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Haplotype diversity generated by ancient recombination-like events in the MHC of Indian rhesus macaques

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Abstract The *Mamu-A*, *Mamu-B*, and *Mamu-DRB* genes of the rhesus macaque show several levels of complexity such as allelic heterogeneity (polymorphism), copy number variation, differential segregation of genes/alleles present on a haplotype (diversity) and transcription level differences. A combination of techniques was implemented to screen a large panel of pedigreed Indian rhesus macaques (1,384 individuals representing the offspring of 137 founding animals) for haplotype diversity in an efficient and inexpensive manner. This approach allowed the definition of 140 haplotypes that display a relatively low degree of region variation as reflected by the presence of only 17 *A*, 18 *B* and 22 *DRB* types, respectively, exhibiting a global linkage disequilibrium comparable to that in humans. This finding contrasts with the situation observed in rhesus macaques from other geographic origins and in cynomolgus monkeys from Indonesia. In these latter populations, nearly every haplotype appears to be characterised by a unique *A*, *B* and *DRB* region. In the Indian population, however, a reshuffling of existing segments generated "new" haplotypes. Since the recombination frequency within the core MHC of the Indian rhesus macaques is relatively low, the various haplotypes were most probably produced by recombination events that accumulated over a long evolutionary time span. This idea is in accord with the notion that Indian rhesus macaques experienced a severe reduction in population during the Pleistocene due to a bottleneck caused by geographic changes. Thus, recombination-like processes appear to be a way to expand a diminished genetic repertoire in an isolated and relatively small founder population.

Keywords MHC · Non-human primates · Macaques · Haplotypes · Microsatellites · Evolution

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Introduction

During the past few decades, the Indian rhesus macaque (*Macaca mulatta*) has been one of the animals of choice for preclinical, biomedical research. Because of an import prohibition regarding these animals, however, researchers often make alternative use of rhesus macaques from China or aim to become self-sustaining by breeding Indian monkeys. The Biomedical Primate Research Centre (BPRC) has chosen the latter possibility mainly because the genetics of the Major Histocompatibility Complex (MHC), which is highly important for diverse immune-related diseases, has been

studied exhaustively in these monkeys for more than 30 years. Additionally, a few groups were assembled with rhesus macaques of Burmese origin. In addition to rhesus macaques, cynomolgus macaques (*Macaca fascicularis*) have emerged as study animals in recent years, and the BPRC houses a small breeding colony of Indonesian cynomolgus monkeys.

As in humans, the highly polymorphic genes of the macaques' MHC encode cell-surface glycoproteins, of which the classical class I (Mamu-A and Mamu-B) and class II (Mamu-DR, Mamu-DQ and Mamu-DP) molecules are equipped with a groove that can accommodate peptides of foreign origin. In the past, the BPRC's Indian-origin rhesus macaque colony was typed for its MHC (Mamu)-A, MHC-B and MHC-DR antigens (Bontrop et al. 1995; Roger et al. 1976; van Es and Balner 1978) by serological methods. Haplotypes were defined by segregation analyses and named according to the founder animals, in which the haplotype was originally observed. Over the last two decades, serotyping has been replaced by molecular techniques like full-length cDNA sequencing for class I (Boyson et al. 1996; Otting et al. 2007; Otting et al. 2005; Otting et al. 2008) and sequencing of the most polymorphic exon 2 of the various class II genes (Bontrop et al. 1999; Doxiadis et al. 2001; Doxiadis et al. 2000; Khazand et al. 1999; Otting et al. 2002; Otting et al. 2000; Otting et al. 1998; Slierendregt et al. 1995; Slierendregt et al. 1994). In Indian rhesus macaques, DP and DQ molecules are encoded by a single pair of genes: namely, *DPA1*/*DPB1* and *DQA1*/*DQB1*, respectively. In contrast, the *A*, *B* and *DR* regions have been subjected to several rounds of duplications (Dawkins et al. 1999; Gaudieri et al. 1997; Kulski et al. 1999). As a consequence, the *A*, *B* and *DRB* macaque regions show diversity in both the number and variety of loci that are present per chromosome/haplotype, a phenomenon referred to as "region configuration polymorphism" (Bonhomme et al. 2008; Doxiadis et al. 2011; Doxiadis et al. 2009b; Otting et al. 2005; Slierendregt et al. 1994). For Indian rhesus macaques, one to three transcribed *A* genes may be present per haplotype, of which *A1* is generally the most polymorphic one characterised by a high transcription level (Otting et al. 2005). The *A* region of rhesus macaques of other origins and of cynomolgus macaques of various origins shows even higher levels of diversity (Budde et al. 2010; Kita et al. 2009; Ma et al. 2009; Otting et al. 2007; Pendley et al. 2008; Saito et al. 2012; Wiseman et al. 2009). The *Mamu-B* region shows still more region configuration polymorphisms reflected by up to ten transcripts per haplotype (Daza-Vamenta et al. 2004), of which only one or two are transcribed at a higher level in B cells and/or PBMCs (Campbell et al. 2009; Greene et al. 2011; Karl et al. 2008; Otting et al. 2005; Otting et al. 2008; Uda et al. 2005). However, expression levels may vary in different cell types as described for distinct leukocyte subsets (Greene et al. 2011). Furthermore, the *DRB* region of rhesus and cynomolgus monkeys contains two to six *DRB* genes per haplotype, two or three of which appear to be transcribed, whereas the others seem to be pseudo-genes (Blancher et al. 2012; Blancher et al. 2006; de Groot et al. 2004; Doxiadis et al. 2012). In contrast to humans, in which only five *DRB* configurations (DR1, DR8, DR51, DR52 and DR53) are described and which all contain a very polymorphic *DRB1* gene, a high number of *DRB* configurations have been defined in macaques with low levels of allelic variation within a given configuration.

Because of the variable number and content of *Mamu-A*, *Mamu-B* and *Mamu-DRB* genes per haplotype, most techniques suitable for their molecular typing are time consuming and laborious. Therefore, microsatellite genotyping methods have been developed based on the presence of polymorphic short tandem repeats (STR), the amplification of which results in length patterns that are characteristic for the various *A*, *B* and/or *DRB* haplotypes. In the case of *Mamu-A*, two microsatellites, D6S2854 and D6S2859, have been used that are localised next to the relevant genes (Doxiadis et al. 2011). The *Mamu-B* region can be defined by four different STRs, MIB6, MIB7, MICA and D6S2793, which are situated in the *B* region of macaques (Bonhomme et al. 2008; Doxiadis et al. 2009b). The most straightforward genotyping can be performed with microsatellite D6S2878, named DRB-STR, which is localised within intron 2 of virtually all *DRB* genes and, thus, serves as an excellent tool for haplotyping both macaque and other primate species (de Groot et al. 2008a; de Groot et al. 2009; Doxiadis et al. 2007a; Doxiadis et al. 2009a; Otting et al. 2012). A combination of all molecular techniques mentioned above has been implemented to screen the Indian and Burmese rhesus macaques and to define their MHC haplotypes. The results of this study will be presented and compared to the *Mhc* haplotypes of Indonesian cynomolgus monkeys.

Materials and methods

Animals and cell lines

The BPRC houses a breeding colony of about 650 rhesus macaques of Indian origin. The colony was founded in the 1970s by more than 140 animals, and it has been pedigreed for more than seven generations. Additionally, the BPRC-bred rhesus macaques of Burmese origin were founded by two alpha males and 13 females and two mixed-bred animals, but breeding of these animals has been discontinued. For the last 10 years, all breeding groups consist of one alpha male and two to six females with their offspring. Lymphoblastoid B-cell lines and genomic DNA (gDNA)

have been available from most of the animals in the colony for the last 20 years so that animals could also be typed retrospectively. Thus, in the present study, 2,377 *Mamu* core haplotypes (*Mhc-A*, *Mhc-B* and *Mhc-DRB*) of 1,384 rhesus macaques of Indian and 145 of Burmese origin could be defined. Extended haplotypes with additional *DQA1*, *DQB1* and *DPB1* information have also been defined on more than 2,000 chromosomes for the Indian rhesus macaques, and *Mamu-A*/*B*/*DRB*/*DQ* haplotypes have been determined on 258 chromosomes of rhesus monkeys of Burmese origin.

Haplotype definition

In the past, *Mhc* haplotypes of Indian rhesus macaques were defined by co-segregation of A and B antigens in at least two animals of the same family and were named according to the founder animal, with prefixes A and B indicating a founder male and prefixes C and D indicating a founder female, respectively. Currently, a haplotype is defined by co-segregation of the same microsatellite (STR) pattern in at least two animals of the same family with STR D6S2878 for *DRB* and STRs D6S2854 and D6S2859 for *Mamu-A*. In addition, in most of the Indian monkeys, segregation of four *B* region-related STRs (MIC6, MIC7, MICA and D6S2793) has been followed. Furthermore, *Mamu-A* and *Mamu-B* as well as *Mamu-DRB* of a given founder haplotype have been ascertained by full-length complementary DNA (cDNA) or by exon 2 gDNA sequencing of selected animals, respectively. In such a way, 176 haplotypes of Indian rhesus macaques based on 137 founder animals have been defined along with 24 haplotypes of Burmese monkeys based on 17 founders.

