DLA diversity in dog breeds. 17th July 2012

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For nearly twenty years I have been investigating diversity in the Major Histocompatibility Complex (MHC) region of the genome in dogs and wolves. The MHC is central to the control of the immune system in all mammals. Research into human immune-mediated diseases has revealed strong associations with the MHC Class II genes, see Box 1. Similar work in dogs has identified MHC associations in most of the canine immune mediated diseases that have been investigated. These diseases include hypothyroid disease, diabetes, immune-mediated haemolytic anaemia, anal furunculosis, Addison's disease and myositis. These are all "complex diseases", which are caused by a combination of many genes plus environmental factors/triggers, see box 2 for more information. The MHC class II genes are highly variable, and at a population level, it is important that there is a wide range of variation, because that means the population can respond to more infections, than a population with few variants.

Box 1: what does MHC-association mean?
Most of the diseases with MHC associations are "complex" diseases. These are diseases that are caused by the interaction of many different genes. Each gene will have a different level of influence on the risk of developing the disease. In human studies, it has been shown that the MHC confers about half the genetic risk for a disease. This means that having a particular MHC type will make it more likely that one develops the disease, but that is by no means certain, because of all the other genes that are involved.

Within the canine MHC, there is a region known as Dog Leucocyte Antigen (DLA) class II, which contains three very variable (polymorphic) genes called DLA-DRB1, DQA1 and DQB1. These genes have many different variants, which we call alleles. We have identified over 250 DLA-DRB1 alleles, 45 DQA1 alleles and 120 DQB1 alleles to date. These numbers increase on an almost daily basis, as we investigate further dog breeds. These three genes are inherited as a set from each parent, so every dog has two sets of these DLA genes, one inherited from the sire and one inherited from the dam. We refer to these "sets" or "combinations" as haplotypes, and in the total dog population, we have identified about 300 different haplotypes. However, within any one breed, there is usually only a limited number of haplotypes. Generally there are about five haplotypes found within a breed: one at high frequency (between 50-70% of dogs will have that haplotype), two at a frequency around 20% and one or two at around 1-5%.

Box 2: What are "environmental" factors/triggers?
The likeliest environmental trigger is a viral infection. When the body responds to the virus, it is possible that there can be a cross-reaction against ones own cells. Thus in diabetes, you start destroying the pancreas, and can then no longer make insulin.
We do not yet know whether it is a particular virus that triggers a certain disease, or whether there are several different virus's involved.

There have been many studies of MHC in wild and/or endangered animal populations, and there is evidence that a certain minimum number of different haplotypes may be necessary for long term survival. If only a few MHC haplotypes exist in a breed or species, the risk of the entire population being wiped out by a new disease is probably very high. For example, we studied a population of Arctic foxes living on a remote island in the Russian far east, and found that all the animals were homozygous for the same haplotype at the MHC. The population was decimated by an epizootic mange outbreak in 1918, and has not really recovered, despite now being protected from hunting. A similar population on a nearby island is thriving, and was shown to have several different MHC haplotypes. However, it is hard to put an actual figure on the number of haplotypes necessary for survival of a species or breed. In another study, we identified eight different MHC haplotypes in the Ethiopian wolf, which is the most endangered canid in the world. This was more diversity than we had expected, considering the limited population size (500 in total) and their isolation in only a few locations in the Bale mountains, plus the various rabies epidemics that have occurred in recent years.

In 2010, the Poodle Club of Canada (PCC) contacted me after they had seen a statement from Dr. Bannasch that only three MHC haplotypes had been found in Standard Poodles, and they were deeply concerned about the implications. The PCC sponsored a study initially funded by a grant from Royal Canin dog food, and subsequently funded by participants The study looked into the number and extent of DLA haplotype variations in Poodles worldwide. Before this study, I had identified six haplotypes in Standard Poodles (in 14 individuals), and one haplotype (which we are now calling *Poodle Haplotype #1),* was by far the most common. Poodle haplotype #1 was also seen in Miniature and Toy Poodles. In Miniatures, 11 haplotypes had been identified, and in Toys, 12 haplotypes. There was some sharing of haplotypes between poodle varieties.

