Immunogenetics (2008) 60:775–781
DOI 10.1007/s00251-008-0327-x

BRIEF COMMUNICATION

High variability in the MHC class II DA beta chain of the brushtail possum (*Trichosurus vulpecula*)

Olivia J. Holland · Phil E. Cowan · Dianne M. Gleeson · Larry W. Chamley

Received: 25 June 2008 / Accepted: 31 July 2008 / Published online: 29 August 2008

Abstract The diversity of class II major histocompatibility complex (MHC) loci was investigated in the brushtail possum, an important marsupial pest species in New Zealand. Immunocontraception, a form of fertility control that generates an autoimmune response, is being developed as a population control method for the possum. Because the immune response is partly under genetic control, an understanding of immunogenetics in possum will be crucial to the development of immunocontraceptive vaccines. MHC molecules are critical in the vertebrate immune response. Class II MHC molecules bind and present exogenously derived peptides to T lymphocytes and may be important in the presentation of immunocontraceptives. We used polymerase chain reaction primers designed to amplify the peptide binding region of possum class II MHC genes to isolate sequences from 49 animals. We have previously described 19 novel alleles from the *DAB* locus in the possum (Holland et al., Immunogenetics 60:449–460, 2008). Here, we report on another 11 novel alleles isolated from possum *DAB*, making this the most diverse marsupial locus described so far. This high level of diversity indicates that *DAB* is an important MHC locus in the possum and will need to be taken into account in the design of immunocontraceptive vaccines.

Keywords Brushtail possum · *Trichosurus vulpecula* · Major histocompatibility complex · MHC · Marsupial · Class II

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The common brushtail possum (*Trichosurus vulpecula*) is a marsupial indigenous to Australia. In New Zealand, the possum is a major invasive pest, causing severe damage to native ecosystems and acting as a vector of bovine tuberculosis (Clout and Ericksen 2000). Various population control methods are being developed for the possum in New Zealand, including immunocontraception (Cowan 2000). The aim of immunocontraception is to generate an autoimmune response that renders the animal infertile (Cowan 2000). Because the immune response is partly under genetic control, an understanding of immunogenetics in the possum will be critical to the development of immunocontraceptive vaccines.

Major histocompatibility complex (MHC) molecules are important in the vertebrate immune response. The eutherian MHC has been well characterised but there is comparatively little known about the MHC in marsupials. Class II MHC molecules bind and present exogenously derived peptides to T lymphocytes. Three classical class II MHC families have been identified in marsupials: *DA* (possibly equivalent

to the eutherian *DR*), *DB* and *DC* (Belov et al. 2004, 2006). Each family consists of genes that encode one or more of the α and β chains that make up the MHC molecule. The peptide binding region (PBR) of class II molecules is formed from the α 1 and β 1 domains of these chains, encoded by exon 2. We have previously described 19 novel alleles from the β chain of the *DA* locus (*DAB*) in the possum (Holland et al. 2008). Here, we report on another 11 novel alleles isolated from possum *DAB*, making this the most diverse marsupial locus described so far.

This work was approved by the Animal Ethics Committee of Landcare Research, Lincoln. All procedures involving animals were carried out in accordance with the 1987 Animal Protection (Codes of Ethical Conduct) Regulations of New Zealand. Forty-nine animals were captured from a wild population in Lewis Pass (42 22.510S,172 24.009E). Mesenteric lymph nodes were collected from the gut connective tissue of 12 of the animals (Table 1). Leukocytes were extracted from lymph nodes by washing nodes with sterile phosphate buffered saline (PBS), stabbing them with a 25-gauge needle attached to a 3-ml syringe and injecting the node with PBS until the cells spilled out. Cells were then transferred to RNAlater (Qiagen) at −20°C. RNA was extracted from cells using the RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesised with the GeneAmp RNA PCR Kit (Applied Biosystems) following the manufacturer's protocol using random hexamer primers. Samples of ear tissue were collected from the remaining 37 possums as previously described (Holland et al. 2008).