Mamu-A and Mamu-B typing by full-length cDNA sequencing

RNA was isolated from lymphoblastoid B cell lines (RNeasy kit, Qiagen, Valencia, CA, USA) and subjected to a One-Step RT-PCR kit (Promega BioSystems, Madison, WI, USA), as recommended by the supplier. In these reactions, the primers 5′MAS: AATTCATGGCGCCCCGAACCCTCCTCCTGG, 3′MAS: CTAGACCACACAAGGCGGCTGTCTCAC, 5′ MBS: ATTCATGGCGCCCCGAACCCTCCTCCTGC and 3′MBS: CTAGACCACACAAGACAGTTGTCTCAG, which are specific for *Mamu-A* and *Mamu-B* transcripts, respectively, were used. The final elongation step was extended to 7 min to generate a 3′ A overhang. The RT-PCR products were cloned by using the InsTAclone kit (Fermentas, St. Leon-Rot, Germany) or the PCR cloning kit (Qiagen, Valencia, CA, USA). After transformation, colonies were picked for plasmid isolations: namely, 16–32 colonies for the *Mamu-A* transcript and 32–64 colonies for the *Mamu-B* transcript, respectively. Sequencing reactions were performed using the BigDye terminator cycle sequencing kit version 3.1, and samples were run on a Genetic Analyzer 3130 (both Applied Biosystems, Foster City, CA, USA). The sequences were analysed with MacVector™ (Oxford Molecular Group).

Mamu-DQA1, Mamu-DQB1, Mamu-DPB1 and Mamu-DRB exon 2 sequencing

Genomic DNA was extracted from EDTA blood samples or from immortalised B-lymphocytes by a standard salting out method. The amplification of exon 2 of *Mamu-DQA1*, *Mamu-DQB1* and *Mamu-DPB1* was performed mainly according to earlier published methods (Doxiadis et al. 2003; Doxiadis et al. 2006), except that the PCR mix contained 5–50 ng of DNA, 2.5 mM of $MgCl_2$, and 0.4 μM of the following primers for *DQB1*: 5′DQB1-intr1 TCC CCG CAG AGG ATT TCG TG, 3′ DQB1-intr2 TGC GGG CGA CGA CGC CTC ACC TC or, alternatively, the following primers for *DPB1*: 5′DPB1new GGA TTA GGT GAG AGT GGT GCC C; 3′DPB1new CRG CCC AAA GCC YTC ACT CAC. The PCR conditions were according to published methods (Doxiadis et al. 2003), with an annealing temperature of 62 °C for *DQB1*. The PCR conditions and primers for amplification of *DRB* exon 2 + STR in intron 2 are described elsewhere (Doxiadis et al. 2007a). The exon 2 sequences of *DQA1*, *DQB1* and *DPB1* were first sequenced directly (Doxiadis et al. 2003) and analysed with the SBTengine programme (GenDx, Utrecht, The Netherlands). *DQA1*, *DQB1* and *DPB1* sequences containing unknown alleles as well as *DRB* sequences were subjected to cloning and sequencing with 15 to 60 clones selected on the basis of the amplicon using pJet cloning kit (Fermentas, Heidelberg, Germany) and on conditions as described above and elsewhere (Penedo et al. 2005). The sequences were analysed with MacVector™ (Oxford Molecular Group) and SeqMan Pro (DNASTAR, Lasergene). The sequence of the new allele *Mamu-DQB1*06new* (preliminary name; accession number KC835255) has been sent to NHP IPD-MHC and is waiting for allele definition. Although *Mamu-DRA1* and *Mamu-DPA1* are also at least oligomorphic, because their polymorphism is localised mostly outside exon 2, the alleles of these genes have not been determined in this study.

Microsatellite genotyping by D6S2878, D6S2854, D6S2859, D6S2793, MIB6, MIB7 and MICA

Mamu-DRB genotyping by microsatellite D6S2878 (DRB-STR) was performed as described earlier (Doxiadis et al. 2007a). Additionally, *Mamu-A* genotyping by STRs D6S2854 and D6S2859 was performed as published previously (Doxiadis et al. 2011; Wiseman et al. 2007). For genotyping of the *Mamu-B* region, four STRs (D6S2793, MIB6, MIB7 and MICA) were selected, and the following

primers, which had been newly designed or published earlier (Wiseman et al. 2007), were used:

D6S2793-F NED-CTACCTCCTTGCCAAACTT GCTATTTGT, D6S2793-R AATAGCCATGAGAAG CTATGTGGGGGA, MIB6-F6-FAM-GATTCTTCAGAG AAGCAGAACC, MIB6-RCTGCAGATTTTCGTATGTAC; MIB7-F VIC-GAGAAGCAGAACCAATAGGGGG, MIB7-R TGTGCCTCATCCAATCAG TGG, MICA-F NED-CCT TTTTTTCAGGGAAAGTGC and MICA-RCCTTACCA TCTCCAGAAACTGC. The labelled primers were synthesised by Applied Biosystems and the unlabelled primers by Invitrogen (Paisley, Scotland). The PCR reaction for D6S2793 was performed in a 20 μl reaction volume containing 1 U of *phusion* hot start high fidelity DNA polymerase (Thermo Fisher Scientific, Breda, The Netherlands) with 3 % DMSO, 0.08 μM of the respective forward and reverse primers, 3.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1× HF buffer (Thermo Fisher Scientific) and 50 ng DNA. For this PCR, a "touchdown" programme was used with 30 s at 98 °C as initial denaturation step, followed by 4 cycles as published earlier (Wiseman et al. 2007). A final extension step was performed at 72 °C for 5 min. For MIB7, the PCR reaction was performed in a 25-μl reaction volume containing 1 U of *Taq* polymerase (Invitrogen, Paisley, Scotland) with 0.24 μM of the forward and reverse primers, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1× PCR buffer II (Invitrogen, Paisley, Scotland) and 50 ng DNA. For MICA and MIB6, a multiplex PCR was used in a 25-μl reaction volume containing 1 U of *Taq* polymerase (Invitrogen, Paisley, Scotland) with 0.48 μM of the forward and reverse primers of MICA, 0.2 μM of the forward and reverse primers of MIB6, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1× PCR buffer II (Invitrogen, Paisley, Scotland) and 50 ng DNA. The cycling parameters for MIB7 and the multiplex PCR of MIB6/MICA were a 5-min 94 °C initial denaturation step, followed by 4 cycles of 1 min at 94 °C, 30 s at 58 °C and 30 s at 72 °C. The programme was followed by 25 cycles of 45 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C. A final extension step was performed at 72 °C for 30 min. The amplified DNA was prepared for genotyping according to the manufacturer's guidelines and was analysed on the ABI 3130 genetic analyser (Applied Biosystems). STR analysis was performed using the GeneMapper programme (Applied Biosystems).

Test of linkage disequilibrium

Global linkage disequilibrium (GLD) between each pair of loci (known phase) was tested with an extension of a Fisher's exact test as implemented in Arlequin 3.5 (Excoffier and Lischer 2010). In brief, the test starts with a contingency table of haplotype counts, where the marginal totals are the allele counts at each locus and then uses a Monte-Carlo Markov Chain approach (Guo and Thompson 1992) to generate tables with the same marginal totals and a probability smaller than or equal to the original table. To assess the null hypothesis of no association (i.e., no linkage disequilibrium) between the loci, a p value equal to the proportion of tables having a probability smaller than or equal to the original table is estimated. The Markov chain was run for 9 million stages with an initial burn in of 10,000.

Additionally, the classical linkage disequilibrium coefficient D (and its significance using a χ^2 test), the standardised linkage disequilibrium coefficient D', and the square of the correlation coefficient r^2 were computed between all pairs of alleles/haplotypes for each pair of loci. For the *A*, *B* and *DRB* regions, which contain multiple loci, the alleles of each region of a certain haplotype (e.g., the *Mamu-A* region that may be represented by several *Mamu-A* genes) were summarised and considered to belong to a unique locus, since no recombination had been observed between them in the population studied. All allelic associations were reported; however, only haplotypes with more than three copies observed in the sample were discussed.

The adjustment of individual p values, which was made in order to take multiple testing into account (for GLD or haplotype linkage disequilibrium (LD)), was performed using the False Discovery Rate (FDR) method for each pair of loci (Benjamini and Yekutieli 2001). This method is less conservative than the usual Bonferroni's correction. However, the extra power comes at the cost of an increase in false positives and, therefore, results should be viewed with caution if only one or a few haplotypes exhibit significant adjusted LD p values for a given pair of loci.

