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DLA-DRB1, DQA1, and DQB1 Alleles and Haplotypes in North American Gray Wolves

Lorna J. Kennedy, John M. Angles, Annette Barnes, Lindsey E. Carmichael, Alan D. Radford, William E. R. Ollier, and George M. Happ

From the Centre for Integrated Genomic Medical Research, University of Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT, UK (Kennedy and Ollier); VetResearch Genetics, 18 Crammond Avenue, Bundeena, NSW 2230, Australia (Angles); Veterinary Clinical Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZJ, UK (Barnes); the Department of Biological Sciences, University of Alberta, Edmonton, AB, T6G 2E9, Canada (Carmichael); the University of Liverpool Veterinary Teaching Hospital, Leahurst, Neston, S Wirral CH64 7TE, UK (Radford); and the University of Alaska, Fairbanks, PO Box 757040, Fairbanks, AK 99775, USA (Happ).

Address correspondence to Dr. L. J. Kennedy at the address above, or e-mail: lorna.kennedy@manchester.ac.uk.

Abstract

The canine major histocompatibility complex contains highly polymorphic genes, many of which are critical in regulating immune response. Since domestic dogs evolved from Gray Wolves (*Canis lupus*), common DLA class II alleles should exist. Sequencing was used to characterize 175 Gray Wolves for DLA class II alleles, and data from 1856 dogs, covering 85 different breeds of mostly European origin, were available for comparison. Within wolves, 28 new alleles were identified, all occurring in at least 2 individuals. Three DLA-DRB1, 8 DLA-DQA1, and 6 DLA-DQB1 alleles also identified in dogs were present. Twenty-eight haplotypes were identified, of which 2 three-locus haplotypes, and many DLA-DQA1/DQB1 haplotypes, are also found in dogs. The wolves studied had relatively few dog DLA alleles and may therefore represent a remnant population descended from Asian wolves. The single European wolf included carried a haplotype found in both these North American wolves and in many dog breeds. Furthermore, one wolf DQB1 allele has been found in Shih Tzu, a breed of Asian origin. These data suggest that the wolf ancestors of Asian and European dogs may have had different gene pools, currently reflected in the DLA alleles present in dog breeds.

The major histocompatibility complex (MHC) is essential for the presentation of foreign peptides to the vertebrate immune system (Brodsky et al. 1996; Weenink and Gautam 1997), with very high levels of polymorphism present in certain genes of the complex. Maintenance of this diversity in the MHC is thought to be due to a combination of pathogen-driven selection and inbreeding avoidance mechanisms (Apanius et al. 1997; Paterson 1998). Several investigators have shown that diversity in the MHC can be a useful tool for management of both wild and captive endangered species, including the desert bighorn sheep (Gutierrez-Espeleta et al. 2001), Przewalski's horse (Hedrick et al. 1999), and the Mexican wolf (Hedrick et al. 2000). In particular, low levels of heterozygosity in the MHC have been suggested to increase susceptibility to infectious diseases (O'Brien et al. 1985), and it has been proposed that criteria for selection in captive-bred populations should include some measure of polymorphism in the MHC.

Similar to other gray wolf populations in North America, the Alaskan gray wolf has undergone historical decline in numbers as a direct result of hunting and displacement from traditional territories. Numbers though have recovered to a current population estimated at some 5000–7000 gray wolves (Stephenson et al. 1995), with wolves distributed evenly over most of the state. Canadian wolves have also experienced periodic persecution and continue to be harvested, but most mainland populations are currently considered large and stable (Usher 1965; Van Zyll de Jong and Carbyn 1999; Hayes and Harestad 2000). This is in contrast to the Mexican and Red wolf populations that survive as small founder populations after approaching extinction. In these populations, loss of diversity in nuclear DNA (Roy et al. 1994; Garcia-Moreno et al. 1996) and in the MHC (Hedrick et al. 2000) have been documented. The Alaskan gray wolf population provides a natural, expanding population for comparison of MHC polymorphism for conservation genetics in the North American gray wolf.

The aims of the current study were to determine the nature and degree of polymorphism for MHC class II loci DLA-DRB1, DQA1, and DQB1 for the Alaskan and Canadian gray wolves in several geographically separated regions.