Exon 2 of possum *DAA*, *DAB*, *DBA* and *DBB* loci was amplified with the polymerase chain reaction (PCR) primers pairs DAAE2F/DAAE2R, DABE2inF/DABE2inR, DBAE2inF_2/DBAE2inR_2 and DBBE2inF/DBBE2inR, respectively. PCR conditions, visualisation, sequencing and analysis of sequences were as previously described (Holland et al. 2008).

Where there were apparent multiple alleles (due to the presence of single nucleotide polymorphisms), ambiguous sequences were amplified by PCR and then cloned into a pGEM-T Easy vector (Promega) following the manufacturer's protocol. Clones containing inserts were sequenced in both directions with the PCR primers as detailed in Holland et al. (2008). Eight to 12 clones were sequenced for each individual PCR.

Phylogenetic tree building was conducted with Bayesian and maximum likelihood analyses. The most appropriate model of molecular evolution was first determined in Modeltest 3.7 (Posada and Crandall 1998). Bayesian analysis was conducted in MrBayes 3.11 (Huelsenbeck and Ronquist 2001) for 10^7 generations. Maximum likelihood analysis was conducted in PAUP* 4.0b10 (Swofford 2002) for ten replicates.

To examine diversity in the PBR of possum class II MHC loci *DAA*, *DAB*, *DBA* and *DBB*, we determined nucleotide sequence from exon 2 in 49 animals. We identified 11 novel alleles in the *DAB* locus, which we have named *TrvuDAB*011701* to *TrvuDAB*012701*, and amplified 16 of the previously identified *DAB* alleles (Table 1; Lam et al. 2001; Holland et al. 2008). No new alleles were found in other loci. All novel *DAB* sequences were 235 nucleotides long with no insertion/deletion events (indels) in the alleles. Across the 11 novel sequences, there were 96 polymorphic sites containing 118 variants. Fifty-six of the 78 codons contained variations; ten of these were synonymous and 46 were non-synonymous at the amino acid level. When these sequences were combined with the 21 known possum *DAB* alleles (Lam et al. 2001; Holland et al. 2008), 140 variants were present in 105 polymorphic sites.

Phylogenetic analysis was conducted on all known possum *DAB* alleles and other marsupial and eutherian MHC loci alleles. Phylogenies constructed using Bayesian and maximum likelihood methods returned identical topologies; therefore, only the Bayesian tree is shown (Fig. 1). As expected, the eutherian *DRB*, *DPB* and *DQB* loci and the marsupial *DAB* and *DBB* loci formed separate clades. All possum *DAB* alleles were found together in a single clade, which also contained other marsupial *DAB* sequences.