Results

Mamu-A haplotyping of Indian rhesus macaques

In Indian rhesus macaques, 13 Mamu-A serotypes and an additional one, called "blank" (−), had been delineated in the past (Fig. 1). Additionally, five *Mamu-A* region configurations (r.c.) have been defined previously according to their number and content of loci, and "major" and "minor" *A* alleles have been determined based on transcription levels (Otting et al. 2007; Otting et al. 2005). The Mamu-A serotypes confer to *A* haplotypes, which are characterised by different lineages of the "major" *A1* locus (Fig. 1, colour coded). The "minor" *A2* locus comprises two lineages, *A2*05* and *A2*24*, whereas two other "minor" loci, *A3* and *A4*, are represented by only one lineage each, *A3*13* and *A4*14*, respectively. Using high-resolution sequencing and additional methods such as microsatellite typing with D6S2854 and D6S2859 the serological "blank" specificity could be subdivided into two well-defined *A* haplotypes (Fig. 1; haplotype 1e: A—a, and 5d: A—b). Furthermore, one other haplotype could by redefined as well (Fig. 1,

Hapl	Serol	*A1* locus	*A2* locus	*A3* locus	*A4* locus	D6S2854	D6S2859	fr (N=2377)
1a	A1	*A1*012:01*	*A2*05:11/19*			null	165/7,195	5.55%
1b	A5	*A1*011:04*	*A2*05*			181,192	187	2.69%
1c	A9	*A1*001:01/02*	*A2*05:04*			177,181	201	11.53%
1d	A22	*A1*007:01/02/03*	*A2*05:07*			181,192	176,186	4.67%
1e	A-a	*A1*025:01*	*A2*05:09*			185,192	187	0.29%
2a	A10	*A1*008:01:01*	*A2*05:03*	*A3*13:03*		173,181,196	171,203	20.40%
2b1	A28a	*A1*003:06*	*A2*24:01*	*A3*13:05*		173,181,185,192,196	159,209/211	4.00%
2b2	A28b	*A1*003:02*	*A2?*	*A3?*		177,192,196	173	
3a	A3	*A1*019:01*	*A2*05:05*		*A4*14*	173,212	189,201	1.18%
3b	A23	*A1*006:02*	*A2*05:01*		*A4*14*	173,181,196	171,185	4.84%
4a	A19	*A1*002:01*		*A3*13:02*		192	(166)	15.82%
4b	A21	*A1*016:02*		*A3*13:06*		181,192	(166)	1.85%
5a	A6	*A1*004:01:01*			*A4*14*	181,185,196	171	23.18%
5b	A27	*A1*026:01*			*A4*14*	181,196	175	1.14%
5c	A33	*A1*023:01*			*A4*14*	180,219	166	2.65%
5d	A-b	*A1*028:01*			*A4*14*	177	166	0.04%
n.d.	A?	n.d.	n.d.	n.d.		192,221	185	0.17%

Fig. 1 *Mamu-A* region configurations/haplotypes of Indian rhesus macaques. The order and positioning of the loci/lineages is schematically drawn and does not represent the actual localisation of the genes on the chromosome. *Solidus* indicates the detection of either one or the other *Mama-A* allele or STR length, whereas *comma* indicates that both STR lengths have been detected. *STR lengths in brackets* could not always be observed. *Question mark* indicates that an allele or locus is expected but (not yet) detected. *n.d.* not defined

haplotype 2b), and an additional haplotype could also be deciphered (Fig. 1, last entry). The associated *A* alleles of the latter, however, have not yet been determined at the nucleotide sequence level. We were able to show that STR typing with both microsatellites resulted in STR-length patterns, which could always be unambiguously related to a certain *Mamu-A* haplotype. In such a way, 17 *Mamu-A* haplotypes could be defined (Fig. 1). Four *A* haplotypes, characterised by the lineages *A1*001*, *A*002*, *A*004* and *A*008*, are observed at a frequency greater than 10 % in the Indian rhesus macaque panel tested (Fig. 1, red-bordered).

Mamu-B haplotyping of Indian rhesus macaques

In the past, 15 B serotypes had been defined in our Indian rhesus macaque colony. These serotypes correspond to 17 region configurations/haplotypes that can be differentiated by molecular methods (Fig. 2). In contrast to the *Mamu-A* region, there may be one, two or three *B* loci per chromosome that encode for a "major" *B* transcript. The total number of loci, however, which encode for a *B* transcript and that are detected by Sanger sequencing, vary from one to seven per haplotype (Otting et al. 2005). Even more "minor" *B* alleles will be observed when high-throughput next-generation sequencing is performed. Furthermore, 40 different *B* lineages/loci have been defined in our Indian rhesus macaques. As a consequence, a *B* haplotype is mostly characterised by a set of *B* lineages/loci, which are jointly inherited together (Fig. 2). However, configuration 17, encoding the "major" allele *B*012:01*, harbours, additionally, a "major" *B*022* (Fig. 2, haplotype 17a) or a "major" *B*038* lineage (Fig. 2, haplotype 17b) or even both (Sauermann et al. 2008). This configuration encodes, furthermore, three "minor" alleles (not shown in Fig. 2) and represents the best-characterised rhesus macaque *B* region, since it is present on one of the chromosomes for which the physical map has been published (Daza-Vamenta et al. 2004; Otting et al. 2005; Shiina et al. 2006).

To simplify *Mamu-B* haplotyping, several microsatellites were tested for their use as *B* region-specific markers. Because the Old World monkey's class I *A* region (Doxiadis et al. 2011; Kulski et al. 2004), and especially the *B* region (Doxiadis et al. 2009b), underwent several duplication processes in their evolutionary history, *B*-related microsatellite typing mostly results in highly complex and ambiguous patterns and, therefore, seems unsuitable as a typing tool. However, genotyping with microsatellites MIB6 and MIB7 (Bonhomme et al. 2008; Doxiadis et al. 2009b) resulted in patterns that are often haplotype-specific (Fig. 2). In addition, two microsatellites (MICA and D6S2793) were chosen. These microsatellites are localised within the MIC genes, which are situated next to the *B*

region. As in the HLA system, each haplotype comprises two *MIC* genes (Averdam et al. 2007; Doxiadis et al. 2007b). In contrast to the situation in humans, however, rhesus macaques encode a *MICB* gene in conjunction with either a *MICA* or a *MICAB* gene, of which the latter is considered to be a fusion product of *MICA* and *MICB* (Doxiadis et al. 2007b). The MICA-STR is positioned within exon 5 of the *MICA* gene, whereas D6S2793 (=MICB) is observed within intron 1 of *MICB* as well as of *MICAB*. Thus, there should be only one MICA-STR present per haplotype, whereas two different STR lengths may be observed for D6S2793, which is indeed the case for the region configurations 5 and 10 (Fig. 2). In contrast to the A-related STRs, the four B-related STRs often show different STR lengths within a given *B* haplotype, which are, however, specific for a certain founder animal. Nevertheless, STR typing with the chosen microsatellites mostly leads to an unambiguous definition of the *Mamu-B* haplotypes. As observed for the *A* region, there are only a few *B* configurations: namely, those characterised by the lineages *B*001*, *B*012* and *B*024*, observed at a frequency greater than 10 % in the panel (Fig. 2, red-bordered).

Mamu-DRB haplotyping

In contrast to *Mhc* class I typing in rhesus macaques, DR serotyping has been proven not to be valuable for high resolution typing, most probably due to the lack of suitable antisera. In the last two decades, cloning and sequencing of the most polymorphic *Mamu-DRB* exon 2 have been performed instead, and 16 *DRB* region configurations have been defined in our Indian rhesus macaque colony (Fig. 3). The number of *DRB* loci varies from two to six per haplotype, and 19 different *DRB* loci/lineages have been defined. Nearly all region configurations contain at least one *DRB6* pseudo-gene, but other lineages, or certain alleles of other lineages as well, might not be transcribed (de Groot et al. 2004). Some loci/lineages, e.g., *DRB3*04* and *DRB1*07*, are named according to human lineages as defined by their exon 2 sequence, but most lineages are macaque specific ("W"). In contrast to the *B* region, loci/lineages are shared by several configurations (Fig. 3, colour coded). As holds true for the *A* and *B* regions, however, allelic variation is rarely observed within a certain *DRB* configuration, with the exception of region configuration 3, which is polymorphic for both *DRB1* loci (Fig. 3a–f).

Microsatellite typing with D6S2878, which is situated in intron 2 of nearly all *DRB* genes and possesses STR-length patterns that are haplotype specific, has recently been proven to be an ideal tool for *DRB* typing in various species (de Groot et al. 2008a; de Groot et al. 2009; Doxiadis et al. 2007a; Doxiadis et al. 2009a; Otting et al. 2012). Thus, microsatellite typing with this STR has been performed and has resulted in the definition of 22 *DRB* haplotypes in Indian rhesus macaques (Fig. 3). Only a few *DRB* haplotypes are highly frequent; 1a, 3a and 4 are present at a frequency greater than 10 % (Fig. 3, red-bordered).

