

Report to the AKC Board of Directors

from the

American Kennel Club Health and Welfare Advisory Panel

**Molecular Genetic Analysis of Backcross Dalmatians Compared to AKC Dalmatians, UK
Dalmatians, Pointers, and Other Breeds**

April 30, 2010

This memorandum contains:

- 1) Comments from the AKC Health and Welfare Advisory Panel**
- 2) Mars Veterinary Report: Interim genetic analysis of selected backcross Dalmatians using principle component analysis**

Comments on the Mars Veterinary Report: Interim genetic analysis of selected backcross Dalmatians using principle component analysis

American Kennel Club Health and Welfare Advisory Panel

In order to provide further scientific evidence on the genetic relationship of the Backcross and AKC Dalmatian populations, and any relationship of the Backcross Dalmatians to the Pointer population, a molecular genetic study using principle component analysis was requested of Mars Veterinary.

The AKC Health and Welfare Advisory Panel would like to thank Mars Veterinary, and especially Drs. Angela Hughes and Neale Fretwell for consenting to do this analysis at no charge. We would also like to thank Ms. Denise Powell for collecting the DNA cheek swabs and providing pedigrees on the Backcross Dalmatians analyzed in the study.

The 27 Backcross Dalmatians included in the study represent dogs eight to twelve generations downline from the single Pointer used to establish the Backcross line. As this is not a study on the SLC2A9 gene causing hyperuricosuria, these include heterozygous LUA, homozygous LUA, and HUA dogs from the Backcross line.

The 27 dogs represent a broad cross-section of the Backcross population. They do not represent all of the Backcross dogs that could eventually be considered for AKC registration. This consideration would require phenotypical evaluation of each proposed dog, but can also include genotypical evaluation, for which Mars Veterinary has offered their assistance.

This study was commissioned to answer a simple question of whether the Backcross Dalmatians retain distinguishable molecular genetic relationships to AKC Pointers. What Mars Veterinary has delivered is a complex analysis of the population structure of the Dalmatian breed based on their stored samples of AKC (US) Dalmatians, KC (UK) Dalmatians, and the submitted Backcross Dalmatian samples. They have run principle component analyses of these versus their stored samples of Pointers and a mixed "All Breed Outgroup" population.

The report findings include:

Page 3: "What can be seen clearly in this analysis is that the Dalmatian breed has significant population stratification based on geographical origin of the samples. Two separate clusters are formed, with dogs from the United Kingdom clearly distinguished from those sampled in the United States by the first principle component of the analysis. This is likely to have been caused by genetic drift in the two population groups (or different selection pressure in the two countries), and also probably means that there has been limited admixture between the two populations. Evidence for similar cryptic population structure between samples from the same breed from different geographies has been observed in multiple breeds by Mars Veterinary previously."

Page 4: "In this analysis the backcross Dalmatians clearly cluster with the other purebred Dalmatian samples and are not intermediate between Pointer and the other purebred Dalmatian clusters."

Page 5: "Note that the backcross Dalmatians cluster closer to, (and hence show more genetic similarity to), the US purebred Dalmatian samples than the UK cluster of samples."

Page 6: "In this analysis the backcross Dalmatians cluster with the Dalmatian_US group samples and all samples are clearly differentiated from the Purebred Pointer samples by the first principle component of the analysis. There is no evidence of any of the backcross Dalmatians being more closely related to the Pointers than some individual samples from the Dalmatian_US group."

Page 7: "What is seen is that the three Dalmatian groups overlap but appear to be clustering together with other samples from the same group rather than fully intermingled. This again suggests that there is cryptic population structure between the three groups of Dalmatians. Note that the Backcross Dalmatians again cluster closer to, and hence show more genetic similarity to, the US purebred Dalmatian samples than the UK cluster of samples. All Dalmatian samples are clearly differentiated from the samples from other breeds as would be expected of a purebred breed cluster."

Page 8: "There is evidence of overlap between the US_Dalmatians and backcross Dalmatians but not between either group and the UK bred Dalmatians. This analysis confirms that the backcross Dalmatians appear to be more closely related to the US bred Dalmatians than the UK bred Dalmatians at the genetic markers tested here."

Page 9: "Although there is some overlap between the two groups – there is some evidence of population stratification between the US bred and backcross Dalmatians in this figure."

Page 10: "There is strong evidence for population stratification between the backcross Dalmatians and the UK bred Dalmatians in this analysis. This is similar to that observed between the UK bred and US bred Dalmatians in figure 1. It is currently unknown what factor or factors in the genetic markers are driving this distinction and this remains to be determined.

Summary

This analysis has shown that the backcross Dalmatians genetically are more like purebred Dalmatians than any other breed group and are more closely related to US-bred Dalmatians than UK-bred ones. There is no evidence of detectable admixture with the Pointer breed in this analysis. There is however clear evidence of cryptic population structure within the Dalmatian breed, with the most striking differences seen between Dalmatians from the UK and the other two groups.