Materials and Methods

Tissue samples from 137 Canadian gray wolves were provided by the Department of Environment and Natural Resources (Government of the Northwest Territories [NTs]) and the Parks Canada DNA Repository (University of Alberta). These samples have also been included in larger studies of wolf population genetic structure (Carmichael et al. 2001), and population delineations used here follow those of Carmichael et al. (2001). In the NTs, 3 of the populations, Northern Richardsons ($n = 19$), Southern Richardsons ($n = 20$), and Tuk-Inuvik ($n = 20$) were located in the McKenzie River Delta (68°N, 135°W). Two NT populations, Paulatuk ($n = 19$) and Great Bear Lake ($n = 11$), were fairly close together (67°N, 126°W), and the other ($n = 20$) was located on Banks Island (72°N, 125°W). Twenty-eight samples were also included from the Kluane National Park area (Yukon Territory). The Kluane and McKenzie River Delta populations are considered boreal forest wolves (Carmichael et al. 2007), with all other populations occupying mainland or island barren-ground tundra.

Tissue samples for 38 gray wolves were accessed from the Alaskan Frozen Tissue Collection at the University of Alaska Museum, Fairbanks Alaska. Latitude and longitude were recorded for the site of tissue acquisition for most of the wolf samples. Samples were divided into 4 groups based on geographical regions in Alaska. Three groups were geographically isolated from the others by natural barriers. The first group ($n = 12$) extended from Prince of Wales Island to Thomas Bay (latitude 55–57°N, 130–134°W) and represented the islands of southeast Alaska. The second group ($n = 18$) was geographically separated from the first and represented central Alaska (latitude 63–65°N, 146–148°W). A third group ($n = 4$) represented part of northern Alaska (latitude 68–69°N, 151–153°W) and was separated from the second group by the Brooks Range. There were also 4 Alaskan samples from unknown locations. A blood sample from a single wolf from Poland was available. For comparison, we had DLA haplotype data for 1856 dogs (Kennedy, Barnes, Happ, Quinnell, Bennett, et al. 2002; Kennedy et al. 2007), from over 85 different breeds, mainly of European origin. DNA was extracted according to recommended protocols using a commercially available kit (DNeasy; Qiagen, Crawley, UK).

DLA-DRB1, DQA1, and DQB1 Typing

Sequence-based typing was performed for exon 2 of DLA-DRB1, DQA1, and DQB1 using locus-specific intronic primers followed by BigDye (Applied Biosystems, Warrington, UK) sequencing. Ambiguous or new DNA sequences were confirmed using a separate polymerase chain reaction (PCR) for subsequent DNA cloning (TA cloning; Invitrogen, Paisley, UK) and sequencing. All the new alleles found in this study fulfill the DLA nomenclature committees criteria for new alleles, which include either DNA cloning and subsequent sequencing of several clones in both directions or sequencing of at least 2 PCRs from homozygous animals in both directions (Kennedy et al. 1999, 2001). Three alleles have not yet met those criteria and remain with local names.

The primers used to amplify exon 2 of DLA-DRB1 were forward DRBF: gat ccc ccc gtc ccc aca g and reverse DRBR3T7: taa tac gac tca cta tag gg cgc ccg ctg cgc tca (Kennedy et al. 2005). The alternative forward primer was DRBIn1: ccg tcc cca cag cac att tc (Wagner, Burnett, Works, and Storb 1996). The primers used to amplify exon 2 of DLA-DQA1 were forward DQAin1: taa ggt tct ttt ctc cct ct and reverse DQAIn2: gga cag att cag tga aga ga (Wagner, Burnett, DeRose, and Storb 1996). The primers used to amplify exon 2 of DLA-DQB1 were forward DQB1BT7: taa tac gac tca cta tag gg ctc act ggc ccg gct gtc tc (Wagner, Burnett, DeRose, and Storb 1996) and reverse DQBR2: cac ctc gcc gct gca acg tg (Kennedy, Barnes, Happ, Quinnell, Bennett, et al. 2002).

Sequencing for typing was performed in one direction only: reverse for DRB1 and DQA1 and forward for DQB1. T7 tailed primers were used in the initial PCRs for DRB1 and DQB1 (the T7 portion is underlined), and these products were sequenced with T7: taa tac gac tca cta tag gg. The primers are intronic and locus specific. The product sizes are 303 bp for DRB1 (309 bp for the alternative primer), 345 bp for DQA1, and 300 bp for DQB1. Sequencing to confirm new alleles was done in both directions, and DNA cloning was performed if animals homozygous for the allele were not identified.

All PCR reactions are performed with 25 ng DNA in a 25-μl reaction containing 1× PCR buffer as supplied by Qiagen (with no extra magnesium), Q solution (Qiagen), final concentrations of 0.1 μM for each primer, and 200 μM each dNTP, with 2 units of *Taq* polymerase (Qiagen HotStarTaq). A negative control containing no DNA template was included in each run of amplifications to identify any contamination. A standard touchdown PCR protocol was used for all amplifications, which consisted of an initial 15 min at 95 °C, 14 touchdown cycles of 95 °C for 30 s, followed by 1 min annealing, starting at 62 °C (DRB1), 54 °C (DQA1), 73 °C (DQB1) and reducing by 0.5 °C each cycle, and 72 °C for 1 min. Then 20 cycles of 95 °C for 30 s, 55 °C (DRB1), 47 °C (DQA1), 66 °C (DQB1) for 1 min, and 72 °C for 1 min plus a final extension at 72 °C for 10 min.

