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Definition of *Mafa-A* and *-B* haplotypes in pedigreed cynomolgus macaques (*Macaca fascicularis*)

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Abstract The major histocompatibility complex (MHC) class I *B* gene/allelic repertoire was investigated in a pedigreed population of cynomolgus macaques of mixed Indonesian/Malaysian origin. The *Mafa-B* alleles detected in this cohort are mostly specific for a given geographic area, and only a small number of alleles appears to be shared with other populations. This suggests the fast evolution of *Mafa-B* alleles due to adaptation to new environments. In contrast to humans, the *B* locus in Old World monkeys displays extensive copy number variation. The *Mafa-B* and previously defined *-A* gene combinations segregate in families and thus allowed the definition of extended haplotypes. In many cases it was possible to assign a particular *Mafa-I* allele to one of these *Mafa-A/B* haplotypes as well. The presence of a large number of stable haplotypes in this cohort of animals, which was pedigreed for up to eight generations, looks promising for developing discriminative MHC typing tools that are less cumbersome. Furthermore, the discovery of 53 unreported *Mafa-B* sequences expands the lexicon of alleles significantly, and may help in understanding the complex organisation of the macaque *B* region.

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Introduction

The cynomolgus macaque (*Macaca fascicularis*), also known as the crab-eating or long-tailed macaque, is widely used as an animal model in biomedical studies. Currently this species is applied as often as the commonly used rhesus macaque (*Macaca mulatta*). Cynomolgus monkeys are used as models for infectious diseases, such as AIDS, SARS and tuberculosis, as well as for transplantation research (McAuliffe et al. 2004; Wiseman et al. 2007; Aoyama et al. 2009; Mee et al. 2009; Reed et al. 2009). Owing to use of macaques in immune-related research, thorough investigations of their major histocompatibility complexes (MHC) are required. The MHC represents a multigene family in which the proteins play a key role in the generation of adaptive immune responses in vertebrate species. The class I and II genes of the MHC display abundant polymorphism that has a profound impact on features such as disease susceptibility, organ transplantation, and reproduction success.

The MHC systems in humans (HLA) and in other primate species have been studied extensively (Bontrop 2006). The orthologues of the classical *HLA-A* and *-B* genes, which are involved in the presentation of intracellularly processed peptides to cytotoxic T cells, are present in the rhesus and cynomolgus macaque (Boyson et al. 1996; Krebs et al. 2005). However, in these animals the genes have undergone several rounds of duplication and display copy number variation (Anzai et al. 2003; Daza-Vamenta et al. 2004; Otting et al. 2005). Whereas in humans only one copy of the *HLA-A* and *-B* genes is

present, in macaques seven *A*-like genes are distinguished. On each haplotype, one polymorphic gene is observed, named *Mamu-A1* or *Mafa-A1*, in combination with one or two oligomorphic genes designated *Mamu-* or *Mafa-A2* up to *-A7*, respectively (Otting et al. 2007; Pendley et al. 2008; Campbell et al. 2009; Kita et al. 2009). The same organisation is also applicable to the pig-tailed macaque (*Macaca nemestrina*) (Lafont et al. 2007; Wu et al. 2008).

The situation for the *HLA-B* orthologues in macaque species is even more complicated. In one rhesus macaque, the MHC region was completely sequenced, yielding one complete haplotype of 5.3 megabase-pairs. On this haplotype, 19 distinct *Mamu-B* genes were present, of which 14 genes have the potential to code for bonafide proteins (Anzai et al. 2003; Daza-Vamenta et al. 2004; Bonhomme et al. 2008; Doxiadis et al. 2009). For the MHC of cynomolgus macaque a BAC-based contig map was constructed (Watanabe et al. 2007). Although the degree of gene multiplication is less than in the rhesus macaque, this contig map still contains 12 distinct *Mafa-B* like loci. Sequencing studies at the cDNA level, however, have shown that only two or three genes per haplotype are transcribed at considerable levels (majors) in rhesus- and in cynomolgus macaques (Krebs et al. 2005; Otting et al. 2005, 2008; Pendley et al. 2008). At least one other *B*-like gene, characterised by low levels of polymorphism and transcription (minors), is present on all haplotypes. It has been designated *Mamu-I, Mafa-I,* and *Mane-I* in the respective species of macaques (Urvater et al. 2000; Robinson et al. 2003). On the completely sequenced MHC-region of the rhesus macaque, this locus is designated as the *Mamu-B3* gene (Daza-Vamenta et al. 2004; Doxiadis et al. 2009).