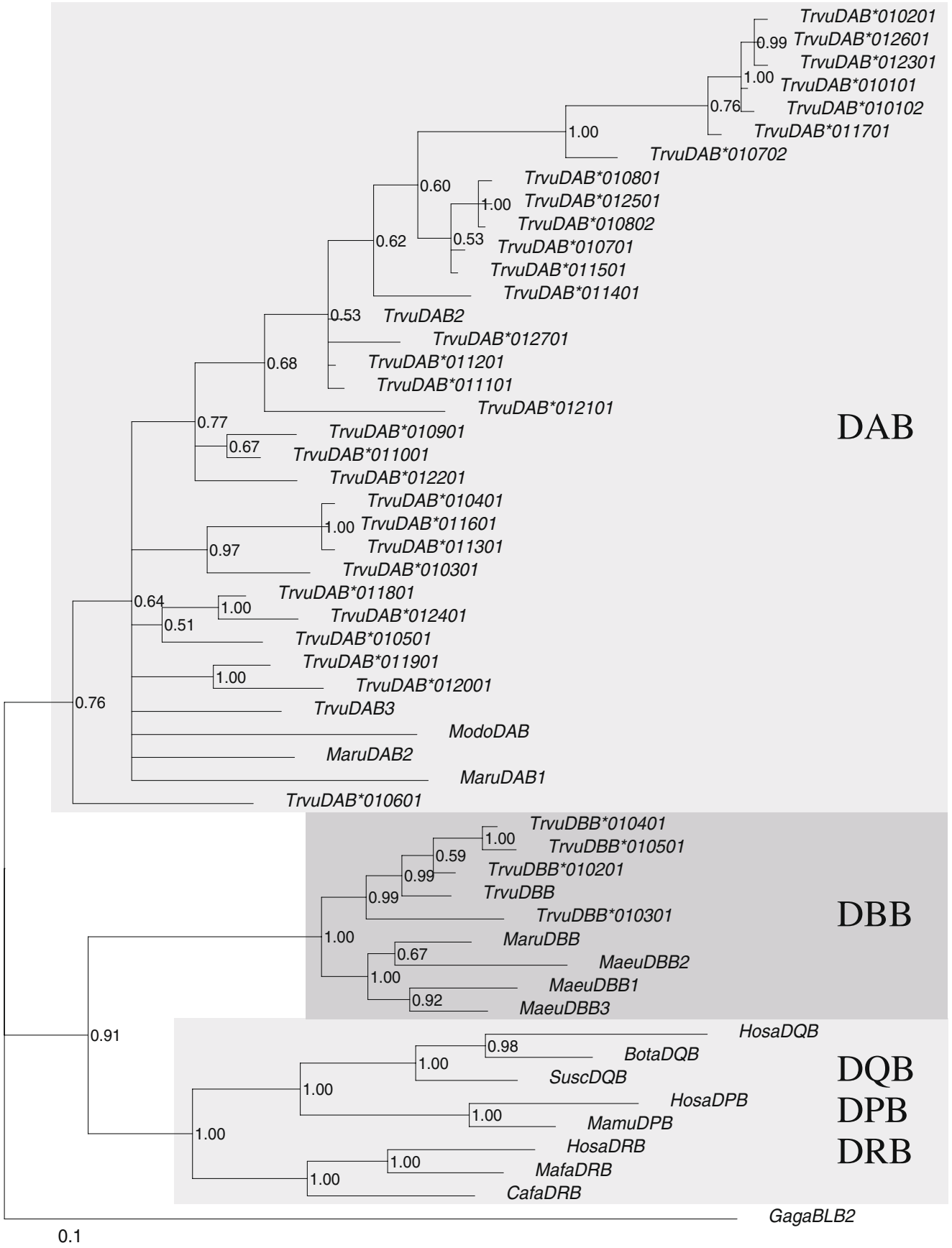
Conserved features found in the MHC class II loci of other marsupial and eutherian species were present in many of the novel possum *DAB* alleles. No structural mutations that could disrupt function, such as stop codons or frameshift mutations, were found in the novel alleles (Fig. 2). All alleles contained conserved paired cysteines (C15, C79) and a potential glycosylation site (NGT, positions 19 to 21), both features of classical class II molecules (Mazerolles et al. 1988; Brogdon et al. 1998; Belov et al. 2004). The potential CD4 interaction site at positions 39 to 42, RFDS (Mazerolles et al. 1988; Brogdon et al. 1998; Belov et al. 2004), was fully conserved in four of the new alleles. The other seven novel *DAB* alleles featured a single amino acid difference in this motif. The phenylanine at position 40 was replace with a leucine in *TrvuDAB*011701* and with a tyrosine in *TrvuDAB*012501*. The aspartic acid at position 41 was an aspargine in *TrvuDAB*011801* and *TrvuDAB*012401* and a tyrosine in *TrvuDAB*012001*. The serine at position 42 was changed to a glycine in *TrvuDAB*012301* and *TrvuDAB*012601*. Many of these changes are non-conservative; however, the marsupial CD4 has unique features (Duncan et al. 2007), and the impact of this variation on the function of these MHC molecules is unknown. It has been suggested that in eutherians, a tryptophan (W61) and an aspargine (N82) bond with the main chain of bound peptides (Brown et al. 1993). In the possum, both positions vary; position 61 can be tryptophan,