PCR samples were purified as follows: 2 units of shrimp alkaline phosphatase (Amersham, Little Chalfont, UK) and 10 units of Exo1 (New England Biolabs, Hitchin, UK) were added to 5 μl of PCR product and the mixture incubated for 1 h at 37 °C followed by for 15 min at 80 °C. Two microliters of a 1 in 10 dilution was used for sequencing. Cycle sequencing was performed using Big Dye Terminator V3 (Applied Biosystems), and samples were sequenced on an Applied Biosystems 3100 or 3730 Genetic Analyzer. Sequencing data were analyzed using MatchTools and MatchTools Navigator (Applied Biosystems).

There is extremely high linkage disequilibrium between DLA-DRB1, DQA1, and DQB1 (particularly between

Table 1. DLA-DRB1/DQA1/DQB1 alleles found in this cohort of 194 gray wolves

New[a] DRB1 alleles identified in wolves $n = 14$	DRB1 alleles also found in dogs $n = 3$	New[a] DQA1 alleles identified in wolves $n = 4$	DQA1 alleles also found in dogs $n = 8$	New[a] DQB1 alleles identified in wolves $n = 9$	DQB1 alleles also found in dogs $n = 6$
03101	00601	01101	00101	01401	00701
03202[b]	00901	01301	00201	03201	008011
03501	02901	01701[c]	00301	03301	01303
03601		bl[d]	005011	03401	02002
03701			00601	04001	02901
03801			01001	04101	03501[e]
04101			012011	04201	
04401			014011[e]	038v	
04501				05501	
049v					
06501[b]					
09101					
09201					
09301					

DRB1*03202, DRB1*06501, and DQA1*01701 were identified in this study but confirmed in other laboratories and submitted to GenBank by those other laboratories (Hedrick et al. 2000; Hedrick et al. 2002; and Seddon and Ellegren 2002), respectively. Accession numbers for the new alleles are DRB1*03101 (AF336108), DRB1*03202 (AF516916), DRB1*03501 (AF336109), DRB1*03601 (AF336110), DRB1*03701 (AF343738), DRB1*03801 (AF343739), DRB1*04101 (AF343742), DRB1*04401 (AF343745), DRB1*04501 (AF343746), DRB1*06501 (AF516917), DRB1*049v (AM408902), DRB1*09101 (AM408903), DRB1*09201 (AM408904), DRB1*09301 (AM408905), DQA1*01101 (AF343733), DQA1*01301 (AF343735), DQA1*014011 (AF336107), DQA1*01701 (AY126647), DQB1*01401 (AF343732), DQB1*03201 (AJ311104), DQB1*03301 (AJ311105), DQB1*03401 (AJ311106), DQB1*03501 (AJ311107), DQB1*04001 (AJ316223), DQB1*04101 (AJ316224), DQB1*04201 (AJ316225), DQB1*038v (AM408906), and DQB1*05501 (AM408907).

[a] All these new alleles were identified in wolves, and have not been seen in dogs to date.

[b] These alleles also found in Red wolves.

[c] This allele also found in coyotes.

[d] A new unidentified allele.

[e] These alleles were identified in this study but have since been found in dogs by LJK.

DQA1 and DQB1), and this can be exploited to assign haplotypes. Thus, three-locus (DLA-DRB1, DQA1, and DQB1) haplotypes were established using an interactive and subtractive approach as described previously (Kennedy, Barnes, Happ, Quinnell, Bennett, et al. 2002; Kennedy, Barnes, Happ, Quinnell, Courtenay, et al. 2002). First, all wolves that were homozygous at all 3 loci were selected, and from these 9 different DLA-DRB1/DQA1/DQB1 haplotype combinations were identified. These haplotypes were also found in heterozygous wolves, and by subtraction, the other haplotype in those wolves could be identified. In many cases, the subtractions revealed haplotypes that had already been identified in homozygous wolves. Second, wolves that were homozygous at only 2 loci were selected. From these wolves, many of the previous haplotypes were confirmed and also several further haplotypes were identified. The remaining wolves were examined using the haplotype data already identified, with further possible haplotypes assigned. There is a theoretical potential for misassignment of haplotypes using this method, but in practice, it is very clear and easy to assign them, and within this data set, there were no wolves for which we were uncertain of the haplotype assignment. Bootstrapping analyses were carried out using Stata (StataCorp 2003). Analyses for dN/dS ratios were performed using single likelihood ancestor counting analysis. Codon-based dN/dS values were calculated using fixed effects likelihood (FEL). Both programs were used online at http://www.datamonkey.org/ (Pond and Frost 2005).

