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Sequence diversity of the MHC DRB gene in the Eurasian beaver (*Castor fiber*)

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Abstract

Major histocompatibility complex (MHC) genes, coding molecules which play an important role in immune response, are the most polymorphic genes known in vertebrates. However, MHC polymorphism in some species is limited. MHC monomorphism at several MHC class I and II loci was previously reported for two neighbouring northern European populations of the Eurasian beaver (*Castor fiber*) and reduced selection for polymorphism has been hypothesized. Here, we analysed a partial sequence of the second exon of the MHC II DRB locus from seven relict European and Asian beaver populations. We detected 10 unique alleles among 76 beavers analysed. Only a western Siberian population was polymorphic, with four alleles detected in 10 individuals. Each of the remaining populations was fixed for a different allele. Sequences showed considerable divergence, suggesting the long persistence of allelic lineages. A significant excess of nonsynonymous substitutions was detected at the antigen binding sites, indicating that sequence evolution of beaver DRB was driven by positive selection. Current MHC monomorphism in the majority of populations may be the result of the superimposition of the recent bottleneck on pre-existing genetic structure resulting from population subdivision and differential pathogen pressure.

Keywords: bottleneck, *Castor fiber*, conservation, MHC class II, positive selection, rodents

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Introduction

The major histocompatibility complex (MHC) is a key element of the vertebrate immune system, responsible for presentation of foreign peptides to T cells (Klein 1986). MHC genes are the most polymorphic genes described in vertebrates, with polymorphism occurring predominantly at residues involved in peptide binding (Brown *et al*. 1988, 1993; Musolf *et al*. 2004). This variation presumably allows the binding of a variety of parasite-derived antigens, and there is growing evidence for an association between MHC-types and susceptibility to parasites (e.g. Hill *et al*. 1991; Kaufman & Wallny 1996; Thursz *et al*. 1997; Carrington 1999; Langefors *et al*. 2001; Froeschke & Sommer 2005; Harf & Sommer 2005). The mechanisms deemed responsible for maintaining polymorphism at MHC genes include frequency-dependent selection (Snell 1968; Borghans *et al*. 2004) and heterozygote advantage (Doherty & Zinkernagel 1975). Frequency dependence arises because the bearers of common alleles become more likely to be evaded by evolving parasites (Trachtenberg *et al*. 2003), while heterozygosity allows presentation of a wider range of pathogen-derived peptides, and thus provides greater resistance to infection (Carrington 1999; Penn *et al*. 2002). It has been suggested that species or populations with low MHC polymorphism may be particularly vulnerable to infection, and consequently may face a higher risk of extinction (O'Brien & Evermann 1988; Hughes 1991).

Whether limited MHC diversity affects population viability and survival remains controversial (Edwards & Potts 1996; Mikko *et al*. 1999; Hedrick 2003). A recent report suggests that selection may retain MHC diversity even when population bottlenecks lead to the loss of genetic variation elsewhere in the genome (Aguilar *et al*. 2004). On the other hand, limited or a lack of MHC polymorphism in the Eurasian beaver (Ellegren *et al*. 1993), moose (Mikko & Andersson 1995), musk ox, roe or fallow deer (Mikko *et al*. 1999), apparently have not hampered population expansion following recent bottlenecks. Beavers are particularly

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striking in this respect as an RFLP (restriction fragment length polymorphism) study of several MHC class I and II loci revealed complete monomorphism in Scandinavian and northern Russian populations, each fixed for a different haplotype, even though the latter (but not the former) showed some minisatellite variability (Ellegren *et al*. 1993).

Due to overhunting, the Eurasian beaver (*Castor fiber*) was eliminated throughout most of its range by the mid-19th century, but several small ($n < 200$) isolated populations survived in various parts of the former range and some of these were then used for re-introductions (Nolet & Rosell 1998; Halley & Rosell 2002). Recent studies of mitochondrial DNA (mtDNA) variation carried out on individuals derived from those relict populations (Ducroz *et al*. 2005; Durka *et al*. 2005) revealed low levels of within-population diversity, probably as a result of bottlenecks that these populations had undergone. These studies also documented an extreme population structure, with no haplotypes shared between populations, so that each of the currently recognized subspecies is characterized by unique mtDNA haplotypes. However, the differentiation in mtDNA is recent, most likely not older than the last glacial period (Durka *et al*. in press).