Hapl	Serol	*B* locus	*B* locus	*B* locus	*B* locus	MIB7	MIB6	MICA	D6S2793	fr (N=2377)
1	B35	B*006:02				243	130	195	256	0.42%
2	B-	B*055				null	null	198	275	0.25%
3	B2	B*047:01	B*038:01			248	122	198	250/273/279	5.09%
4	B14	B*008:01	B*006:01			null	null	192/198	248/250	7.61%
5	B24	B*024:01	B*019:01			226/262	null/114	198	248/234,250/250/273	13.97%
6	B29	B*015:04	B*044:01			219,249/219,221,247	110/110,112	192/198/201	250/256/273	3.91%
7	B13	B*048:01	B*041:01	B*064:01		null	null	198	248/262/271	7.91%
8	B18	B*055:01	B*058:02	B*063:01		null	null	198	248/256/277	2.02%
9	B20	B*028:01	B*021:01	B*068:06		null/248/250	null/114	189/198	250/256/275	1.43%
10	B25	B*069:01	B*050:02	B*065:01		233,241/ 241,250	null	198	232,248/248/273	1.18%
11	B26	B*001:01	B*007:01/02/03	B*030:02		239, 248, 250, 253, 255	122, (138)	198/201	248/250/273/283	21.37%
12	B31	B*066:01	B*067:01	B*068:01		244, (253)	null	195/198	275	0.34%
13	B32	B*036:01	B*037:01	B*045:01		219,233/219,256	110	189	256/262/275	6.86%
14	B34	B*043:01	B*027:01	B*030:03		216,246	142/145	198	250/267/271	2.61%
15	n.d.	B*075:01/3	B*069:03	B*068:02		246	122	198	250	1.56%
16	B17	B*017:01	B*029:01:02	B*060:02	B*061:01	248,(259)	116	192/198/201	248/250/275/283	6.77%
17a	B11a	B*012:01	B*022:01	B*030:01	B*049:01	241,(244), 249/249	null/122/138/140	192/198	248/250/256/267/279	16.70%
17b	B11b	B*012:01	B*038:01	B*030:01	B*049:01	241,244,249 or 251	138	198	273	

Fig. 2 *Mamu-B* region configurations/haplotypes of Indian rhesus macaques. The order and positioning of the loci/lineages is schematically drawn and does not represent the actual localisation of the genes on the chromosome. *Solidus* indicates the detection of either one or the other STR length, whereas *comma* indicates that both STR lengths have been detected. *STR lengths in brackets* could not always be observed. *n.d.* not defined

Hapl	DRB	STR	DRB	STR	DRB	STR	DRB	STR	DRB	STR	DRB	STR	fr (N=2377)
1a	DRB1*04:06	205	DRB5*03:01	169									12.07%
1b	DRB1*04:11	223	DRB5*03:04	153									0.88%
2	DRB3*04:03	192	DRB*W3:05	225									9.00%
3a	DRB1*03:03	195	DRB1*10:07	196	DRB6*01:03	(202-8)							14.39%
3b	DRB1*03:12	195	DRB1*10:07	201	DRB6*01:03	n.d.							1.14%
3c	DRB1*03:17	193	DRB1*10:08	196	DRB6*01:07	168							0.21%
3d	DRB1*03:18	181	DRB1*10:03	201	DRB6*01:07	182							0.17%
3e	DRB1*03:06	224	DRB1*10:07	197	DRB6*01:03	(202)							3.49%
3f	DRB1*03:06	224	DRB1*10:03	207	DRB6*01:07	182							6.94%
4	DRB1*03:09	237	DRB6*01:01	194	DRB*W2:01	261							19.69%
5	DRB1*04:03	227	DRB*W5:01	208	DRB6*01:07	182							3.91%
6	DRB*W3:03	193	DRB*W4:01	187	DRB6*01:14	216							3.74%
7	DRB1*04:07	193	DRB3*04:09	236	DRB6*01	206							1.01%
8	DRB5*03:07	169	DRB*W28:01	(242)	DRB6*01	196	n.d.	219					1.56%
9	DRB1*04:04	223	DRB*W3:07	195	DRB*W7:02	188	DRB6*01:02	164					2.31%
10	DRB1*07:01	197	DRB3*04:05	235	DRB5*03:03	169	DRB6*01:23	184					6.39%
11	DRB*W25:01	214/6	DRB*W20:02	216	DRB6*01:06	205	DRB6*01:12	214					3.49%
12	DRB1*03:18	181	DRB*W6:03	269	DRB6*01:18	220	n.d.	192					0.13%
13	DRB1*03:18	181	DRB*W6:04	201	DRB*W6:03	275	DRB6*01:04	222	DRB6*01:05	188			1.30%
14	DRB3*04:10	182	DRB*W4:02	187	DRB*W27:01	225	DRB6*01:07	182	DRB6*01:16	206			1.01%
15	DRB*W6:06	199	DRB*W21:04	213	DRB*W26:03	177	DRB6*01:22	236	DRB6*01:11	178			4.38%
16	DRB1*03:10	239	DRB*W1:01	223	DRB*W6:02	279	DRB*W6:09	223	DRB6*01:06	192	DRB6*01:18	210	2.78%

Fig. 3 *Mamu-DRB* region configurations/haplotypes of Indian rhesus macaques. The order and positioning of the loci/lineages is schematically drawn and does not represent the actual localisation of the genes on the chromosome. *Solidus* indicates the detection of either one or the other STR length. Here, *STR lengths in brackets* indicate that the STR lengths have been defined by sequencing, not by STR typing. *n.d.* not defined

Mhc-A–B–DRB haplotyping

In the present study, we were able to analyse 1,383 Indian rhesus macaques (*N* haplotypes=2,377) for their *A–B–DR* region by various molecular techniques, which has resulted in the definition of 176 *Mhc* core haplotypes, 140 of which are different (Suppl. Table 1). Thus, most of the haplotypes are unique to one founder animal. Although the majority of these haplotypes are rare (1 %), 12 haplotypes are observed at a frequency between 2 and 4 %, and two *A–B–DRB* combinations are more frequent (5.6 and 8.5 %), but none exceeds 10 % (Table 1). Whereas the most frequent haplotype (*A1*002:01–B*001:01*, *B*007:01–DRB3*04:03*, *DRB*W3:05*) has been observed in three unrelated founder animals, the second most frequent combination (*A1*004:01:01–B*19:01*, *B*024:01–DRB1*03:09*, *DRB6*01:01*, *DRB*W2:01*) has been introduced into the breeding colony only once. As such, the latter frequency is the result of the breeding success of one single alpha male. Although the number of different *Mamu-A*, *Mamu-B* and *Mamu-DRB* types is limited within the Indian rhesus macaque population tested (17, 18 and 22, respectively, see Figs. 1, 2 and 3), the number of different *Mamu-A–B–DRB* core haplotypes is high. Indeed, *Mamu-A* can combine with diverse *B* and *DRB* haplotypes and vice versa (Suppl. Table 1). An example is given for the most frequent *Mamu-A* type, *A1*004:01:01*, *A4*14*, which has been observed to segregate with a total of 23 different *B–DRB* combinations in the Indian rhesus macaque population (Fig. 4A). Comparable results are gained if the most frequent *B* (*B*001:01*, *B*007:01/02/03*, *B*030:02*) and *DRB* (*DRB1*03:09*, *DRB6*01:01*, *DRB*W2:01*) haplotypes are analysed and can be detected with 26 different *A–DRB* and 23 *A–B* combinations, respectively (Fig. 4B and C).

Extended haplotypes in Indian rhesus macaques

In addition to the core *Mhc*, polymorphism at the adjacent *DQA1*, *DQB1* and *DPB1* loci have been defined in Indian rhesus macaques. *DQA1* and *DQB1* show comparable allelic variation levels with 15 and 17 alleles observed, respectively (Suppl. Table 2). Furthermore, *Mamu-DQA1* and *Mamu-DQB1* segregate as strongly linked pairs (Fig. 5), of

Table 1 *Mamu-A–B–DRB* haplotype frequencies (>2 %) of Indian rhesus macaques

founder	*A1**	*B**	*B**	*DRB*	*DRB*	*DRB*	*DRB*	fr (%) (N=2377)
C 3620, D 597	*001:01/02*	*012:01*	*038:01*	*1*04:06*	*5*03:01*			2.02
A 1435	*001:01/02*	*041:01*	*048:01*	*1*03:03*	*1*10:07*	*6*01:03*		2.19
A 2777, B 2957, D 2849	*002:01*	*001:01*	*007:01*	*3*04:03*	*W3:05*			8.50
A 3026, D 2838	*003:06*	*036:01*	*037:01*	*1*04:06*	*5*03:01*			2.19
D 2837	*004:01:01*	*012:01*	*022:01*	*6*01:14*	*W3:03*	*W4:01*		2.02
D Yusa	*004:01:01*	*017:01*	*029:01:02*	*1*03:03*	*1*10:07*	*6*01:03*		2.86
A 600	*004:01:01*	*019:01*	*024:01*	*1*03:09*	*6*01:01*	*W2:01		5.60
C 426	*004:01:01*	*048:01*	*064:01*	*1*04:06*	*5*03:01*			2.40
A 3070	*007:03*	*019:01*	*024:01*	*6*01:12*	*W25:01	*6*01:06	*W20:02	3.32
B 1435, C 3633, D 2407	*008:01:01*	*001:01*	*007:03*	*1*03:03*	*1*10:07*	*6*01:03*		2.57
B 2774	*008:01:01*	*036:01*	*037:01*	*1*04:03*	*6*01:07*	*W5:01		3.53
A 2957	*008:01:01*	*038:01*	*047:01*	*1*03:06*	*1*10:07*	*6*01:03*		3.49
B 2775	*012:01*	*006:01*	*008:01*	*1*07:01*	*3*04:05*	*5*03:03*	*6*01:23*	3.62
C 2414, C 2419	*023:01*	*012:01*	*022:01*	*1*03:06*	*1*10:03*	*6*01:07*		2.23
								46.53