Further analysis may be able to reveal additional insights in the analysis of the backcross data set, including individual chromosome analysis reporting, but these comparisons have not been possible to date given the limited timescale available to perform this report in time for the meeting for which it is intended."

The Mars Veterinary report provides an excellent explanation of their methods and results. All three groups of studied Dalmatians form a tight cluster separate from all other breeds, including Pointers.

Due to Mars' complex analysis, they have been able to separate the within breed differences between the UK Dalmatians, US (AKC) Dalmatians, and the Backcross Dalmatians reported as within breed population stratification. This is not surprising, and has been observed with several other breeds. With this complex analysis, breeds can often be separated based on their molecular genetic similarities and differences between families and geographically isolated populations (US versus European dogs).

The US Dalmatians and Backcross Dalmatians overlap in their molecular genetic signatures, but still retain differences based on differences between dogs with different family/pedigree backgrounds. Any matings between Backcross Dalmatians and AKC Dalmatians are more similar than matings between either group and UK Dalmatians.

This is an interim report produced by Mars Veterinary to provide material for discussion by the Dalmatian club at their round table meeting May 2, 2011. Mars Veterinary has offered to continue assisting the Dalmatian breed in this analysis and with further molecular genetic studies that may assist the breed in a resolution of this issue.

Respectfully submitted to the AKC Board of Directors by the AKC Health and Welfare Advisory Panel:
Dr. Sandra Barker, Dr. Jerold Bell, Mr. Eddie Dziuk, Dr. John Hamil, Dr. Joan Hendricks, Dr. Linda Lord, Dr. Patricia Olson, Dr. Elaine Ostrander, Dr. Frances Smith

Interim genetic analysis of selected backcross Dalmatians using Principle Component Analysis

Objective

Mars Veterinary has been provided with buccal cell samples on cytology swab brushes from each of 27 backcross Dalmatian dogs from Ms. Denise Powell. The intent was to compare genetic marker data for 321 SNP markers ascertained from each of these samples to the complementary genetic marker data held for a number of purebred Dalmatian dogs in Mars Veterinary's reference database. This comparison was requested by Dr. Jerold Bell on behalf of the AKC Health and Welfare Advisory Panel to the AKC Board.

Methods

A single batch of 27 buccal swab samples was received at the laboratory of Mars Veterinary's genotyping supplier, where DNA was extracted from each sample using our standard methodology, and typed against 321 single nucleotide polymorphism (SNP) markers using the Sequenom Platinum genotyping technology. Eight multiplex reactions were used to genotype each sample, and the SNP genotype data for each locus was scored automatically using the latest version of the Sequenom Mass Array analysis platform software. Data for each sample was provided to Mars Veterinary in a .csv file format and was analysed in this report using an in-house program designed to perform comparisons of multiple input data sets using the methodology of principle component analysis (PCA).

PCA summarizes the data by the greatest contributing factors to variance in the data. The effect is to cluster together the most similar samples in the dataset and to separate the more distantly related samples. PCA is a method of simplifying the data in order to focus on the most important factors (principle components) that cause differences between dogs. When plotted in three dimensions, the different axes representing the most important principle components with the x axis being PCA 1 (most different factor), y axis PCA 2 (second most different factor) and the z axis being PCA 3 (third most important factor). Often, its operation can be thought of as revealing the internal structure of the data in a way which best explains the variance in the data.

Results

The SNP data for the 27 backcross Dalmatian samples were uploaded into our the PCA analysis software package and a number of different analyses were made comparing this backcross Dalmatian data set against SNP data from other canine purebred dog samples (in multiple combinations of comparisons). These analyses were designed to investigate the relationship of these backcross samples to the reference samples in Mars Veterinary's database from the following different purebred dog breed clusters previously identified. The results of this analysis are contained in a series of figures in the following pages. Breed clusters used in the comparison were:

- i) Purebred Dalmatians sampled from US bred dogs (Dalmatian_US)
- ii) Purebred Dalmatians sampled from UK bred dogs (Dalmatian_UK)
- iii) Purebred Pointers (Pointer)
- iv) An All breed reference outgroup containing a single dog from each of 226 unique breed clusters (All Breed Outgroup) in the Mars Veterinary reference breed database

Table 1: Backcross Dalmatian DNA samples typed

The 27 dogs typed and used in this analysis were identified to Mars Veterinary as:

P570-200	Hartley
P536-626	Will
P524-763	Daisylani
P490-214	Bart
P445-710	Holly
P635-341	Koda
P654-595	Buster
P524-759	Classy
P667-483	Black Devon
P607-294	Miles
P598-850	Lyra
P654-604	Marley
P658-366	Blackie
P615-984	Selia
P658-367	Koda II
P458-163	Ecco
P632-709	Betty
P458-160	Layla
P538-871	Ty
P667-484	Yellow Daisy
P507-566	Mac
P651-785	Hannah
P614-472	Bridgette
P602-640	Lila
P651-781	Maks
P652-256	Willow
P561-280	Tommy

Figure 1: Three dimensional PCA plot of the two different sets of Purebred Dalmatians in Mars Veterinary's reference breed database (Dalmatian_US v Dalmatian_UK).

