Immunogenetics (2019) 71:13–23
https://doi.org/10.1007/s00251-018-1080-4

ORIGINAL ARTICLE

Limited MHC class II gene polymorphism in the West African chimpanzee is distributed maximally by haplotype diversity

Nel Otting [1,2] · Natasja G. de Groot [1,2] · Ronald E. Bontrop [1,2,3]

Received: 26 June 2018 / Accepted: 22 August 2018 / Published online: 29 August 2018

Abstract

Chimpanzees have been used for some time as an animal model in research on immune-related diseases in humans. The major histocompatibility complex (MHC) region of the chimpanzee has also been the subject of studies in which the attention was mainly on the class I genes. Although full-length sequence information is available on the *DRB* region genes, such detailed information is lacking for the other class II genes and, if present, is based mainly on exon 2 sequences. In the present study, full-length sequencing was performed on *DQ*, *DP*, and *DRA* genes in a cohort of 67 pedigreed animals, thereby allowing a thorough analysis of the MHC class II repertoire. The results demonstrate that the number of MHC class II lineages and alleles is relatively low, whereas haplotype diversity (combination of genes/alleles on a chromosome) seems to have been maximised by crossing-over processes.

Keywords Chimpanzee · MHC · Nonhuman primates

Introduction

Chimpanzees (*Pan troglodytes*) in the wild are found across a west-east belt in equatorial Africa, where they live in various habitats including dry savannahs and rainforests. Based on geographic barriers, chimpanzees have been classified into four subspecies: the western (*P. t. verus*), the central (*P. t. troglodytes*), the eastern (*P. t. schweinfurthii*), and a group (*P. t. vellerosus or P. t. elliotti)* that is found around the river Sanaga in Cameroon (Groves 2001). Although there are no apparent morphological differences, this division is confirmed by genetic studies on mitochondrial DNA, Y-chromosomes, and microsatellites (Becquet et al. 2007; Gagneux et al. 1999; Stone et al. 2002). From an evolutionary perspective, chimpanzees are considered the closest living relatives of humans, and the two species share at least 98.7% of their non-repetitive DNA (Prado-Martinez et al. 2013). The split between chimpanzees and humans is estimated to have happened about five million years ago (de Groot et al. 2017; Gagneux et al. 1999). Like humans, chimpanzees are susceptible to infections such as HIV-1, various hepatitis viruses, and the *Plasmodium falciparum* parasite (Balla-Jhagjhoorsingh et al. 1999; Bettauer 2010; Pacheco et al. 2013; Wroblewski et al. 2015). The animals were once used as animal models in studies on human infectious diseases, but because of their cognitive similarity to humans, it is no longer considered ethical to experiment on chimpanzees (Gagneux et al. 2005).

The major histocompatibility complex (MHC) plays a pivotal role in the adaptive arm of the immunological defence system. The polymorphic genes and the encoded proteins are divided into two main classes. The MHC class I proteins are present on all nucleated cells and control intracellular infections by binding peptides and presenting them to cytotoxic T cells. MHC class II proteins are expressed on professional antigen-presenting cells belonging to the white blood cell lineage. They are also peptide-binding receptors that activate helper T lymphocytes and thus regulate responses against pathogens of extracellular origin. The orthologues of the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00251-018-1080-4) contains supplementary material, which is available to authorized users.

✉ Nel Otting
otting@bprc.nl

[1] Department of Comparative Genetics and Refinement, Biomedical Primate Research Centre, Lange Kleiweg 161, 2288 GJ Rijswijk, The Netherlands

[2] IPD-MHC database Nonhuman Primate Nomenclature Committee, Rijswijk, The Netherlands

[3] Department of Biology, Theoretical Biology and Bioinformatics, Utrecht University, Utrecht, The Netherlands

human classical polymorphic class I genes, named *HLA-A*, *-B*, and *–C*, are also present in the chimpanzee and are named *Patr-A*, *-B*, and *-C*, respectively (Anzai et al. 2003; Bontrop 2006; Lawlor et al. 1988; Maibach et al. 2017; Mayer et al. 1988; Parham and Ohta 1996). However, in comparison to humans, a reduction in families of class I alleles has been observed (de Groot et al. 2000, 2008; McAdam et al. 1995). This decline in the MHC class I repertoire, and the fact that chimpanzees are relatively resistant to AIDS (Balla-Jhagjhoorsingh et al. 1999), indicates that this great ape has experienced a selective sweep by SIV or a related retrovirus (de Groot et al. 2002; de Groot and Bontrop 2013).

The MHC class II proteins are heterodimers of alpha and beta polypeptide chains, which are encoded by two distinct genes, *A* and *B*. In humans, these genes are *HLA-DRA/-DRB*, *HLA-DQA/–DQB*, and *HLA-DPA/-DPB* pairs. The orthologues are present in the chimpanzees and are named *Patr-DRA*, *-DRB*, *-DQA*, *-DQB*, *-DPA*, and *DPB*. The *DRB* gene is duplicated, and two to four genes may be present on the human chromosome (Bohme et al. 1985). The human *DRB* haplotypes are grouped into five region configurations, based on the number and combination of different *DRB* genes (Doxiadis et al. 2000). The chimpanzees from the former pedigreed colony at the Biomedical Primate Research Centre (BPRC) were investigated for the *Patr-DRB* region by sequencing and microsatellite analyses (de Groot et al. 2009; Doxiadis et al. 2012). In this panel, 29 *DRB* haplotypes were observed that group into nine region configurations. The number of *Patr-DRB* genes per region configuration varies from two to five. At present, little is known about polymorphism of the other MHC class II genes in the chimpanzee. The sequences that are archived in the nonhuman primate part of the IPD-MHC database comprise only exon 2-related data (de Groot et al. 2008; Gyllensten et al. 1996; Otting et al. 1998). In the present study, we aimed at extending the 29 *DRB* haplotypes with full-length alleles of *Patr-DRA*, *-DQA*, *-DQB*, *-DPA*, and *-DPB* genes, thus providing a complete MHC class II haplo-map for this cohort of chimpanzees.

Materials and methods

Sampling

The BPRC pedigreed chimpanzee colony was started with 36 founder animals, most of which were wild-born, and were captured in Sierra Leone in the late 1970s. The colony extended to roughly 200 animals over three generations, up until they were dispersed to European zoos and sanctuaries. The colony was pedigreed based on the segregation of polymorphic MHC markers, defined by several methodologies (Bontrop et al. 1995; Slierendregt et al. 1993). Although they are no longer housed at BPRC facilities, lymphoblastoid B cell lines, PBMCs, and DNA samples from most of the animals are still available. For investigations of the class II genes, the B cells or PBMCs of 67 animals were selected in a way such that all formerly defined *DRB* haplotypes were represented (de Groot et al. 2009) and that each animal had a parent or offspring in the panel.

The majority of the founder animals belonged to the subspecies *P. t. verus*, though four female founders of the subspecies *P. t. troglodytes* and their crossbreed offspring were present in the colony. Two naturally SIV-infected *P. t. schweinfurthii* chimpanzees had been given shelter at the BPRC, but they were never allowed to produce offspring. The composition of the cohort in the study was 18 *P. t. verus* founders, 36 *P. t. verus* offspring, two *P. t. troglodytes* founders, nine *P. t. verus/troglodytes* hybrids, and two *P. t. schweinfurthii* animals.

RNA isolation and amplification

RNA was isolated from 10 to 20 million thawed B cells or PBMCs using the RNeasy kit (Qiagen, Valencia, Ca, USA). First-strand cDNA syntheses were performed on the RNA samples with the RevertAid-kit cDNA synthesis kit as recommended by the supplier (Thermo Fisher Scientific, Waltham, MA, USA). For the amplification reactions, some primers were copied from earlier studies on macaques (O'Connor et al. 2007), and some primers were self-designed (Table 1). The PCRs were performed using Phusion Hot Start II DNA polymerase in 50 ul mixtures as suggested by the supplier (Thermo Fisher Scientific).

The amplification conditions consisted of an initial step of 30 s at 98 °C, was followed by 25 cycles of 98 °C for 10 s, 55 °C for 15 s, and 72 °C for 30 s, and the last extension step was extended to 1 min. The PCR products were run on a 1% agarose gel, excised from this gel, and purified using the GeneJet gel extraction kit (Thermo Fisher Scientific).