which *DQA1*01:04/DQB1*06:01*, *DQA1*26:01/DQB1*18:01* and *DQA1*26:02/DQB1*18:11* are the most frequent combinations in our cohort, with 17, 19 and 21 %, respectively. In our panel, *DPB1* is the least polymorphic gene with 13 alleles, of which two are observed at a very low frequency (Suppl. Table 2, grey) and are only present in two unrelated founder animals. Extended *Mhc* haplotypes (*N*=2,156) have been defined for 138 of the 176 founder haplotypes, and 127 have turned out to be unique (Suppl. Table 3). The most common *A–B–DRB* haplotype, *A1*002:01–B*001:01*, *B*007:01–DRB3*04:03*, *DRB*W3:05*, which has been observed in three founders (Table 1; A 2777, B 2957 and D 2849), can be subdivided into two haplotypes on the basis of their *DPB1* alleles (Table 2, grey coloured). Both are present at nearly equal frequencies in the colony. Comparably, most of the common haplotypes that are represented by more than one founder can be subdivided by *DQ–DP* typing and, consequently, some of the founder *A–B–DRB–DQ–DP* haplotypes are observed at less than 2 % (Suppl. Table 3) and are, therefore, not listed in Table 2. Both haplotypes of one founder animal (2957) are segregating with a close high frequency (~4 %, Table 2), indicating that both haplotypes have been introduced at equal levels in the breeding colony.

Linkage disequilibrium of *Mhc* alleles of Indian rhesus macaques

Since the number of different extended haplotypes is high in comparison to the relatively low degree of classes I (*A*, *B*) and II (*DRB*, *DQ* and *DP*) haplotypes/alleles, we analysed the patterns of linkage disequilibrium in the founder population both by a test of global linkage disequilibrium (GLD) between each pair of loci/regions and by assessing the linkage disequilibrium (LD) of individual alleles/haplotypes. The *p* values of individual haplotypes were obtained by an extended exact test and were adjusted for multiple testing (as described in "Materials and methods").

The global tests (Table 3) reveal strong associations amongst the class II loci/regions, and, more particularly, amongst *DRB*, *DQA1*, *DQB1* and *DPB1*, which appear to form a tight linkage group, where each locus/region is in highly significant GLD ($P<0.001$) with the others. *A* and *B* ($P<0.001$), and *B* and *DRB* ($P<0.05$) regions are also in significant GLD. These observations are consistent with relative genomic positions and physical distances amongst the loci (Daza-Vamenta et al. 2004).

The analysis of haplotype LD confirms the strong linkage for class II specificities, as many individual class II haplotypes exhibit significant linkage disequilibrium. For *DQA1–DQB1*, all tandems are in significant LD at the 1 % level, as is true for all but one *DRB–DQA1* and *DRB–DQB1* pair. *DPB1*, which is also in significant GLD with the other class II loci, shows a lower proportion of significant allelic association (1 % level) with the other loci (4/15 with *DQA1*; 5/14 with *DQB1*; and 4/15 with *DRB*) (Suppl. Table 4). The general pictures for linkage disequilibria presented by both methods (i.e., GLD and individual LD) are in close agreement, even if the adjusted *p* values that remain significant for the *A–B* haplotypes after multiple test adjustments refer to rare haplotypes with fewer than three copies (Suppl. Table 4, green coloured).

Mamu-A, *-B* and *-DRB* haplotyping of Burmese macaques

In contrast to the situation involving Indian rhesus macaques, Mamu-A and Mamu-B serotyping of monkeys of other origins could not be undertaken because of the lack of suitable antisera. As a result, only molecular typing data are available for rhesus macaques of Burmese origin. Within the offspring (# 145 animals) of two alpha male founders (4049 and 4052), 13 females, and two Indian/Burmese mix-bred animals, 15 different *Mamu-A* haplotypes could be observed (Table 4). Because no "minor" *A* loci have been detected for some of the Burmese *A* haplotypes, we did not define a locus/haplotype name. One haplotype, however, is identical to the Indian macaque haplotype 5a (Fig. 1 and Table 4, bold), and two others are probably identical as well. Unlike in Indian rhesus macaques, a region configuration lacking the *A1* locus could be observed (Table 4, haplotype 11). Additionally, a duplication of the "major" *A1* locus and configurations with another "major" *A* locus (*A7*) have been defined (Table 4, haplotypes 10 and 9). Furthermore, haplotypes are detected with other "minor" *A* loci such as *A5* or *A6*, which have not yet been observed in Indian rhesus macaques (Table 4, haplotypes 7, 8 and 11) (Doxiadis et al. 2011).

Comparable results have been gained for the *Mamu-B* region of Burmese animals (Table 5). Here, 16 different *B* configurations/haplotypes have been defined by STR typing and full-length sequencing. In addition, two haplotypes could be determined by microsatellite typing but not yet by sequencing. Two *B* region configurations are probably identical in Burmese and Indian rhesus macaques (Fig. 2 and Table 5, haplotypes 8 and 11). Two *B* alleles (*B*066:01* and *B*067:01*) appear to be part of three different configurations. *B*066:01* is observed with *B*067:01* and *B*068:01* in Burmese macaques (Table 5, haplotypes 26 and 27), whereas in Indian macaques, there is one *B* haplotype that combines all three *B* alleles (Fig. 2, haplotype 12). Therefore, it is conceivable that an ancient recombination process built up that haplotype 12 in Indian rhesus macaques or, vice versa, the two Burmese haplotypes. However, the possibility cannot be excluded that the respective *B* loci of the Burmese rhesus macaques have been missed by Sanger sequencing and will show up in next-generation sequencing methods.

Microsatellite typing and subsequent sequencing has allowed definition of 15 different Burmese *DRB* haplotypes (Table 6), three of which are identical to *DRB* haplotypes of Indian rhesus macaques (Fig. 3 and Table 6; haplotypes 1a, 3a and 3f, bold), and one is nearly identical (Fig. 3, haplotype 15 and Table 6: haplotype 15b). Haplotype 3f is frequently observed in both populations. Only one allele (*DRB1*03:09*), which is part of the most common Indian *DRB* region configuration 4 (Fig. 3), is observed together with two different alleles on haplotype 22 in Burmese

A

Mamu-A	Mamu-B	Mamu-B	Mamu-DRB	Mamu-DRB	Mamu-DRB	Mamu-DRB	Mamu-DRB
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*04:04	DRB6*01:02	DRB*W3:07	DRB*W7:02	
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*04:06	DRB5*03:01			
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*04:07	DRB3*04:09	DRB6*01		
A1*004:01:01	B*012:01	B*022:01	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*012:01	B*038:01	DRB1*03:06	DRB1*10:03	DRB6*01:07		
A1*004:01:01	B*012:01	B*022:01	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*004:01:01	B*012:01	B*022:01	DRB1*03:10	DRB6*01:18	DRB*W1:01	DRB*W6:02	DRB*W6:09
A1*004:01:01	B*012:01	B*022:01	DRB1*04:04	DRB*W3:07	DRB*W7:02	DRB6*01:02	
A1*004:01:01	B*012:01	B*022:01	DRB6*01:14	DRB*W3:03	DRB*W4:01		
A1*004:01:01	B*015:04	B*044:01	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*017:01	B*029:01:02	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*019:01	B*024:01	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*004:01:01	B*036:01	B*037:01	DRB1*03:12	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*038:01	B*047:01	DRB1*03:06	DRB1*10:03	DRB6*01:07		
A1*004:01:01	B*048:01	B*064:01	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*048:01	B*064:01	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*048:01	B*064:01	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*004:01:01	B*048:01	B*064:01	DRB1*04:06	DRB5*03:01			
A1*004:01:01	B*050:02	B*065:01	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*004:01:01	B*050:02	B*065:01	DRB1*03:18	DRB6*01:05	DRB*W6:04	DRB*W6:11	DRB6*01:04
A1*004:01:01	B*055:01	B*058:02	DRB1*03:09	DRB6*01:01	DRB*W2:01		

