeding population, and  $F_{IS}$  is a measure of inbreeding.  $N_a$  and  $N_e$  are highest for BYT and lowest for the BWT, but these values are highly dependent on the number of dogs tested. The number of actual and effective alleles for a particular population is accurate only when the population tested is large enough to represent all of the alleles and their frequency for each variety of Biewer.

Based on values for all 104 Biewers, the mean number of alleles found at each locus is 6.39, which is similar to many pure breeds. The number of the alleles ( $N_e$ ) that contribute the most to overall heterozygosity is 3.35, which is also in line with a number of pure breeds. The expected heterozygosity ( $H_e$ ) is the value that would be anticipated if the allele frequencies were in Hardy-Weinberg equilibrium (HWE), i.e., a condition achieved when mate selection is totally random. The values for  $H_o$  and  $H_e$  are nearly equal, indicating that the population is in near Hardy-Weinberg equilibrium. FIS is a coefficient of inbreeding and calculated from the  $H_e$  and  $H_o$  ( $1-H_o/H_e$ ). A value of -1.0 would mean offspring were products of parents that were totally unrelated at all genomic STR loci, while a value of +1.0 would mean that the offspring were products of parents that were genetically identical. The FIS for the total Biewer population was -0.056, which indicates that there is a small excess of individuals that are more outbred than the population as a whole.

A comparison of genetic assessment values for the three varieties of Biewer show no significant differences. As is expected, the average number of alleles ( $N_a$ ) increases as the population size increase, because more dogs are going to yield additional alleles, especially alleles that occur at low frequency.  $H_o$  and  $H_e$  values for each variety are similar to the other varieties and to each other, yielding FIS values that are also not significantly different.

**Table 2.** Genetic assessment of Biewer (n=104) based on allele frequencies at 33 genomic STRs

<b>Pop</b>		<b>N</b>	<b><math>N_a</math></b>	<b><math>N_e</math></b>	<b><math>H_o</math></b>	<b><math>H_e</math></b>	<b>FIS</b>
<b>BWR</b>	<b>Mean</b>	25	5.242	3.183	0.684	0.648	-0.056
	<b>SE</b>		0.200	0.167	0.031	0.026	0.027
<b>BWT</b>	<b>Mean</b>	29	5.333	3.004	0.656	0.628	-0.045
	<b>SE</b>		0.245	0.181	0.028	0.023	0.024
<b>BYT</b>	<b>Mean</b>	50	5.788	3.374	0.685	0.674	-0.019
	<b>SE</b>		0.253	0.167	0.021	0.020	0.015
<b>Total</b>	<b>Mean</b>	104	6.394	3.346	0.677	0.669	-0.014
	<b>SE</b>		0.261	0.180	0.022	0.021	0.012

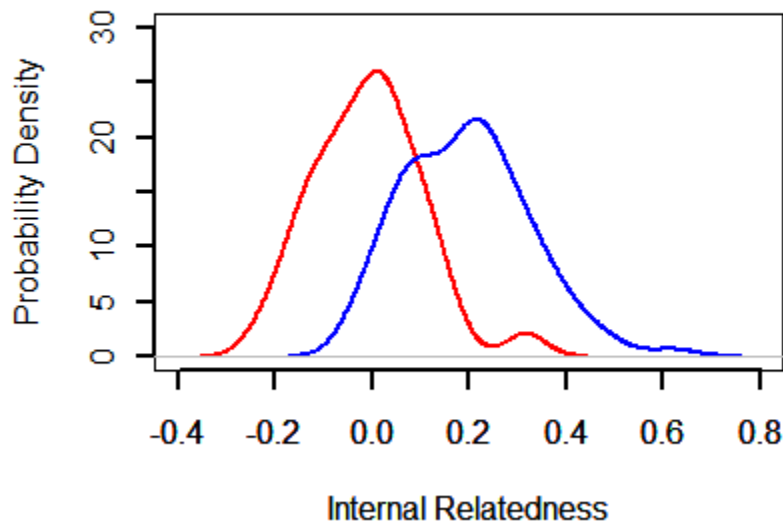
## **B. The use of genomic allele frequencies to determine internal relatedness**

### *1. Internal relatedness of individuals in the BYT, BWR and BYT/BWR populations*

Genetic assessments such as that presented in Table 2 are an indicator of population-wide heterozygosity and do not reflect the genetic diversity of individuals within the population. The genetic diversity of an individual is largely determined by the diversity inherited from each of the 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 uses allele frequencies to give more weight to rare and uncommon alleles. On average, the lower the IR, the more outbred the individual, and the higher the score, the more inbred. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with a value of -1.0 would have parents that were totally different at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were identical at every locus. A

value of +0.25 would be seen in offspring of parents that were full siblings, provided that the parents of the full siblings were randomly bred. IR values >0.25 would occur when the parents of the full sibling parents were themselves highly inbred.