Sequencing and analyses

Direct sequencing reactions on the PCR products were performed in the forward and reverse directions, using the BigDye terminator cycle sequencing kit, and the samples were run on a Genetic Analyzer 3500 capillary system (Thermo Fisher Scientific). The forward and reverse PCR primers, listed in Table 1, were also used as sequencing primers for the respective PCR products. The resulting peak patterns were analysed with MacVector™ software, version 15.5.0 (Cambridge, UK).

New alleles, based on at least two independent PCR reactions, were submitted to the ENA database (www.ebi.ac.uk/ena) and to the nonhuman primate section of the IPD-MHC database for official designations (de Groot et al. 2012; Maccari et al. 2017).

Table 1 Primers used for the amplification of MHC class II full-length sequences. Some primers have been copied from an earlier study, and others are self-designed. Y = C/T and R = A/G

Primer	Sequence	Designer
DPA-F1	5′-CTCATCACTGTTCCTGTGCTC	Otting
DPA-R1	5′-TCCTAAGTCCTCTTCTGTTCAG	O'Connor et al. 2007
DPA-R2	5′-CTGTTCAGATATTTTGTCACC	Otting
DPB-F2	5′-GCAGCTCTTTTCATTTTGCC ACCC	Otting
DPB-R2	5′-GTCCTGGAACCAGGTGCTAACG	O'Connor et al. 2007
DPB-R3	5′-GCTTGTTCTAAGGCACATTAGTC	Otting
DQA-F1	5′-GAGGCTGCCTTGGGAAGA	Otting
DQA-F2	5′-CTGAGGCTGCCTTGGGAAGAA	O'Connor et al. 2007
DQA-F3	5′-CTGCTRAGGCTGCCTGGGGAAG	Otting
DQA-R1	5′-ACCTTCCCTTCCAGGATGGG	O'Connor et al. 2007
DQA-R2	5′-TTAGGTAGCTGGGTGGCTTACT	O'Connor et al. 2007
DQB-F2	5′-CCACTACTTTTCCCTTCGTCT	O'Connor et al. 2007
DQB-R2	5′-GGCAGGGACAAGTAGGCATT	O'Connor et al. 2007
DRA-F2	5′-CYGAGCTCTACYGACTCCCAA	Otting
DRA-R2	5′-TGGGGTGGCTATAGGGCTGG	O'Connor et al. 2007

Results

MHC class II allele discovery and nomenclature

Direct sequencing of the MHC class II PCR products on the Genetic Analyser 3500 led to peak patterns that display double peaks at particular positions because of heterozygosis. In cases where only one allele was present, a second PCR with another primer combination was performed, to minimise the chance that a second allele had been missed. This resulted often in samples with two products. First the 'homozygous' samples were analysed, followed by a comparison of peak patterns of related animals that shared one allele. With this strategy, it was possible to determine unambiguously the full-length alleles in the panel of animals. As a result, the application of an extra cloning step was not necessary. A total of 48 full-length class II alleles were detected for the five different MHC class II genes in the chimpanzee panel, 30 of which were extensions of published exon 2 sequences (Table 2).

The Patr-DRA gene

Five *Patr-DRA* alleles encoding three proteins were detected, and two alleles were extensions of the exon 2 sequences, *Patr-DRA*01:01* and *01:02*, that had already been listed in the IPD database. Since the deduced proteins encoded by these alleles are identical, the names—in accordance with the nomenclature protocol—were adjusted by two extra digits to become *Patr-DRA*01:01:01* and *01:01:02*. To avoid confusion, the former **01:02* designation was skipped, and the new sequences received the allele numbers *Patr-DRA*01:03* and **01:04*. The *DRA*01:03:02* allele was found in three animals of the *P. t. troglodytes* descent, whereas the *DRA*01:04* appeared to be specific for *P. t. schweinfurthii* (Table 2). It is noted that the *Patr-DRA* gene shows no polymorphism in the exon 2 region that encodes the part of the molecule that forms the scaffolding of the antigen-binding alfa-1 domain. This suggests that synonymous point mutations accumulated over time but that purifying selection was operative on the main functional elements.

Patr-DQA and -DQB alleles

For the *Patr-DQA* gene, nine full-length alleles were observed, encoding seven different proteins (Fig. 1). Seven alleles are extensions of known exon 2 sequences, whereas *DQA1*20:04:01* and **20:04:02* had not previously been documented. Again, the data suggest regional differences: the *DQA1*05:01* allele appears to be specific for the *P. t. troglodytes* subspecies, whereas *DQA1*20:03* was only observed in a *P. t. schweinfurthii* animal.

The nine *DQA* alleles in the panel cluster into three lineages, of which *DQA1*01* and **05* are shared with humans, as represented in the bar charts covering four primate species (Fig. 2). Members of the *DQA1*01* and **05* lineages are also present in the olive baboon (*Papio anubis*) that lives in equatorial Africa, and in various macaque species that have their habitat all over Asia (Otting et al. 2016, 2017). As a representative sample for the macaque species, the data of the long-tailed macaque (*Macaca fascicularis*) is included in Fig. 2. The widespread presence of these two lineages indicates that they must be very old and predate the separation of *Hominoidea* and *Cercopithecoidea*. Thus far, the *DQA1*20* lineage, as defined by the distinct clustering in phylogenetic analyses on full-length sequences, has only been observed in the chimpanzee. The **20* lineage groups in the vicinity of the *HLA-DQA1*02* and *03* branches, which suggests a common ancestry of these lineages. It would be interesting to explore the class II DQ region of other great apes on the presence of the *DQA1*20* lineage.

Eleven full-length *Patr-DQB* alleles representing nine allotypes were found (Fig. 1), eight of which were extensions of known exon 2 sequences. Three *DQB* alleles were specific for the *P. t. troglodytes* animals and their offspring, and two alleles were detected only in *P. t. schweinfurthii* (Table 2). The chimpanzees share the *DQB1*03* and **06* lineages with humans, and **06* is also present in Old World monkeys (Fig. 2). Furthermore, the **15* lineage is shared with macaques and baboons. Notable is the large number of *HLA-DQB1* alleles in comparison to *HLA-DQA1*, whereas in the investigated nonhuman primates, these numbers are comparable. In Fig. 2, the actual number of *HLA-DQB* alleles is tenfold higher. Nevertheless, it is not expected that additional lineages

Table 2 New chimpanzee MHC class II alleles detected in this study. For each allele, the official designation, a reference animal, and the accession number are provided. Some alleles are extensions of exon 2 sequences, listed under a previous name in the IPD-MHC database. The numbers in the last three columns indicate the frequencies of the alleles in the cohort. ve = *P. t. verus.* tr = *P. t. troglodytes.* sc = *P. t. schweinfurthii*