The PCC has provided two groups of DNA samples of dogs from North America (although many had foreign origins) for DLA analysis. The first group (31 Standard Poodles) was selected for good health and diverse pedigrees, from both mainstream and obscure lines. The second group (62 Standard Poodles) also included some randomly selected dogs that the owners volunteered. In addition, the Animal Health Trust (AHT) in the UK provided information on the Standard Poodle DNA samples they had collected and a group of 50 of was selected for DLA testing, again looking for diversity based on pedigree. The hope was to identify as many haplotypes as possible.

After the DLA analysis of the PCC group 1 and the AHT group, we had identified 14 haplotypes in Standard Poodles. These haplotypes are shown in the table below. The colors have been added for visualization. The colors make it easy to see that Poodle haplotypes #3 and #4 differ from each other and from #1 only by one allele in the DRB1 position, while haplotype #2 is different from #1 in all alleles.

Note that although there are 12 different DRB1 alleles, 6 different DQA1 alleles and 10 different DQB1 alleles, there are only 14 haplotypes or combinations of these alleles found in Standard Poodles.

Table 1: Poodle Haplotypes found in Standard Poodles as of 2011.

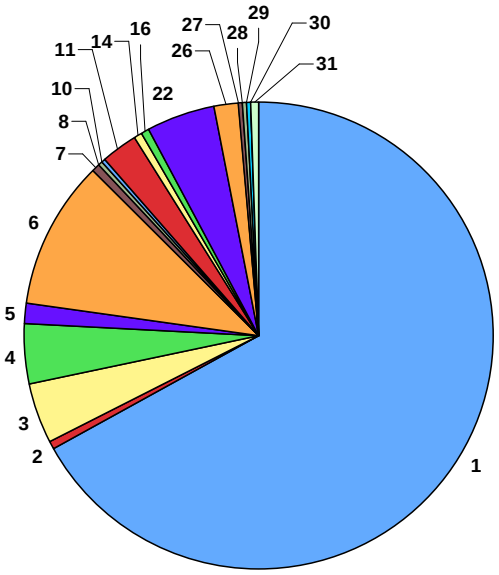
Haplotype	DRB1	DQA1	DQB1	Also in
1	01501	00601	02301	mini & toy
2	00601	05011	00701	mini
3	01502	00601	02301	mini
4	01503	00601	02301	mini
5	00101	00101	00201	
6	01501	00901	00101	
7	00101	00101	03601	mini & toy
8	01301	00101	00201	mini & toy
11	00901	00101	08011	mini
14	00201	00901	00101	toy
22	02001	00401	01303	(UK only)
26	01201	00401	13017	
27	01101	00201	01302	(UK only)
28	01801	00101	00802	(UK only)

All these haplotypes are found at different frequencies, and Figure 1 shows the frequency distribution of the various haplotypes in Standard Poodles.
Presumably, as a breed, Standard Poodles are better off having 14 haplotypes than only having three or six haplotypes. We may not want to lose any haplotypes existing in the breed, because these

Box 3: What is heterozygote advantage or homozygote disadvantage?
The best place to start reading about this is the Wikipedia entry, which uses the example of sickle cell anaemia and malaria.

may represent genetic diversity. The natural reaction might be that we need to decrease the incidence of haplotype #1 and increase the incidence of the rarer haplotypes. However there is significant debate in the scientific community about this and the possible unintended consequences. Perhaps the rarer haplotypes are rare for a good reason such as an association with a particular disease, which is currently at a very low frequency in poodles. And perhaps haplotype #1 is so common because it is the "healthiest". There again, haplotype #1 may be so common because of a bottleneck in the breed or heavy use of popular sires that carried it. There is some evidence that having two copies of the same haplotype (homozygous) may result in an animal that is less able to respond to immune challenges compared to having two different haplotypes (heterozygous). See box 3 for information about heterozygote advantage. There is also evidence from human studies that when a couple are homozygous for the same MHC haplotype, there is an increased risk of spontaneous abortion. There is also some evidence that when animals can choose their mating partners, they tend to avoid partners that have the same MHC type as themselves.