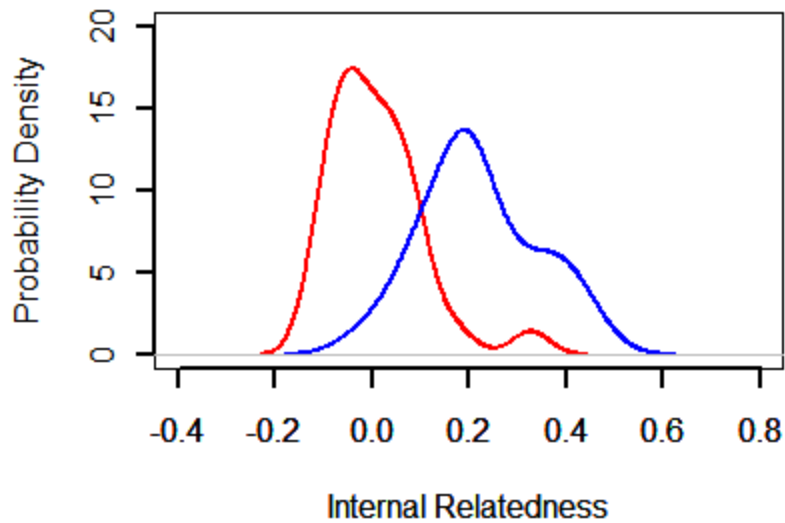
Figure 2 is a graphic portrayal of IR (red line) values for all 104 Biewer, regardless of variety. The most outbred dog in the population has an IR value of -0.241 while the most inbred dog in the population had an IR value of 0.327. The mean IR value for the population was -0.008, which meant that one-half of the population was more outbred, and one-half more inbred. One quarter of the population had values between -0.008 and -0.241 and one quarter had values between 0.058 and 0.327. Therefore, the 104 Biewer tested were balanced by dogs either more inbred or outbred than the mean and with very few dogs that were more inbred than what would be expected from a breeding of full siblings from the population.



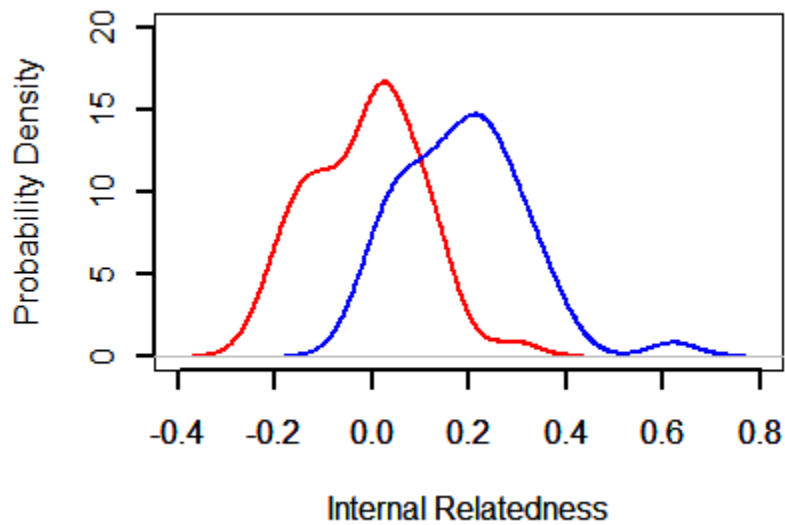
**Fig. 2.** Distribution of IR estimated in 104 Biewer based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (IRVD-blue line). Lost diversity was determined by comparing allele frequencies at the same loci between BWR and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.

The IR values for the three varieties of Biewer were also graphed separately (Figs. 3-5). The BWT had the tightest (narrowest) IR curve with less highly outbred dogs and fewer highly inbred dogs (Fig.3.). The BYT and BWR (Fig. 4, 5) had broader IR curves than the BWT due to the presence of dogs that were both more inbred and more outbred than dogs found among the BWT. This difference could also reflect the numbers of dogs in each group that were tested, or the way dogs were selected from each variety for testing. The BYT and BWR were also more genetically diffuse on the PCoA plot (Fig. 1).

