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The killer cell Ig-like receptors (KIR) modulate immune responses through interactions with MHC class I molecules. The *KIR* region in large cohorts of rhesus and cynomolgus macaque populations were characterized, and the experimental design enabled the definition of a considerable number of alleles (n = 576) and haplotypes, which are highly variable with regard to architecture. Although high levels of polymorphism were recorded, only a few alleles are shared between species and populations. The rapid evolution of allelic polymorphism, accumulated by point mutations, was further confirmed by the emergence of a novel *KIR* allele in a rhesus macaque family. In addition to allelic variation, abundant orthologous and species-specific *KIR* genes were identified, the latter of which are frequently generated by fusion events. The concerted action of both genetic mechanisms, in combination with differential selective pressures at the population level, resulted in the unparalleled rapid evolution of the *KIR* gene region in two closely related macaque species. The variation of the *KIR* gene repertoire at the species and population level might have an impact on the outcome of preclinical studies with macaque models. ***The Journal of Immunology*****, 2020, 204: 1770–1786.**

Natural killer cells provide an early defense mechanism against infectious diseases and tumor formation by their ability to recognize and kill cells with aberrant MHC class I expression (1–3). This immune surveillance is modulated by killer cell Ig-like receptors (KIR), which are expressed on NK cells and subsets of T cells (4–6). These gene products are transmembrane receptors consisting of two or three extracellular domains, which can facilitate ligand interaction, and a long or short cytoplasmic tail that can utilize an intracellular ITIM or ITAM, respectively (3, 7). In humans, the gene family encoding the *KIR* genes is located on chromosome 19 q13.4, and its complexity is reflected by allelic polymorphism, gene copy number variation (CNV), chromosomal recombination, and alternative splicing (8–11).

Comparison of the *KIR* gene cluster in humans and other primate species suggests a first round of expansion to occur between 30 and 45 million years ago (12), which involved two progenitor genes. The *KIR3DX1* lineage is nowadays represented by a single copy in primates but expanded in cattle, whereas the *KIR3D* progenitor gene was subjected to diversification by tandem duplications, deletions, and recombinations (13, 14). This expansion resulted in a head-to-tail gene cluster encoding a broad repertoire of *KIR* genes, the overall architecture of which is conserved in primates. Species-specific diversification, however, may have resulted in differential lineage expansions and sequence variation, which is reflected by few *KIR* orthologs that are shared between distantly related primate species. Primate *KIR* genes are phylogenetically classified into lineages based on receptor structure and ligand specificity. In humans, lineage I includes *KIR2DL4* and *KIR2DL5*, lineage II *KIR3DL1/L2/S1*, the expanded lineage III *KIR2DL1-3*, *KIR2DS1-5*, and the pseudogenes, and lineage V *KIR3DL3*, respectively. The initial expansion of lineage III members can be traced back to orangutans, and its emergence seems to have coevolved with the presence of *HLA-C*–like genes, which are present on ~50% of the contemporary orangutan *MHC* haplotypes (15). In chimpanzees and humans, the lineage III *KIR* genes expanded further, and their genomic clusters comprise 17 and 13 *KIR* genes, respectively, but only four genes are considered orthologs (14). Old-World monkeys, like macaques (genus *macaca*), expanded mainly lineage II *KIR* genes (*KIR3D*), which may be associated with their expanded MHC class I repertoire (16, 17).

Macaques are geographically the most widespread nonhuman primates (NHP) that diversified from the human and great ape lineage ~25 million years ago and include ~20 species that share a habitat spanning from northeast Africa to Asia. Rhesus and cynomolgus macaques (*Macaca mulatta*, *Macaca fascicularis*) are closely related species that diverged from each other ~1–3 million years ago. Rhesus macaques are distributed across South, East, and Southeast Asia, whereas cynomolgus macaques mainly inhabit the mainland and islands of Southeast Asia. Geographically distinct populations, such as the Indian, Burmese, and Chinese rhesus macaques and the insular cynomolgus macaques, emerged by means of natural barriers and resulted in intraspecific variation. The Isthmus of Kra, which is the narrowest part of the Malaysian peninsula, separates the cynomolgus macaques that inhabit the mainland of Southeast Asia in a northern (Cambodia, Thailand, and Vietnam) and southern (Malaysian peninsula) population, and it is suggested that this geographical barrier restricts gene flow (18, 19). In Indochina, rhesus and cynomolgus macaques may come across

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The sequences presented in this article have been submitted to the European Nucleotide Archive (https://www.ebi.ac.uk/ena/) under accession number PRJEB33481.