MHC monomorphism accompanied by fingerprint polymorphism in the Russian population led Ellegren *et al*. (1993) to suggest that beavers may experience reduced selection on MHC polymorphism. To test if the pattern of sequence variation indeed supports the hypothesis of reduced selection on MHC diversity, we investigated sequence variation at an MHC class II locus in seven indigenous populations of the Eurasian beaver throughout its range. If selection on MHC was important in the history of the beaver, and provided that at least some allelic variation has been retained, we expect divergent allelic lineages to be present in the beaver populations as commonly observed in other species (reviewed in Hedrick *et al*. 2001; Garrigan & Hedrick 2003; Musolf *et al*. 2004). Moreover, a signature of positive selection, a higher rate of nonsynonymous than synonymous substitutions, particularly at antigen-binding sites, is expected (Bernatchez & Landry 2003; Garrigan & Hedrick 2003). Screening MHC polymorphism in multiple populations over the vast Eurasiatic range of the beaver should distinguish between these alternative hypotheses.

The second exon of the DRB locus, coding the residues involved in antigen presentation, was chosen for sequence analysis, as it is the most polymorphic human class II locus (Janeway *et al*. 1999). High polymorphism was also reported in a number of other mammals (e.g. Nino-Vasquez *et al*. 2000; Ditchkoff *et al*. 2001; Richman *et al*. 2001; Kennedy *et al*. 2002; Musolf *et al*. 2004; Schaschl *et al*. 2004). Thus, due to linkage disequilibrium with other MHC II loci, DRB should provide information on the overall level of MHC II haplotype diversity.

Materials and methods

Samples

We analysed 76 individuals from seven populations (Table 1, Fig. 1), representing all currently recognized subspecies of the Eurasian beaver (Gabryś & Ważna 2003; Ducroz *et al*. 2005; Durka *et al*. 2005). Most of the animals studied were also scored for mtDNA sequence variation by Ducroz *et al*. (2005) and Durka *et al*. (2005). All these samples either represent indigenous beaver populations, or are known to be derived from indigenous populations. Thus we avoided sampling admixed populations established

Table 1 Samples assessed for sequence variation at a 171-bp fragment of MHC DRB locus. *N*, number of individuals studied; ssp, subspecies undetermined, populations derived from *Castor fiber belorussicus* and *Castor fiber orientoeuropaeus* which due to extensive translocations most likely lost their distinctness (Durka *et al*. in press). In case of the only polymorphic population, *Castor fiber pohlei*, the numbers in parentheses following allele names are numbers of individuals where a particular allele was found; the number of individuals sampled per locality is given in parentheses following a locality name. Two putative pseudogenes are not included (see Results)

No.	Subspecies	Country	*N*	Alleles	Localities
1	*C. f. galliae*	France	4	*Cafi-DRB*07*	Bracieux, Vertou, Escrignelles, Lestioux
2	*C. f. albicus*	Germany	19	*Cafi-DRB*01*	Dessau (2), Dübener Heide (1),Eisleben (1), Havelberg (1), Havelland (2), Mark Brandenburg (1), Schwarze Elster (7), Senftenberg, Tangermünde (3)
3	*C. f. fiber*	Norway	18	*Cafi-DRB*02*	Bø
4	*C. f.* ssp.	Poland	10	*Cafi-DRB*03*	Pasłęka river (5), Kętrzyn (5)
5	*C. f. pohlei*	Russia	10	*Cafi-DRB*06(8)* *Cafi-DRB*08(7)* *Cafi-DRB*09(6)* *Cafi-DRB*10(2)*	Kondiskyj Zakaznik
6	*C. f. tuvinicus*	Russia	8	*Cafi-DRB*05*	Azas River
7	*C. f. birulai*	Mongolia	7	*Cafi-DRB*04*	Bulgan-Gol