Each colored dot represents a single dog from the Mars Veterinary database. What can be seen clearly in this analysis is that the Dalmatian breed has significant population stratification based on geographical origin of the samples. Two separate clusters are formed, with dogs from the United Kingdom clearly distinguished from those sampled in the United States by the first principle component of the analysis. This is likely to have been caused by genetic drift in the two population groups (or differential selection pressure in the two countries), and also probably means that there has been limited admixture between the two populations. Evidence for similar cryptic population structure between samples from the same breed from different geographies has been observed in multiple breeds by Mars Veterinary previously.

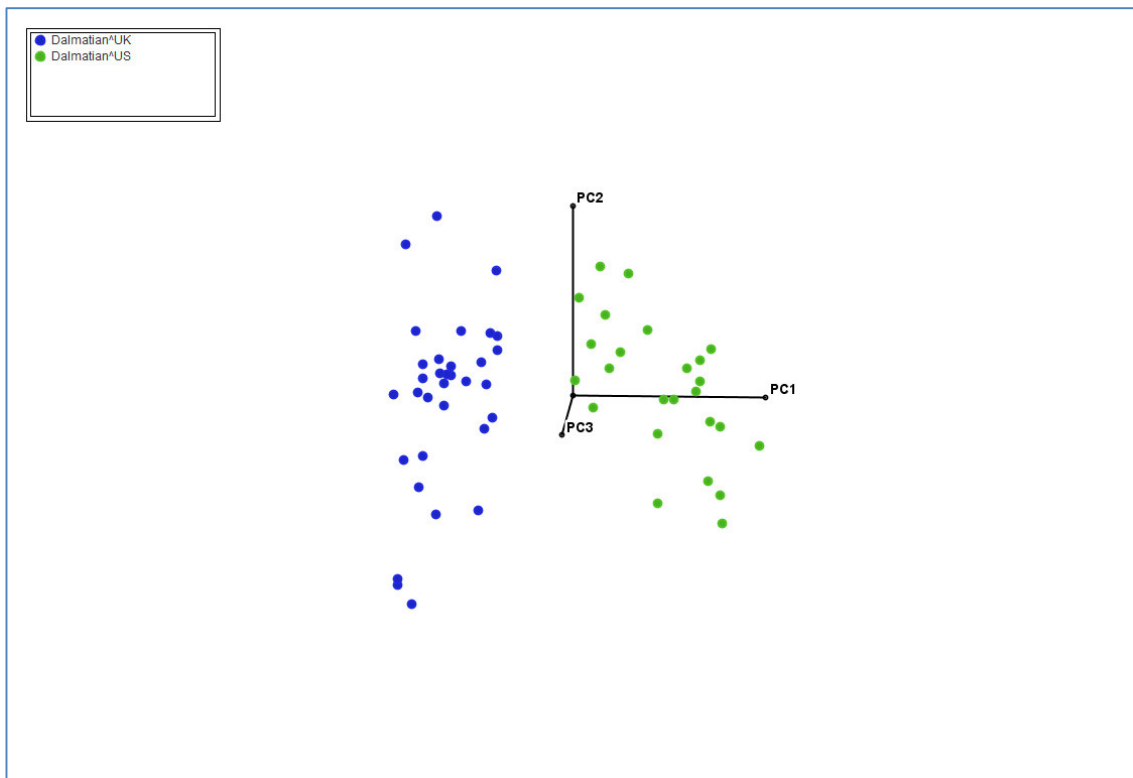


Figure 2: Three dimensional PCA plot showing the two different sets of Purebred Dalmatians in Mars Veterinary's reference breed database (Dalmatian_US & Dalmatian_UK) together with Pointers, the backcross Dalmatians (called decoded in the key) and an all breed outgroup.

In this analysis the backcross Dalmatians clearly cluster with the other purebred Dalmatian samples and are not intermediate between Pointer and the other purebred Dalmatian clusters.