Results

In this study, 14 new DLA-DRB1 alleles, 4 new DLA-DQA1 alleles, and 9 new DLA-DQB1 alleles were identified (see Table 1). Nucleotide and amino acid sequence data are available from http://www.ebi.ac.uk/ipd/mhc/dla/index.html. Two other alleles, DQA1*014011 and DQB1*03501, identified in this study of wolves have since been identified in dogs.

One allele, DLA-DRB1*09301, was only amplified in wolves that were homozygous for the allele and not in heterozygous wolves. When DNA was mixed from 2 different homozygous wolves, 12 times as much DNA from the wolf with DRB1*09301 had to be added in order to detect the allele by sequencing the PCR product. Using a different forward primer (DRBIn1) resolved this issue. These 2 forward primers overlap, with DRBF being upstream to DRBIn1, and we therefore assume that the putative point mutation preventing this allele from amplifying must lie in the portion of DRBF that does not overlap with DRBIn1. Unfortunately, as this is the upstream part of DRBF, it will require the design of another primer to identify the mutation. Similarly, 2

Table 2. Overall DLA-DRB1/DQA1/DQB1 haplotype frequencies

DRB1	DQA1	DQB1	Gene frequency (%)	Number of homozygous wolves $n = 35$	Number of heterozygous wolves $n = 140$
00601	**005011**	**00701**	6.29	4	14
00601	**00601**	03301	0.57	0	2
00601	bl	03301	2.29	0	8
00901	**00101**	**008011**	4.00	0	14
02901	**00301**	04101	1.43	0	5
03101	01101	01401	10.86	8	22
03101	01101	04001	6.29	2	18
03202	**00201**	**02901**	2.86	0	10
03202	**005011**	04201	6.00	0	21
03501	**00201**	**01303**	7.43	4	18
03601	**012011**	**03501**	4.29	3	9
03701	**00601**	03301	4.86	4	9
03701	bl	03301	3.71	0	13
03801	**00101**	**008011**	3.43	0	12
03801	**005011**	**01303**	2.57	0	9
04101	01301	03201	2.29	0	8
04401	**00201**	**01303**	2.86	2	6
04401	01701	038v	1.14	0	4
04501	**014011**	03401	5.14	1	16
049v	**005011**	**00701**	0.57	0	2
06501	**014011**	03401	0.57	0	2
09101	**00601**	**02002**	3.43	0	12
09201	**00601**	**02002**	3.71	0	13
09301	**01001**	05501	12.29	7	29
Other	Single	Haplotypes	1.14	0	4

Alleles in **bold** are also found in dogs. bl, no DQA1 allele detected.

haplotypes appeared to lack a DQA1 allele (see DQA1*bl in Tables 1 and 2). We have not yet been able to confirm whether these haplotypes carry an unidentified DQA1 allele or whether the gene is missing. The latter explanation seems unlikely, as there are 2 wolves that are homozygous for one of these haplotypes. A similar situation has occurred in a group of dogs from Russia that appear to lack a DQB1 gene on one haplotype (Kennedy et al. 2007). It is most likely that this apparent lack of DQA1 and DQB1 alleles represent allele dropout, as shown for DRB1, and the current primers need to be modified. However, these allele dropouts are easily detected by the presence of unusual haplotype combinations.

In total, 28 DLA-DRB1/DQA1/DQB1 haplotypes were identified (see Table 2). Thirty-five wolves were homozygous for all 3 loci. Nine haplotypes were identified in homozygous gray wolves and confirmed in heterozygous wolves. Fifteen further haplotypes were identified in at least 2 heterozygous animals, and 4 other haplotypes were identified in single heterozygous animals.

Seven haplotypes occurred in the overall population at a frequency of >5%, 14 occurred at a frequency between 1–5%, and 3 occurred at a frequency of <1%. Four other haplotypes were only found in single heterozygous animals.

Two of the 28 three-locus haplotypes are commonly found in domestic dogs, whereas 7 of the 17 DQA1/DQB1 haplotypes are found in dogs. When considering DQ alleles that have been previously identified in dogs, no new combinations of alleles were seen.

An analysis of haplotypes found in the different regions was performed (Table 3). There were major differences in the number of haplotypes found and the haplotype frequencies between the different groups. The samples from the Canadian Northwest showed the most variation, but this is not surprising because there were 109 wolves in this group compared with approximately 20 in all the others except 2. Several haplotypes were only found in the northwest Canada group (see Table 3).