Figure 1: DLA Haplotypes found in Standard Poodles



As an aside: it would be interesting to know whether spontaneous abortions are common in dogs, and also whether when you put two dogs (that are identically homozygous for their MHC) together to mate, they show little interest in each other.

We have now completed DLA testing on the PCC group 2 (62 dogs) and also have been able to access other DLA data generated by Dr Niels Pedersen (University of California at Davis). In addition, I have been able to type many more poodles from our biobank of samples. Altogether we now have DLA data for 405 poodles, which have been divided into several different groups, see Table 2.

Table 2: Number of poodles that have been DLA typed.

Poodle Variety	Origin	No. tested
Standard	UK	93
Unknown/Not reported	UK	19
Standard	N America	154
Miniature	UK	30
Toy	UK	15
Pudl	Croatia	5
Addison's case	UK	29
Addison's case	N America	2
Sebaceous adenitis case	UK	24
Sebaceous adenitis case	N America	34

Altogether we identified 35 different DLA class II haplotypes within this group of poodles, see Table 3. (N.B. This table does not include the poodles affected with Addison's disease or sebaceous adenitis.) There is a complicated pattern of sharing of haplotypes between the different poodle varieties. Some haplotypes are found in most types of poodles e.g. #1 and 11. Haplotype #1 is found at high frequency in all Poodle varieties, and many dogs carry two copies of this haplotype (i.e. they are homozygous). Other haplotypes are shared between two different varieties: e.g. #4 is found in Standard and miniatures, while #7, 8, 9, 32 and 36 are found in both miniatures and toys. You can see that some of the North American standard poodles have haplotypes #7 or #8. This is because there are eight poodles within this group which are actually part-crosses between standard and miniature or toy poodles.

Some haplotypes appear to have geographical origins: there are haplotypes found only in the UK, only in North America and only in Croatia. Using this haplotype information, it should be possible to assign the most likely variety for most of the poodles of unknown variety.
Since the 81 dogs (in PCC group 1 and the AHT group) were selected for either health and vigour or from less well known lines, we expected to find a larger number of haplotypes than in the general Standard Poodle population. The pcc1/aht group had 14 different haplotypes, with two only being found in single dogs, while pcc2 had 12 different haplotypes with four only being found in single dogs (data not shown). So this could be interpreted as the pcc1/aht group having 12 haplotypes versus the pcc2 group with eight, as one tends to discount haplotypes found only once in a breed. These single haplotypes may be present in dogs that are not truly "purebred" dogs, or at least descended from such a dog.

Table 3: DLA haplotype frequencies (freq %), plus the numbers of dogs homozygous (No hom) for each haplotype, in different varieties of Poodle