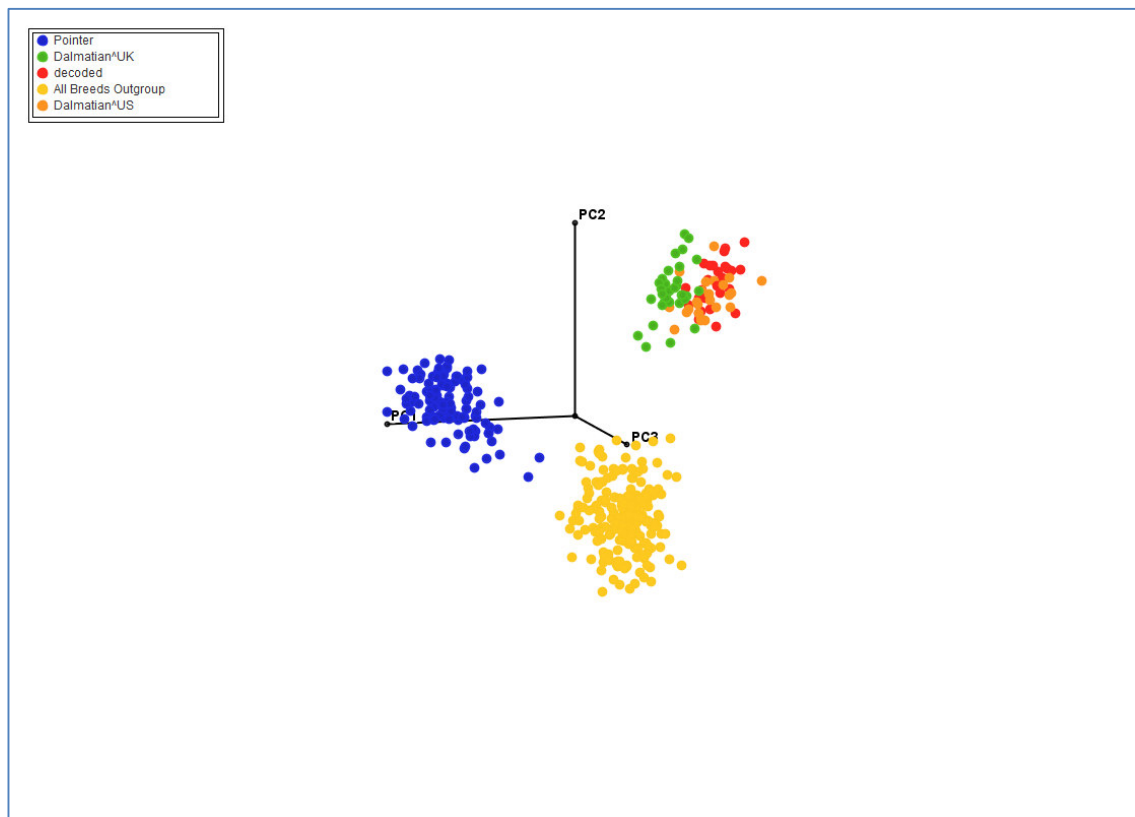


Figure 3: Another angle of the 3-D plot showing the same analysis as shown in Figure 2. Three dimensional PCA plot showing the two different sets of Purebred Dalmatians in Mars Veterinary's reference breed database (Dalmatian_US & Dalmatian_UK) together with the Pointers, the backcross Dalmatians (called decoded in the key) and an all breed outgroup.

This angle shows the clear separation between the 3 tested Dalmatian groups and the Pointer and All breed outgroup samples. What can also be seen is that the three Dalmatian groups overlap but appear to be clustering together with other samples from the same group rather than fully intermingled. This suggests that there is likely cryptic population structure between the 3 groups of Dalmatians. Note that the backcross Dalmatians cluster closer to, (and hence show more genetic similarity to), the US purebred Dalmatian samples than the UK cluster of samples.