Allele	Previous name	Remarks	Animal	Accession	ve	tr	sc
*Patr-DRA*01:01:01*	*Patr-DRA*0101*	Extension	Diana	LT899688	16	1	
*Patr-DRA*01:01:02*	*Patr-DRA*0102*	Extension	Liesbeth	LT899689	39		
*Patr-DRA*01:03:01*		New	Cor	LT899691	61	7	2
*Patr-DRA*01:03:02*		New	Sanne	LT899690		3	
*Patr-DRA*01:04*		New	Noah	LT908062			1
*Patr-DQA1*01:01*		Extension	Karin	LT622879	28	2	
*Patr-DQA1*05:01*		Extension	Victoria	LT622880		4	
*Patr-DQA1*05:02:01*		Extension	Sherry	LT622881	7		
*Patr-DQA1*05:02:02*		Extension	Dylan	LT622882	10	4	1
*Patr-DQA1*05:03*		Extension	Yoko	LT622883	6		
*Patr-DQA1*20:01*		Extension	Marco	LT899692	6	1	
*Patr-DQA1*20:03*		Extension	Noah	LT963443			1
*Patr-DQA1*20:04:01*		New	Louise	LT622884	21		
*Patr-DQA1*20:04:02*		New	Caro	LT622885	38		
*Patr-DQB1*03:02:01*		Extension	Dylan	LT607052	22		
*Patr-DQB1*03:02:02*		New	Cor	LT607051	59	1	
*Patr-DQB1*03:03:01*		Extension	Brigitte	LT607053		3	
*Patr-DQB1*03:03:02*		New	Victoria	LT607054		4	
*Patr-DQB1*03:04*		Extension	Noah	LT908064			1
*Patr-DQB1*03:05*		Extension	Lady	LT607055	5		2
*Patr-DQB1*03:06*		New	Riet	LT908065		1	
*Patr-DQB1*06:01*		Extension	Pearl	LT607056	4		
*Patr-DQB1*06:02*		Extension	Hilko	LT607057	24	2	
*Patr-DQB1*15:01*		Extension	Ingrid	LT899694	2		
*Patr-DQB1*15:03*		Extension	Nico	LT908063			2
*Patr-DPA1*01:01*	*Patr-DPA1*0201*	Extension	Sherry	LT607049	63	1	1
*Patr-DPA1*01:02*		New	Ruben	LT607047	10		
*Patr-DPA1*01:03*		New	Dirk	LT622895	3		
*Patr-DPA1*01:04*		New	Lady	LT607046	8		
*Patr-DPA1*01:05*	*Patr-DPA1*0301*	Extension	Edith	LT607044	25		1
*Patr-DPA1*01:06*		New	Hilko	LT607045	6		
*Patr-DPA1*01:07*		New	Victoria	LT607050		1	
*Patr-DPA1*01:08*		New	Sanne	LT607048		4	
*Patr-DPA1*01:09*		New	Brigitte	LT622894		2	
*Patr-DPA1*01:10*		New	Bart	LT904766		3	
*Patr-DPB1*01:07*	*Patr-DPB1*11*	Extension	Lady	LT622891	8		
*Patr-DPB1*01:09*	*Patr-DPB1*13*	Extension	Karin	LT622890	30		
*Patr-DPB1*01:11*	*Patr-DPB1*16*	Extension	Sherry	LT622887	23		
*Patr-DPB1*01:12*	*Patr-DPB1*17*	Extension	Liesbeth	LT622892	30		
*Patr-DPB1*01:13:01*	*Patr-DPB1*22*	Extension	Louise	LT622889	22		
*Patr-DPB1*01:13:02*		New	Nico	LT908060			1
*Patr-DPB1*01:16*	*Patr-DPB1*30*	Extension	Victoria	LT622886		4	
*Patr-DPB1*01:17*	*Patr-DPB1*31*	Extension	Brigitte	LT622888		1	
*Patr-DPB1*01:18*	*Patr-DPB1*32*	Extension	Debbie	LT622893	1		
*Patr-DPB1*01:19*		New	Noah	LT908061			1
*Patr-DPB1*02:01*	*Patr-DPB1*19*	Extension	Bart	LT904984		3	
*Patr-DPB1*02:02*	*Patr-DPB1*20*	Extension	Brigitte	LT904985		2	
*Patr-DPB1*03:04*	*Patr-DPB1*04*	Extension	Renee	LT904983	1		

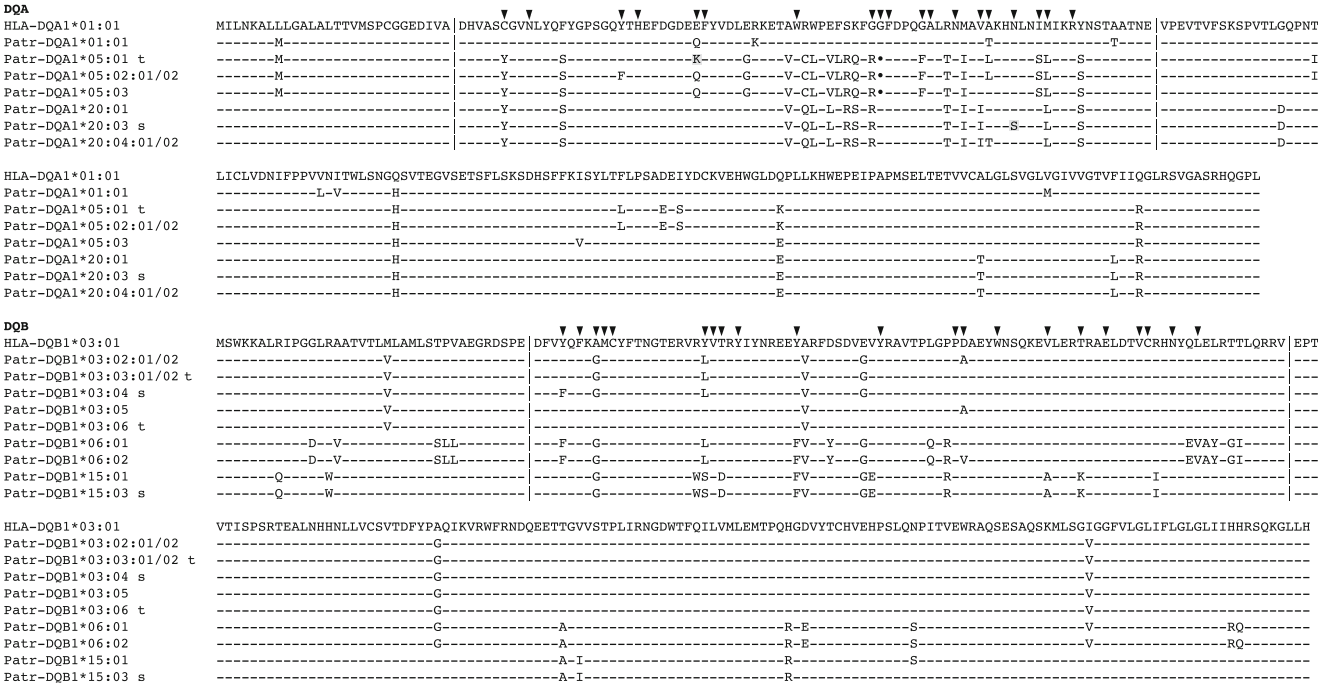


Fig. 1 The deduced protein sequences of the chimpanzee *DQA* and *DQB* alleles found in this study. For comparison, HLA consensus sequences are included. The alpha-1 and beta-1 domains of the proteins, encoded by exon 2, are *between* the vertical lines. The triangles above the HLA sequences indicate residues that contribute to the binding properties and the specificity of the class II molecule (Reche and Reinherz 2003). The shaded amino acids lysine (K) and serine (S) are observed only in *P. t. troglodytes* and *P. t. schweinfurthii*, respectively. The amino acid deletion in the *DQA1*05* alleles is represented by dots. t, the allele is found only in *P. t. troglodytes*; s, the allele is found only in *P. t. schweinfurthii*

next to *Patr*03*, **06*, and **15* will be found for the chimpanzees when more animals are investigated.

Patr-DPA and -DPB alleles

Ten full-length *Patr-DPA* alleles were detected, and although the variation is low, most differences are non-synonymous, and each allele encodes a unique protein (Fig. 3). Four of these new alleles were detected in *P. t. troglodytes* animals and their offspring. For the *Patr-DPB* gene, 13 full-length alleles were detected, 11 of which confirmed exon 2 sequences in the databases. In the1990s, a number of these exon 2 sequences were detected in the present cohort of chimpanzees, by means of first-generation sequencing on acrylamide gels. Full-length sequencing of the same individuals revealed the flaws inherent in the old technique, and the reading/typing errors of seven false alleles were rectified and updated in the databases (Suppl. Table 1). Four *DPB* alleles were present exclusively in *P. t. troglodytes* and their progeny, whereas two other alleles were detected only in the *P. t. schweinfurthii* chimpanzees (Table 2). In humans, the allelic variation of the *DPB* gene was generated by point mutations, but the exchange of small sequence motifs by recombination played a more prominent role (Doxiadis et al. 2001; Gyllensten et al. 1996). The rapid evolution has hampered the division of *HLA-DPB* alleles into distinct lineages, and as a consequence, each allele has received its own lineage number, up to 702 at the moment. This is in contrast to the situation in Old World monkeys, in which the *DPB* alleles show a clear division into lineages (Otting et al. 2016, 2017). Thus far, for the *Patr-DPB* exon 2 alleles that were archived in the databases, the same nomenclature rule was used as for *HLA-DPB* alleles, and each allele received its own lineage number. However, phylogenetic analyses revealed that the *Patr-DPB* alleles cluster into three groups (data not shown). These observations have led to a renaming of the *Patr-DPB* alleles, based on three lineages (Suppl. Table 1).