						Poodle	standard		unclassified		standard		miniature		toy		pudl	
						Origin	UK		UK		N America		UK		UK		Croatia	
						No dogs tested	93		19		154		30		15		5	
haplo #	DRB1	DQA1	DQB1	total hom	total %	Comment	No hom	freq %	No hom	freq %	No hom	freq %	No hom	freq %	No hom	freq %	No hom	freq %
1	01501	00601	02301	168	61.5	all	39	65.6	5	34.2	65	63.6	8	45.0	4	36.7	1	20.0
6	01501	00901	00101	6	9.5	standard: UK	3	15.6	1	5.3	1	9.1						
3	01502	00601	02301	1	4.1	standard		1.6		7.9		5.5		1.7				
26	01201	00401	013017	1	1.1	standard: N Am					1	2.9						
4	01503	00601	02301	2	4.1	stnd, mini	1	7.0			1	3.2		6.7				10.0
22	02001	00401	01303		3.8	standard, lower freq		4.8		5.3		3.9						
11	00901	00101	008011		2.6	all, lower freq		1.1				3.9		3.3		3.3		
5	00101	00101	00201	1	1.7	standard, low freq		1.6	1	10.5		1.9		1.7				
27	01101	00201	01302		0.4	stnd: minor haplo		1.1				0.3						
7	00101	00101	03601	2	1.9	mini, toy			1	5.3		1.0	1	10.0		6.7		
8	01301	00101	00201		1.2	mini, toy				2.6		0.3		5.0		13.3		
9	01501	00601	02601	2	1.1	mini, toy			1	7.9			1	6.7		3.3		
32	00302	00101	00802		0.5	mini, toy								5.0		3.3		
36	111v	00601	02002		0.4	mini, toy								1.7		3.3		
2	00601	005011	00701		0.9	mini		1.1		2.6		0.6		3.3				
10	010011	00201	01501		0.5	mini						0.3		5.0				
12	01501	00601	02002	1	0.5	mini								3.3			1	20.0
35	00802	00301	00401		0.1	mini								1.7				
14	00201	00901	00101	1	0.5	toy					1	1.0				3.3		
16	01501	00601	04901		0.5	toy				2.6		0.6				3.3		
13	08401	00901	00101		0.4	toy										3.3		20.0
21	01702	00201	01303		0.4	toy				2.6						6.7		
20	00301	00101	00802		0.2	toy				2.6						3.3		
15	01501	00601	00301		0.1	toy										3.3		
18	01801	00101	00201		0.1	toy										3.3		
33	01501	00101	03601		0.1	toy										3.3		
17	01502	00601	02601		0.4	minor haplo												10.0
31	00106	00101	00201	1	0.4	minor haplo: N Am					1	1.0						
29	10301	00101	00802		0.1	minor haplo: N Am						0.3						
30	01501	00601	02201		0.1	minor haplo: N Am						0.3						
28	01801	00101	00802		0.1	minor haplo		0.5										
25	02601	00101	008011		0.1	minor haplo: Croatia												10.0
34	08401	00601	02301		0.1	minor haplo: Croatia												10.0
23	02301	00301	00501	1	0.2	minor haplo			1	5.3								

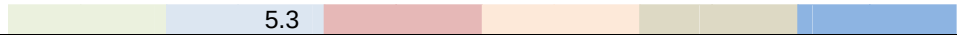
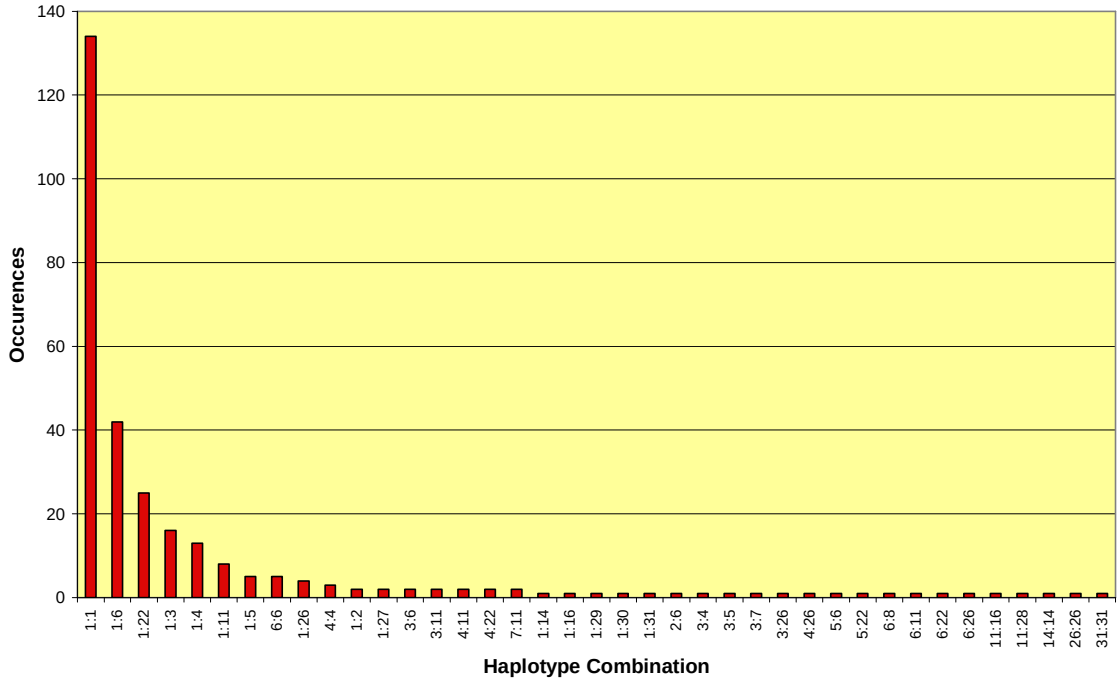
24	07601	00601	02301	0.2	minor haplo	5.3	