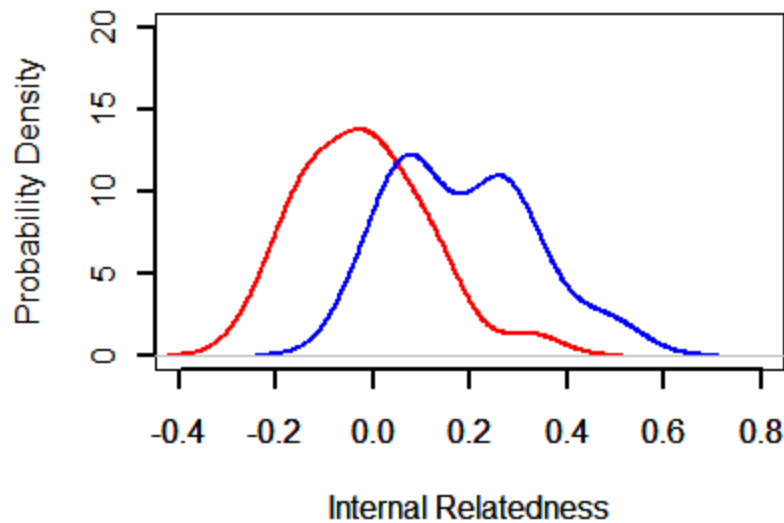




**Fig. 3.** Distribution of IR estimated in 29 BWT based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (IRVD-blue line). Lost diversity was determined by comparing allele frequencies at the same loci between BWT and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.



**Fig.4.** Distribution of IR estimated in 50 BYT based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (IRVD-blue line). Lost diversity was determined by comparing allele frequencies at the same loci between BWR and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.



**Fig. 5.** Distribution of IR estimated in 25 BWR based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (IRVD-blue line). Lost diversity was determined by comparing allele frequencies at the same loci between Biewer and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.

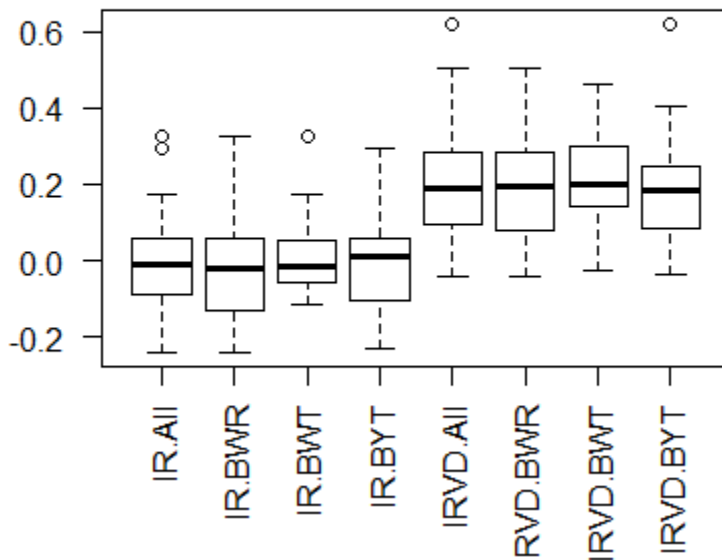
## *2. Measuring genetic diversity lost during breed evolution*

The IR values can be adjusted in such a way as to provide a qualitative estimate of total genetic diversity that has been lost during evolution of a breed from its most genetically diverse ancestors to present time. This is done by using allele frequencies obtained from DNA of present day village dogs from the Middle East, SE Asia and Island Pacific nations, which closely reflect the ancestors of dogs prior to extensive genetic manipulations by humans. These dogs are the single most random bred and genetically diverse population of dogs that has been studied to date. The adjusted IR value is known as IR-village dogs or IRVD.

The IRVD values for various populations of Biewer are shown by the blue line in Figs. 2-5. The IRVD curve of the 104 Biewer ranged from -0.040 to 0.621 with a mean of 0.195. A value of 0.25 would be seen among a litter of village dogs resulting from a full sibling mating, with the siblings coming from a randomly mating population. Values  $\geq 0.25$  occur in offspring of full sibling parents that come from an inbred population. The more inbred the population, the higher the IRVD. The loss of genetic diversity observed in Biewer as a result of breed creation, as represented by high IRVD values, is not unique and similar IR/IRVD profiles have been seen in other breeds that began with a small founder population (e.g., Alaskan Klee Kai, Akita, and Black Russian Terrier).