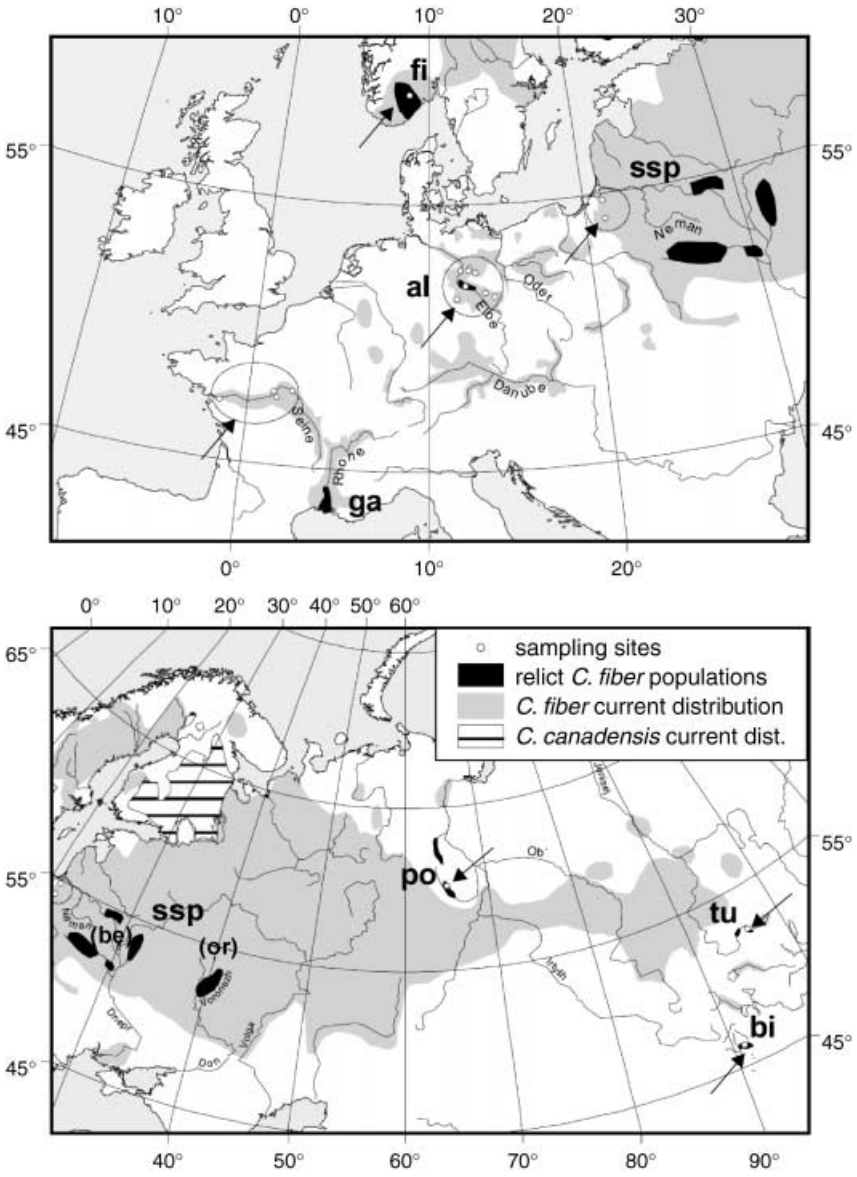

Fig. 1 The Euarsian beaver populations scored for variation at the MHC DRB gene fragment (arrows and circles). Abbreviations: al, *Castor fiber albicus*; be, *C. f. belorussicus*; bi, *C. f. birulai*; fi, *C. f. fiber*; ga, *C. f. galliae*; or, *C. f. orientoeuropaeus*; po, *C. f. pohlei*; tu, *C. f. tuvinicus*; vi, *C. f. vistulanus*; ssp, subspecies undetermined, populations derived from *C. f. belorussicus* and *C. f. orientoeuropaeus* which due to extensive translocations most likely lost their distinctness (Durka *et al*. 2005).

in some areas through the re-introduction of mixed-stock animals (e.g. Schwab & Lutschinger 2001).

DNA isolation, PCR and SSCP

Total genomic DNA was extracted following a modification of the cetyltrimethyl ammomium bromide (CTAB) method (Winnepenninckx *et al*. 1993) from small tissue fragments or hairs preserved in 70% ethanol. Primers JS1 and JS2 (Schad *et al*. 2004) were used to amplify a 171-bp (excluding primers) fragment of the second DRB exon.