Table 4 shows an analysis of haplotypes within different populations in Northwestern Canada. In general, there are about 15 haplotypes within each population, except for the one from Banks Island, which has only 4. All except 2 haplotypes are found in more than one population. There is no accepted single measure of diversity (Gillespie 1998). Two possible measures are the number of alleles at a locus and the level of heterozygosity of alleles. Thus, if we compare the 18 wolves from central Alaska and the 12 wolves from SE Alaska, we see that they have 8 and 7 haplotypes, respectively, suggesting similar levels of diversity. However, when you consider the frequencies of the different haplotypes in each population, it is clear that the wolves from central Alaska are much less diverse because of one very frequent haplotype, DLA-DRB1*03101/DQA1*01101/DQB1*01401. We compared the different populations by sampling random populations of the same size 1000 times from the total wolf population ($n = 175$) and calculating the expected number of haplotypes for each size of population. For the packs

Table 3. DLA-DRB1/DQA1/DQB1 haplotype frequencies by region

DRB1	DQA1	DQB1	Overall gene frequency (%) $n = 175$	Alaska N $n = 4$	Alaska Central $n = 18$	Alaska SE $n = 12$	Alaska Unknown $n = 4$	Canada NT $n = 109$	Canada Yukon $n = 28$
00601	005011	**00701**	6.29	12.50				8.72	3.57
00601	00601	03301	0.57			8.33			
00601	bl	03301	2.29			8.33		2.75	
00901	**00101**	**008011**	4.00					5.50	3.57
02901	**00301**	04101	1.43			4.17			7.14
03101	01101	01401	10.86	12.50	66.67		25.00	3.67	5.36
03101	01101	04001	6.29		2.78			5.05	17.86
03202	**00201**	**02901**	2.86			4.17		3.67	1.79
03202	**005011**	04201	6.00		8.33		12.50	5.50	8.93
03501	**00201**	**01303**	7.43	12.50	8.33	45.83	12.50	3.67	3.57
03601	**012011**	**03501**	4.29	37.50	2.78		25.00	0.92	12.50
03701	**00601**	03301	4.86	12.50	5.56		12.50	5.05	3.57
03701	bl	03301	3.71					5.05	3.57
03801	**00101**	**008011**	3.43					5.50	
03801	**005011**	**01303**	2.57		2.78	4.17		2.29	3.57
04101	01301	03201	2.29		2.78			2.29	3.57
04401	**00201**	**01303**	2.86					0.92	14.29
04401	01701	038v	1.14					1.83	
04501	**014011**	03401	5.14			25.00	12.50	4.13	3.57
049v	**005011**	**00701**	0.57					0.92	
06501	**014011**	03401	0.57					0.92	
09101	**00601**	**02002**	3.43					5.05	1.79
09201	**00601**	02002	3.71					5.96	
09301	**01001**	**05501**	12.29					19.72	
Other	Single	Haplotypes	1.14	12.50				0.92	1.79
Total	Number	Haplotypes	28	6	8	7	6	24	17
Probability[a]				0.31	0.001	0.001	0.31	0.08	0.06

Alleles in **bold** are also found in dogs. bl, no DQA1 allele detected.

[a] The probability, calculated by bootstrapping, of the population being less diverse than expected.

in NTs, samples were taken from the total from that region ($n = 109$). The probabilities for each population are shown in the bottom line of Tables 3 and 4. These probabilities suggest that the wolves in central and SE Alaska are significantly less diverse than expected, whereas the population from the Yukon are borderline significantly less diverse than expected. All the other populations have lower diversity than expected but are nonsignificant. Within the packs in the NTs, all except the Tuk-Inuvik pack are less diverse than expected, with the group from Banks Island being significantly less diverse ($P < 0.001$). The Tuk-Inuvik pack is slightly more diverse than expected.

Phylogenetic trees for DLA-DRB1, DQA1, and DQB1 (data not shown) indicate that gray wolf alleles were present throughout the trees constructed using domestic dog alleles and are not limited to a few branches.

Nonsynonymous (dN) substitutions were significantly greater than synonymous (dS) substitutions for DLA-DRB1, DLA-DQA1, and DLA-DQB1 alleles in both wolves and dogs. The overall dN/dS ratios were 1.9 and 1.8 for DLA-DRB1 in dogs and wolves, 1.4 and 2.2 for DLA-DQA1 in dogs and wolves, and 1.6 and 2.8 for DLA-DQB1 in dogs and wolves, respectively. The nonsynonymous substitutions occurred within recognized DLA hypervariable regions and points of antigen contact, with the pattern of substitutions very similar to that seen in the domestic dog. Figure 1 shows the dN/dS values for each codon based on 77 DLA-DRB1 alleles found in dogs and 23 DLA-DRB1 alleles found in wolves. There are no significant differences between the plots.

