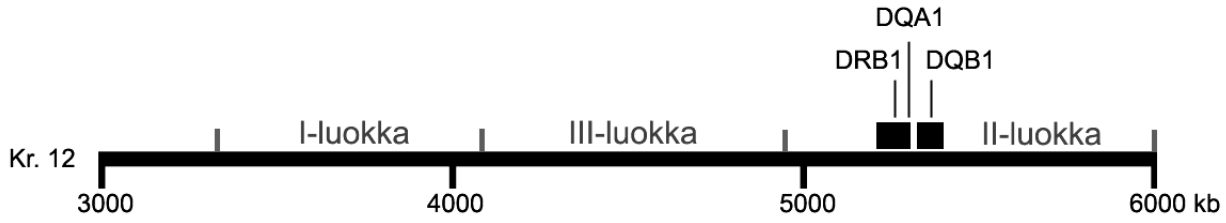
MHC CLASS II DIVERSITY IN ICELANDIC SHEEPDOG

In small closed dog populations inbreeding cannot be avoided and that causes homozygosity to increase. Homozygosity is harmful especially when an individual inherits a gene for inherited sickness from both parents. Homozygosity can also be harmful in characters, like immune defence and MHC, which require different alleles to function effectively.

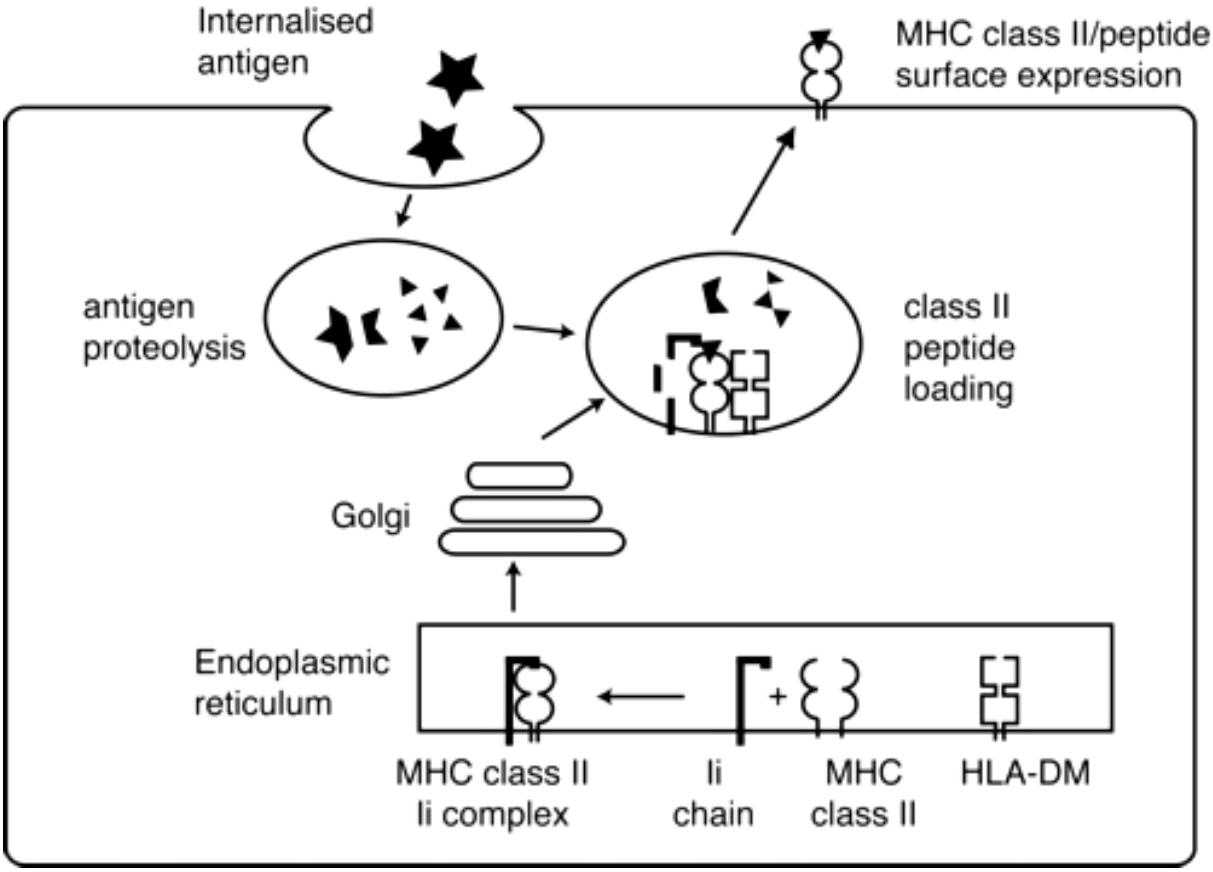
MHC and DLA

MHC is a shortening of ”major histocompatibility complex”. The name applies to one of the MHC-gene functions, which raised interest in the MHC as a research area about thirty years ago. MHC-genes play an important role in recognizing self from non-self - individual’s own tissues from foreign tissues, for example in transplantation. Rejection reactions after organ transplantation are caused by MHC-gene products. MHC hasn’t changed much during evolution and is similar in all mammals. Canine MHC genes are given the prefix DLA (”dog leucocyte antigen”).

Dog MHC is in chromosome 12 (picture 1). MHC-genes are divided into three classes based on the function of their gene products. In this study class II genes DLA-DRB1, DLA-DQA1 and DLA-DQB1 were studied from Icelandic sheepdog. The function of the proteins coded by these genes is shown in picture 2. The class II genes code for proteins, which take part in the beginning of the immune reaction. They recognize and present parts of different kinds of antigens to other cells, which take part in the immune reaction. Because the amount of different kinds of antigens is large, it is important that many MHC-alleles are conserved both in individual and population levels. Low amount of alleles can lower the population’s defence against different antigens. A homozygous individual might be in greater risk of getting an infectious disease or autoimmune problems.



Picture 1. Dog’s MHC is situated in chromosome 12. MHC-classes I, III and II are in the chromosome next to each other. The genes DRB1, DQA1 and DQB1, which were studied from Icelandic sheepdog, belong to the class II and are located close to each other. (luokka = class in Finnish, kb = kilobase)