Table 1 Tissues types sampled and *DAB* alleles found in individual possums

		alleles																															
possums	sample type	*TrvuDAB3*	*TrvuDAB2*	*TrvuDAB*010101*	*TrvuDAB*010102*	*TrvuDAB*010201*	*TrvuDAB*010301*	*TrvuDAB*010401*	*TrvuDAB*010501*	*TrvuDAB*010601*	*TrvuDAB*010701*	*TrvuDAB*010702*	*TrvuDAB*010801*	*TrvuDAB*010802*	*TrvuDAB*010901*	*TrvuDAB*011001*	*TrvuDAB*011101*	*TrvuDAB*011201*	*TrvuDAB*011301*	*TrvuDAB*011401*	*TrvuDAB*011501*	*TrvuDAB*011601*	*TrvuDAB*011701*	*TrvuDAB*011801*	*TrvuDAB*011901*	*TrvuDAB*012001*	*TrvuDAB*012101*	*TrvuDAB*012201*	*TrvuDAB*012301*	*TrvuDAB*012401*	*TrvuDAB*012501*	*TrvuDAB*012601*	*TrvuDAB*012701*
2328	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2329	ear tissue	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2330	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2332	ear tissue	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2343	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2349	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2350	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2381	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
2392	ear tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
2395	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2397	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
2398	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2451	ear tissue	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2456	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2460	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2470	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
3040	ear tissue	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2401	ear tissue	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2405	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2410	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2411	ear tissue	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2413	ear tissue	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2414	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2415	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2417	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2418	ear tissue	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2423	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2439	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2440	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2443	ear tissue	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
2444	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2445	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2449	ear tissue	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
2523	ear tissue	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
2583	ear tissue																																
3276	ear tissue	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0289/2402	ear tissue	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2202	lymph nodes	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2235	lymph nodes	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
2237	lymph nodes	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
2215	lymph nodes	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
2226	lymph nodes	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0
2227	lymph nodes	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
2263	lymph nodes	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
2265	lymph nodes	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2282	lymph nodes	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
2300	lymph nodes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2234	lymph nodes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
2276	lymph nodes	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Individual 2583 was not successfully amplified for any *DAB* alleles

1 The allele was detected, *0* the allele was not detected


TrvuDAB*010201
0.99 TrvuDAB*012601
TrvuDAB*012301
1.00 TrvuDAB*010101
0.76 TrvuDAB*010102
TrvuDAB*011701
1.00 TrvuDAB*010702
0.60 TrvuDAB*010801
1.00 TrvuDAB*012501
TrvuDAB*010802
0.62 0.53 TrvuDAB*010701
TrvuDAB*011501
TrvuDAB*011401
0.53 TrvuDAB2
TrvuDAB*012701
0.68 TrvuDAB*011201
TrvuDAB*011101
TrvuDAB*012101
0.77 TrvuDAB*010901
0.67 TrvuDAB*011001
TrvuDAB*012201
TrvuDAB*010401
1.00 TrvuDAB*011601
0.97 TrvuDAB*011301
TrvuDAB*010301
TrvuDAB*011801
0.64 1.00 TrvuDAB*012401
0.51 TrvuDAB*010501
TrvuDAB*011901
1.00 TrvuDAB*012001
0.76 TrvuDAB3
ModoDAB
MaruDAB2
MaruDAB1
TrvuDAB*010601
DAB
TrvuDBB*010401
1.00 TrvuDBB*010501
0.59 TrvuDBB*010201
0.99 TrvuDBB
0.99 TrvuDBB*010301
1.00 MaruDBB
0.67 MaeuDBB2
1.00 MaeuDBB1
0.92 MaeuDBB3
DBB
0.91 HosaDQB
0.98 BotaDQB
1.00 SuscDQB
1.00 HosaDPB
1.00 MamuDPB
1.00 HosaDRB
1.00 MafaDRB
1.00 CafaDRB
DQB
DPB
DRB
GagaBLB2
0.1

