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Genetic diversity of immune response genes in English Setters

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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.

Table 1 shows the DLA haplotype profiles for English, Irish and Gordon Setters. It is clear that some haplotypes are found in all varieties, but there are also unique haplotypes within each variety.

Table 1: DLA haplotype frequencies in setter varieties.

	Haplotype				Breed	Setter (English)		Setter (Irish)		Setter (Gordon)	
					No tested	127	127	115	115	108	108
#	DRB1	DQA1	DQB1	comment		No hom	% haplo	No hom	% haplo	No hom	% haplo
1	00101	00101	00201	all		**61**	**67.3**	**27**	**51.7**		**12.5**
2	00601	005011	00701	all		**12**	**24.8**	**1**	**7.8**		0.9
3	00103	00101	00201	all			0.4	**1**	**9.6**	**1**	**7.4**
4	00901	00101	008011	all			**1.2**		**8.3**	**3**	**12.5**
5	02001	00401	01303	all			0.4		**1.7**	**3**	**15.3**
6	00102	00101	00201	E, I			0.4	1	0.9		
7	006set	005011	00701	E only			**1.6**				
8	01502	00601	02301	E only			0.4				
9	01301	00101	00201	E only		**1**	**3.1**				
10	00104	00101	00201	E only			0.4				
11	00801	00301	00401	I only					**1.3**		
12	00903	00101	008011	I only					0.4		
13	00501	00301	00501	I, G				**1**	**17.8**		**1.4**
14	01801	00101	00201	I, G					0.4	**1**	**1.9**
15	01801	00101	00802	G only						**9**	**31.0**
16	04901	01001	01901	G only						**3**	**8.8**
17	01501	00601	02301	G only							**6.5**
18	10201	00101	00201	G only							**1.9**

No hom = number of homozygous dogs with the haplotype. % haplo = haplotype frequency.
Haplotypes in **bold** occur in more than one dog. E = English setter. I = Irish setter. G = Gordon setter

The English setter has five main haplotypes plus a further five haplotypes that were found in single dogs only. There is one haplotype at high frequency (#1), one at 24.8% (#2) and three others at low frequency (#3,5,6,8,10). This is a typical profile for a purebred dog breed. Two of the haplotypes found in a single dog (#5,6) are also found in Irish setters, so these two dogs may have been mis-classified. Irish setters have seven main haplotypes, with the commonest haplotype having a lower frequency than in English setters. Gordon setters have ten main haplotypes, with a range from 31-1.4%. These three breeds have a complicated pattern of haplotype sharing. From a diversity perspective, Gordon setters have the best DLA profile, Irish setters have a reasonable profile, while there is some cause for concern in the English setter.

Within English setters there is one major haplotype that has a frequency of 67%, which means that 88% of English setters will carry one or two copies of this haplotype. 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 setters. Or, 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 an animal having two different haplotypes (heterozygous). See box 3 for information about heterozygote advantage. There

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.

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.
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.

When we consider country of origin within each variety of setters, it is interesting to see that some haplotypes are more widespread than others. For example, haplotype 1 is equally common in English setters from Europe and the USA, but that all the other haplotypes are either found in Europe **or** the USA but not both. Whereas in Irish setters there is more sharing of haplotypes between Europe and the USA, but greater variation of actual haplotype frequencies.

Table 2: DLA haplotype profiles in setters from different countries.

	Country			Europe		USA		Europe		USA		Sweden	
	Breed			Setter (English)		Setter (English)		Setter (Irish)		Setter (Irish)		Setter (Gordon)	
	No tested			108	108	19	19	45	45	98	98	98	98
	Haplotype												
No	DRB1	DQA1	DQB1	No hom	% haplo	No hom	% haplo	No hom	% haplo	No hom	% haplo	No hom	% haplo
1	00101	00101	00201	51	67.6	1	65.8	**6**	**38.9**	**23**	**50.5**		10.2
2	00601	005011	00701	**12**	**29.2**			1	3.3	1	8.7		0.5
3	00103	00101	00201		0.5			**14**	**48.9**	**1**	**8.7**	1	8.2
4	00901	00101	008011				**7.9**				9.7	2	10.2
5	02001	00401	01303				**2.6**		3.3		0.5	3	16.8
6	00102	00101	00201		0.5			1	2.2				
7	006set	005011	00701		**1.9**								
8	01502	00601	02301		0.5								
9	01301	00101	00201			**1**	**21.1**						
10	00104	00101	00201				**2.6**						
11	00801	00301	00401								1.5		
12	00903	00101	008011								0.5		
13	00501	00301	00501						**2.2**	**1**	**19.9**		1.5
14	01801	00101	00201						1.1				
15	01801	00101	00802									9	34.2
16	04901	01001	01901									3	9.7
17	01501	00601	02301										6.6
18	10201	00101	00201										2.0

No hom = number of homozygous dogs with the haplotype. % haplo = haplotype frequency.
Haplotypes in **bold** occur at different frequencies in different countries.

I have already mentioned that it has been observed that both mammals and birds will preferentially select mates with dissimilar MHC. Perhaps our dogs 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!

These results suggest that some carefully controlled cross breeding between different varieties of setter could improve genetic diversity. However, the MHC is **not** the best measure of overall diversity within a breed. MHC data should be used alongside data from panels of markers across the genome, in order to assess genetic diversity within a breed and within indivduals.

English setters have a high susceptibility to develop hypothyroid disease, and it has been shown that haplotype #1 is associated with this disease. We collected many samples at an English Setter show last year and this increased our numbers of this breed considerably. Table 3 compares the presence of the DLA risk haplotype #1 (DLA-DRB1*00101/DQA1*00101/DQB1*00201) with hypothyroid status. Overall, the presence of haplotype #1, either one (heterozygous) or two copies (homozygous), is the same in cases and controls, at 85.7%. However, there is a significant difference between the 71.4% cases versus 43.8% controls that are homozygous for this haplotype.

Table 3: Frequency of hypothyroid risk haplotype in English setters

Status of risk haplotype	Presence of risk haplo	No copies of risk haplo	cases n=21	% cases	controls n=98	% controls
hom or het	+/+ or -/-	at least 1	18	85.7	84	85.7
homozygous	**+/+**	**2**	**15**	**71.4**	**43**	**43.8**
heterozygous	+/-	1	3	14.3	41	41.8
not present	-/-	0	3	14.3	14	14.3

Hom or het = homozygous or heterozygous. Haplo = haplotype

As I have already mentioned, it would seem natural to try and decrease the frequency of haplotype #1 in English setters. However, this would exclude most of the dogs in the breed, so that is not the answer. This is because the MHC is only part of the genetic risk for hypothyroid disease, and there will be many other genes that contribute susceptibility.

Over the last four years we have been part of a large European funded project, called LUPA, which has aimed to identify genetic markers for many canine diseases. As part of this study we have performed whole genome scans using hypothyroid cases and healthy controls from several different breeds. [We could not use English setters in this part of the study, as we did not have enough cases to provide the necessary power in the experiment.] The whole genome scans identified several regions of the genome that were associated with hypothyroid disease in Rhodesian Ridgebacks Doberman and Boxer. We have been able to confirm some of these associations using replication groups from a further ten breeds, including English setters.

Currently we are looking for further funding, so that we can perform fine-mapping in those confirmed regions of association, in order to identify some of the actual genes that confer susceptibility for hypothyroid disease apart from the MHC.