The sequencing of macaques from different geographic areas has shown that each population has its own characteristic set of *Mamu/Mafa-A* and *-B* alleles, and only a few alleles are shared between cohorts/populations (Krebs et al. 2005; Otting et al. 2008; Campbell et al. 2009). This is in contrast to the data that were observed for the MHC class II sequences obtained from these species (Otting et al. 2002; Doxiadis et al. 2006; O'Connor et al. 2007; de Groot et al. 2008). Moreover, the interspecies sharing of MHC class I alleles in rhesus and cynomolgus macaques is in the same order of magnitude as the intraspecies sharing (Otting et al. 2007).

We have access to cynomolgus macaques that have been pedigreed for eight generations, and the origin of the animals was determined based on mtDNA analyses. In an earlier study, we showed that the *Mafa-A* alleles are mostly unique for this population. Hence, a unique set of *Mafa-B* alleles is expected to be present in the same animals. The question is whether these *B*-alleles segregate in a stable linkage to the already described *Mafa-A* sequences in these animals. Should this be the case, MHC typing on this cohort may be then performed using less cumbersome techniques: for instance, those based on microsatellite or SNP analyses.

Furthermore, expanding the lexicon of *Mafa-B* alleles may provide more insight into the organisation of the macaque B-region, and may help in the definition of different lineages and loci, resulting in a more appropriate nomenclature.

Materials and methods

Animals and cell lines

The cynomolgus macaques used in this study had originally been kept at the University of Utrecht, where the animals were housed in social groups for up to eight generations. Recently, however, the colony of 135 animals was transferred to the new facilities at the Biomedical Primate Research Centre (BPRC), for the purpose of behavioural studies. The BPRC had access to blood samples drawn during health-checks, and lymphoblastoid cell-lines were established. The origin of the animals was determined by mitochondrial DNA (12S rRNA) analyses (Doxiadis et al. 2003; de Groot et al. 2008), and the founder animals appear to have originated either in the Indonesian islands or in continental Malaysia.

cDNA, cloning, and sequencing

For all animals used in this study RNA was isolated from lymphoblastoid B-cells (Rneasy kit, Qiagen) and subjected to One-Step RT-PCR, as recommended by the supplier (Qiagen or Promega). The primers 5′MBS: AATTCATGGCGCCCCGAACCCTCCTCCTGC and 3′ MBS: CTAGACCACACAAGACAGTTGTCTCAG were used that anneal specifically to *Mhc-B* transcripts in macaques (Boyson et al. 1996). Furthermore, for a subset of the animals the generic class I primers 5′ GGACTCA G A A T C T C C C C A G A C G C C G A G and 3′ TCTCAGTCCCTCACAAGGCAGCTGTC were used. The final elongation step was extended to 30 min to generate a 3′dA overhang. The RT-PCR products were cloned using the InsT/Aclone kit (Fermentas) or the PCR cloning kit (Qiagen). After transformation, 32 to 48 colonies were picked for plasmid isolation. Sequencing reactions were performed using the BigDye terminator cycle sequencing kit, and samples were run on an automated capillary sequencing system (Applied Biosystems Genetic Analyzer 3100).

Phylogenetic analyses and nomenclature

Sequences were analysed using Sequence Navigator Software version 1.0.1 (Applied Biosystems) and MacVector™ version 10.6.0 (Oxford Molecular Group), followed by manual adjustments. After the alignments of all *Mamu*- and *Mafa-B* exon 1–4 sequences using the MacVector software, version 10.6.0, phylogenetic analysis was performed with the *phylogeny.fr* pipeline (Dereeper et al. 2008) using maximum likelihood (ML) of the software PhyML 3.0 with the substitution model HKY85 with 4 categories, the gamma shape parameter of 0.407, a transition/transversion ratio of 2.130, and a SH-like approximate Likelihood-Ratio Test (aLRT) for statistical test of branch support. For tree rendering the pipeline uses the program TreeDyn 198, and the output tree is rooted using the mid-point rooting method.