Discussion

This is the first study of the MHC class II in Alaskan and Canadian gray wolf to identify and define three-locus DLA-DRB1, DQA1, and DQB1 haplotypes. MHC haplotype information for population studies offers significant advantages compared with single-locus allele frequencies, as a lack of recombination between genetic loci in the MHC leads to conservation of haplotypes (Degli-Esposti et al. 1992). The presence of a common haplotype between 2 isolated populations suggests a common ancestry, but gene flow alone can produce the same pattern.

There is a lack of variation of DLA alleles and haplotypes in Alaska, but in Canada, there appears to be a much higher level of variation. This may merely reflect the difference in the numbers of wolves tested from each region (although nothing is known about the relatedness of the Alaskan samples) or may actually represent the level of variation within those regions, perhaps relating to differences in severity of

Table 4. DLA-DRB1/DQA1/DQB1 haplotype frequencies by pack in northwest Canada

				Boreal forest			Barren ground		Island
DRB1	DQA1	DQB1	Overall gene frequency (%) $n = 109$	Northern Richardsons $n = 19$	Southern Richardsons $n = 20$	Tuk-Inuvik $n = 20$	Great Bear Lake $n = 11$	Paulatuk $n = 19$	Banks Island $n = 20$
00601	**005011**	**00701**	8.72	18.42	17.50	7.50	4.55	2.63	
00601	00601	03301							
00601	bl	03301	2.75	10.53		5.00			
00901	**00101**	**008011**	5.50			12.50	13.64	10.53	
02901	**00301**	04101							
03101	01101	01401	3.67	5.26	10.00			5.26	
03101	01101	04001	5.05	7.89		2.50	4.55	2.63	12.50
03202	**00201**	**02901**	3.67	2.63	5.00	7.50		2.63	
03202	**005011**	04201	5.50	2.63	2.50	5.00	18.18	13.16	
03501	**00201**	**01303**	3.67	5.26		7.50	9.09	2.63	
03601	**012011**	**03501**	0.92			2.50	4.55		
03701	**00601**	03301	5.05	2.63	10.00			2.63	
03701	bl	03301	5.05	18.42	12.50	2.50	9.09	2.63	
03801	**00101**	**008011**	5.50	5.26	2.50	2.50		5.26	15.00
03801	**005011**	**01303**	2.29	2.63	2.50	7.50			
04101	01301	03201	2.29		2.50	7.50		2.63	
04401	**00201**	**01303**	0.92			5.00			
04401	01701	038v	1.83		2.50	2.50		5.26	
04501	**014011**	03401	4.13	2.63	15.00	2.50		2.63	
049v	**005011**	**00701**	0.92			5.00			
06501	**014011**	03401	0.92		2.50		4.55		
09101	**00601**	**02002**	5.05	5.26	12.50	5.00		5.26	
09201	**00601**	**02002**	5.96			2.50	13.64	10.53	12.50
09301	**01001**	05501	19.72	7.89	2.50	7.50	13.64	23.68	60.00
Other	Single	Haplotypes	0.92	2.63			4.55		
Total	Number	Haplotypes	24	15	14	19	11	16	4
Probability[a]				0.18	0.06	0.08[b]	0.15	0.23	0.001

Alleles in **bold** are also found in dogs. bl, no DQA1 allele detected.

[a] The probability, calculated by bootstrapping, of the population being less diverse than expected.

[b] This population is more diverse than expected.

historical persecution and thus population bottlenecks in Alaska relative to Canada. Bootstrapping showed that the wolf populations in central and SE Alaska as well as those of the Yukon and Banks Island were significantly less diverse than expected. Supporting this idea is the observation that Banks Island wolves, the least variable of those surveyed here, were severely reduced or extirpated in the 1950s and only began to recover in the 1970s (Usher 1965; Miller 1995). More interesting is the fact that the greatest number of haplotypes occurred in the Canadian boreal forest populations, which also possessed haplotypes found in no other population (Tables 3 and 4). This result is consistent with both habitat-based isolation of wolf ecotypes in this region (Carmichael LE, unpublished data) and with colonization of the Canadian barren grounds by wolves previously isolated in southern glacial refugia (Carmichael LE, unpublished data).