We used single strand conformation polymorphism (SSCP) for initial screening of the samples, with the aim of identifying putative homo- and heterozygotes, which were further characterized at the DNA sequence level, either by direct sequencing of polymerase chain reaction (PCR) product, or, in case of individuals displaying more than one putative allele, by cloning PCR products and sequencing multiple clones.

Twenty-microlitre PCRs contained 2 μL of 10 × PCR buffer with $(NH_4)_2SO_4$ (Fermentas), 2.0 mM $MgCl_2$, 1 μM of the FAM-labelled JS1 and HEX-labelled JS2 primers, 0.2 mM of each dNTP and 0.5 U of *Taq* polymerase (Fermentas). The cycling scheme was 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 3 min.

One microlitre of 10–20 × diluted PCR product was mixed with 9.5 μL of Hi-Di formamide (ABI) and 0.5 μL of 0.3 M NaOH. The mixture was then denatured at 90 °C for

2 min and immediately placed on ice. Then, 0.3 μL of GenScan 500 ROX standard (ABI) mixed with 0.7 μL of Hi-Di formamide was added. SSCP analysis was performed using 5% GenScan polymer (ABI) with the addition of 10% glycerol in an ABI 3100 genetic analyser. TBE(1×) with 10% glycerol was used as running buffer. Electrophoresis was conducted at 25 °C, samples were injected for 22 s, injection voltage was set to 1 kV and electrophoresis voltage to 15 kV. Samples were run for 25 min. Chromatograms were analysed with GENEMAPPER 3.5 software (ABI).

Direct sequencing, cloning and allele identification

In case of homozygous individuals detected by SSCP analysis, at least four putative homozygotes of each type were directly sequenced from the PCR product. PCRs were performed as above, but using nonlabelled JS1 and JS2 primers. The PCR product was purified with Clean-Up columns (A&A Biotechnology) and sequenced on both strands using BigDye Terminator Kit 1.1 or 3.1 and primers JS1 and JS2; the sequencing reaction products were purified using DyeEx 2.0 spin columns (QIAGEN), and run on an ABI 3100 genetic analyser.

All putative heterozygotes were cloned using the pGEM T-Easy vector system (Promega). Recombinant clones were detected by blue/white screening and plasmid DNA minipreps were prepared with Plasmid Mini Kit (A&A Biotechnology). Multiple clones (9.3 per individual on average) containing inserts were sequenced from each putative heterozygote using the M13 forward primer. Sequences were checked by eye and aligned manually in BIOEDIT 7 (Hall 1999). Some individuals with putative rare alleles were also cloned from a second, independent PCR. As PCR- and cloning-generated artefacts are common in analyses of polymorphic genes, particularly in case of MHC genes (for a discussion, see Jarvi *et al*. 2004), we employed conservative criteria for allele identification. We treated sequences obtained in clones derived from at least two independent PCRs as confirmed alleles, regardless if they occurred in the same or different individuals. Other sequence variants, which might have represented polymerase errors and PCR- or cloning-generated recombinants, were excluded from the analyses. In designating allele names we followed the nomenclature rules set by Klein *et al*. (1990).

Analysis

The average pairwise nucleotide distances (Kimura 2-parameter model or K2P), Poisson-corrected amino acid distances as well as the average rates of synonymous (d_S) and nonsynonymous (d_N) substitutions per site were computed in MEGA3 (Kumar *et al*. 2004) using the Nei–Gojobori method with the Jukes–Cantor correction for multiple substitutions (Nei & Gojobori 1986); their standard errors were obtained through 1000 bootstrap replicates. The one-tailed *Z*-test, as implemented in MEGA was performed in order to test if positive selection shaped the evolution of the assayed fragment of the DRB second exon. We analysed synonymous vs. nonsynonymous substitutions at all amino acid positions, antigen binding sites (ABS) and non-ABS. The location of the putative ABS and non-ABS was inferred from the human MHC II molecule structure (Brown *et al*. 1993).