B

Mamu-A1	Mamu-B	Mamu-B	Mamu-DRB	Mamu-DRB	Mamu-DRB	Mamu-DRB	Mamu-DRB
A1*001:01/02	B*001:01	B*007:01/02/03	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*001:01/02	B*001:01	B*007:01/02/03	DRB1*03:06	DRB1*10:03	DRB6*01:07		
A1*001:01/02	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*001:01/02	B*001:01	B*007:01/02/03	DRB1*03:18	DRB1*10:03	DRB6*01:07		
A1*001:01/02	B*001:01	B*007:01/02/03	DRB1*07:01	DRB3*04:05	DRB5*03:03	DRB6*01:23	
A1*002:01	B*001:01	B*007:01/02/03	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*002:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*002:01	B*001:01	B*007:01/02/03	DRB1*03:10	DRB6*01:18	DRB*W1:01	DRB*W6:02	DRB*W6:09
A1*002:01	B*001:01	B*007:01/02/03	DRB3*04:03	DRB*W3:05			
A1*003:02	B*001:01	B*007:01/02/03	DRB1*04:06	DRB5*03:01			
A1*003:06	B*001:01	B*007:01/02/03	DRB1*03:10	DRB6*01:18	DRB*W1:01	DRB*W6:02	DRB*W6:09
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*04:04	DRB6*01:02	DRB*W3:07	DRB*W7:02	
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*04:06	DRB5*03:01			
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*04:07	DRB3*04:09	DRB6*01		
A1*006:01	B*001:01	B*007:01/02/03	DRB*W6:06	DRB*W21:04	DRB*W26:03	DRB6*01:22	DRB6*01:11
A1*006:02	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*006:02	B*001:01	B*007:01/02/03	DRB*W6:06	DRB*W21:04	DRB*W26:03	DRB6*01:22	DRB6*01:11
A1*007:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01		
A1*007:02	B*001:01	B*007:01/02/03	DRB1*04:06	DRB5*03:01			
A1*008:01:01	B*001:01	B*007:01/02/03	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*008:01:01	B*001:01	B*007:01/02/03	DRB*W3:03	DRB*W4:01	DRB6*01:14		
A1*012:01	B*001:01	B*007:01/02/03	DRB1*03:03	DRB1*10:07	DRB6*01:03		
A1*012:01	B*001:01	B*007:01/02/03	DRB1*07:01	DRB3*04:05	DRB5*03:03	DRB6*01:23	
A1*026:01	B*001:01	B*007:01/02/03	DRB*W6:06	DRB*W21:04	DRB*W26:03	DRB6*01:22	DRB6*01:11

C

Mamu-A1	Mamu-B	Mamu-B	Mamu-DRB	Mamu-DRB	Mamu-DRB
A1*001:01/02	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*002:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*002:01	B*012:01	B*022:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*002:01	B*015:04	B*044:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*002:01	B*019:01	B*024:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*012:01	B*022:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*015:04	B*044:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*019:01	B*024:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*048:01	B*064:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*050:02	B*065:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*004:01:01	B*055:01	B*058:02	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*006:02	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*006:02	B*019:01	B*024:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*006:02	B*027:01	B*030:03	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*007:01	B*001:01	B*007:01/02/03	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*008:01:01	B*038:01	B*047:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*011:04	B*006:01	B*008:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*012:01	B*012:01	B*022:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*016:02	B*006:01	B*008:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*016:02	B*019:01	B*024:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*023:01	B*019:01	B*024:01	DRB1*03:09	DRB6*01:01	DRB*W2:01
A1*025:01	B*015:04	B*044:01	DRB1*03:09	DRB6*01:01	DRB*W2:01

Fig. 4 Combinations of the most frequent *Mamu-A*, *Mamu-B*, or *Mamu-DRB* haplotype. **A** *Mamu-A1*004:01:01* combinations with all different *Mamu-B* and *Mamu-DRB* haplotypes. **B** *Mamu-B*001:01:01* combinations with all different *Mamu-A* and *Mamu-DRB* haplotypes. **C** *Mamu-DRB1*03:09* combinations with all different *Mamu-A* and *Mamu-B* haplotypes

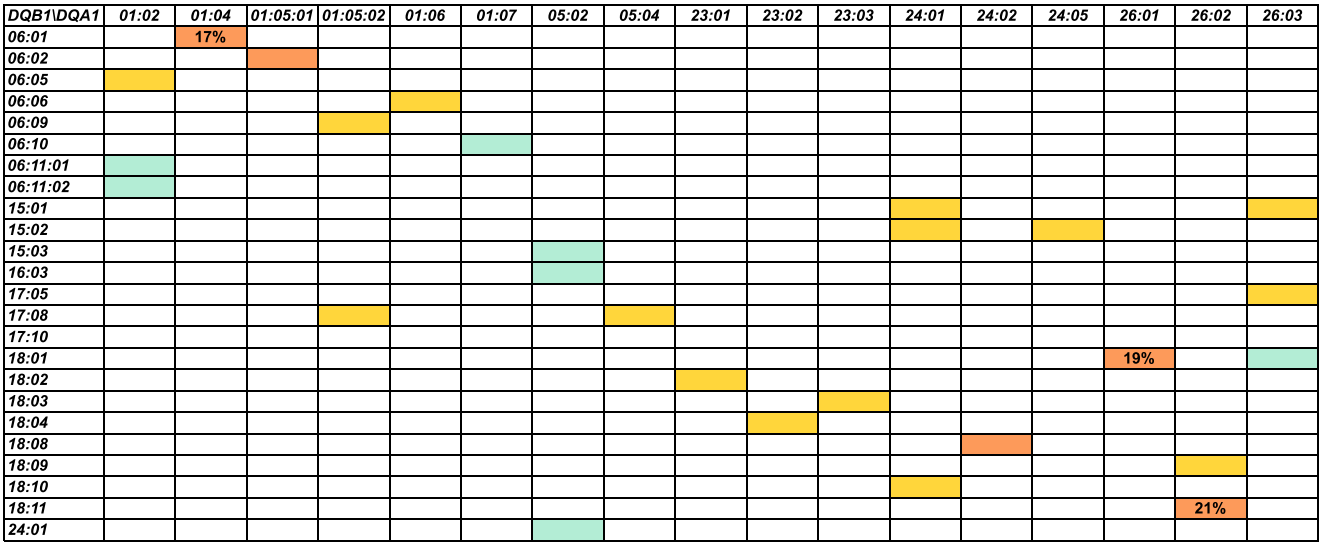

Fig. 5 *DQA1*/*DQB1* pairs observed in Indian and Burmese rhesus macaques. *DQA1*/*DQB1* pairs, which are observed in Indian or Burmese macaques only, are depicted in *yellow* or *turquoise*, respectively, whereas *DQA1*/*DQB1* tandems, which are observed in both populations, are depicted in *orange*

macaques. Since both haplotypes are the most frequent ones in the respective macaque populations, these haplotypes may also be built up by recombination-like events.

Extended haplotypes in Burmese rhesus macaques

In addition to the core MHC, the class II loci *DQA1* and *DQB1* have also been defined in Burmese rhesus macaques. Eleven *DQA1* and 13 *DQB1* alleles have been observed, which are similar or sometimes identical to the *DQ* alleles that are detected in Indian animals. Twelve *Mamu-DQA1*/*DQB1* pairs could be defined, of which those that are observed frequently in Indian monkeys are also present in Burmese macaques (Fig. 5, orange blocks). Most *DQ* combinations, however, are different if one compares monkeys of Burmese and Indian origins, confirming the observation that *DQ* alleles and combinations are strongly origin specific (Fig. 5, yellow and turquoise blocks) (Doxiadis et al. 2003). In total, 24 founder haplotypes of Burmese monkeys could be defined for their *Mamu-A*/*B*/*DRB*/*DQ* content, 22 of which represent

Table 2 Extended *MamuMhc* haplotype frequencies (>2 %) of Indian rhesus macaques

founder	*A**	*B**	*B**	*DRB*	*DRB*	*DRB*	*DRB*	*DQA1**	*DQB1**	*DPB1**	fr (%) (N=2156)
D 597	*001:01/02*	*012:01*	*030:01*	*1*04:06*	*5*03:01*			*26:02*	*18:11*	*05:01*	2.04
A 1435	*001:02*	*041:01*	*048:01*	*1*03:03*	*1*10:07*	*6*01:03*		*26:01*	*18:01*	*06:02*	2.41
B 2957	*002:01*	*001:01*	*007:01*	*3*04:03*	*W3:05			*24:01*	*18:10*	***01:01***	4.45
A 2777, D 2849	*002:01*	*001:01*	*007:01*	*3*04:03*	*W3:05			*24:01*	*18:10*	***15:01***	4.92
A 3026, D 2838	*003:06*	*036:01*	*037:01*	*1*04:06*	*5*03:01*			*26:02*	*18:11*	*05:01*	2.41
D 2837	*004:01:01*	*012:01*	*022:01*	*W3:03	*W4:01	*6*01:14		*23:01*	*18:02*	*02:01*	2.23
C 426	*004:01:01*	*041:01*	*048:01*	*1*04:06*	*5*03:01*			*26:02*	*18:11*	*07:01*	2.64
D Yusa	*004:01:01*	*017:01*	*029:01:02*	*1*03:03*	*1*10:07*	*6*01:03*		*26:01*	*18:01*	*07:01*	3.15
A 600	*004:01:01*	*019:01*	*024:01*	*1*03:09*	*W2:01	*6*01:01*		*01:04*	*06:01*	*15:01?*	6.17
A 3070	*007:03*	*019:01*	*024:01*	*6*01:12*	*W25:01	*6*01:06*	*W20:02	*26:02*	*18:11*	*08:01*	3.66
A 2957	*008:01:01*	*038:01*	*047:01*	*1*03:06*	*1*10:07*	*6*01:03*		*26:01*	*18:01*	*06:04*	3.85
B 2774	*008:01:01*	*036:01*	*037:01*	*1*04:03*	*W5:01	*6*01:07*		*24:02*	*18:08*	*01:01*	3.90
B 2775	*012:01*	*006:01*	*008:01*	*1*07:01*	*3*04:05*	*5*03:03*	*6*01:23*	*26:03*	*15:01*	*05:01*	3.99
C 2414	*023:01*	*012:01*	*022:01*	*1*03:06*	*1*10:03*	*6*01:07*		*01:02*	*06:05*	*07:02*	2.13
											47.96