The Patr-DQ and -DP tandems

The parent/offspring combinations in the panel allowed us to define the combinations of alleles—also named haplotypes—that segregate per chromosome. First, the pairing of the *A* and *B* alleles for the *DQ* tandems was investigated (Fig. 4). Both the *Patr-DQA* and *-DQB* alleles are grouped into three lineages, thus indicating that theoretically nine different lineage combinations may be present. However, in this panel of chimpanzees, only four separate *DQ* lineage pairs were observed. The alleles of the *DQA1*01* lineage combine exclusively with *DQB1*06* alleles, a combination that is also observed in humans,

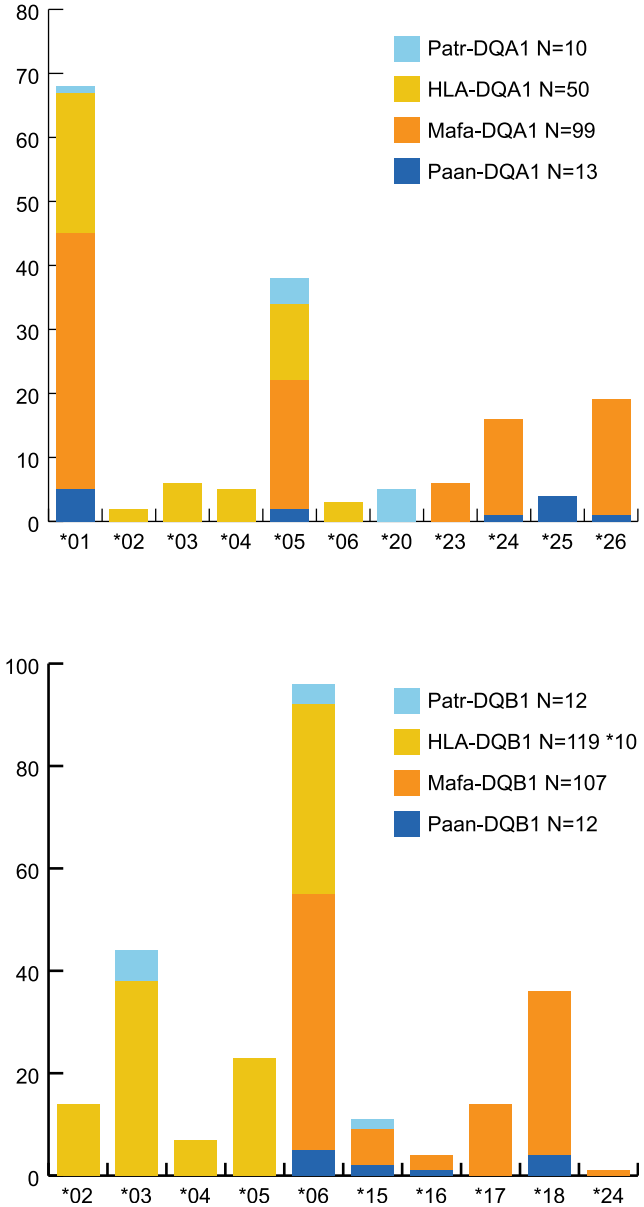


Fig. 2 The lineage distribution of *DQ* alleles in chimpanzees (Patr), humans (HLA), long-tailed macaques (Mafa), and olive baboons (Paan). On the *X*-axis, the lineage numbers are given, whereas the *Y*-axis represents the number of alleles (*N*) present in the IPD-MHC database. To adjust to the magnitudes of the other species in the figure, 10 divide the actual number of *HLA-DQB* alleles

macaques, and olive baboons (Fig. 4) (Otting et al. 2016, 2017). The *DQA1*01/DQB1*06* pairing predates the separation of hominoids and Old World monkeys and confirms the trans-species model of evolution (Doxiadis et al. 2001; Klein 1987). The other combinations are less strict: the *DQA1*20* lineage pairs only with *DQB1*03* alleles, though the latter also combines with the *DQA1*05* lineage. In particular, many lineage combinations are observed in humans. It should be mentioned, however, that the HLA data in Fig. 4 are based on hundreds of human MHC haplotypes gathered from 217 populations around the world (http://allelefrequencies.net/).

Variation among the *Patr-DPA* alleles is low, and alleles cluster into one lineage. Most *DPA* alleles combine with only one *DPB* allele, though three of them, *DPA1*01:01*, **01:02*, and **01:05*, pair with more than one *DPB* allele. This indicates that intra-region recombination may promote the birth of new pairing combinations.

Extending chimpanzee haplotypes with DR data

Based on kinship in the chimpanzee panel, the *DQ* and *DP* pairs were merged with the *DRA* alleles. Subsequently, these *DRA/DQ/DP* sets were combined with the *DRB* results from an earlier study conducted in our laboratory (Fig. 5) (de Groot et al. 2009). Based on their gene content, the *Patr-DRB* region configurations in this study are indicated by Roman numerals I to IX and are listed in Fig. 5 by the allele-names of the *DRB1* locus. Whenever polymorphism was observed within one of these region configurations, a lower-case letter was introduced to mark the further differentiation. For example, the haplotypes from Ia up to Id have the same *DRB* gene content or region configuration, but show allelic variation in their *DRB3*, *DRB5*, or *DRB6* genes.

For all *DRB* haplotypes in the *P. t. verus* and *P. t. troglodytes* groups, the complete MHC class II haplotypes could be defined. One exception is haplotype Ih, for which it was not possible to determine the exact *DP* combination. For a number of *DRB* haplotypes, more than one *DRA/DQ/DP* set was observed. For example, three and two sets that differ only for their *DP* alleles were found, respectively, for the Ia and Ic haplotypes. The IIa, VII, IVa, and IVb *DRB*-haplotypes also show variation among the *DP* genes. This is not surprising, since the *DP* region is more remote from the *DR* region on the chromosome in comparison to the *DQ* region. However, differences in the *DQA* alleles are also seen, for example, in the *DRB*-haplotypes If and Ig. Even the *DRA* sequences are not fixed within one *DRB* type, as is evident in haplotypes Ib and Ig. The variation, however, is based on only one nucleotide. A unique *DRA/DQ/DP* combination was found that is listed in Fig. 5 as 'orphan combination'. The corresponding *DRB* type for this combination is unknown. The two *P. t. schweinfurthii* animals, Niko and Noah, had no relatives in the colony, and the *DRA/DQ/DP* combinations could not be assigned unambiguously to the *DRB* types (Fig. 5). The animal Noah appeared to be homozygous for the *DRB* region; however, two different *DRA* alleles are observed in this animal. Heterozygosity in the *DQ* region was also observed in this animal.