### 3. Statistical comparison of IR and IRVD values for BWR, BWT, and BYT

It is possible to use IR and IRVD values to make a statistical comparison of the three varieties of Biewer. This comparison, although using the same allele and allele frequency data reported in Table 2, weighs the contribution of rarer alleles more highly than common alleles and is therefore an approximation of the genetic similarities of BWT, BWR and BYT parents are to each other. Such a comparison also compensates for variables such as population size that might skew the final interpretations. Figure 3 is a box and whisker plot of IR and IRVD data from the three varieties of Biewer. The three varieties are statistically identical based on ANOVA analysis of IR and IRVD data (adjusted P values = 0.89 - 0.99). Therefore, this is another test that demonstrates a high degree of relatedness between the three Biewer varieties.



**Figure 3.** A statistical comparison of IR vs IRVD values of three Biewer varieties (n=104).

### C. DLA Class I and II Haplotype frequencies

The DLA consists of four gene rich regions making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases. 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. Specific alleles at the four STR loci associated with the DLA88 are linked together in various combinations, forming specific haplotypes (Table 3). Groups of genes and their alleles inherited as a block, rather than singly, are called

haplotypes. 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 also inherited as a single block or haplotype (Table 4). One haplotype comes from each of the parents. The linkages between alleles within Class I or II regions are very strong; while linkages between regions of the DLA that are more distant from each other, such as Class I and II, are weaker. There are almost two million base pairs separating the class I and II regions, thus allowing for some genetic recombination to occur between DLA class I and II haplotypes. Because there is much less recombination within the DLA region, the inheritance of DLA haplotypes can be used to indicate potential artificial genetic bottlenecks resulting from small founder numbers, popular sire effects, geographic isolation, catastrophes, etc. If the genetic bottleneck occurred many generations ago, and is followed by a period when random breeding resumes, the population will appear genetically heterogeneous based on the 33 genomic STR markers, but the imprints of that bottleneck will be reflected by a higher than expected frequency of certain DLA class I and II haplotypes.

### *1. DLA class I and II haplotypes identified in Biewer dogs and their relative frequency*

We have identified 16 different STR-associated DLA Class I and 13 DLA Class II haplotypes among the 104 Biewer dogs tested (Table 3). This is a comparatively small number of haplotypes compared to breeds such as the Standard Poodle, Miniature Poodle and Italian Greyhound, but in line with the Akita, Alaskan Klee Kai, Havanese, and Black Russian Terrier. All of the latter breeds started with a relatively small number of founders. The number of haplotypes may increase with the testing of more Biewer dogs, but it is doubtful whether the increase will be large. The comparatively low number of DLA class I and II haplotypes in the Biewer dogs tested is an indication that the breed has evolved from a relatively small founder population, or that many of the original founders have been lost from the population over time.

All of the DLA class I and II haplotypes observed in the Biewer dogs tested have been identified previously in other breeds, demonstrating that the ancestry of Biewer dogs is shared by many breeds, including village dogs from which all pure breeds evolved. The more common DLA class I and II haplotypes (shaded) were shared at similar frequency between the three varieties of Biewer that were tested. Moreover, three DLA class I haplotypes (1012, 1030, and 1040) and four class II haplotypes (2003, 2016, 2023 and 2040) tended to predominate in all three varieties. The variation in less common haplotypes between the three Biewer varieties can be explained by the relatively small numbers of each type of dog tested. As more dogs are tested, many of the zero values are expected to disappear.

DLA class I and II haplotype are inherited as blocks of linked genes from each parent by descent with limited recombination. Therefore, it appears that Biewer dogs descended from not only a small founder population, but from a population that consisted of a high proportion of dogs that possessed these specific DLA haplotypes. This is another indication that the three varieties were derived from the same founder population and have not genetically deviated from that foundation stock.

**Table 3:** DLA Class I and II haplotype and their frequencies in Biewer dogs of three varieties as well as a small number of Yorkshire Terrier and Biro Biewer.