A phylogenetic tree using the neighbour-joining method was constructed from K2P nucleotide distances in MEGA in order to establish phylogenetic relationships of the beaver MHC DRB alleles. A formal model selection procedure is not presented as complex models did not perform better than this simple measure of nucleotide distance. As trans-specific polymorphism is common for MHC II genes (Hedrick 2001; Garrigan & Hedrick 2003; Musolf *et al*. 2004) we included a representative set of homologous rodent sequences in the analysis, as well as human, rhesus, cat, dog and sheep sequences (Fig. 2). Robustness of the obtained topology was assessed through 1000 bootstrap replicates.

Results

We detected 10 unique alleles among the 76 beavers analysed (GenBank Accession nos DQ060686–95). Twenty-six of 171 (15.2%) nucleotide and 15 of 57 (26.3%) amino acid positions were variable (Table 2). No insertions/deletions or stop codons were detected. The number of pairwise nucleotide differences between pairs of alleles ranged from 1 (*Cafi-DRB*06* vs. *Cafi-DRB*07*) to 15 (*Cafi-DRB*01* vs. *Cafi-DRB*05*) and the number of amino acid differences from 1 (*Cafi-DRB*06* vs. *Cafi-DRB*07*) to 13 (*Cafi-DRB*03* vs. *Cafi-DRB*08*). The average pairwise K2P distances and Poisson-corrected amino acid distances computed for all sites as well as for the ABS and non-ABS are given in Table 3. In two populations (*Castor fiber birulai* and *Castor fiber pohlei*), a divergent sequence was detected in addition to the DRB alleles (GenBank Accession no. DQ060696, 35–38% divergence relative to the beaver DRB sequences), representing most likely a pseudogene, as evidenced by a single nucleotide deletion and the presence of a stop codon in the middle part of the sequence. This putative pseudogene was detected, along with presumably functional DRB alleles in all *C. f. birulai* and two *C. f. pohlei* individuals. A second putative pseudogene sequence (GenBank Accession no. DQ060697), also characterized by a single nucleotide deletion and a stop codon was detected in two *C. f. birulai* individuals. Its nucleotide divergence from DRB alleles ranged from 28% to 35% and from the first pseudogene 40%. These putative pseudogene sequences were not included in the following analyses.