◀ **Fig. 1** Phylogenetic tree of MHC class II β chain exon 2 alleles. The chicken (*GagaBLB*) is used as an outgroup. Sequences are given a label containing a species code and locus name. The *scale bar* gives the number of expected changes per site. Marginal posterior probabilities greater than 0.50 are given for each clade. NCBI accession numbers for sequences are: Marsupial: *Trichosurus vulpecula—TrvuDAB1* (AF312030), *TrvuDAB2* (AF312029), *TrvuDBB* (AY271265), *Macropus rufogriseus—MaruDAB1* (M81624), *MaruDAB2* (M81626), *MaruDBB* (M81625), *Monodelphis domestica—ModoDAB* (AF010497), *Macropus eugenii—MaeuDBB1* (AY438039), *MaeuDBB2* (AY438038), *MaeuDBB3* (AY438041); Eutherian: *Homo sapiens—HosaDRB* (NM_021983), *HosaDPB* (NM_002121), *HosaDQB* (NM_002123), *Macaca fascicularis—MafaDRB* (DQ381751), *Macaca mulatta MamuDPB* (AB219104), *Canis familiaris*: *CafaDRB* (NM_001014768), *Bos Taurus—BotaDQB* (AY911331), *Sus scrofa—SuscDQB* (AB009659) and Avian: *Gallus gallus—GagaBLB2* (EF579812)

tyrosine, leucine or glycine and position 82 can be aspargine or tryptophan. Variation at position 61 has been previously reported in marsupial species, and it is unknown what affect differences in the amino acids at these positions may have on peptide binding (Lam et al. 2001; Siddle et al. 2007).

More variations were observed in the *DAB* locus than in other possum MHC class II loci investigated. In the loci *DAA*, *DBA* and *DBB*, no novel sequences were identified (data not shown). In total, five alleles have been isolated in the possum from *DAA*, 12 from *DBA*, five from *DBB* and 32 from *DAB* (Belov et al. 2004; Holland et al. 2008). Comparing the level of polymorphism in the PBRs of the α and β chain loci shows the *DAB* locus had the highest number of substitutions (Fig. 3). In the α chain loci, *DAA* had a single polymorphic amino acid position out of the 82 available (0.012) and in *DBA* 26 of the 70 available positions (0.371) were polymorphic. In the β chain loci, 49 of the 78 available positions (0.628) were polymorphic in *DAB*, compared to 23 of the available 90 positions (0.256) in *DBB*. More than two substitutions were found in only one position of the *DBB* locus but in 18 positions of the *DAB* locus. Polymorphic positions found in possum *DAB* agree well with those identified in human *DRB* (Brown et al. 1993), suggesting a similar arrangement of polymorphic pockets occurs in the possum.