In summary, based on the rearrangements among the *DQ* and *DP* alleles, almost all *DRA/DQ/DP* combinations listed in Fig. 5 are unique. Only six identical sets are

```
Patr-DPA
HLA-DPA1*01:03          MRPEDRMFHIRAVILRALSLAFLLSLRGAGAIKA|DHVSTYAAFVQTHRPTGEFMFEFDEDEMFYVDLDKKETVWHLEEFGQAFSFEAQGGLANIAILNNNLNTLIQRSNHTQATNDPPEVTVFPKEPVEL
Patr-DPA1*01:01          --------------F-------------------|-------M-------------------Q-----------------R--------------------I----------A-|--------------
Patr-DPA1*01:02          --------------F-------------------E|-------M-------------------Q-----------------R--------------------I----------A-|--------------
Patr-DPA1*01:03          -H------------F-------------------E|-------M-------------------Q-----------------R--------------------I----------A-|--------------
Patr-DPA1*01:04          ------I-------F-------------------|-------M-------------------Q-----------------R--------------------I----------A-|--------------
Patr-DPA1*01:05          --------------F-------------------|-------M-----------------------------------------R--------------------I----------A-|--------------
Patr-DPA1*01:06          --------------F-------------------|-------T-----------------------------------------R--------------------I----------A-|--------------
Patr-DPA1*01:07 t        ------I-------F-------------------|-------M-----------------------------------------R--------------------I----------A-|--------------
Patr-DPA1*01:08 t        ------I----V--F-------------------|-------M-----------------------------------------R--------------------I----------A-|--------------
Patr-DPA1*01:09 t        --------------F-------------------|--------------------------------------------------R--------------------I----------A-|--------------
Patr-DPA1*01:10 t        --------------F-------------------|--------------------------------------------------R--------------------I----------A-|--------------

HLA-DPA1*01:03          GQPNTLICHIDKFFPPVLNVTWLCNGELVTEGVAESLFLPRTDYSFHKFHYLTFVPSAEDFYDCRVEHWGLDQPLLKHWEAQEPIQMPETTETVLCALGLVLGLVGIIVGTVLIIKSLRSGRDPRAQGTL
Patr-DPA1*01:01          ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:02          ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:03          ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:04          ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:05          ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:06          ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:07 t        ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:08 t        ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-
Patr-DPA1*01:09 t        ----------------------------P-----------------------------------K-----------------------R--------------------I------------Q---P-
Patr-DPA1*01:10 t        ----------------------------P-----------------------------------K-----------------------R--------------------I----------------P-

Patr-DPB
HLA-DPB1*01:01          MMVLQVSAAPRTVALTALLMVLLTSVVQGRATPE|NYVYQGRQECYAFNGTQRFLERYIYNREEYARFDSDVGEFRAVTELGRPAAEYWNSQKDILEEKRAVPDRVCRHNYELDEAVTLQRRV|QPKVNVSP
Patr-DPB1*01:07          ---------S-----------------------|----------------------------------------------D---------L---------GM-----------------|--------
Patr-DPB1*01:09          ---------S-----------------------|-----------------Y----------------------------------------L---------M-------N---------|--------
Patr-DPB1*01:11          ---------S-----------------------|----------------------------------------------------------L---------I-----------------|--------
Patr-DPB1*01:12          ---------S-----------------------|------V-----------------------------------------------------L---E-----GM-------N---------|--------
Patr-DPB1*01:13:01/02    ---------S-----------------------|------V-----------------------------------------------------L---------I-----------------|--------
Patr-DPB1*01:16 t        ---------S-----------------------|---------------Y----------------------------------------L---------I-------N---------|--------
Patr-DPB1*01:17 t        ---------S-----------------------|-----V-----------------------N-----------------D---------L---------I-----------------|--------
Patr-DPB1*01:18          ---------S-----------------------|-----V-----------------------------------------------L---------I-----------------|--------
Patr-DPB1*01:19 s        ---------S-----------------------|-----V-----------Y-----------------------------D---------L---------M-----------------|--------
Patr-DPB1*02:01 t        ----------------------------------|--LF-----------------------FV-----MR----------D---------L---------M-------VG-M------|--R-----
Patr-DPB1*02:02 t        ---------S-----------------------|--LF-----------------------F-------R----------D---------L---------M-------VG-M------|--R-----
Patr-DPB1*03:04          ---------S-----------------------|--LF-V-----------------------FV-----------------------L---E------M-----------------|--R-----

HLA-DPB1*01:01          SKKGPLQHHNLLVCHVTDFYPGSIQVRWFLNGQEETAGVVSTNLIRNGDWTFQILVMLEMTPQQGDVYICQVEHTSLDSPVTVEWKAQSDSAQSKTLTGAGGFVLGLIICGVGIFMHRRSKKVQRGSA
Patr-DPB1*01:07          ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:09          ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:11          ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:12          ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:13:01/2     ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:16 t        ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:17 t        ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*01:18          ----------------------------------------------------------------------------------------------------R--------------------L----------
Patr-DPB1*01:19 s        ----------------------------------------------------------------------------------------------------R-----------------------------
Patr-DPB1*02:01 t        ---------------------------------R----------------------------------T-----------------------R-----------------------------
Patr-DPB1*02:02 t        ---------------------------------R----------------------------------T-----------------------R-----------------------------
Patr-DPB1*03:04          ---------------------------------R----------------------------------T-----------------------R-----------------------------
```

Fig. 3 The deduced protein sequences of the chimpanzee *DPA* and *DPB* alleles found in this study. For comparison, HLA consensus sequences are included. The alpha-1 and beta-1 domains of the proteins, encoded by exon 2, are *between* the vertical lines. The triangles above the HLA sequences indicate residues that contribute to the binding properties and the specificity of the class II molecule (Reche and Reinherz 2003). The shaded amino acid alanine (A) in *DPA* and the shaded *DPB1*02* lineage are only seen in *P. t. troglodytes*.t, the allele is found only in *P. t. troglodytes*; s, the allele is found only in *P. t. schweinfurthii*

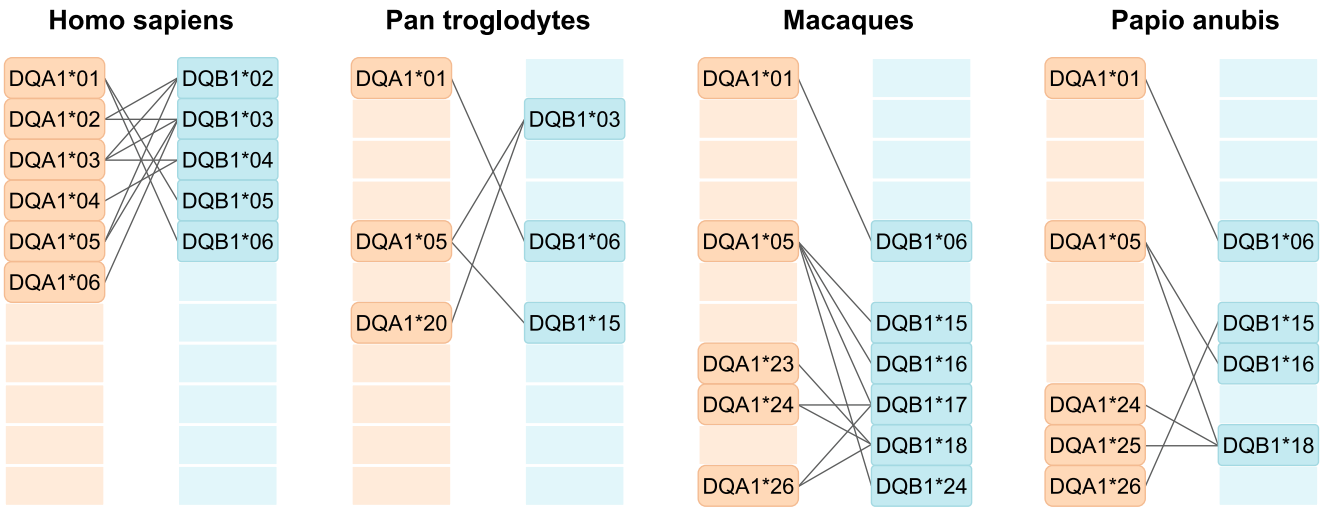
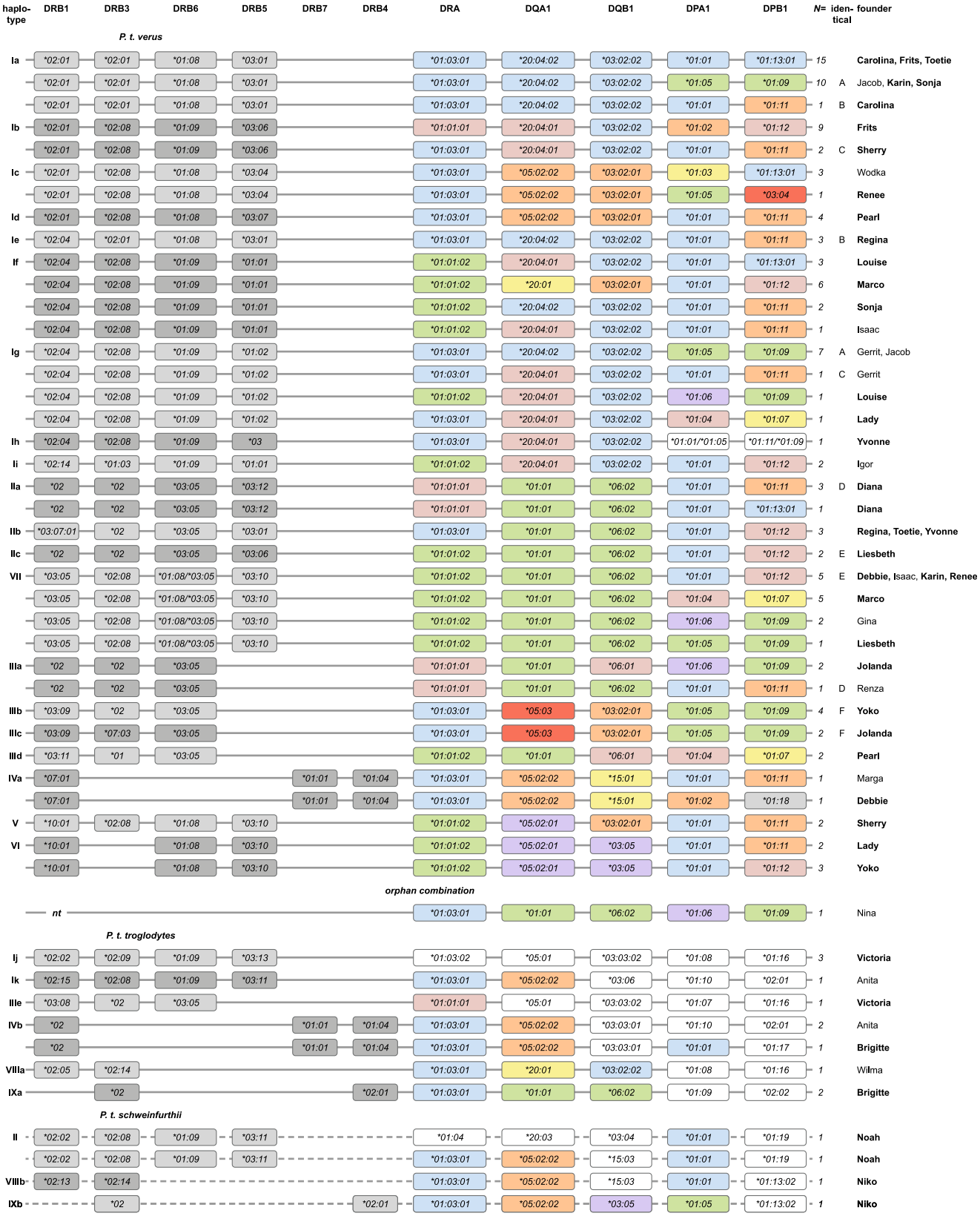