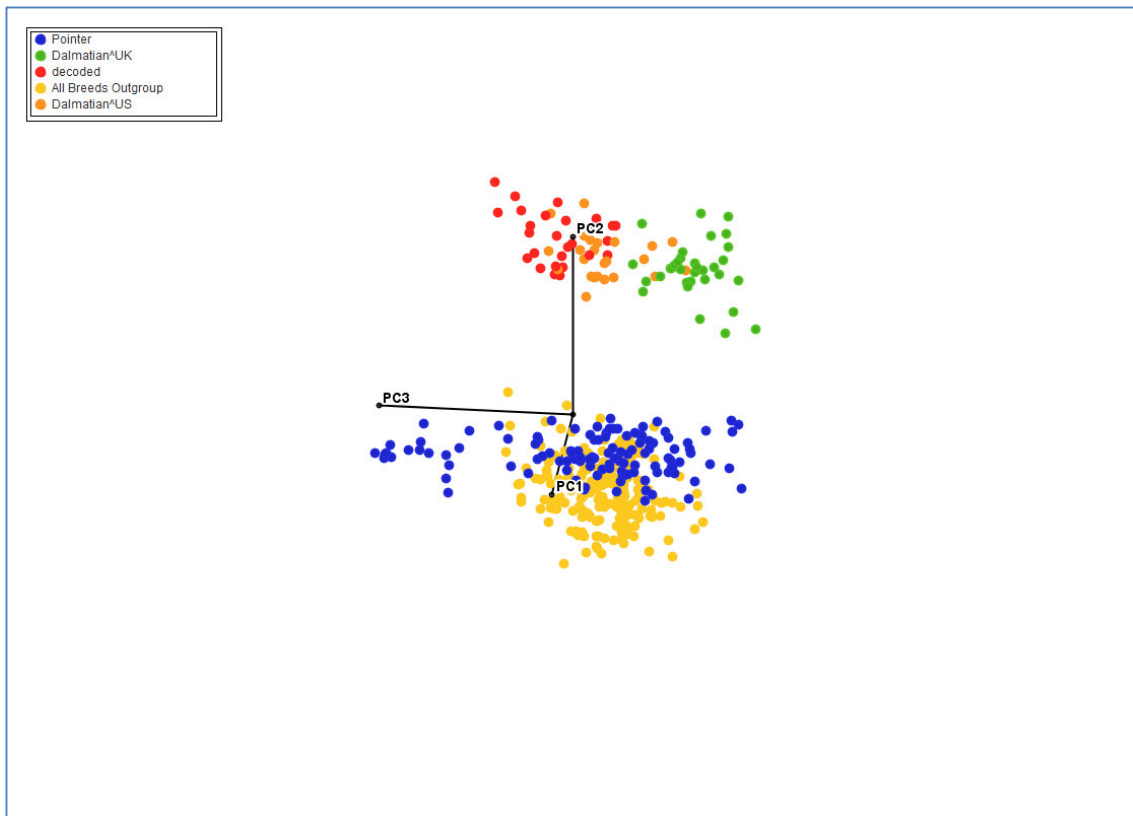


Figure 4: Three dimensional PCA plot showing the Dalmatian_US cluster only together with the Pointers, and the backcross Dalmatians (called Query Dalmatians in the key).

In this analysis the backcross Dalmatians cluster with the Dalmatian_US group samples and all samples are clearly differentiated from the Purebred Pointer samples by the first principal component of the analysis. There is no evidence of any of the backcross Dalmatians being more closely related to the Pointers than some individual samples from the Dalmatian_US group.

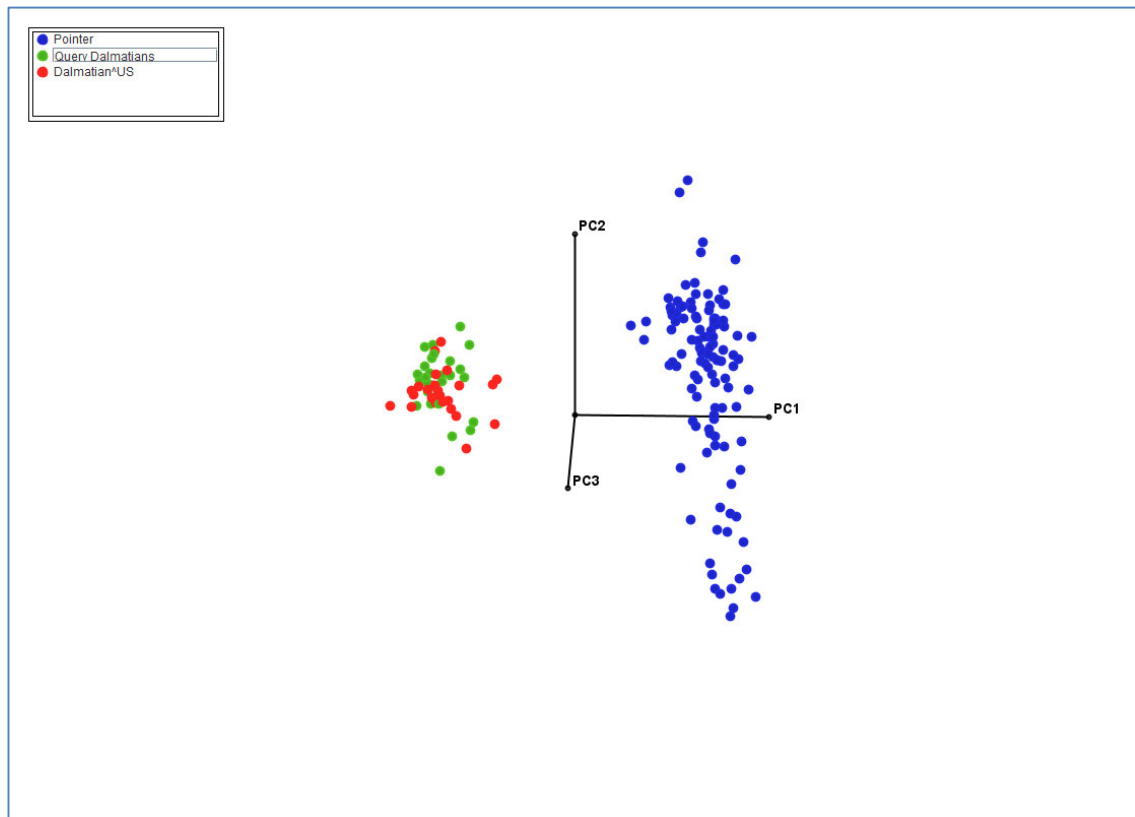


Figure 5: Three dimensional PCA plot showing the two different sets of purebred Dalmatians in Mars Veterinary's reference breed database (Dalmatian_US & Dalmatian_UK) together with the backcross Dalmatians (called decoded in the key and in subsequent figures) and an all breed outgroup.