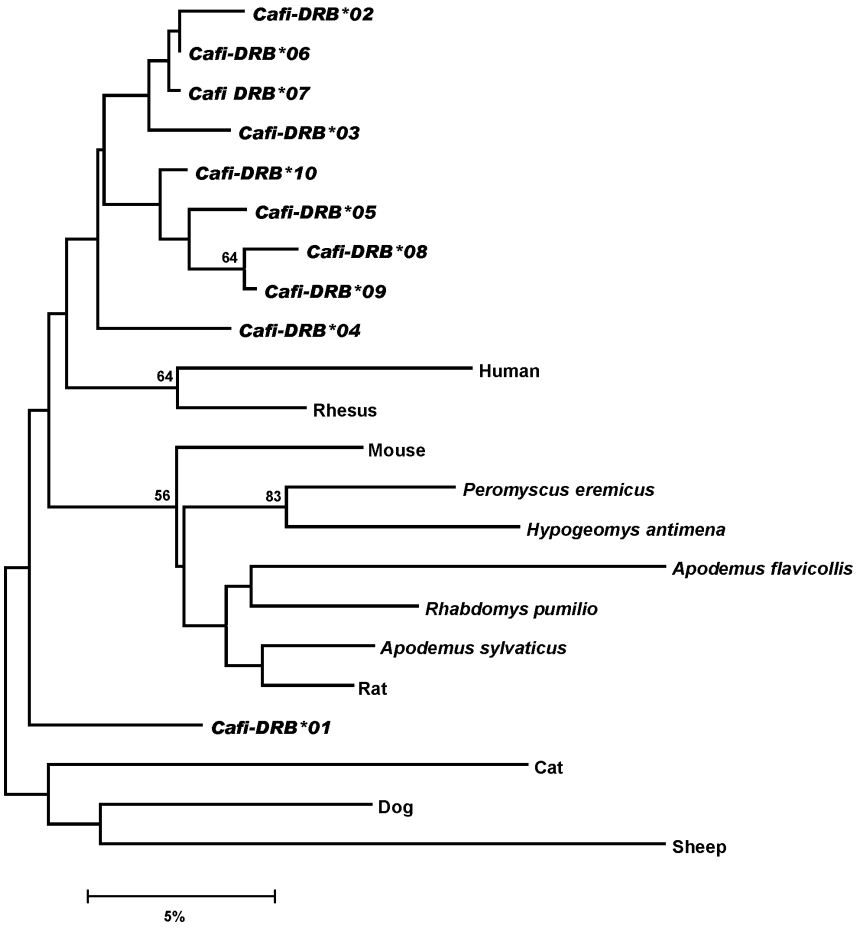

Fig. 2 A neighbour-joining phylogenetic tree constructed for a 171-bp fragment of the MHC DRB locus from a matrix of Kimura 2-parameter nucleotide distances (K2P). In addition to 10 beaver alleles (*Cafi-DRB*01–10*), the following sequences were included: *Apodemus flavicollis* (GenBank Accession no. AY699761) *Apodemus sylvaticus* (AY699728), cat (AJ428212), dog (AF016912), human (AJ293695), *Hypogeomys antimena* (AJ416075), mouse (U88920), *Peromyscus eremicus* (AY219813), rat (AJ003232), *Rhabdomys pumilio* (AY928324), rhesus (AJ534301) and sheep (AY230000). Bootstrap values ≥ 50% (1000 replicates) are shown.

Table 2 Amino acid sequences of MHC DRB alleles detected in the Eurasian beaver. Dots indicate identity to the reference sequence. Putative antigen binding sites (ABS) (Brown *et al*. 1993) are marked with asterisks

	* * * ** * * ** * * ** * *
*Cafi-DRB*01*	ERVRFLNRYV YNREEFVRFD SDVGEFRAVT ELGRPDAEYW NGQKDLLERK RAAVDTV
*Cafi-DRB*02*	....L.D..F .....Y.... ....V..DA ....
*Cafi-DRB*03*	......D..F H.G..N.... ....V..DA ......F
*Cafi-DRB*04*	......D..F .....Y.... .....Y.... ....RS..S. .....I..DA ......Y
*Cafi-DRB*05*	......E.HI .....H.... .....Y.... .....I..S. .....V..DA ......Y
*Cafi-DRB*06*	....L.D..F .....Y.... ....V..DA ......F
*Cafi_DRB*07*	....L.D..F .....H.... ....V..DA ......F
*Cafi-DRB*08*	......E.HI .....H.... .....Y.... .....I..S. ....QR ......Y
*Cafi-DRB*09*	......E.HI .....H.... .....Y.... ....QR ......Y
*Cafi-DRB*10*	......E.HI .....H.... .....Y.... ....V..DA ......F

The geographic distribution of DRB alleles showed extreme structure. No alleles were shared by any pair of populations. Each of the European populations was fixed for a unique allele (Table 1). The same is true for Asiatic *Castor fiber tuvinicus* and *C. f. birulai* (not taking into account the putative pseudogenes). The only population showing polymorphism at the DRB locus was *C. f. pohlei*, where we found a total of four alleles in 10 individuals. In four beavers from this population, three alleles per individual were detected. Generally, there was no close similarity in allele sequences between geographically proximate populations.

The phylogenetic relationships among the beaver alleles were poorly resolved. With the exception of *Cafi-DRB*01*,

Table 3 The average nucleotide and amino acid distances among the Eurasian beaver MHC DRB alleles. Standard errors (in parentheses) where obtained through 1000 bootstrap replicates. Nucleotide distance is corrected for multiple substitutions using the Kimura 2-parameter model (K2P), amino acid distance is corrected using expectations from Poisson distribution. ABS, putative antigen binding sites as determined by Brown *et al*. (1993). Distances are given as percentages per site

K2P nucleotide distance			Poisson-corrected amino acid distance		
All sites	ABS	Non-ABS	All sites	ABS	Non-ABS
6.5 (1.3)	18.4 (4.8)	2.8 (1.1)	14.2 (3.6)	43.5 (13.3)	6.0 (2.7)

Table 4 The average rates of nonsynonymous substitutions per nonsynonymous site (d_N), synonymous substitutions per synonymous sites (d_S), with standard errors obtained through 1000 bootstrap replicates in parentheses, and the results of the *Z*-test of positive selection. d_S and d_N values are given as percentages per site

Sites	d_N	d_S	*Z*	*P*
All	8.0 (2.2)	2.3 (1.2)	2.792	0.003
ABS	23.8 (7.9)	4.5 (3.0)	2.525	0.006
Non-ABS	3.3 (1.7)	1.6 (1.3)	0.894	0.187

all alleles formed one cluster. However, this grouping did not receive considerable bootstrap support (Fig. 2). In a tree constructed using only synonymous variation, all beaver alleles grouped together, although with only moderate 54% bootstrap support (tree not shown).