The gray wolf MHC class II alleles in the present study were designated under DLA nomenclature (Kennedy et al. 1999, 2001) rather than the alternative *Calu* nomenclature as proposed in 1990 (Klein et al. 1990). At the time the *Calu* nomenclature was proposed for the gray wolf (*Canis lupus*), the domestic dog was considered to belong to a separate species (*Canis familiaris*). However, hybridization between the gray wolf and domestic dog is now recognized in North America, leading to reclassification of the domestic dog as a subspecies of the gray wolf (*C. lupus sp. familiaris*). This is consistent with previous molecular studies examining the origins of the domestic dog from the gray wolf (Vila et al. 1999; Wayne and Ostrander 1999) and with data presented in this paper. The exon 2 domains of the MHC class II genes DLA-DRB1, DQA1, and DQB1 in the gray wolf are identical in structure to the DLA of domestic dogs and have similar sites for polymorphism at each of the MHC class II loci. It is only possible to label them as "gray wolf" or "domestic dog" because we know a priori which animal they have came from. Although there is also evidence from Italy demonstrating wolf–dog hybridization to be rarer than commonly supposed (Vila et al. 2003; Verardi et al. 2006), this does not help with the nomenclature issue.

Phylogenetic trees for DLA-DRB1, DQA1, and DQB1 showed that gray wolf alleles do not cluster independently from the published domestic dog DLA alleles (Kennedy et al. 1999, 2001). This phenomenon has been described in primates and termed "transspecies polymorphism" (Klein 1987; Cooper et al. 1998). Seventeen of the 44 alleles found in the gray wolves have also been

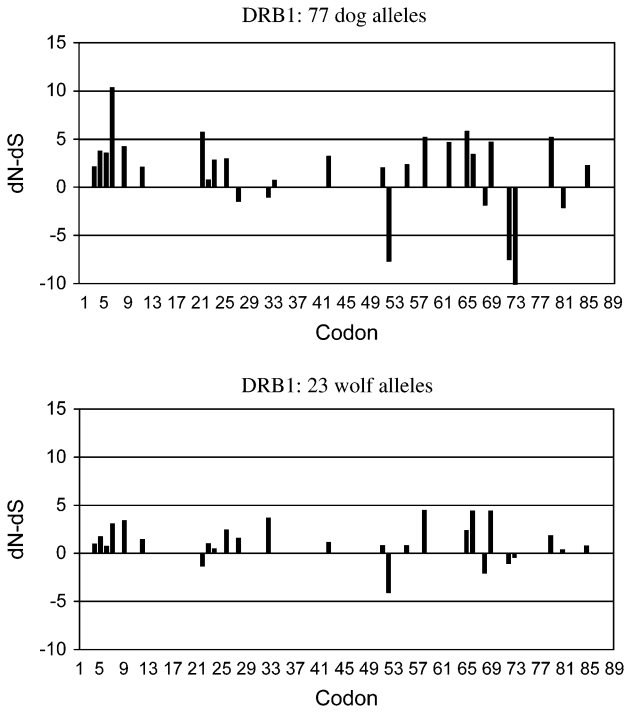

Figure 1. Plots of normalized dN–dS for DLA-DRB1 dog and wolf alleles. The 3 hypervariable regions comprise codons 8–16, 26–38, and 56–74.

found in the domestic dog. We also found 2 haplotypes DLA-DRB1*00601/DQA1*005011/DQB1*00701 and DLA-DRB1*00901/DQA1*00101/DQB1*008011 in the gray wolves that are seen in many breeds of the domestic dog. The single European wolf we have collected from Poland is homozygous for the first (and most frequent in dogs) of these haplotypes. The number of alleles in common and the preservation of 2 three-locus haplotypes and 7 DQA1/DQB1 haplotypes support a recent origin of the domestic dog from the gray wolf. Recent gene flow from wild dogs to gray wolves is another possible cause for the presence of these identical alleles. There was less sharing of alleles between wolves and dogs than we expected considering that the wolf is the ancestor of the domestic dog. Perhaps these wolves are a remnant population descended from Asian wolves, whereas most of the dog breeds we have studied are of European origin. Interestingly, one wolf DLA-DQB1 allele has since been found in Shih Tzu, a breed of Asian origin. These data suggest that the wolf ancestors of Asian and European dogs may have had different gene pools, currently reflected in the DLA alleles present in dog breeds.

The apparent greater conservation/maintenance of DLA-DQA/DQB *cis* combinations between dogs and wolves supports the idea that there is strong evolutionary/selective pressure for maintaining DQ combinations that "work well," that is, provide an appropriate regulated immune response. It is clear from the DQA1/DQB1 combinations seen in both this study and the 1856 domestic dogs DLA typed (Kennedy, Barnes, Happ, Quinnell, Bennett, et al. 2002; Kennedy et al. 2007) that there are a set of "permissible" combinations, and it is fundamental for biological success that there is linkage between certain DQA1 and DQB1 combinations. Each DQA1 allele is only found in combination with 1 or 2 DQB1 alleles, with one combination being extremely common. Similarly, each DQB1 allele is only found in combination with one or 2 different DQA1 alleles, and, again, there is usually one combination that is very much more common.