"?" indicates that the allele has not (yet) been ascertained; "/" indicates that one or the other allele has been defined

Table 3 Global linkage disequilibrium between each pair of *Mhc* loci of Indian rhesus macaques

	A	*B*	*DRB*	*DQA1*	*DQB1*	*DPB1*
A		**	–	–	–	–
B	*P*=0.0000		*	–	–	–
DRB	*P*=0.05143	*P*=0.04293		**	**	**
DQA1	*P*=0.09050	*P*=0.76651	*P*=0.000		**	**
DQB1	*P*=0.07317	*P*=0.14292	*P*=0.000	*P*=0.000		**
DPB1	*P*=0.12290	*P*=0.09208	*P*=0.000	*P*=0.000	*P*=0.000	

Below diagonal: FDR-adjusted *p* probability values obtained after 9,000,000 steps of the Markov chain procedure (see "Materials and methods"). Above diagonal: ** indicates significant linkage disequilibrium at 1 %, * indicates significant linkage disequilibrium at 5 % and – indicates no significant linkage disequilibrium

different combinations (Table 7). There is only one Burmese *Mhc* haplotype (B 4052, Table 7, bold), of which the *A*, *B*, *DRB* and *DQ* types are also detected separately in Indian macaques. The same *A–B–DRB–DQ* combination on one haplotype, however, has not been observed in the studied Indian cohort. Thus, no extended Burmese *Mhc* haplotype is identical to haplotypes observed in Indian rhesus macaques.

Discussion

Although a lower number of Burmese rhesus macaques has been studied in comparison to Indian rhesus monkeys, the total of *A*, *B*, or *DRB* haplotypes encountered in both populations is nearly identical (e.g., 17 Indian *Mamu-A* [Fig. 1] versus 15 Burmese *Mamu-A* [Table 4] haplotypes). The number of different Indian *Mamu-A*, *Mamu-B* or *Mamu-DRB* haplotypes is even lower when compared to those in cynomolgus macaques. In a population of Indonesian cynomolgus monkeys, which was much smaller than the Indian rhesus macaque panel, more *Mafa-A*, *Mafa-B* and *Mafa-DRB* haplotypes have been observed (e.g., 17 Indian *Mamu-A versus* 29 *Mafa-A* haplotypes) (Otting et al. 2012). Furthermore, *Mhc* typing of Chinese rhesus macaques as well as Burmese macaques from other breeding colonies has shown that the high degree of *Mamu-A* and *Mamu-B* variation detected in these animals is comparable to the variation observed in cynomolgus monkeys (Li et al. 2012; Liu et al. 2012; Naruse et al. 2010; Otting et al. 2007; Otting et al. 2008). In contrast, the low degree of *A*, *B* and *DRB* variation

Table 4 *Mamu-A h*aplotypes of Burmese rhesus macaques

Hapl	*A1*	*A1/A7*	*A2*	A4-A6	D6S2854	D6S2859	ori	fr (%) (N=258)
1d?	*A1*007:05*		*A2*05?*		192	173		0.78
?	*A1*032:02:01*		?		181,184,192,196	167		3.49
?	*A1*041:01*		?		181,192	176		2.33
?	*A1*050:01*		?		173,181,192	171,236		9.69
?	*A1*051:01*		?		173,181	167,201		3.88
?	*A1*056:03*		?		188	175		1.16
?	*A1*110: 01*		?		177,192	145		6.20
2a?	*A1*008:01:02*		?		173,181,196	171,203	In?	19.3
5a	***A1*004:01:01***			***A4*14:03***	**181,185,196**	**171**	**In**	6.98
7	*A1*049:04*			*A5*30:02*	190,196,216	171,189		3.88
7?	*A1*049:02*			?	190,196,216	171,189		1.55
8	*A1*007:01*			*(A6*01:02)*	181,185,192	176,186		6.59
9	*A1*059:01*	*A7*02:01*			177,181,212	185		2.33
10	*A1*043:01*	*A1*065:01*			173,185,192,196	169,176,201		6.59
11			*A2*05:26*	*A6*01:01*	192	199		25.19

hapl haplotype, *n.d.* not defined, *Ori* origin, *In* Indian, "*In*?" indicates that the haplotype may be the same as in Indian rhesus macaques, "?" indicates that the respective allele, locus/lineage, or haplotype cannot be not ascertained or is not known, "()" indicate that an allele has not always been detected, "/" indicates that one or the other STR length has been observed depending on the founder haplotype

Table 5 *Mamu-B* haplotypes of Burmese rhesus macaques

Hapl	*B**	*B**	*B**	*B**	MIB7	MIB6	MICA	D6S2793	ori	fr (%) (N=255)
18	***066:01***	?			239, 251	null	198	258		3.53
19	*004:01*	*063:01?*			216, 221, 246	112, 133	198	275		3.92
20	*007:03*	*039:01*			249/253	114, 122/4	198	258/273		14.51
21	*038:02*	*101:01*			244	112	189	273		6.27
22	*044:03*	*036:01:01*			224, 253	112	192	256		3.53
23	*050:02*	*065:01*			null	null	198	279		2.35
24	*056:01*	***067:01***			253	130	192	269		0.39
25	*063:02*	***067 01***			238, 245, 251	null	198	248		3.92
26	***066:01***	***067:01***			247, 268	null	198	242		7.84
27	***066:01***	***068:01***			246, 268	null	198	260		25.49
8?	*055:01*	*058:02*	?		n.d.	n.d.	n.d.	n.d.	In?	1.18
11?	*001:01:01*	*007:02*	?		239	114, 122	198	269	In?	3.92
28	*003:03:03*	*043:01*	*073:01*		239, 246	114	189	273		7.06
29	*021:02*	*028:02:01*	*061:05*	*068:04*	246, 257	null	201	242		6.67
30	*064:02*	*098:01*	*099:01*	*100:01*	246	null	198	275		1.96
31	*068:02*	*075:01*	*069:03*		null	122	198	250		5.10
?	n.d.	n.d.			239, 253	null	192	248		0.78
?	n.d.	n.d.			253	null	192	null		1.57

hapl haplotype, *n.d*. not defined, *Ori* origin, *In* Indian, "*In*?" indicates that the haplotype may be the same as in Indian rhesus macaques, "?" indicates that the respective allele, locus/lineage, or haplotype cannot be not ascertained or is not known, "()" indicate that an allele has not always been detected, "/" indicates that one or the other STR length has been observed depending on the founder haplotype

Table 6 *Mamu-DRB* haplotypes of Burmese rhesus macaques

Hapl	*DRB*	STR	*DRB*	STR	*DRB*	STR	*DRB*	STR	*DRB*	STR	ori	fr (%) (N=258)
1a	***1*04:06***	**209**	***5*03:01***	**169**							**In**	1.55
17	*1*03:21*	181	*1*03:23*	209								10.85
18	*4*01:02*	289	*5*03:06*	173								6.20
3a	***1*03:03***	**195**	***1*10:07***	**196**	***6*01:03***	**(202)**					**In**	6.59
3f	***1*03:06***	**228**	***1*10:03***	**199**	***6*01:07***	**182**					**In**	**10.85**
19	*1*03:26*	222	*1*10:18*	188	*6*01:07*	182						2.33
20	*6*01:12*	212	**W4:03*	n.d.	**W25:06*	202						1.16
21	*1*03:21*	181	*1*03:22*	209	*1*10:03*	175						4.26
22	*1*03:09*	229	*6*01:09*	226	**W25:07*	181						25.19
23	**W20:02*	230	*6*01:12*	222	n.d.	205						4.65
24	*1*03:18*	181	**W6:03*	271	*6*01:18*	215	n.d.	190				0.39
25	*1*04:17*	229	*6*01:07*	183	**W5:02*	n.d.	*6*01*	208				10.85
26	*1*03:26*	214	*1*07:03:03*	207	*6*01:24*	168	**W26:04*	177	*6*01:20*	208		5.43
27	*1*07:04*	231	*4*01:04*	220	*6*01:18*	186	*6*01*	186	**W25:05*	185		7.75
15b	**W6:06*	213	**W21:04*	213	**W26:04*	177	*6*01:22*	232	*6*01:08*	176	In	1.94

STR (202) has been defined by sequencing

hapl haplotype, *n.d*. not defined, *Ori* origin, *In* Indian, "*In*?" indicates that the haplotype may be the same as in Indian rhesus macaques, "?" indicates that the respective allele, locus/lineage, or haplotype cannot be not ascertained or is not known, "()" indicate that an allele has not always been detected, "/" indicates that one or the other STR length has been observed depending on the founder haplotype