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Abbreviations used in this article: CNV, copy number variation; KIR, killer cell Ig-like receptor; NHP, nonhuman primate.

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each other, and bidirectional introgression is substantiated by shared genetic features (20–22). For example, ancestral haplotypes of the highly polymorphic MHC class I region are encountered in rhesus macaques and cynomolgus macaques (16, 23, 24), whereas extensive allele sharing had been documented for the *MHC* class II genes (25).

Several sequencing platforms have been used to characterize the macaque *KIR* gene region, particularly in Indian rhesus macaques (10, 26–30). Data on the *KIR* gene cluster and repertoire in other rhesus macaque populations is limited. For cynomolgus macaques, only the Mauritian animals were characterized thoroughly (31–33). This population was founded by a few animals that were introduced to the island by human interference ~500 y ago and, therefore, have a restricted *KIR* gene content.

Rhesus and cynomolgus macaques are used as preclinical models for many infectious and autoimmune diseases, as the immune response and pathologies reflect the human situation (34, 35). The origin of macaques, however, vary between different research facilities and might impact the disease phenotype, which is, for example, reported for SIV/AIDS studies in Indian and Chinese rhesus macaques (36, 37). The presence or absence of certain *KIR* genes, in combination with the MHC class I ligands, have been associated with disease susceptibility in both humans (6) and macaques (38, 39). A comprehensive overview of the *KIR* gene content and repertoire of different natural macaque populations is, however, lacking, despite the potential refinement for macaque models. Therefore, we set out to analyze the *KIR* transcriptomes of cohorts of rhesus and cynomolgus macaques of different geographical origins, which probably experienced varying selective pressures. Our observations illustrate in both highly related macaque species and populations an unparalleled form of rapid evolution of *KIR* genes that is propelled by point mutations and complex chromosomal recombinations, which generate novel gene entities and result in highly variable haplotype architectures.

Materials and Methods

Samples and origin

Forty-six rhesus macaques, comprising seven families, and 70 cynomolgus macaques, comprising 11 families, were selected from the self-sustaining colony housed at the Biomedical Primate Research Centre. During the annual health checks, EDTA or heparin whole blood samples were obtained, and PBMCs were isolated from the latter. PBMC samples from 16 Chinese rhesus macaques, comprising seven families, were obtained from the Biomedical Primate Research Centre Bio-bank.

The geographical origin of most rhesus macaques was known based on importation records, such as the families from the Indian, Chinese, and Burmese populations. Additional transcriptome data of Indian rhesus macaques was incorporated from a previous *KIR* study conducted by our laboratory (10). The geographical origin of the cynomolgus macaques was mainly deduced by phylogenetic comparison of mitochondrial 12S rRNA gene segments (40). With regard to this data, we defined three cynomolgus macaque populations, which originated from the mainland of Malaysia, from the Indonesian and Malaysian islands, and from Mauritius. The mainland population was further divided into populations north and south of the Isthmus of Kra. The origin of three cynomolgus macaques (Ji0603077, J15028, J16019) could not be determined unambiguously. In addition, previously reported KIR haplotypes of Mauritian cynomolgus macaques were added to the analysis (33).

RNA isolation and KIR transcriptome amplification

Total RNA was extracted directly from EDTA whole blood samples or from $\pm 15 \times 10^6$ PBMCs with RNeasy Mini Kit (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions. First-strand cDNA was synthesized with the RevertAid First Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) using oligo(dT)18 primers. Genomic DNA was extracted from EDTA whole blood samples by a standard salting-out procedure, or from $\pm 15 \times 10^6$ PBMCs with an AllPrep RNA/DNA Mini Kit (Qiagen) according to the manufacturer's instructions.