The apparent cryptic population structure in Dalmatians (seen in figure 1 and 3) is again evident here and is revealed by the second principal component. What is seen is that the three Dalmatian groups overlap but appear to be clustering together with other samples from the same group rather than fully intermingled. This again suggests that there is cryptic population structure between the three groups of Dalmatians. Note that the backcross Dalmatians again cluster closer to, and hence show more genetic similarity to, the US purebred Dalmatian samples than the UK cluster of samples. All Dalmatian samples are clearly differentiated from the samples from other breeds as would be expected of a purebred breed cluster.

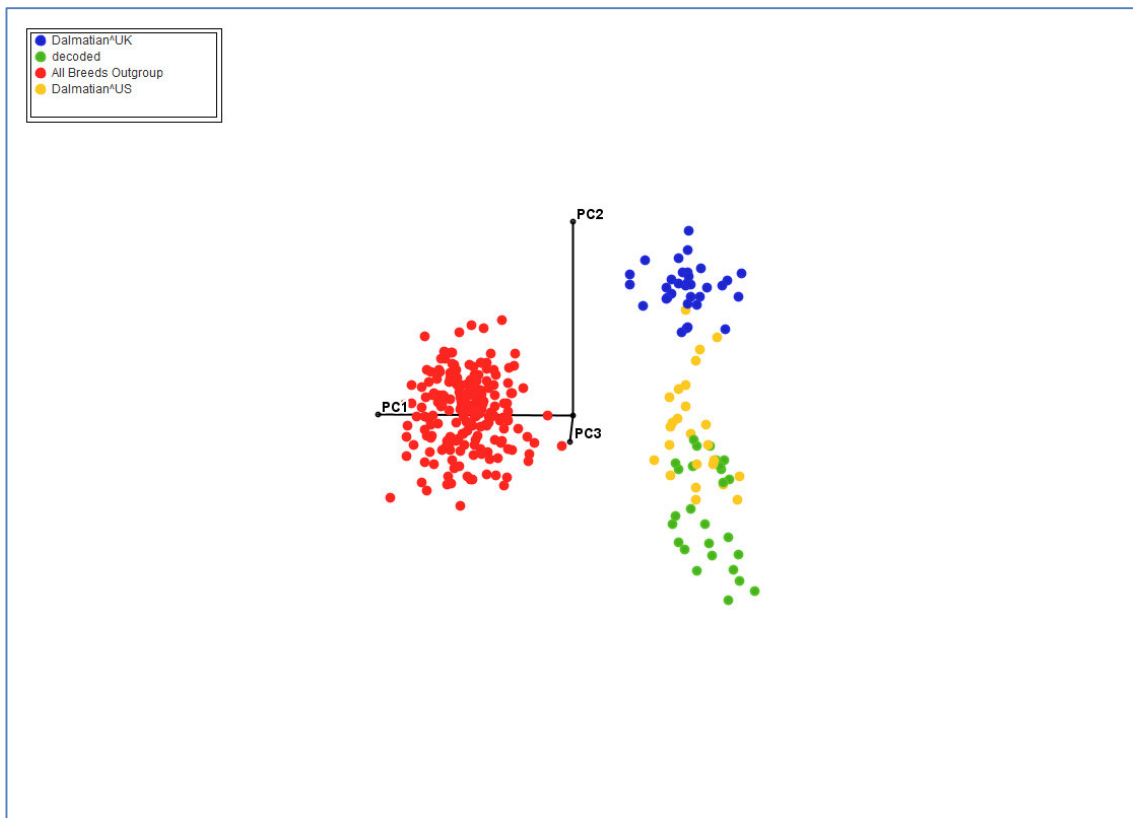


Figure 6: Three dimensional PCA plot showing the two different sets of purebred Dalmatians in Mars Veterinary's reference breed database (Dalmatian_US & Dalmatian_UK) together with the backcross Dalmatians (called decoded in the key and in subsequent figures).

This analysis again shows that the three Dalmatian groups are beginning to show separation into three distinct clusters, with UK Dalmatians differentiated by the first principle component and the backcross and US Dalmatians separated by the second principle component. There is evidence of overlap between the US_Dalmatians and backcross Dalmatians but not between either group and the UK bred Dalmatians. This analysis confirms that the backcross Dalmatians appear to be more closely related to the US bred Dalmatians than the UK bred Dalmatians at the genetic markers tested here. This is confirmed by the comparisons shown in Figures 7 and 8 below.