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               5    10   15   20   25   30   35   40   45   50   55   60   65   70   75   80   85   90
TrvuDAB2       EHFTEQGKSECHFVNGTETVRFVERHVYNREEFMRFDSDVGEYVALTELGRRQAEYYNSQKDYMESRRGQVDNYCRHNYEVIEPFSVRRR 90
TrvuDAB3       ....g.f.y..........h.......i...........a...f.........h..lw..r.eil.g..a...t-................ 89
TrvuDAB*010101 ----myst...y......h.q....yi...q..l....a......v.....pt..nw.rd..ll...ta..wf..vw.kla-------- 78
TrvuDAB*010102 ----myst...y......h.q....yi...q..l....a......v.....pt..nw.rd..ll...ta..wf..vw.kla-------- 78
TrvuDAB*010201 ----myst...y......h......yi...q..l...ga......v.....pt..nw.rd..ll...ta..wf..vw.kla-------- 78
TrvuDAB*010301 ----lyst...........h....d.yf.....yv........f..v.....pd...g...eil.de.ae..r........s-------- 78
TrvuDAB*010401 ----lyst...........r..ymdkyf......v.y.........v..r..pd...w...eil.r..as..tf.......f-------- 78
TrvuDAB*010501 ----..a............r..l.q.yi.....yv........f..v.....gi..nl...eil.rl.aa..t.......g.-------- 78
TrvuDAB*010601 ----..h.a..........s..y.v.fi.....ya.y..n.........r..pd...w..r.eil.rl..e..tv..n....s-------- 78
TrvuDAB*010701 ----..l............h..l.g.fi......v.y.........v......d..nw.rd...l.d...e..r..k...k.--------- 77
TrvuDAB*010702 ----..l............h..l.g.fi......v.y.........v......d..nw.rd..ll...ta..wf..vw.kla-------- 78
TrvuDAB*010801 ----..l............h..l.g.fi......v.y......f.........d..nw.rd...l.d...e..r..k...k.s-------- 78
TrvuDAB*010802 ----..l............h..l.g.fi......v.y......f.........d..nw.rd...l.d...e..r..k...k.s-------- 78
TrvuDAB*010901 ----...............r....d.yi.....yv........f..v..r...s......r...l.de.as..t.........-------- 78
TrvuDAB*011001 ----...............rl...d.yi.....yv........f..v.....ps......l...l.g..ta..t........f-------- 78
TrvuDAB*011101 ----..l....y.......................................v........nw.rd...l..k...............-------- 78
TrvuDAB*011201 ----..l....y.......................................v........nw.rd...l.d...e..r..k...k.s-------- 78
TrvuDAB*011301 ----lyst...........r..ymd.yf......v.y.........v..r..pd...w..r.eil.r..as..tf.......f-------- 78
TrvuDAB*011401 ----myst...y......h.q....yi...q..l.l..a......v......d..nw.rd...l.d...e..r..k...k.s-------- 78
TrvuDAB*011501 ----..l............h..l.g.fi......v.y.........v......d..nw.rd...l.d...e..r..k...k.s-------- 78
TrvuDAB*011601 ----lyst...........r..ymd.yf......v.y.........v..r..pd...w...eil.r..as..tf.......f-------- 78
TrvuDAB*011701 ----mhst...y......h.q....yi...q..l.l..a......v......d..nw.rd..ll...ta..wf..vw.kla-------- 78
TrvuDAB*011801 ----.lv............r..l.q.yi.....y...n.....f..a......s...g..r.eil.rl.aa..t.......ls-------- 78