Full-length KIR transcripts were obtained by amplification of total cDNA with a *KIR2DL04*-specific and two *KIR1D/KIR3D*-generic primer sets in accordance with a previously reported protocol (10). These primer sets were cross-reactive for the different rhesus and cynomolgus macaque populations. PCR products were size-selected by gel electrophoresis ($\pm$1250 bp) and purified using a GeneJet Gel Extraction Kit (Invitrogen). The samples were pooled and purified twice using AMPure XP Beads (Beckman Coulter, Woerden, the Netherlands) at a 1:1 bead-to-DNA volume ratio. The DNA concentration of purified pooled samples (>1 μg total DNA) was measured using the Qubit dsDNA HS Assay Kit and Qubit 2.0 Fluorometer (Thermo Fisher Scientific).

PacBio SMRTbell libraries were generated according to Pacific Biosystems "Procedure and Checklist - Amplicon Template Preparation and Sequencing," and sequencing was performed using a PacBio Sequel platform with a 10 h video time using sequencing kit versions 2.0, 2.1, and 3.0, which was performed by the Leiden Genome Technology Center.

PacBio data analysis

Circular consensus sequences were selected for high-read quality (value of 0.99 or higher), and demultiplexed based on unique barcoding.

Geneious Prime 2019 software was used to map the circular consensus sequences to a database, consisting of reported rhesus macaque and novel cynomolgus macaque KIR sequences, to identify 100% matching reads (100% overlap, 0% mismatch, maximum ambiguity = 1). The unused reads of related animals were grouped and were de novo assembled. The consensus of each de novo contig was trimmed for the primer sequence, and phylogenetically aligned with the rhesus and cynomolgus macaque database. De novo sequences were confirmed when shown to segregate or when identified in two separate PCRs and were subsequently submitted to the European Nucleotide Archive and assigned an accession number (https://www.ebi.ac.uk/ena/).

Macaque KIR nomenclature

The nomenclature of the KIR transcripts in rhesus and cynomolgus macaques follows the general guidelines of the KIR nomenclature report for NHP (41). In brief, the name of the gene indicates the number of domains (1D, 2D, or 3D) and the signaling function (S or L). The inclusion of a "W" implies a workshop gene, which indicates a gene that is divergent on the basis of phylogenetic analysis but lacks sufficient reliability because of the low frequency or because of the absence of genomic sequencing or family studies. The inclusion of "Q" indicates that it is questionable whether the transcripts are feasible. Two digits distinguish the different genes, and an asterisk followed by three digits distinguishes alleles. Two additional digits indicate synonymous variation.

Novel cynomolgus macaque *KIR* sequences were compared with a database of 342 reported rhesus macaque sequences (10, 26, 29, 30) and newly identified transcripts by phylogenetic analysis, using the Neighbor-Joining Tree-Building Method (best tree mode) in MacVector software (MacVector, Cambridge, U.K.). Phylogenetic clusters were confirmed by the Maximum Likelihood Comparison and Neighbor-Joining Tree-Building Methods in MEGA7 software, and all methods provided similar trees. Rhesus and cynomolgus macaque *KIR* sequences that clustered together with a close phylogenetic distance were considered orthologs and received matching *KIR* gene names. Clusters of *Mafa-KIR* sequences that diverged from the other sequences according to sequence comparison and phylogenetic analysis received a workshop number. In addition, workshop numbers were assigned to cynomolgus macaque *KIR* genes that were thought to be the result of recombination events, as these are considered novel entities. In contrast, recombinant *KIR* genes in rhesus macaques are named after an allele of the gene that contributes the largest gene segment, as is described for this species in the NHP nomenclature report (41). The previously reported 46 *Mafa-KIR* sequences (33), all of which originated from the Mauritian cynomolgus macaque population, were also named.