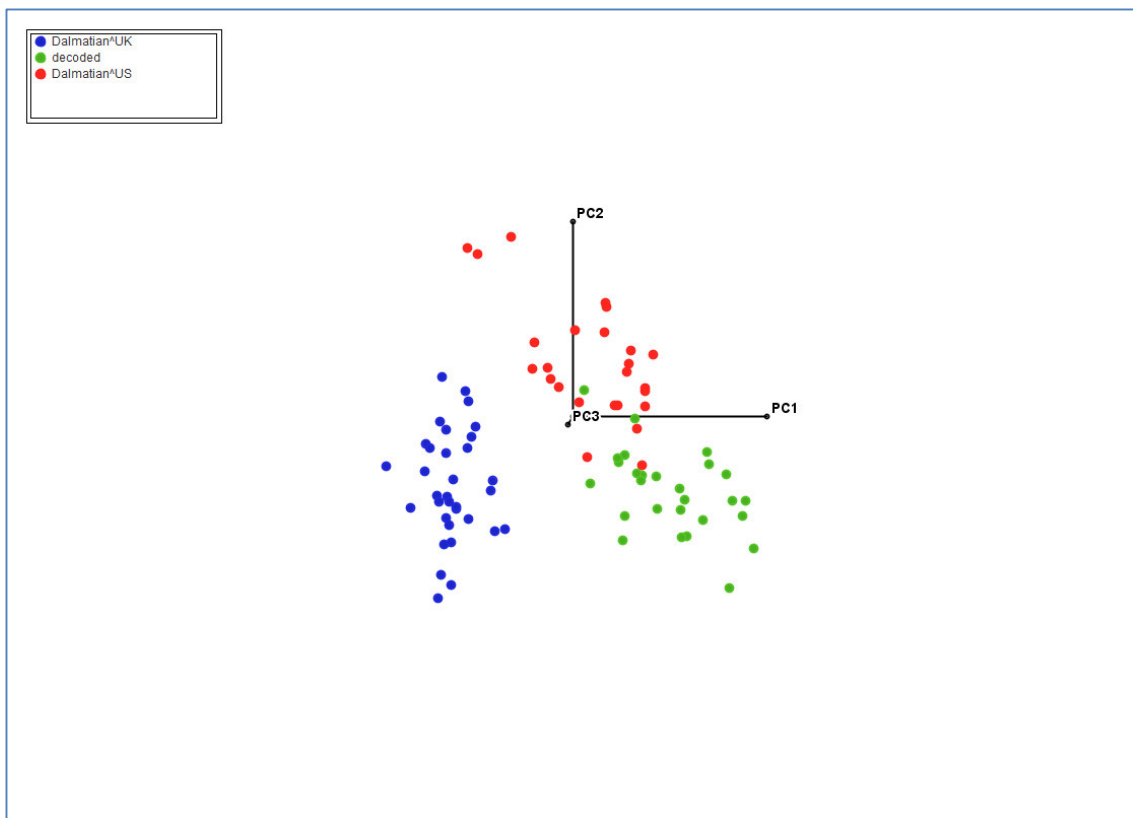


Figure 7: Three dimensional PCA plot showing the Dalmatian_US breed cluster together with the backcross Dalmatians (called decoded in the key and in subsequent figures).

Although there is some overlap between the two groups – there is some evidence of population stratification between the US bred and backcross Dalmatians in this figure.