TrvuDAB*011901 ----..f............r..ymq.yf.....yv........f.dvs....s.......efl.r..ta..w........s-------- 78
TrvuDAB*012001 ----..f...........gr..ymq.yf.....iv..y.....f.......gi..nl...eil.r..ta..w...s....s-------- 78
TrvuDAB*012101 ----.lv.f.............y.d.yi...q..v....a......v.....pi..nw.rd..ll.de.as..t.......lf-------- 78
TrvuDAB*012201 ----q.q.a..y..........y.d.yi.....yv...........v..r..pd.....l........aa..t........s-------- 78
TrvuDAB*012301 ----myst...y......h.q....yi...q..l...ga......v.....pt..nw.rd..ll...ta..wl..vw.kla-------- 78
TrvuDAB*012401 ----myst...y......r..l.q.yi.....y...n.....f..v......s...g..r.eil.rl.aa..t.......ls-------- 78
TrvuDAB*012501 ----..l............h....g.fi......v.y......f.........d..nw.rd...l.d...e..r..k...k.s-------- 78
TrvuDAB*012601 ----myst...y......h.q....yi...q..l...ga......v.....pt..nw.rd..ll...ta..wf..vw.kla-------- 78
TrvuDAB*012701 ----..a...................yf.....yv...........v.....pd...w.......k................-------- 78
HosaDRB        pr.l..a.c....l.....r.wnli.yi..q..ya.yn..l...q.v....pd...w.....ll.r..ae..t...y..g.v.s.t.q.. 90
                         C                                              B                 C  B
```

Fig. 2 Alignment of deduced amino acid class II *DAB* sequences of the possum and human *DRB*. Alpha helix and beta pleated sheet structures determined for *TrvuDAB*010101* are shown above the sequences with a *helix symbol* and *arrow*, respectively. Alpha helix and beta-pleated sheet structures were determined by investigating secondary structure models. A potential glycosylation site (NGT) and potential CD4 interaction site (RFDS; Mazerolles et al. 1988; Brogdon et al. 1998; Belov et al. 2004) are *boxed*. Conserved cysteine residues are indicated with a *C* and peptide binding residues (Brown et al. 1993) are indicated with a *B* below the sequence. *Dots* indicate sequence agreement with *TrvuDAB2*; *dashes* indicate gaps in the sequence introduced to optimize the alignment

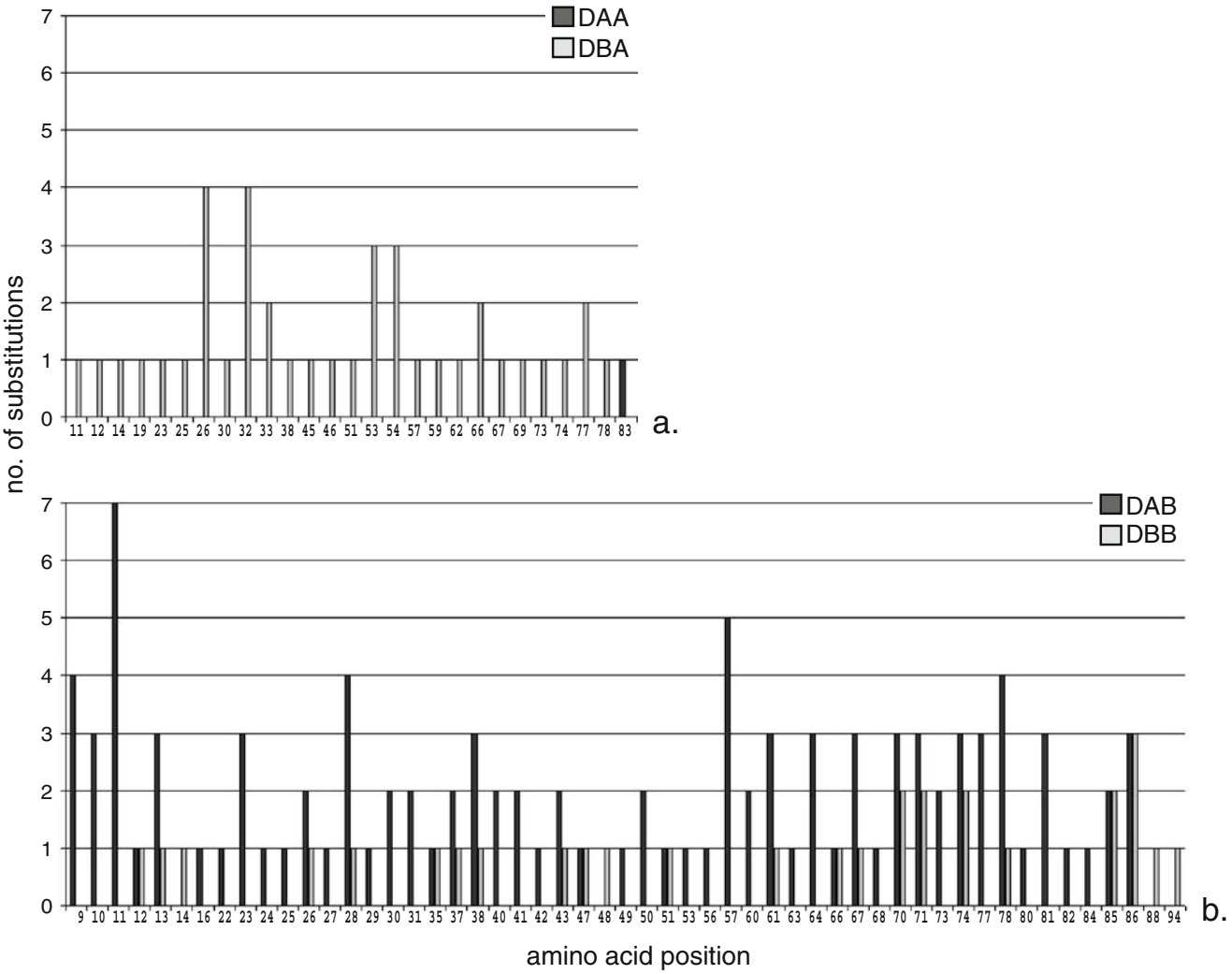

Fig. 3 Substitutions in polymorphic sites of the PBR in the class II MHC loci of the possum. **a** Alpha chain loci *DAA* and *DBA*; **b** beta chain loci *DAB* and *DBB*. Amino acid position is numbered from the beginning of the β 1 domain (Brown et al. 1993)

Up to six *DAB* alleles were found in individual possums (Table 1). Some species are known to possess multiple copies of MHC genes, an example being the nine *DRB* loci known from humans (Campbell and Trowsdale 1993; Marsh et al. 2000). The presence of greater than two alleles in an individual suggests that possums have multiple *DAB* genes. We have previously found that multiple genes are likely to be present in possum *DAB*, *DBA* and *DBB* loci (Holland et al. 2008).