Table 7 *Mhc* haplotype frequencies of Burmese rhesus macaques

Founder	*A1/A2*	*A1/A7*	*A4-A6*	*B**	*B**	*B**	*B**	*DRB*	*DRB*	*DRB*	*DRB*	*DRB*	*DQA1**	*DQB1**	Fr (%) (N=258)
B 4049	*A1*004:01:01*		*A4*14:03*	*003:03:03*	*043:01*	*073:01*		*1*03:21*	*1*03:23*				*01:04*	*06:01*	6.98
C 4104	*A1*007:01*			*056:01*	*067:01*			*1*03:18*	*W6:03*	*6*01:18*	?		*24:05*	*06:01*?	0.39
C 2412	*A1*007:01*?			*68:02*	*075:01*	*069:03*		*4*01:02*	*5*03:06*				*01:07*	*06:10*	5.04
C 2387	*A1*007:01*		*A6*01:02*	n.d.	n.d.			*4*01:02*	*5*03:06*				*01:07*	*06:10*	1.16
C 4064*	*A1*007:05*			*066:01/*	*067:01/*	*056:01/*	*063:02*	*1*03:21*	*1*03:22*	*1*10:03*			*01:04*	*06:01*	0.78
B 4052	***A1*008:01:02***			***001:01:01***	***007:02***			***1*03:06***	***1*10:03***	***6*01:07***			***26:02***	***18:11***	3.88
C 4072	*A1*008:01:02*			*007:03*	*039:01*			*1*03:06*	*1*10:03*	*6*01:07*			*26:02*	*18:11*	4.65
D 4065	*A1*008:01:02*			*007:03*	*039:01*			*1*04:17*	*6*01:07*	*W5:02	*6*01:20*?		*24:02*	*18:08*	7.36
C 4050	*A1*008:01:02*			*044:03*	*036:01:01*			*1*03:21*	*1*03:22*	*1*10:03*			*01:04*	*06:01*	3.49
D 4064*	*A1*032:02:01*			*066:01/*	*067:01/*	*056:01/*	*063:02*	*1*04:17*	*6*01:07*	*W5:02	*6*?		*24:02*	*18:08*	3.49
C 4109	*A1*041:01*			*050:02*	*065:01*			*1*03:06*	*1*10:03*	*6*01:07*			*01:02*	*06:11:01*	2.33
A 4052	*A1*043:01*	*A1*065:01*		*021:02*	*028:02:01*	*061:05*	*068:04*	*1*03:03*	*1*10:07*	*6*01:03*			*26:01*	*18:01*	6.59
C 4078	*A1*049:02*			n.d.	n.d.			*1*04:06*	*5*03:01*				*26:02*	*18:11*	1.55
D 4074	*A1*049:04*		*A5*30:02*	*063:02*	*067:01*			*1*03:21*	*1*03:23*				*26:03*	*17:10*	3.88
D 4050	*A1*050:01*			*064:02*	*098:01*	*099:01*	*100:01*	*6*01:08*	*6*01:22*	*W6:06	*W21:04	*W26:04	*05:02*	*15:03*	1.94
D 4101, C 4095	*A1*050:01*			*066:01*	*067:01*			*1*07:04*	*4*01:04*	*6*01:18*	*6*01*	*W25:05	*05:02*	*24:01*	7.75
D 4078	*A1*051:01*			*004:01*	*063:01*?			*6*01:12*	*W20:02	?	*5*03:01*?		*05:02*	*16:03*	3.88
D 4077	*A1*056:03*			*055:01*	*058:02*			*6*01:12*	*W4:03	*W25:06			*01:02*	*06:11:02*	1.16
C 4077	*A1*059:01*	*A7*02:01*		*007:03*	*039:01*			*1*03:26*	*1*10:18*	*6*01:07*			*01:07*	*06:06L*	2.33
C 4090	*A1*110: 01*			*038:02*	*101:01*			*6*01:12*	*W20:02	?			*05:02*	n.d.	0.78
C 4065	*A1*110:01*			*038:02*	*101:01*			*1*03:26*	*1*07:03:03*	*6*01:24*	*W26:04	*6*01:20*	*01:05:01*	*06:02*	5.43
A 4049, C 4074	*A2*05:26*		*A6*01:01*	*066:01*	*068:01*			*1*03:09*	*6*01:09*	*W25:07			*26:01*	*18:01*	25.19

n.d. not defined, "?" indicates that the respective allele has not yet been defined or ascertained, *DQB1*06:06L* is a newly defined allele (submitted to NHP IPD-MHC database for allele definition), **B* alleles of animal 4064 could not be assigned to haplotypes, thus "/" indicates that one and/or the other B alleles belong to the respective haplotype

observed in Indian rhesus macaques is also detected for single-nucleotide polymorphisms (SNPs) in these monkeys (Ferguson et al. 2007; Smith and McDonough 2005). All results are in accord with the notion that the Chinese population expanded by a factor of more than three and separated from the Indian population ~162 thousand years ago. After separating, the Indian population maintained its ancestral population size until ~50 to 20 thousand years ago, when it was reduced by a factor of four, probably owing to a severe bottleneck due to geographic events like desiccation and/or a subsequent onset of glacial conditions (Hernandez et al. 2007; Smith and McDonough 2005). Thus, the reduced number of *A*, *B* and *DRB* haplotypes in Indian rhesus macaques most likely does not reflect a sampling bias. However, this possibility cannot be completely excluded without sampling of wild-caught rhesus monkeys from various parts of India.

According to the low number of *A*, *B* and *DRB* region variation, one would expect the number of core *Mhc-A/B/DRB* haplotypes defined in Indian rhesus monkeys to be lower than the number of haplotypes observed in Burmese rhesus or in Indonesian cynomolgus macaques. This, however, is not the case; the Indonesian cynomolgus macaque colony, based on ~28 founder animals, possesses 32 different *Mhc* haplotypes, and in Indian rhesus macaques, originating from ~140 founders, a comparable number of different haplotypes (i.e. 140) have been defined. This approximation can be confirmed by a random sampling of 32 of the 176 Indian *Mhc* founder haplotypes, 30 of which turned out to be dissimilar (Suppl. Table 5). Thus, the number of different *Mhc* haplotypes in Indian rhesus macaques is nearly as high as in Indonesian cynomolgus monkeys. However, nearly every haplotype has a unique *Mafa-A*, *Mafa-B* and *Mafa-DRB* pattern due to allelic variation and different region configurations, whereas in Indian rhesus macaques *Mhc* haplotypes generate their diversity by a recombination of a relatively small number of *A*, *B* and *DRB* segments.

Additionally, the GLD between each pair of *Mhc* loci/regions of Indian rhesus macaques shares some similarities with certain *Mhc* loci in humans with respect to a highly significant GLD between *DRB*, *DQA* and *DQB*. Moreover, as in humans, GLD was detected between *A* and *B* on the one hand, and *B* and *DRB* on the other hand. However, Indian rhesus macaques showed significant GLD between *DPB1* and the other three class II loci/regions, whilst in humans, such a linkage has not been consistently observed between *DPB1* and any other loci (Sanchez-Mazas et al. 2000). This may be due to differences in recombination hotspots. As no pairs of loci exist with more than three haplotypes, no meaningful GLD can be detected in cynomolgus monkeys. As observed in our cynomolgus macaque panel of Indonesian origin, *Mhc* haplotypes with unique *A*, *B*, and *DRB* patterns are also described in cynomolgus macaques of other origins and in Chinese rhesus macaques (Karl et al. 2008; Otting et al. 2007; Saito et al. 2012). These results confirm LD measurements made by the correlation coefficient of alleles from frequency-matched SNPs. The SNP data showed that in Indian rhesus macaques, the LD extends much further than LD observed in European humans, whereas the Chinese rhesus macaque population shows little LD, even for SNPs that are physically very close (Hernandez et al. 2007). Although the different *Mhc* haplotypes of Indian rhesus monkeys are most probably a result of recombination-like events between the *A*, *B* and *DR* regions, only ten crossing-over events have been mapped within the core MHC of the >2,300 Indian haplotypes analysed, of which five are *A–B* and four are *B–DRB* recombinations (one remained undefined). Thus, the recombination rate in Indian rhesus macaques seems to be slightly lower for *A–B* and much lower for *B–DRB* than in humans, where recombination frequencies of 0.21 and 0.94 for *A–B* and *B–DRB*, respectively, have been described (Martin et al. 1995a, b). As a consequence, the observed Indian *Mhc* haplotypes appear to be old entities, which were already present in the founder animals. Thus, recombination-like events were gathered over long evolutionary time spans, one of which most likely started to occur in the distant past and within a reduced founder population. There is no evidence that recombination frequencies in Indian rhesus macaques as such differ from those in other macaque species or in rhesus macaques of other origins. Additionally, selection pressure may have given advantage to the offspring of animals in which a recombination had taken place. This phenomenon is also observed in cynomolgus macaques from the island of Mauritius, where seafarers introduced a small founder population of animals ~500 years ago. Six highly frequent *Mhc* haplotypes appear to account for almost all *Mhc* diversity in those monkeys, in which the offspring after ~100 generations show about 30 % recombinants of these original *Mhc* haplotypes (Mee et al. 2009; Wiseman et al. 2007). Similar observations were made in chimpanzees, which experienced a selective sweep targeting the MHC region (de Groot et al. 2008b; de Groot et al. 2002). Thus, recombination-like processes appear to be a mechanism whereby a diminished genetic repertoire in an isolated and small founder population may be expanded. As a consequence, at the population level, the animals possess different haplotypes warranting the possibility that a wide range of adaptive immune responses may be directed to one and the same, as well as to various, pathogens.

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