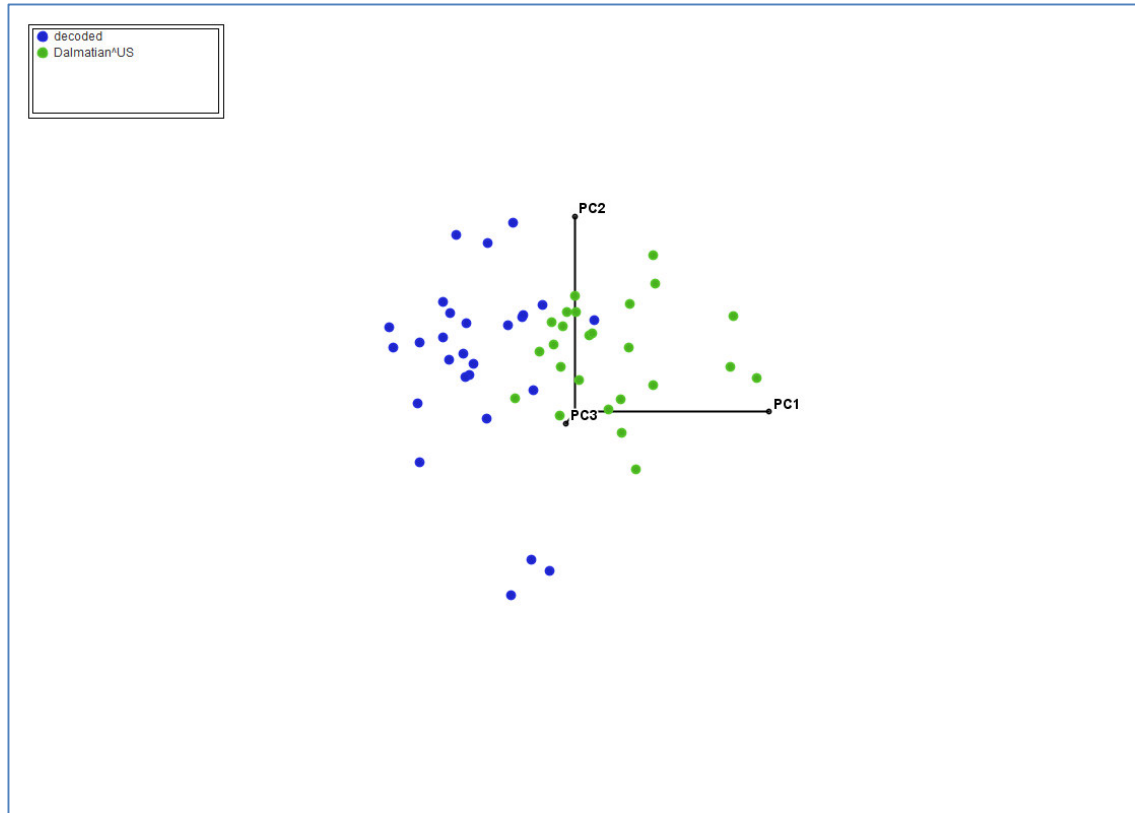
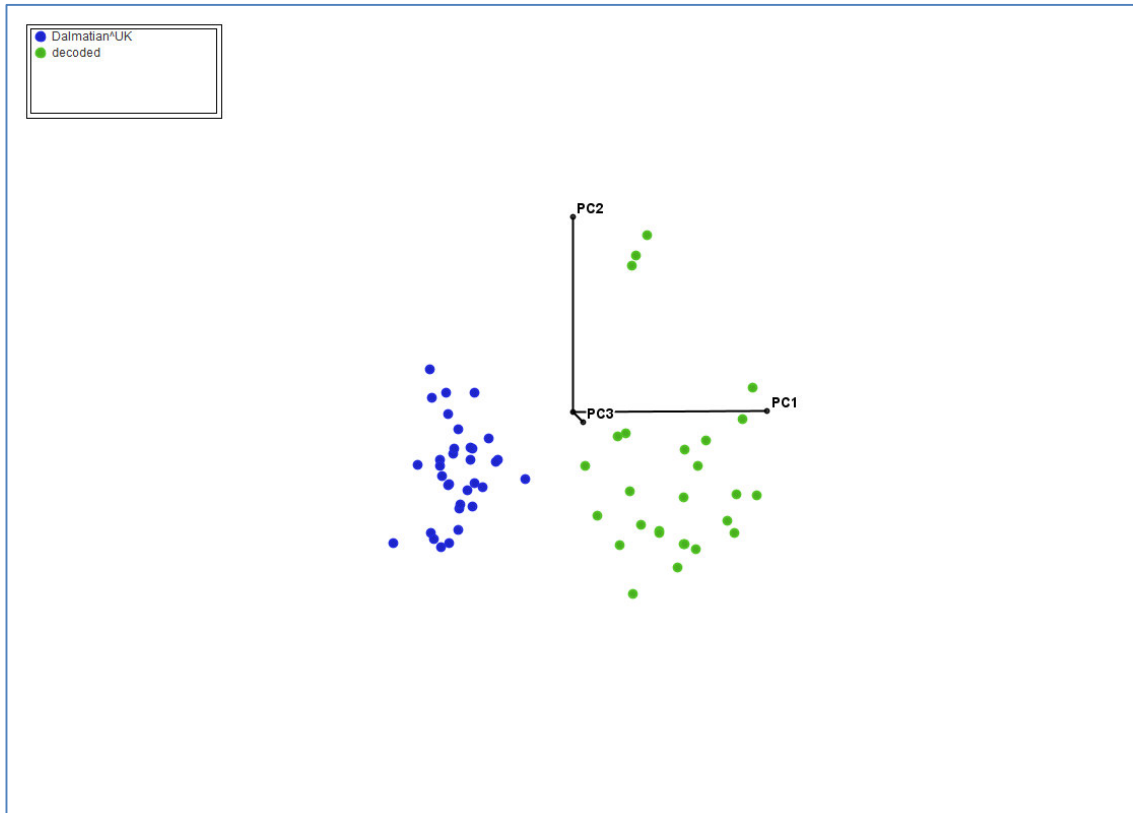


Figure 5: Three dimensional PCA plot showing the Dalmatian_UK cluster together with the backcross Dalmatians (called decoded in the key and in subsequent figures).

There is strong evidence for population stratification between the backcross Dalmatians and the UK bred Dalmatians in this analysis. This is similar to that observed between the UK bred and US bred Dalmatians in figure 1. It is currently unknown what factor or factors in the genetic markers are driving this distinction and this remains to be determined.



Summary

This analysis has shown that the backcross Dalmatians genetically are more like purebred Dalmatians than any other breed group and are more closely related to US-bred Dalmatians than UK-bred ones. There is no evidence of detectable admixture with the Pointer breed in this analysis. There is however clear evidence of cryptic population structure within the Dalmatian breed, with the most striking differences seen between Dalmatians from the UK and the other two groups.

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