<b>DLA Class I Haplotype Frequencies (Updated Oct 8, 2019)</b>						
<b>DLA1 #</b>	<b>STR types</b>	<b>Biewer (n=120)</b>	<b>Biewer Yorkshire Terrier (n=53)</b>	<b>Biewer Terrier (n=107)</b>	<b>Yorkshire Terrier (n=16)</b>	<b>Biro Biewer (n=3)</b>
1005	389 371 277 181	0.029	0.009	0.014	--	--
1007	380 372 281 182	0.004	0.009	--	0.03	--
1008	386 373 289 182	0.008	--	--	0.06	--
1012	388 369 289 188	0.192	0.151	0.229	0.28	0.2
1014	375 373 287 178	0.025	0.009	0.070	0.09	--
1016	382 371 277 178	0.025	0.019	0.019	0.03	--
1019	380 373 287 185	0.071	0.085	0.033	0.09	--
1030	380 373 293 178	0.458	0.349	0.472	0.31	0.5
1035	386 373 277 184	--	0.009	--	--	--
1040	380 371 277 186	0.108	0.208	0.075	--	--
1043	393 381 277 183	0.008	0.009	0.023	--	--
1046	376 379 291 180	0.033	--	0.047	--	--
1068	380 373 287 181	--	0.009	--	--	--
1087	380 371 277 178	0.004	--	--	--	--
1092	376 379 277 181	--	0.038	--	0.06	--
1104	386 373 289 186	0.013	0.019	0.005	--	--

1105	382 379 277 178	0.004	--	--	--	0.3
1115	386 371 277 182	--	--	0.005	--	--
1117	376 373 277 180	--	--	0.005	--	--
1118	376 377 277 180	0.004	0.019	--	--	--
1130	380 373 287 178	0.008	0.009	--	--	--
1131	388 379 277 181	0.004	0.047	0.005	0.03	--

**DLA Class II Haplotype Frequencies (Updated Oct 8, 2019)**

<b>DLA2 #</b>	<b>STR types</b>	<b>Biewer (n=120)</b>	<b>Biewer Yorshire Terrier (n=53)</b>	<b>Biewer Terrier (n=107)</b>	<b>Yorkshire Terrier (n=16)</b>	<b>Biro Biewer (n=3)</b>
2003	343 324 282	0.233	0.208	0.252	0.38	0.2
2005	339 322 280	0.046	0.009	0.047	--	--
2013	345 327 284	0.054	0.142	0.065	--	--
2016	339 323 284	0.079	0.094	0.056	0.09	--
2021	339 324 268	0.004	--	--	--	--
2023	341 323 282	0.458	0.349	0.472	0.31	0.5
2037	341 327 280	0.025	0.009	0.070	0.09	--
2040	345 327 280	0.071	0.085	0.028	0.03	--

2044	343 324 268	--	0.038	--	0.06	--
2053	343 324 280	--	0.009	--	--	--
2074	341 324 284	0.004	0.019	0.005	--	--
2080	339 325 276	0.004	0.009	--	0.03	--
2081	343 322 282	0.013	0.019	0.005	--	--
2082	339 325 268	--	0.009	--	--	--
2083	339 324 282	0.004	--	--	--	0.3
2084	339 323 268	0.004	--	--	--	--

### III. How will you be given the results of DNA-based genetic diversity testing on your dog.

After a sample is submitted for genetic testing, the identity of the dog and owner will be replaced by a laboratory barcode identifier. This identifier will be used for all subsequent activities and each owner will be provided with a certificate that reports the internal relatedness, genomic STR genotypes and DLA class I and II haplotypes for the dog(s) tested. The internal relatedness value for the dog being tested is related to the population as a whole.



### IV. What should you do with this information?

The goal for Biewer breeders should be to produce a greater and greater proportion of puppies with IR scores less than 0, which is near the mean IR value for all three varieties that were tested. This can be achieved by selecting parents that are as different as possible in their genomic STR alleles and allele frequencies. It is also important to rebalance diversity within the DLA region whenever possible and to avoid homozygous alleles or DLA haplotypes. Even though genetic diversity is limited, it is nonetheless important to properly manage the diversity that exists. Maintaining existing diversity will require using different combinations of breeding stock, including even those from inbred lines with high IR values. IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. A breeding pair with identical IR values can have genetically distinct parents and 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 is much different in genomic allele and allele frequencies and DLA haplotypes, 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 as a result of genetic recombination. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

In brief, potential sires and dams should be first screened for genetic differences in the genome and in the DLA regions by first comparing allele differences at each STR locus, and then at the



DLA class I and II haplotypes. Some thought should be given to rare vs common alleles. This information is included on all certificates and on the website. This preliminary comparison will identify promising pairings and if desired, genetic information on the potential sires and dams can then be used to calculate actual IR expectations for their puppies. 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 improving genetic diversity in puppies should be paramount. Diversity could be increased by outcrossing to classic or parti-colored Yorkshires, but this is likely to be soundly rejected. Nevertheless, because Biewers were presumably derived only from Yorkshire terriers with the Piebald gene in the recent past, it should be relatively easy to find Yorkshire terriers with similar genotypes as Biewers.