Figure 2 shows the numbers of dogs with each haplotype combination (we call this the "genotype"). You can clearly see the excess of dogs having a genotype that includes haplotype #1.

Figure 2: DLA Haplotype combinations found in Standard Poodles



Despite the careful selection of the pcc1/aht dogs for health and vigour, there was very little difference between the groups in terms of their haplotype frequency profiles. Both groups have the same large proportion of dogs (c60%) with two copies of haplotype #1. The only significant difference is that haplotype #6 has a frequency of around 20% in UK dogs, but only about 10% in dogs from North America.

There are nine haplotypes that are commonly found in the Standard poodle. Haplotype #1 is the most frequent in Standard poodles and the same percentage, 42%, of both UK (39/93) and North American dogs (65/154) are homozygous for this haplotype. A haplotype frequency of 65% means that 87% of standard poodles have either one or two copies of this haplotype. Haplotype #4 is more frequent in the UK than North America, but otherwise the haplotype profiles are very similar between these two groups.

If we consider the haplotype profiles of miniature and toy poodles, they have 14 and 15 different haplotypes respectively, of which five and three respectively are shared with standard poodles.

Rather than try to increase the frequency of the currently low frequency haplotypes within Standard poodles, it seems to me that it would be possible to introduce more genetic diversity into the Standard poodle by crossing with miniature and/or toy poodles. However, such a strategy would need careful genetic monitoring, so as to avoid further problems down stream. Any such matings should only be between dogs that have been genetically profiled for a variety of markers distributed throughout the genome, which may include the MHC, but should not be exclusively the MHC.
Each breed health group could have make considered advice available, but only after considerable discussion, and with consultation with genetic counselors.

I have already mentioned that it has been observed that both mammals and birds will preferentially select mates with dissimilar MHC. Perhaps our Poodles would as well if we let them do the selecting! It might be wise to breed a bitch with two copies of haplotype #1 to a dog with at least one other haplotype. And certainly it would be wise to find the lines with rare haplotypes and made sure they survive. But at this point, I would not be comfortable offering advice on to how to use DLA haplotype information to make breeding decisions. There is much more to a dog than his MHC!

Owners of the dogs who participated in this study can receive the results of their dog's DLA haplotype testing. Results have been provided owners of the dogs in the PCC groups. The list of the AHT selected dogs can be provided to the Standard Poodle Club if requested. If you have supplied a DNA sample to the AHT, you can contact Molly Windebank to see if your dog was one of those selected and, if so, how to request the results.

Although this study has ended, if you would like to have your dog's DLA tested, it can be done through Genoscoper, or you can send saliva or blood (EDTA) samples direct to me, Lorna Kennedy. The charge I make is less than genoscoper, but currently I have a much longer turn-around period.