We detected clear signs of positive selection acting on the assayed fragment of the DRB locus in the evolutionary history of beaver. The rate of nonsynonymous substitutions was almost four times higher than the rate of synonymous substitutions ($P = 0.003$, *Z*-test of positive selection) when all nucleotide positions were considered, and over five times higher ($P = 0.006$, *Z*-test of positive selection) when only ABS were included (Table 4). We did not find a significant excess of nonsynonymous substitutions for non-ABS (Table 4).

Discussion

We have found a considerable sequence variation at MHC DRB exon 2 in relict beaver populations across the species range. However, all populations, except one, were monomorphic. Our data thus corroborate results of the RFLP-based survey by Ellegren *et al*. (1993) who found monomorphism at DRB and other MHC loci in the Scandinavian population. Nevertheless, each monomorphic population was fixed for a different allele, and the western Siberian population (*Castor fiber pohlei*) retained four alleles. No alleles were shared between populations.

Amplification of divergent alleles in various populations makes it highly unlikely that the consensus primers used in our study failed to amplify a substantial fraction of alleles or amplified different loci in individual populations. Of course we cannot exclude the possibility that certain alleles did not amplify, but this would not be a systematic phenomenon, unlikely to explain the lack of polymorphism in six of seven populations.

Three alleles per individual were found in four *C. f. pohlei* beavers, indicating that some haplotypes in this population consist of at least two DRB loci. The presence of more than one DRB locus, with haplotypes sometimes differing in the number of copies, appears to be widespread among mammals, and has been reported, e.g. in horses (Fraser & Bailey 1996), primates (Bontrop *et al*. 1999; Khazand *et al*. 1999), cattle (Lewin *et al*. 1999), cats (Kennedy *et al*. 2002), sea lions (Bowen *et al*. 2004), and hairy-footed gerbils (Harf & Sommer 2005). Because of interlocus allelic exchange known to occur at MHC loci, assigning alleles to individual loci is not possible without more detailed genomic information. Therefore, in our phylogenetic analysis we treated all alleles detected in *C. f. pohlei* as representing the DRB locus.

The allele nucleotide sequences were highly divergent with up to 12.7% K2P distance between *Cafi-DRB*1* and *Cafi-DRB*5* alleles. For comparison, the maximum divergence between mtDNA control region haplotypes was 5.9% K2P distance (Durka *et al*. 2005), showing clearly that MHC DRB variants are considerably older, especially given the much higher mutation rate in the mammal mtDNA control region compared to the nuclear genome. Our phylogenetic analysis (Fig. 2) produced a poorly resolved tree, which is not surprising, given relatively short sequence length and very high polymorphism. The lack of evidence for trans-specific polymorphism and the fact that the beaver alleles fall outside of a moderately supported rodent group may be explained by the antiquity of the beaver lineage which split from its closest living relatives 90–100 million years ago (Adkins *et al*. 2003).

Analysis of synonymous and nonsynonymous substitutions provided strong evidence for positive selection acting on the MHC DRB exon 2 in the past. The d_N/d_S ratio was significantly higher than unity, and the ratio was even higher when only putative antigen binding sites (ABS) were included. In contrast, the d_N did not significantly differ from d_S when only non-ABS sites were considered. Thus, the inference made by Ellegren *et al*. (1993) that beavers experience 'reduced selection pressure for MHC polymorphism' was not supported by our results. Instead, the monomorphism seen in all but one population could be a result of recent population bottlenecks. Low variation at MHC loci was attributed to recent bottlenecks in a number of species (e.g. Mikko & Andersson 1995; Mikko *et al*. 1999;