Fig. 4 The pairing of *DQA* and *DQB* lineages. The lines between the *DQA* and *DQB* blocks represent the pairing of lineages on the chromosomes of human, chimpanzees, macaques, and olive baboons


haplo-type
DRB1
DRB3
DRB6
DRB5
DRB7
DRB4
DRA
DQA1
DQB1
DPA1
DPB1
N=
iden-tical
founder
P. t. verus
Ia *02:01 *02:01 *01:08 *03:01 *01:03:01 *20:04:02 *03:02:02 *01:01 *01:13:01 15 Carolina, Frits, Toetie
*02:01 *02:01 *01:08 *03:01 *01:03:01 *20:04:02 *03:02:02 *01:05 *01:09 10 A Jacob, Karin, Sonja
*02:01 *02:01 *01:08 *03:01 *01:03:01 *20:04:02 *03:02:02 *01:01 *01:11 1 B Carolina
Ib *02:01 *02:08 *01:09 *03:06 *01:01:01 *20:04:01 *03:02:02 *01:02 *01:12 9 Frits
*02:01 *02:08 *01:09 *03:06 *01:03:01 *20:04:01 *03:02:02 *01:01 *01:11 2 C Sherry
Ic *02:01 *02:08 *01:08 *03:04 *01:03:01 *05:02:02 *03:02:01 *01:03 *01:13:01 3 Wodka
*02:01 *02:08 *01:08 *03:04 *01:03:01 *05:02:02 *03:02:01 *01:05 *03:04 1 Renee
Id *02:01 *02:08 *01:08 *03:07 *01:03:01 *05:02:02 *03:02:01 *01:01 *01:11 4 Pearl
Ie *02:04 *02:01 *01:08 *03:01 *01:03:01 *20:04:02 *03:02:02 *01:01 *01:11 3 B Regina
If *02:04 *02:08 *01:09 *01:01 *01:01:02 *20:04:01 *03:02:02 *01:01 *01:13:01 3 Louise
*02:04 *02:08 *01:09 *01:01 *01:01:02 *20:01 *03:02:01 *01:01 *01:12 6 Marco
*02:04 *02:08 *01:09 *01:01 *01:01:02 *20:04:02 *03:02:02 *01:01 *01:11 2 Sonja
*02:04 *02:08 *01:09 *01:01 *01:01:02 *20:04:01 *03:02:02 *01:01 *01:11 1 Isaac
Ig *02:04 *02:08 *01:09 *01:02 *01:03:01 *20:04:02 *03:02:02 *01:05 *01:09 7 A Gerrit, Jacob
*02:04 *02:08 *01:09 *01:02 *01:03:01 *20:04:01 *03:02:02 *01:01 *01:11 1 C Gerrit
*02:04 *02:08 *01:09 *01:02 *01:01:02 *20:04:01 *03:02:02 *01:06 *01:09 1 Louise
*02:04 *02:08 *01:09 *01:02 *01:03:01 *20:04:01 *03:02:02 *01:04 *01:07 1 Lady
Ih *02:04 *02:08 *01:09 *03 *01:03:01 *20:04:01 *03:02:02 *01:01/*01:05 *01:11/*01:09 1 Yvonne
Ii *02:14 *01:03 *01:09 *01:01 *01:01:02 *20:04:01 *03:02:02 *01:01 *01:12 2 Igor
IIa *02 *02 *03:05 *03:12 *01:01:01 *01:01 *06:02 *01:01 *01:11 3 D Diana
*02 *02 *03:05 *03:12 *01:01:01 *01:01 *06:02 *01:01 *01:13:01 1 Diana
IIb *03:07:01 *02 *03:05 *03:01 *01:03:01 *01:01 *06:02 *01:01 *01:12 3 Regina, Toetie, Yvonne
IIc *02 *02 *03:05 *03:06 *01:01:02 *01:01 *06:02 *01:01 *01:12 2 E Liesbeth
VII *03:05 *02:08 *01:08/*03:05 *03:10 *01:01:02 *01:01 *06:02 *01:01 *01:12 5 E Debbie, Isaac, Karin, Renee
*03:05 *02:08 *01:08/*03:05 *03:10 *01:01:02 *01:01 *06:02 *01:04 *01:07 5 Marco
*03:05 *02:08 *01:08/*03:05 *03:10 *01:01:02 *01:01 *06:02 *01:06 *01:09 2 Gina
*03:05 *02:08 *01:08/*03:05 *03:10 *01:01:02 *01:01 *06:02 *01:05 *01:09 1 Liesbeth
IIIa *02 *02 *03:05 *01:01:01 *01:01 *06:01 *01:06 *01:09 2 Jolanda
*02 *02 *03:05 *01:01:01 *01:01 *06:02 *01:01 *01:11 1 D Renza
IIIb *03:09 *02 *03:05 *01:03:01 *05:03 *03:02:01 *01:05 *01:09 4 F Yoko
IIIc *03:09 *07:03 *03:05 *01:03:01 *05:03 *03:02:01 *01:05 *01:09 2 F Jolanda
IIId *03:11 *01 *03:05 *01:01:02 *01:01 *06:01 *01:04 *01:07 2 Pearl
IVa *07:01 *01:01 *01:04 *01:03:01 *05:02:02 *15:01 *01:01 *01:11 1 Marga
*07:01 *01:01 *01:04 *01:03:01 *05:02:02 *15:01 *01:02 *01:18 1 Debbie
V *10:01 *02:08 *01:08 *03:10 *01:01:02 *05:02:01 *03:02:01 *01:01 *01:11 2 Sherry
VI *10:01 *01:08 *03:10 *01:01:02 *05:02:01 *03:05 *01:01 *01:11 2 Lady
*10:01 *01:08 *03:10 *01:01:02 *05:02:01 *03:05 *01:01 *01:12 3 Yoko
orphan combination
nt *01:03:01 *01:01 *06:02 *01:06 *01:09 1 Nina
P. t. troglodytes
Ij *02:02 *02:09 *01:09 *03:13 *01:03:02 *05:01 *03:03:02 *01:08 *01:16 3 Victoria
Ik *02:15 *02:08 *01:09 *03:11 *01:03:01 *05:02:02 *03:06 *01:10 *02:01 1 Anita
IIIe *03:08 *02 *03:05 *01:01:01 *05:01 *03:03:02 *01:07 *01:16 1 Victoria
IVb *02 *01:01 *01:04 *01:03:01 *05:02:02 *03:03:01 *01:10 *02:01 2 Anita
*02 *01:01 *01:04 *01:03:01 *05:02:02 *03:03:01 *01:01 *01:17 1 Brigitte
VIIIa *02:05 *02:14 *01:03:01 *20:01 *03:02:02 *01:08 *01:16 1 Wilma
IXa *02 *02:01 *01:03:01 *01:01 *06:02 *01:09 *02:02 2 Brigitte
P. t. schweinfurthii
II *02:02 *02:08 *01:09 *03:11 *01:04 *20:03 *03:04 *01:01 *01:19 1 Noah
*02:02 *02:08 *01:09 *03:11 *01:03:01 *05:02:02 *15:03 *01:01 *01:19 1 Noah
VIIIb *02:13 *02:14 *01:03:01 *05:02:02 *15:03 *01:01 *01:13:02 1 Niko
IXb *02 *02:01 *01:03:01 *05:02:02 *03:05 *01:05 *01:13:02 1 Niko