Several of the alleles identified in our gray wolves have also been detected in other Canadian gray wolves (Hedrick et al. 2000) and European gray wolves (Seddon and Ellegren 2002, 2004), whereas other alleles have been found in red wolves (Hedrick et al. 2000, 2002), Mexican wolves (Hedrick et al. 2000, 2002), and still others in coyotes (Hedrick et al. 2000, 2002; Seddon and Ellegren 2002). It is not unexpected to find allele and haplotype sharing between central Alaskan and Canadian gray wolves as both populations are known to migrate significant distances based on radio-collaring of packs (Stephenson et al. 1995). Table 5 lists the equivalent allele names used in the above papers and the official DLA names where assigned. Table 5 also indicates which DLA-DRB1 alleles have been found in which subspecies, domestic dog, gray wolf, red wolf, Mexican wolf, and coyote. It is clear that as more animals from each species are DLA typed, and more new alleles are found, that the extent of allele sharing between species also increases.

The significance of decreased variation in class II DLA for the central Alaskan wolves is uncertain partly due to the small sample size but also to the random nature of sample submissions into the Alaskan Frozen Tissue Collection at the University of Alaska Museum. The high frequency of one haplotype, DLA-DRB1*03101/DQA1*01101/DQB1*01401, gray wolves from central Alaska does suggest a significant founder effect in this region. Eight of 18 wolves were homozygous for this haplotype. Dispersal of wolf packs within large geographical areas is known to occur, with significant gene flow between these wolf packs (Meier et al. 1995). Seven other haplotypes were also detected in the central Alaskan gray wolf population, and it is possible that over time the degree of homozygosity in the MHC class II may lessen through pack dispersal. However, other data suggest that climate and habitat barriers can act very effectively to isolate wolf populations (Carmichael et al. 2001; Geffen et al. 2004).

Low levels of polymorphism in the MHC have been documented in other terrestrial wild populations with no apparent adverse consequences, examples including wild ruminants (Mikko and Andersson 1995; Ellegren et al. 1996; Mikko et al. 1999), beaver (Ellegren et al. 1993), and wild rodents (Figueroa et al. 1986; Nizetic et al. 1988). Caution should be exercised when interpreting these examples, as other populations with limited MHC polymorphism have been suggested to be vulnerable to infectious diseases (O'Brien et al. 1985). The Alaskan and Canadian gray wolf populations provide an opportunity to examine another species for their DLA diversity.

Table 5. Equivalent names for DRB1 alleles in published papers and the related canids in which each has been found

Official name	Hedrick full length[b]	Hedrick partial sequences[c]	Seddon full length[d]	Dog	Gray wolf	Mexican wolf	Red wolf	Coyote
DRB1*00601		Calu-1		y	y	y		
DRB1*00901				y	y			
DRB1*01501	Cala-17			y				y
DRB1*02901				y	y			
DRB1*03202	Caru-1	Caru-1, Cala-10, Calu-11			y		y	y
DRB1*03501		Calu-5			y	y		
DRB1*03601								
DRB1*03701		Calu-7			y			
DRB1*03801		Calu-3			y	y		
DRB1*04101		Calu-9			y			
DRB1*04201		Cala-1						y
DRB1*04301		Calu-2				y		
DRB1*04401		Calu-8			y			
DRB1*04501		Cala-2			y			y
DRB1*04502	Cala-15							y
DRB1*06301	Caru-3	Caru-3					y	
DRB1*06401	Caru-4	Caru-4, Cala-12					y	
DRB1*06501	Caru-2	Caru-2, Cala-11			y		y	
DRB1*06601	Cala-14							y
DRB1*06701	Cala-18		Cala-DRB1*09					y
DRB1*09101					y			
DRB1*09301		Calu-6			y			
DRB1*09201		Calu-10			y			
a		Cala-7						y
a		Cala-3						y
a		Cala-4						y
a		Calu-4				y		
a		Cala-5						y
a		Cala-6						y
a		Cala-8						y
a	Cala-13							y
a	Cala-16							y
a			Calu-DRB1*12		y			
a			Calu-DRB1*13		y			
a			Calu-DRB1*14		y			
a			Calu-DRB1*15		y			
a			Calu-DRB1*16		y			
a			Cala-DRB1*10		y			

[a] Alleles without an official DLA number do not fulfill the nomenclature requirements. Usually they have only been found in a single animal, and there are no confirmatory sequences from other laboratories.

[b] Hedrick et al. (2002).

[c] Hedrick et al. (2000).

[d] Seddon and Ellegren (2002).

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