We have found a large amount of diversity in possum MHC genes. The MHC is known to be highly variable (Marsh et al. 2000). As a relatively recently introduced population, possums in New Zealand might be expected to have limited genetic variation. However, at least 35 importations of possums to New Zealand were made during the 1800s (McDowall 1994). Importations were from a range of locations in eastern Australia and Tasmania and that there was much human assisted mixing of populations in the 50 years after introductions (Clout and Ericksen 2000). This large number of independent importations means considerable genetic variation may have been present at the outset of possum colonisation of New Zealand, with the variability we have identified reflecting pre-importation variation. Supporting this contention, high variability in possum microsatellite markers has also been found in New Zealand (Taylor et al. 2004), indicating wide genetic diversity in New Zealand possums.

The 11 novel possum *DAB* alleles we identified with the 21 previously isolated possum *DAB* alleles (Lam et al. 2001; Holland et al. 2008) raises the total alleles now reported from this locus to 32. Therefore, *DAB* is the most diverse marsupial locus described so far. The large number of

substitutions and high ratio on non-synonymous changes found in these alleles suggests that this locus is under positive selection for diversity. Such high levels of diversity indicate that *DAB* is an important MHC locus in the possum.

The wide diversity in the MHC of the New Zealand possum population may need to be taken into account in future work aimed at developing immunocontraceptive vaccines for use in possum population control, and means care will need to be taken to ensure any contraceptive vaccines are effective for a wide range of possum MHC haplotypes. We are currently investigating how individual differences in response to experimental immunocontraceptive vaccines (Cui and Duckworth 2005; Duckworth et al. 2007) are impacted by genetic diversity in *DAB* and other MHC loci. The nucleotide sequences reported in this article have been submitted to the GenBank nucleotide sequence database and have been assigned accession numbers ranging from EU849585 to EU849595.

Acknowledgements We thank Dr Janine Duckworth, Susie Scobie, Jane Arrow, Dr Robyn Howitt and Dr Weihong Ji for providing samples. The research was funded by the New Zealand Foundation for Research, Science, and Technology Contract C10X0501.

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