Picture 2. Schematic representation of the MHC class II protein function in the antigen presenting cell. MHC II protein binds antigen peptide and presents it on the cell surface. Ii and DM help the MHC II protein to load antigen peptide. (Kelly 2008)

DLA diversity of the Icelandic sheepdog

58 Icelandic sheepdogs from Finland took part in the study. Eight DRB1 alleles, five DQA1 alleles and six DQB1 alleles were found. One DRB1 allele had not been found from dogs in previous DLA studies. It is quite common to find new alleles, when new breeds are studied. The results are shown in table 1. Also another breed Löwchen (Little lion dog) was typed at the same time and its' results are shown for the sake of comparison. 72 Löwchens were studied and the breed had one allele less than Icelandic sheepdog in DRB1 and DQA1 loci. In DQB1 locus Löwchen had one allele more than Icelandic sheepdog.

Icelandic sheepdog's heterozygosity (H) was 79,3 % in DRB1 locus, 76,8 % in DQA locus and 87,7 % in DQB1 locus. Löwchen had lower heterozygosity in all loci. Kennedy et al. (2007a) studied about 80 dog breeds and the average heterozygosity in their study was 66,5 % in DRB1 locus, 60,3 % in DQA1 locus and 67,1 % in DQB1 locus. Icelandic sheepdog has higher values in all DLA loci. This result is probably due to the Finnish breeding habits - many dogs are imported and dogs from different family lines are mixed. It seems that breeding stocks in different countries have formed differentiated lines. Some lines were already found in Oliehoek's (1999) pedigree study. Different DLA alleles have become common in different lines and mixing these lines in Finnish stock causes high heterozygosity. This phenomenon is temporary. Heterozygosity will become lower, because there aren't many more new families to use in breeding.

DRB1, DQA1 and DQB1 loci form a three-allele combination, which is inherited together and called a haplotype. 10 different haplotypes were found in Icelandic sheepdog. The haplotypes and their frequencies are shown in Table 2. Only 8 haplotypes were found in Löwchen, so Icelandic

sheepdog has higher diversity also in this aspect. Haplotype heterozygosity in Icelandic sheepdog is 94,5 %, which is high compared to Löwchen's heterozygosity, 74,3 %, and 80 breeds' average, 75 %. This is very good news for the Icelandic sheepdog.