Although much emphasis is given to “Biewer purity”, the real issue is whether the breed is suffering unduly from the typical types of heritable disorders found in small breeds in general. It is possible to maintain health even in an inbred population, providing that the founding stock was healthy and the original diversity remains intact and in the same balance. The fact that Biewer dogs are not allowed in all shows may actually be beneficial, as this avoids typical artificial genetic bottlenecks often associated with championships. Breeders of the Biewer dogs that were tested appear to be doing a very good job of random mate selection and this should definitely be a continuing practice.

Breeders who do not have access to computer programs to predict IR values of puppies based on IR values of sire and dam can do this manually. 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. The same should apply to the DLA class I and II haplotypes. This information is included on all certificates and on the breed-wide data on the VGL website.

## **V. Summary**

### *1. What genetic assessments tell us about the History of Biewer dogs?*

There are two questions involving genetics that seem to be of concern to the various Biewer groups. One is whether Biewers are a breed distinct from Yorkshire terriers. The answer is dependent on how a breed is defined. Anytime a small group of dogs is selected from a larger population because of relatively minor phenotypic differences and then bred in isolation from the parent breed for a number of generations, MDS and PCoA clusters will drift away from each other. This can be seen in Japanese and American Akita and in American and European Italian greyhounds. Although the drift can be very apparent when comparing different varieties of a breed, the varieties will come back together as a single group in MDS or PCoA plots when these varieties are tested against a genetically and phenotypically distinct breed. Therefore, phenotypic differences that differentiate Biewers from Yorkshire terriers are of equal or greater importance than genotypic differences. Given the history of how the Biewer was created, there are undoubtedly present day Yorkshire terriers that would be similar to modern Biewers by this genetic diversity panel and at least some of them also phenotypically identical.

The second genetic issue concerns the purity of the various Biewer varieties that now claim direct descent from the original Biewer dogs, without genetic introductions of other Yorkshire terrier lines or even breeds. Although we did not address the issue of the origin of the Piebald gene, which is doable, we can say with certainty that the three varieties of Biewer that we tested were not significantly different from each other by a number of genetic parameters. All three varieties possess the same genomic and DLA markers and in approximately the same frequency. This would not occur by chance, so we must assume that all three of the Biewer types that we tested descended from the same small group of founders and that the purity of the Biewer line has been maintained.

## *2. Health problems of Biewer dogs*

Biewers tend to be long-lived (12-15 years+), as is typical for most small dogs. Like other toy breeds, Biewers have also inherited many of the ancestral traits associated with their size. The question is whether the incidence of these deleterious traits exceeds that of dogs in general. If certain ancestral disorders are significantly greater than expected for dogs in general, it is reasonable to place blame on limited genetic diversity and to act to increase breed-wide diversity. If the incidence is comparable to dogs in general, increasing diversity may have a limited affect. Complex or polygenic disorders observed in Biewers include tracheal collapse syndrome and bronchitis, patellar luxation, portal-hepatic shunts, Legg-Calve-Perthe's syndrome (aseptic necrosis femoral head), and eye problems associated with hair irritation (distichiasis). Many small breeds including Biewers also suffer from periodontitis and early tooth loss and are prone to hypoglycemia when stressed or diseased, especially as puppies.

At least two simple recessive deleterious traits of ancestral origin, and therefore found in a number of breeds, occur in Biewers. These include mutations in a gene known as *SOD1* that increases the risk for degenerative myelopathy and a mutation in a urate transporter gene responsible for an increased incidence of uric acid stones in the bladder. Interestingly, Biewers appear to be reasonably free of the many autoimmune disorders that affect so many pure breeds and in spite of having evolved from a small founder population. This is probably a reflection of a comparatively low incidence of autoimmune disease in the founding dogs and the successful attempts of Biewer breeders to avoid further inbreeding of the founding bloodline. It is uncertain what effect eliminating these mutations from Biewer dogs will have on overall genetic diversity. If the incidence of carriers is high, the effect could be significant and if it is low, the effect will be minimal.