The *Mafa-B* alleles have been named according to published nomenclature proposals (Klein et al. 1990; Ellis et al. 2006); however, the number of *Mafa-B* lineages has exceeded 100, and for the lineage numbers three digits have been introduced. In this document, the designations that were published previously are extended with a zero, and contain five ciphers. Two alleles that are highly similar receive the same lineage number, but any difference is indicated by the allele number (fourth and fifth cipher). If two alleles have synonymous base-pair differences, they receive an identical allele number, and the difference is indicated by a sixth and seventh digit. For example, *Mafa-B*0950101* has synonymous differences in comparison to *Mafa-B*0950102* and non-synonymous ones as compared to *Mafa-B*09502*. The novel alleles were submitted to the EMBL-EBI database (accession numbers FM212793-FM212843, FM246485-FM246500, FN423784, FN546179, and FN546180) and to the Non-human primate section of the IMGT/MHC Immuno Polymorphism Database (Robinson et al. 2003).

Results and discussion

Mafa-B, *-I* and *-A* sequences

In the cohort of 115 cynomolgus macaques, three to eight different *Mafa-B* sequences per animal were detected, with varying levels of transcription. The sequences, of which at least three identical clones were present, were reported as alleles. In most cases, these alleles were also confirmed in different animals. In total, 69 *Mafa-B* alleles were present, of which 16 were previously described by other research groups (Uda et al. 2005; Lafont et al. 2007; Pendley et al. 2008; Wu et al. 2008; Campbell et al. 2009; Kita et al. 2009). The other 53 sequences have been submitted to EMBL-EBI and to the MHC-NHP database (Robinson et al. 2003), and have been catalogued. A list of the *Mafa-B* alleles is provided, including the accession numbers, and the reference animals (Table 1).

In most animals, sequences that are alleles of the *Mafa-I* gene, the equivalent of the oligomorphic *Mamu-I* locus, were detected (Urvater et al. 2000). The *Mamu-I/Mafa-I* locus has the characteristics of a nonclassical, with low levels of polymorphism and transcription. Only 12 *Mafa-I* sequences met the criterion of three identical clones, and eight of them have been submitted as novel alleles (Table 1). Nevertheless, the *Mafa-I* gene appears to be present on all haplotypes.

The *Mhc-A* region-derived class I alleles of the animals in this cohort were sequenced in an earlier study (Otting et al. 2007). In those analyses, primers were used that are specific for *Mhc-A* alleles in macaques. However, in some animals the *Mafa-A1* locus was not amplified by this primer set. In the present study, RT-PCR was performed with macaque *Mhc-B*-specific primers, and with the generic class I primers for those animals previously lacking a *Mafa-A1* sequence. The use of these generic primers resulted in the detection of six new alleles for the *Mafa-A1* locus, and one for both the *Mafa-A2* and the *-A5* genes (Table 1), and as such extends the earlier reported data.

Mafa-A, *-B,* and *-I* haplotypes

Since pedigree data were available, it was possible to determine the combinations of *Mafa-B* alleles on one chromosome (haplotype). Most haplotypes contain two major alleles, in combination with one or two alleles with lower levels of transcription, or minors, as based on the number of picked clones within a PCR sample. Unfortunately, it can not be excluded that some alleles are incorrectly considered as minors due to primer inconsistencies. Haplotypes with only one or three majors were also observed. Moreover, it was possible to extend these *Mafa-B* combinations with specific *-A* region configurations/haplotypes that were described in an earlier study (Otting et al. 2007). Combinations of *Mafa-A* and *-B* alleles that are segregating, and are observed in at least two related animals are listed (Table 2). Although more than one *Mafa-A* gene is present on the cynomolgus chromosome, only alleles of the highly polymorphic *Mafa-A1* locus are provided for sake of convenience. Six additional *Mafa-A/B* haplotypes were seen in only one animal, whereas nine sequence combinations were ambiguous, and are not listed in the table. Further analyses on these animals, and on their offspring are needed to find out if these are recombinations.