◀ **Fig. 5** The MHC class II haplotypes present in the cohort of BPRC chimpanzees. The *DRB* haplotypes are extracted from an earlier study (de Groot et al. 2009). Two shades of grey indicate the separate *DRB* haplotypes, because for some of them more than one *DRA/DQ/DP* combination was observed. Each class II allele in the *P. t. verus* group received a *colour*, to illustrate the recombination. *N* represents the number of animals in which a haplotype is found. All *DRA/DQ/DP* combinations are unique, with the exception of six sets, as in indicated from A up to F. The founding animals are listed, and those that were analysed in this study are presented in bold face. The four haplotypes of the *P. t. schweinfurthii* animals, Noah and Niko, are deduced and are not confirmed by segregation, as indicated by the dashed lines. The order of genes in the figure is arbitrary and may not represent the actual order on the chromosome. Nt is not typed

present, as indicated from A up to F. The number of *DRB* haplotypes within the main *P. t. verus* group has been extended from 20 to 38 whole MHC class II haplotypes, one of which is not typed for *DRB*.

Discussion

In this study, we observed some differences between the MHC alleles of the distinct chimpanzee populations/subspecies. Some of these differences may have an impact on their immune responses, since they encode for specific amino acids at antigen-binding positions of the class II molecule (Reche and Reinherz 2003). Examples are the lysine (K) in the *P. t. troglodytes* allele *DQA1*05:01* and serine (S) in the *P. t. schweinfurthii* allele *DQA1*20:03* (Fig. 1). Furthermore, the *DPB1*02* lineage is observed only in *P. t. troglodytes*, and the encoded β1-region differs profoundly from those encoded by *DPB1*01* alleles (Fig. 3). Analyses of the MHC class II alleles may represent tools to distinguish chimpanzees of different genetic backgrounds. For zoos—in particular with regard to the issue of conservation biology—this represents a promising prospect.

In this cohort, the number of animals, other than the West African chimpanzees, is low. For this reason, we focused our discussion on the latter group. The main *P. t. verus* group was started with 32 animals—originating from Sierra Leone—that represent 64 chromosomes comprising an MHC region. The panel included 18 of these founder animals that embody 36 chromosomes. They are represented in bold face in the last column of Fig. 5. By studying the offspring as well, we were able to deduce both of the haplotypes on the chromosomes in three other founder animals—Jacob, Isaac, and Gerrit. For six other parental chimpanzees, only one class II region was construed by segregation, of which Nina had the 'orphan' haplotype with the missing *DRB* typing. Including these 12 deduced founder allele combinations, a total of 48 MHC class II regions—rather than the theoretically number of 64—had actually been inherited and spread in the investigated cohort. To summarise, 20 *DRB* haplotypes were extended with *DRA/DQ/DP* sets to 38 whole MHC class II haplotypes, present on 48 inherited chromosomes. Of these haplotypes, 33 are unique and five are shared by two or more founders, as indicated by their names in the last column of Fig. 5. The present results show that the West African chimpanzee MHC class II region displays a high level of combination diversity. At first glance, this was unexpected, since in *P. t. verus* only three *Patr-DRA*, seven *-DQA*, six *-DQB*, six *–DPA*, and seven *-DPB* alleles were found. Moreover, the difference between alleles is often based on only one nucleotide. This low number of class II alleles, and the similarity of alleles within lineages, is in accordance with the reduction of the MHC class I repertoire documented for these animals (de Groot et al. 2002, 2010). Thus, the tentative selective sweep may also have had an impact on the class II region. The strongest impact of the selective sweep targeted the *Patr-B/C* genes (de Groot et al. 2008), and the MIC region (de Groot et al. 2005). Therefore, the reduction of the MHC class II repertoire may have resulted from a linkage phenomenon. The fact that the majority of the West African chimpanzee class II haplotypes are unique, despite the low number of alleles of various genes, suggests that haplotype diversity was generated by crossing-over events, after the selective sweep. The crossing-over effects become more prominent when the class I data of these animals are included. In the present cohort, 12 *Patr-A*, 11 *-B*, and 8 *-C* alleles had previously been documented (de Groot et al. 2000, 2010). When the class I data is added to the five class II haplotypes that are shared within the cohort, additional variation is observed (data not shown). The number of 33 unique class II haplotypes extends to 40 unique whole MHC regions on 48 founder chromosomes. This study demonstrates that with a limited number of alleles an impressive number of haplotypes can be generated, thereby securing MHC diversity at the population level in a species that has undergone a selective sweep.

Acknowledgements The authors wish to thank F. van Hassel for preparing the figures and D.M. Devine for editing this manuscript.

References

Anzai T, Shiina T, Kimura N, Yanagiya K, Kohara S, Shigenari A, Yamagata T, Kulski JK, Naruse TK, Fujimori Y, Fukuzumi Y, Yamazaki M, Tashiro H, Iwamoto C, Umehara Y, Imanishi T, Meyer A, Ikeo K, Gojobori T, Bahram S, Inoko H (2003) Comparative sequencing of human and chimpanzee MHC class I regions unveils insertions/deletions as the major path to genomic divergence. Proc Natl Acad Sci U S A 100:7708–7713. https://doi.org/10.1073/pnas.1230533100

Balla-Jhagjhoorsingh SS, Koopman G, Mooij P, Haaksma TG, Teeuwsen VJ, Bontrop RE, Heeney JL (1999) Conserved CTL epitopes shared between HIV-infected human long-term survivors and chimpanzees. J Immunol 162:2308–2314

Becquet C, Patterson N, Stone AC, Przeworski M, Reich D (2007) Genetic structure of chimpanzee populations. PLoS Genet 3:e66. https://doi.org/10.1371/journal.pgen.0030066

Bettauer RH (2010) Chimpanzees in hepatitis C virus research: 1998-2007. J Med Primatol 39:9–23. https://doi.org/10.1111/j.1600-0684.2009.00390.x

Bohme J, Andersson M, Andersson G, Moller E, Peterson PA, Rask L (1985) HLA-DR beta genes vary in number between different DR specificities, whereas the number of DQ beta genes is constant. J Immunol 135:2149–2155

Bontrop RE (2006) Comparative genetics of MHC polymorphisms in different primate species: duplications and deletions. Hum Immunol 67:388–397. https://doi.org/10.1016/j.humimm.2006.03.007

Bontrop RE, Otting N, Slierendregt BL, Lanchbury JS (1995) Evolution of major histocompatibility complex polymorphisms and T-cell receptor diversity in primates. Immunol Rev 143:33–62

de Groot NG, Bontrop RE (2013) The HIV-1 pandemic: does the selective sweep in chimpanzees mirror humankind's future? Retrovirology 10:53. https://doi.org/10.1186/1742-4690-10-53

de Groot NG, Garcia CA, Verschoor EJ, Doxiadis GG, Marsh SG, Otting N, Bontrop RE (2005) Reduced MIC gene repertoire variation in West African chimpanzees as compared to humans. Mol Biol Evol 22:1375–1385. https://doi.org/10.1093/molbev/msi127

de Groot NG, Heijmans CMC, Bontrop RE (2017) AIDS in chimpanzees: the role of MHC genes. Immunogenetics 69:499–509. https://doi.org/10.1007/s00251-017-1006-6

de Groot NG, Heijmans CM, de Groot N, Doxiadis GG, Otting N, Bontrop RE (2009) The chimpanzee Mhc-DRB region revisited: gene content, polymorphism, pseudogenes, and transcripts. Mol Immunol 47:381–389. https://doi.org/10.1016/j.molimm.2009.09.003

de Groot NG, Heijmans CM, de Groot N, Otting N, de Vos-Rouweller AJ, Remarque EJ, Bonhomme M, Doxiadis GG, Crouau-Roy B, Bontrop RE (2008) Pinpointing a selective sweep to the chimpanzee MHC class I region by comparative genomics. Mol Ecol 17:2074–2088. https://doi.org/10.1111/j.1365-294X.2008.03716.x