Miller & Lambert 2004; Seddon & Ellegren 2004). In the Australian bush rat, patterns of MHC variation, which included MHC monomorphism of some island populations, were congruent with those for mtDNA and microsatellites (Seddon & Baverstock 1999). Such congruence, occurring in spite of the historical presence of positive selection acting on MHC in this species, implies the dominant influence of genetic drift and structure of the founding population on the current pattern of genetic variation in this species (Seddon & Baverstock 1999; Hinten *et al*. 2003). Similarly, the data from mtDNA suggest that a strong population bottleneck is responsible for reduced genetic variation in the beaver. Overall variation was low, each population was either fixed for a single unique mtDNA haplotype, or showed a few closely related haplotypes. No haplotypes were shared between populations (Durka *et al*. 2005). It has been postulated, on the basis of the pattern of mtDNA sequence variation, that the recent anthropogenic bottleneck was superimposed on pre-existing genetic structure, which could result from watersheds between major Eurasian rivers acting as dispersal barriers for the beaver (Durka *et al*. 2005). The pattern of MHC DRB variation, with different alleles fixed in most populations, seems to be in line with this explanation. Differential pathogen pressure in these partially isolated populations, causing shifts in allele frequencies or even allelic composition, could contribute to structuring of prebottleneck MHC variation.

Explanations other than population bottlenecks have been also proposed to explain low MHC variation, including reduced parasite load, stable niche and solitary lifestyle (reviewed in Edwards & Potts 1996). Behavioural inbreeding avoidance mechanisms were also suggested to affect MHC variation through nonrandom mating with respect to MHC (Penn & Potts 1999). The lack of barriers against inbreeding postulated in the beaver to result from their restricted dispersal and family structure (Ellegren *et al*. 1993) could facilitate fixation of MHC alleles in bottlenecked populations.

Previously, all beaver subspecies were proposed to be designated as management units (MU) (Durka *et al*. in press). Since each subspecies exhibited distinct MHC alleles which where not shared among relict populations, their MU status is corroborated by our data.

While we have found compelling evidence for selection acting on MHC diversity in the past, it remains evident that low (if any) MHC polymorphism did not prevent the expansion of this species in Europe during the 20th century (Nolet & Rosell 1998; Halley & Rosell 2002). For example, the Scandinavian population increased from *c*. 100 individuals to more than 150 000 over the last century and comparable growth was also recorded for other populations that we found monomorphic at the MHC DRB locus (Nolet & Rosell 1998). Likewise, other species with limited MHC polymorphism, namely moose and the roe deer, expanded in Scandinavia over the past century, which led Mikko *et al*. (1999) to question the significance of MHC to species survival. How can the evidence for strong positive selection commonly found in MHC sequences (reviewed in Bernatchez & Landry 2003; Garrigan & Hedrick 2003) be reconciled with many examples of the apparent lack of effect of MHC diversity on population survival? One possibility is that there is indeed only a weak link between strong selection on MHC polymorphism and population dynamics. This would be the case if selection on MHC resulted mostly from intraspecific competition rather than from the effect of MHC on survival. Many parasites (in the wide sense) are debilitating, but not necessary lethal. Individuals with MHC variants preventing infection may have a large competitive advantage over parasitized conspecifics, even if the parasites do not have a large effect on survival in uncompetitive situations. This would cause strong selection on MHC genes without much effect on population dynamics, which in turn may be determined mostly by the carrying capacity of the environment. Bearing this in mind, caution should be exercised before dismissing the significance of MHC variation for conservation programmes. Severe infections do influence population dynamics of endangered species (e.g. Work *et al*. 2000; Hedrick *et al*. 2001; Haydon *et al*. 2002; van de Bildt *et al*. 2002; Naugle *et al*. 2004), and given the growing evidence for the association between MHC type and resistance to infections (e.g. Hill *et al*. 1991; Kaufman & Wallny 1996; Thursz *et al*. 1997; Carrington 1999; Langefors *et al*. 2001; Froeschke & Sommer 2005; Harf & Sommer 2005), there may be many situations where MHC diversity could affect population survival. Therefore, it seems reasonable to preserve MHC diversity in populations, especially if this is possible without compromising other aims of conservation programmes (Hedrick 2002, 2003). With respect to the Eurasian beaver, this could be achieved by special protection of the *C. f. pohlei*, as it is the only population that retained polymorphism at the DRB locus.

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