The number of at least 24 distinct haplotypes is high, though they appear to be stable entities in this population of macaques; recombination was seldom observed. Only one case of crossing over between the *Mhc-A* and *-B* region is

Table 1 The MHC class I alleles detected in the cohort of 115 animals. The alleles that were published before are depicted in bold. For the *Mafa-A* loci only the new alleles are listed. The abbreviations ind, mau, chi, fil, and vie stand for Indonesian, Mauritian, Chinese, Filipino, and Vietnamese, respectively

Designation	Accession number	origin	Reference animals
*Mafa-B*00303*	FM212793		kippa, cuba
Mafa-B*00601	AB195436	??	dobo, laba
*Mafa-B*00602*	FM212794		upupa
*Mafa-B*00704*	FM212795		anastasia, francisca
*Mafa-B*0110102*	FM212796		alfa, vivaa
Mafa-B*01201	AB195442/EU203690	??/ind	alfa, vivaa
*Mafa-B*01302*	FM212797		upupa
*Mafa-B*01602*	FM212839		ratata, sayonara
*Mafa-B*01603*	FM212798		k2
*Mafa-B*01802*	FM212840		freya, riva
Mafa-B*02201	AB195452	??	bilboa
*Mafa-B*02302*	FM212799		pagwa, mokka
Mafa-B*02702	EF442022	mau	alfa, nausikaa
*Mafa-B*02704*	FN546179		sumatra
*Mafa-B*03202*	FM212800		walhalla, kota
Mafa-B*03301	AY958128	vie	ratata, sayonara
Mafa-B*03601	AY958131	vie, chi	linea, nigra
*Mafa-B*03702*	FM212801		k2
*Mafa-B*04003*	FM212802		rastafa
Mafa-B*0440101	AY958141	mau	clint, geisha
*Mafa-B*0440102*	FM212841		dojo, dadaa
*Mafa-B*04404*	FM212803		alfa, kraa
Mafa-B*04501	AY958143/EU203717	mau/ind	vivaa, hippo
Mafa-B*04601	AY958144	mau	clint, geisha
*Mafa-B*04602*	FM212804		dojo, dadaa
Mafa-B*04701	AY958145	mau	pagwaa, weldraa
*Mafa-B*0480102*	FM212805		kippa
*Mafa-B*04902*	FM212806		riva, milva
Mafa-B*05001	AY958149	mau	alfa, vivaa
Mafa-B*05101	AY958150 /EU203718	mau/ind	vivaa, hippo
*Mafa-B*05102*	FM212807		blo, canada
*Mafa-B*05402*	FM212808		alfa, geisha
Mafa-B*05501	EF442021	mau	salsaa
*Mafa-B*05505*	FM212809		dojo, dadaa
*Mafa-B*05506*	FM212810		freya, riva
*Mafa-B*05507*	FM212811		trespa, vodafo
*Mafa-B*05704*	FM212812		walhalla, kota
Mafa-B*05901	EU203723, EU392117	ind/fil	jawa, nanaea
*Mafa-B*0630102*	FM212813		k65
*Mafa-B*06302*	FM212814		kippa
Mafa-B*07101	EU203681	ind	gayo
Mafa-B*07201	EU203684	ind	geisha
*Mafa-B*08802*	FN546180		jura
*Mafa-B*09401*	FM212815		pagwaa, weldraa
*Mafa-B*09402*	FM212816		pedro, gayo
*Mafa-B*0950101*	FM212817		hoeba, geisha
*Mafa-B*0950102*	FM212818		ganza, zazaa

Table 1 (continued)