Inbreeding coefficient (F_{IS}) is the level of heterozygosity compared to Hardy-Weinberg equilibrium. When the level of heterozygosity is low (and the level of homozygosity high), the inbreeding coefficient is positive - this is a sign of inbreeding. When the level of heterozygosity is high, the inbreeding coefficient is negative - this is a sign of outbreeding. The inbreeding coefficient in Icelandic sheepdog was between - 9,4 % and - 10,0 % in different loci. The only statistically significant difference was in DQB1 locus. The result means that there is outbreeding in DQB1 locus and higher heterozygosity than expected compared to allele frequencies. This is an unexpected result at first sight, but goes together with the earlier assumption that differentiated lines are mixed in Finland.

One haplotype was found only in dogs imported from Middle Europe and Denmark and their offspring and one haplotype was found only in one Danish born dog. These results support the idea that different haplotypes have became common in different areas.

Table 1. Allele number in DRB1, DQA1 and DQB1 loci, heterozygosity (H) and inbreeding coefficient (F_{IS}). Statistical significance: * $p < 0,05$.

Locus	Allele number		Icelandic Sheepdog		Löwchen	Average of 80 breeds
	Icelandic	Löwchen	H (%)	F_{IS} (%)	H (%)	H (%)
DRB1	8	7	79,3	-9,4	69,0	66,5
DQA1	5	4	76,8	-10,0	16,7	60,3
DQB1	6	7	87,7	-9,4*	62,9	67,1
Average			81,3	-9,6*	49,5	

Table 2. Haplotypes and their frequencies in Icelandic sheepdog. Different alleles are named with numbers. 011v is a new allele, which was found first time from Icelandic sheepdog.

DRB1	DQA1	DQB1	Frequency (%)
01501	00601	00301	24,5
02301	00301	00501	19,1
01501	00601	02301	17,3
01301	00101	00201	10,9
011v	00201	01303	9,1
01503	00601	00301	7,3
01501	00901	00101	4,5
00201	00901	00101	3,6
00101	00101	00201	2,7
00901	00101	00201	0,9
		total	99,9

Final words

One should keep in mind that especially the results in heterozygosity and haplotype frequencies tell only about the current situation in Finnish population. Because many dogs have been imported to Finland from different family lines and used in breeding, the heterozygosity is probably higher than in the breed as a whole. On the other hand, because many dogs from different lines gave samples to this study, the allele and haplotype numbers are very likely quite close to the real numbers. Of course some probably rare alleles and haplotypes can still be "hiding" in the breed.

The results of the DLA typing were sent to the dog owners and also to the Finnish breeding committee (regarding the dogs, whose owners had given permission). The results can be used as one criterion, when choosing breeding animals. It is important to keep the rare alleles in the breed. It is not recommended to mate two dogs, which are homozygous for the same alleles. It might be possible to use DLA haplotype results to find out relationships between different dog breeds. It is possible to do DLA typing (also for foreign dogs) in HT Diagnostics laboratory, www.canigen.com.

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Literature:

Kelly, A. 2008: MHC class II antigen presentation. http://www.path.cam.ac.uk/pages/kelly/

Kennedy, L. J., Barnes, A., Short, A., Brown, J. J., Lester, S., Seddon, J., Fleeman, L., Francino, O., Brkljacic, M., Knyazev, S., Happ, G. M. & Ollier, W. E. R. 2007a: Canine DLA diversity: 1. New alleles and haplotypes. – Tissue antigens. 69: 272-288.

Kennedy, L. J., O'Neill, T., House, A., Barnes, A., Kyöstilä, K., Innes, J., Fretwell, N., Day, M. J., Catchpole, B., Lohi, H. & Ollier, W. E. R. 2007b: Risk of anal furunculosis in German Shepherd dogs is associated with the major histocompatibility complex. – Tissue Antigens. 71: 51-56.

Niskanen, A. 2008: MHC II -luokan geenien monimuotoisuus Suomen susipopulaatiossa (Canis lupus) ja islanninlammaskoirapopulaatiossa (Canis familiaris). Pro gradu -tutkielma. Oulun yliopisto, biologian laitos, 69 s.

Oliehoek, P. 1999: Inbreeding, effective population size, mean kinship and cluster analysis in the Icelandic Sheepdog as a small population. Student thesis. Wageningen University, 52 s.