de Groot NG, Heijmans CM, Zoet YM, de Ru AH, Verreck FA, van Veelen PA, Drijfhout JW, Doxiadis GG, Remarque EJ, Doxiadis II, van Rood JJ, Koning F, Bontrop RE (2010) AIDS-protective HLA-B*27/B*57 and chimpanzee MHC class I molecules target analogous conserved areas of HIV-1/SIVcpz. Proc Natl Acad Sci U S A 107:15175–15180. https://doi.org/10.1073/pnas.1009136107

de Groot NG, Otting N, Arguello R, Watkins DI, Doxiadis GG, Madrigal JA, Bontrop RE (2000) Major histocompatibility complex class I diversity in a West African chimpanzee population: implications for HIV research. Immunogenetics 51:398–409

de Groot NG, Otting N, Doxiadis GG, Balla-Jhagjhoorsingh SS, Heeney JL, van Rood JJ, Gagneux P, Bontrop RE (2002) Evidence for an ancient selective sweep in the MHC class I gene repertoire of chimpanzees. Proc Natl Acad Sci U S A 99:11748–11753. https://doi.org/10.1073/pnas.182420799

de Groot NG, Otting N, Robinson J, Blancher A, Lafont BA, Marsh SG, O'Connor DH, Shiina T, Walter L, Watkins DI, Bontrop RE (2012) Nomenclature report on the major histocompatibility complex genes and alleles of Great Ape, Old and New World monkey species. Immunogenetics 64:615–631. https://doi.org/10.1007/s00251-012-0617-1

Doxiadis GG, Hoof I, de Groot N, Bontrop RE (2012) Evolution of HLA-DRB genes. Mol Biol Evol 29:3843–3853. https://doi.org/10.1093/molbev/mss186

Doxiadis GG, Otting N, de Groot NG, Bontrop RE (2001) Differential evolutionary MHC class II strategies in humans and rhesus macaques: relevance for biomedical studies. Immunol Rev 183:76–85

Doxiadis GG, Otting N, de Groot NG, Noort R, Bontrop RE (2000) Unprecedented polymorphism of Mhc-DRB region configurations in rhesus macaques. J Immunol 164:3193–3199

Gagneux P, Moore JJ, Varki A (2005) The ethics of research on great apes. Nature 437:27–29. https://doi.org/10.1038/437027a

Gagneux P, Wills C, Gerloff U, Tautz D, Morin PA, Boesch C, Fruth B, Hohmann G, Ryder OA, Woodruff DS (1999) Mitochondrial sequences show diverse evolutionary histories of African hominoids. Proc Natl Acad Sci U S A 96:5077–5082

Groves CP (2001) Primate taxonomy. Smithsonian Institution Press, Washington (D.C.)

Gyllensten U, Bergstrom T, Josefsson A, Sundvall M, Erlich HA (1996) Rapid allelic diversification and intensified selection at antigen recognition sites of the Mhc class II DPB1 locus during hominoid evolution. Tissue Antigens 47:212–221

Klein J (1987) Origin of major histocompatibility complex polymorphism: the trans-species hypothesis. Hum Immunol 19:155–162

Lawlor DA, Ward FE, Ennis PD, Jackson AP, Parham P (1988) HLA-A and B polymorphisms predate the divergence of humans and chimpanzees. Nature 335:268–271. https://doi.org/10.1038/335268a0

Maccari G, Robinson J, Ballingall K, Guethlein LA, Grimholt U, Kaufman J, Ho CS, de Groot NG, Flicek P, Bontrop RE, Hammond JA, Marsh SG (2017) IPD-MHC 2.0: an improved inter-species database for the study of the major histocompatibility complex. Nucleic Acids Res 45:D860–D864. https://doi.org/10.1093/nar/gkw1050

Maibach V, Hans JB, Hvilsom C, Marques-Bonet T, Vigilant L (2017) MHC class I diversity in chimpanzees and bonobos. Immunogenetics 69:661–676. https://doi.org/10.1007/s00251-017-0990-x

Mayer WE, Jonker M, Klein D, Ivanyi P, van Seventer G, Klein J (1988) Nucleotide sequences of chimpanzee MHC class I alleles: evidence for trans-species mode of evolution. EMBO J 7:2765–2774

McAdam SN, Boyson JE, Liu X, Garber TL, Hughes AL, Bontrop RE, Watkins DI (1995) Chimpanzee MHC class I A locus alleles are related to only one of the six families of human A locus alleles. J Immunol 154:6421–6429

O'Connor SL, Blasky AJ, Pendley CJ, Becker EA, Wiseman RW, Karl JA, Hughes AL, O'Connor DH (2007) Comprehensive characterization of MHC class II haplotypes in Mauritian cynomolgus macaques. Immunogenetics 59:449–462. https://doi.org/10.1007/s00251-007-0209-7

Otting N, Doxiadis GG, Versluis L, de Groot NG, Anholts J, Verduin W, Rozemuller E, Claas F, Tilanus MG, Bontrop RE (1998) Characterization and distribution of Mhc-DPB1 alleles in chimpanzee and rhesus macaque populations. Hum Immunol 59:656–664

Otting N, van der Wiel MK, de Groot N, de Vos-Rouweler AJ, de Groot NG, Doxiadis GG, Wiseman RW, O'Connor DH, Bontrop RE (2017) The orthologs of HLA-DQ and -DP genes display abundant levels of variability in macaque species. Immunogenetics 69:87–99. https://doi.org/10.1007/s00251-016-0954-6

Otting N, van der Wiel MK, Doxiadis GG, Bontrop RE (2016) Fifty-one full-length major histocompatibility complex class II alleles in the olive baboon (Papio anubis). HLA 88:270–271. https://doi.org/10.1111/tan.12906

Pacheco MA, Cranfield M, Cameron K, Escalante AA (2013) Malarial parasite diversity in chimpanzees: the value of comparative approaches to ascertain the evolution of Plasmodium falciparum antigens. Malar J 12:328. https://doi.org/10.1186/1475-2875-12-328

Parham P, Ohta T (1996) Population biology of antigen presentation by MHC class I molecules. Science 272:67–74

Prado-Martinez J, Sudmant PH, Kidd JM, Li H, Kelley JL, Lorente-Galdos B, Veeramah KR, Woerner AE, O'Connor TD, Santpere G, Cagan A, Theunert C, Casals F, Laayouni H, Munch K, Hobolth A, Halager AE, Malig M, Hernandez-Rodriguez J, Hernando-Herraez I, Prufer K, Pybus M, Johnstone L, Lachmann M, Alkan C, Twigg

D, Petit N, Baker C, Hormozdiari F, Fernandez-Callejo M, Dabad M, Wilson ML, Stevison L, Camprubi C, Carvalho T, Ruiz-Herrera A, Vives L, Mele M, Abello T, Kondova I, Bontrop RE, Pusey A, Lankester F, Kiyang JA, Bergl RA, Lonsdorf E, Myers S, Ventura M, Gagneux P, Comas D, Siegismund H, Blanc J, Agueda-Calpena L, Gut M, Fulton L, Tishkoff SA, Mullikin JC, Wilson RK, Gut IG, Gonder MK, Ryder OA, Hahn BH, Navarro A, Akey JM, Bertranpetit J, Reich D, Mailund T, Schierup MH, Hvilsom C, Andres AM, Wall JD, Bustamante CD, Hammer MF, Eichler EE, Marques-Bonet T (2013) Great ape genetic diversity and population history. Nature 499:471–475. https://doi.org/10.1038/nature12228

Reche PA, Reinherz EL (2003) Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms. J Mol Biol 331:623–641

Slierendregt BL, Kenter M, Otting N, Anholts J, Jonker M, Bontrop RE (1993) Major histocompatibility complex class II haplotypes in a breeding colony of chimpanzees (Pan troglodytes). Tissue Antigens 42:55–61

Stone AC, Griffiths RC, Zegura SL, Hammer MF (2002) High levels of Y-chromosome nucleotide diversity in the genus Pan. Proc Natl Acad Sci U S A 99:43–48. https://doi.org/10.1073/pnas.012364999

Wroblewski EE, Norman PJ, Guethlein LA, Rudicell RS, Ramirez MA, Li Y, Hahn BH, Pusey AE, Parham P (2015) Signature patterns of MHC diversity in three Gombe communities of wild chimpanzees reflect fitness in reproduction and immune defense against SIVcpz. PLoS Biol 13:e1002144. https://doi.org/10.1371/journal.pbio.1002144