Designation	Accession number	origin	Reference animals
*Mafa-B*09502*	FM212819		mamba, voila
*Mafa-B*09503*	FM212820		linea, nigra
*Mafa-B*09601*	FM212821		upupa
*Mafa-B*09602*	FM212822		pagwa, mokka
*Mafa-B*09801*	FM212823		mamba, voila
*Mafa-B*09901*	FM212824		upupa, jawa
*Mafa-B*10001*	FM212825		laba, joshua
*Mafa-B*10002*	FM212826		trespa, vodafo
*Mafa-B*10101*	FM212827		pagwa, mokka
*Mafa-B*10201*	FM212828		anastasia, francisca
*Mafa-B*10301*	FM212829		alfa, kraa
*Mafa-B*10401*	FM212830		vodafo, juga
*Mafa-B*10501*	FM212831		walhalla, kota
*Mafa-B*10601*	FM212832		vip, rastafa
*Mafa-B*10701*	FM212833		blo, canada
*Mafa-B*10801*	FM212842		ganza, stoa
*Mafa-B*10901*	FM212834		kippa, cuba
*Mafa-B*11001*	FM212843		ganza, stoa
*Mafa-B*11101*	FM212835		ratata, sayonara
*Mafa-B*11201*	FM212836		pagwa, mokka
*Mafa-B*11301*	FM212837		clint, roza
*Mafa-B*11401*	FM246492		laba, joshua
Mafa-I*0109	AB195465	??	trespa, vodafo
Mafa-I*0110	DQ979884	mau	vivaa, hippo
Mafa-I*0111	DQ979885	mau	yabaa, linea
*Mafa-I*011302*	FM246495		walhalla, kota
*Mafa-I*0115*	FM246493		freya, riva
*Mafa-I*0116*	FM246494		giacomo
*Mafa-I*0117*	FM246496		pagwa, mokka
*Mafa-I*0118*	FM246497		kippa, cuba
*Mafa-I*0119*	FM246498		cornea, francisca
*Mafa-I*0120*	FM246499		vip, rastafa
Mafa-I*110101	DQ979886	mau	gayo, nausikaa
*Mafa-I*110102*	FM246500		pagwaa, weldraa
*Mafa-A1*01805*	FM246486		laba, joshua
*Mafa-A1*01806*	FM246489		cornea, salvadoro
*Mafa-A1*06204*	FM246490		ontarijo
*Mafa-A1*07103*	FM246487		joshua
*Mafa-A1*09203*	FM246488		kippa
*Mafa-A1*10401*	FM246491		upupa
*Mafa-A2*0534*	FM246485		cornea
*Mafa-A5*3004*	FN423784		kippa, sjerpa

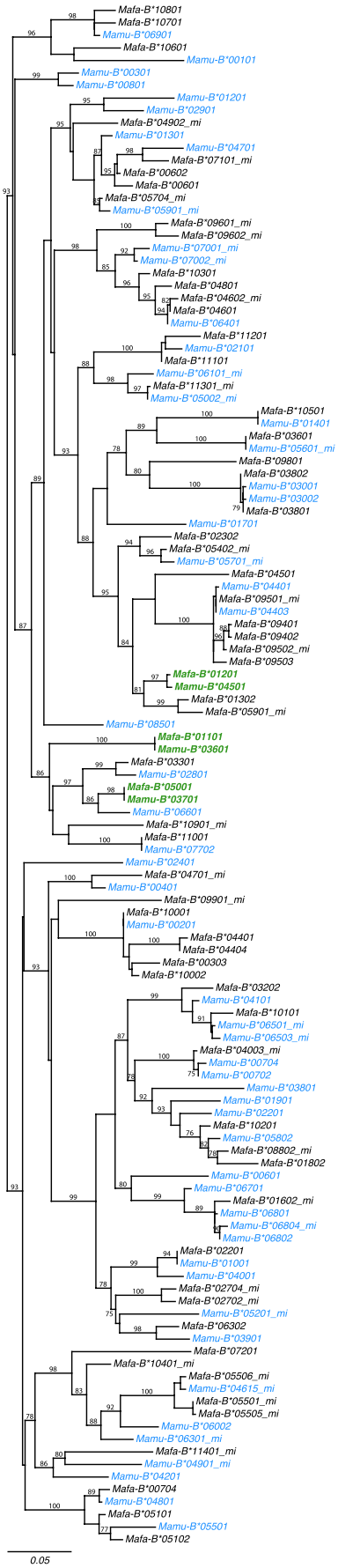
seen; *Mafa-A1*03101* is present in conjunction with two different *Mafa-B* combinations (7 and 8 in Table 2). For eleven of the 24 *Mafa-A/-B* haplotypes it was possible to add an associated *Mafa-I* allele. Preliminary studies with *DRB*-microsatellites (Doxiadis et al. 2007; de Groot et al. 2008) indicate that the *Mafa-A/B* haplotypes are also linked to DRB-STR patterns. Further investigation should reveal whether in the future these animals and their offspring can be typed for the class I alleles by means of this extremely fast and accurate typing technique.

Table 2 *Mafa-A/Mafa-B/Mafa-I* combinations in this cohort of animals. For *Mafa-A*, only the highly polymorphic *Mafa-A1* locus is listed

	Mafa-A	*Mafa-B* major			*Mafa-B* minor		*Mamu-I*	N
1	*A1*00101*	*B*03601*	*B*09503*					4
2	*A1*00301*	*B*0950102*						2
3	*A1*00702*	*B*10002*			*B*05507*		*I*0109*	23
4	*A1*01002*	*B*00601*			*B*08802*			5
5	*A1*01003*	*B*04404*	*B*10301*					15
6	*A1*01805*	*B*10001*			*B*11401*			11
7	*A1*03101*	*B*02302*	*B*10101*	*B*11201*	*B*09602*		*I*0117*	5
8	*A1*03101*	*B*09402*					*I*110101*	6
9	*A1*03102*	*B*09401*			*B*04701*		*I*110102*	9
10	*A1*04002*	*B*10801*						7
11	*A1*05801*	*B*10601*					*I*0120*	7
12	*A1*05901*	*B*00303*			*B*10901*		*I*0118*	17
13	*A1*06001*	*B*0110102*	*B*01201*	*B*05001*				2
14	*A1*06301*	*B*0440101*			*B*05501*	*B*04601*		17
15	*A1*06302*	*B*04501*	*B*05101*				*I*0110*	7
16	*A1*06401*	*B*09801*			*B*09502*		*I*0116*	20
17	*A1*0650102*	*B*03301*	*B*11101*		*B*01602*	*B*02704*	*I*0119*	6
18	*A1*06602*	*B*01802*			*B*05506*	*B*04902*	*I*0115*	18
19	*A1*06801*	*B*0440102*			*B*05505*	*B*04602*		6
20	*A1*06901*	*B*11001*	*B*0950101*					5
21	*A1*07001*	*B*00704*	*B*10201*					5
22	*A1*07101*	*B*03202*	*B*10501*		*B*05704*		*I*011302*	9
23	*A1*07201*	*B*05102*	*B*10701*					7
24	*A1*09203*	*B*06302*			*B*0480102*			2

In our cohort of animals, 16 *Mafa-B* alleles were detected that were already described in studies on other cynomolgus populations. To determine whether these alleles were arranged in haplotypes that are shared between populations a comparison was made. Three of these combinations were observed. Pendley and coworkers have already described the sharing of *B*01201/B*05001/2* and *B*04501/B*05101* allele-combinations in Indonesian and Mauritian cohorts (Pendley et al. 2008), which illustrates that the Mauritian animals originate in the archipelago. Probably the animals were introduced to the island by merchant ships in the Dutch Golden Age (Sussman and Tattersall 1986). Both haplotypes are present in our animals, and are listed, respectively, as 13 and 15 in Table 2. The first one was extended to *A1*06001/B*0110102/B*01201/B*05001*. In Pendley's cohort this combination of *Mafa-B* alleles is seen in an animal that also transcribes *Mafa-A1*06003*. This allele differs by two basepairs from *Mafa-A1*06001*. With genotyping based on reference-strand conformational analysis (RSCA), Krebs and co-workers found the combination *B*0430101/B*0440101/B*0460101* in a cohort of Mauritian animals (Krebs et al. 2005). In our animals, however, we observed this set without *B*0430101* (Table 2, haplotype 14). It is possible that this allele is a minor, and therefore was probably missed in our cloning procedures. In the RSCA study the *B*0430101* peak is also low in comparison to the peaks of the other two alleles. The three shared haplotypes were present in cohorts that, like most of our animals at the BPRC, originate in the Indonesian Islands. Sharing of haplotypes with the recently described Filipino cynomolgus macaques was not observed (Campbell et al. 2009).

The restricted sharing of alleles in different populations of cynomolgus macaques, and moreover the recombination of similar alleles into other haplotypes in these populations, suggests that the diversity within the *Mafa-B* region has been the result of recombination and reshuffling of *B*-like loci during evolution. The cohort under study has been pedigreed for up to 8 generations, however, the haplotypes listed are observed in maximal five generations of related animals. The finding that within this cohort only one crossing between *Mafa-A* and *Mafa-B* is observed may be due to this relatively small number of generations. The fact that each cohort of animals has its own set of alleles and haplotypes necessitates investigation of the MHC for each cohort under study. Only within a breeding colony are the haplotypes more or less stable and predictable, and once the haplotypes are inventoried, robust MHC typing based on microsatellite analyses may be performed. Recombinations can be traced by using microsatellites spanning the whole MHC region.



◀ **Fig. 1** Phylogenetic analyses of *Mafa-B* alleles detected in this study and *Mamu-B* alleles in known haplotypes of Indian and Chinese rhesus monkeys. The analyses are based on the exon 2, 3 and 4 sequences. Alleles that seem identical in this three may have basepair differences in other exons. The two species of macaques are indicated by different colors. *Mafa/Mamu-B* alleles of the one shared haplotype are depicted in green. The extension of allele-names with mi means that these are minors; alleles with relatively low transcription

Comparison to rhesus macaques

To investigate the presence of shared alleles between cynomolgus and rhesus macaques, phylogenetic analyses were performed on all 182 *Mafa-B* and 206 *Mamu-B* sequences, published thus far. Only transcribed alleles, based on analyses of cDNA were included. A subset of these analyses, comprising the exons 2, 3 and 4 of the alleles detected in the present cynomolgus macaques and alleles of known haplotypes in rhesus macaques, is displayed in Fig. 1. The phylogenetic tree shows that cynomolgus and rhesus sequences are fully intertwined, and each clade contains alleles of both species. In total, 17 sets of alleles were observed that are identical for all exons (Table 3), and this number is in the same order of magnitude as the shared alleles among different populations of cynomolgus macaques. For instance, 25 *Mafa-B* alleles were described in the cohort Filipino cynomolgus macaques, of which three alleles were shared with animals of Indonesian origin (Campbell et al. 2009). Next to these shared alleles are several that differ by only one or two basepairs. Identity at the predicted amino-acid level for these alleles was not investigated.

The sharing of haplotypes between cynomolgus and rhesus macaques was also investigated. Only one combination of three *Mafa-B* sequences was present in the rhesus macaque, and interestingly this was the *B*0110102/B*01201/B*05001* combination mentioned above. Moreover, this *Mamu-B*03601/2/B*04501/2/B*03701* haplotype is one of four combinations that is shared by Indian and Chinese rhesus macaques, apart from a few basepair differences. The cynomolgus version differs by three basepairs in *Mafa-B*01201* from the Indian rhesus macaque haplotype. The presence of the haplotype in different cohorts of rhesus monkeys and in the Indonesian/Mauritian cynomolgus macaques suggests that its ancestor was already present before the separation of both species. The stability of the haplotype during macaque evolution may have been caused by a significant advantage in the combat of intercellular pathogens. It is also possible that the sharing of the haplotype results from hybridisation. Molecular studies have revealed that introgression from rhesus macaques to cynomolgus monkeys has occured into the Indo-Chinese peninsula (Bonhomme et al. 2009).

Table 3 Shared alleles in cynomolgus and rhesus macaques

Mafa-B	*Mamu-B*
*B*0110102*	*B*0360101*
B*02201	*B*01001*
B*0310101	*B*0010101*
B*0350101	*B*0280201*
B*03601	*B*05601*
B*0380101	*B*0300302*
B*0400101	*B*00703*
B*0410101	*B*01801*
B*05001	*B*03701*
*B*05506*	*B*04608*
B*06701	*B*07601*
B*08701	*B*09901*
*B*0950101*	*B*04403*
*B*09503*	*B*04404*
*B*10501*	*B*01401*
*B*10701*	*B*06903*
*B*11001*	*B*07702*

The alleles that were published earlier are depicted in bold. The shaded *Mafa-B* alleles were published earlier and are not detected in our cohort of animals.

Similarities in the genetics of the macaque MHC class I regions are at this stage unfortunately not reflected in the nomenclature for *Mhc-B* alleles. The recent renaming of *Mhc-A* alleles has led to a nomenclature in which the distinct *A* loci and lineage numbers are compatible for all macaque species. For the *Mhc-B* region in macaques, however, it is not yet possible to assign the transcribed alleles to distinct *B* loci on the chromosome (e.g. *Mamu-B1, -B2, -B3* etc.). However, it would be useful to adjust the lineage numbers (first three ciphers after the asterisk) so that the same numbers refer to similar sequences in different macaque species. Should more information become available in the near future on the number and order of *B* genes/loci on the macaque haplotypes, the allele designations may then easily be extended by a cipher following the *B*, without affecting the lineage numbers.

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