Macaque KIR haplotype origin and gene frequencies

The origin of each *KIR* haplotype was categorized per macaque population. The populational origin of the rhesus macaques determined the haplotype origin, as none of the rhesus macaques had parents from different populations. The *KIR* haplotypes defined in cynomolgus macaques that had their roots in the mainland of Malaysia (north or south), the Malaysian/Indonesian islands, or in Mauritius were categorized based on the defined origin. In cynomolgus macaques with mixed roots (parents from the mainland and from islands), the origin of the *KIR* haplotypes was determined by the sequencing of parental genomic DNA, the origin of which was known,

using an *Mafa-KIR3DL20* exon 4–specific primer set (forward: 5′-GAAGAGACGGTCATCCTGCAGT-3′; reverse: 5′-ACTCCCCCTATGTGTTGTCAGC-3′) and an *Mafa-KIR1D* exon 4–specific primer set (forward: 5′-GAAGAGACGGTCATCCTGCAGT-3′; reverse: 5′-ACTCCCCCTATGTGTTGTCAGC-3′). Thermal cycling conditions were denatured at 98°C for 2 min, followed by 32 cycles of 98°C for 20 s, 63°C for 25 s, and 72°C for 1 min. Amplicons of ~180 bp were size-selected by gel electrophoresis and purified using a GeneJet Gel Extraction Kit (Invitrogen). Sanger sequencing was used, and the populational haplotype origin could be determined on the basis of three single-nucleotide polymorphisms.

The frequency of a *KIR* gene in rhesus and cynomolgus macaques, or in one of the populations, was determined based on the presence of at least a single copy on a haplotype, the origin of which was determined, rather than on the presence of the gene in an individual.

Results

Definition of rhesus and cynomolgus macaque populations and their KIR transcriptomes

The *KIR* transcriptomes of 62 rhesus and 70 cynomolgus macaques covering different populations were subjected to analysis (Fig. 1) (10). All macaque samples belong to families that comprised two or more individuals, which allowed us to confirm the segregation of alleles but also to define haplotypes (Figs. 2, 3). The origin of the rhesus macaques was documented thoroughly and included Burmese (n = 14), Chinese (n = 16), and Indian (n = 32) origins (Fig. 1). Based on the phylogeny of mitochondrial DNA sequences (40), origins of the cynomolgus macaques were mapped to the mainland of Southeast Asia (n = 26), the Malaysian/Indonesian islands (n = 4), or Mauritius. The mainland population could be further divided into populations north (n = 23) and south (n = 3) of the Isthmus of Kra (Fig. 1). For 19 cynomolgus macaques, a mixed origin was documented, whereas for 21 animals only the origin of a single parent could be determined. To expand our population panel, we included previously reported *KIR* transcriptome data from 30 Indian rhesus macaques (10) and 30 Mauritian cynomolgus macaques (33). Altogether, three rhesus and four cynomolgus macaque populations were subjected to comparison for their *KIR* repertoire.

Allele discovery: abundant levels of species-specific allelic variation in macaques

Up to now, 342 distinct rhesus macaque *KIR* alleles that were mainly isolated from Indian animals have been identified (10, 26, 29, 30). In the current cohort that comprises 32 Indian rhesus macaques, again, another 48 unreported *KIR* alleles were discovered, indicating extensive allelic variation within this population. All Indian rhesus macaque *KIR* alleles could be clustered into 22 different *KIR* genes (Table I). From the Burmese and Chinese cohorts, 73 and 117 novel *KIR* alleles were isolated, respectively, which clustered to previously reported but also newly discovered *KIR* gene entities (Table I). During the course of this study, 34 rhesus macaque *KIR* genes were defined, which comprised 238 novel alleles, and 64 reported *Mamu-KIR* alleles were confirmed (Supplemental Fig. 1). The emergence of one of the novel alleles was observed in rhesus macaque R04104, which is expected to be homozygous for the *KIR* region, as it ought to receive two copies of *Mamu-KIR3DL05*006:01* via the H21-A haplotype (Fig. 2). However, one copy of the *Mamu-KIR3DL05*006:01* allele shows nonsynonymous mutations at two positions in the D1 domain (T > C and G > T), thereby generating a novel allele, designated *Mamu-KIR3DL05*032*. This de novo allele segregated with its corresponding haplotype (H21-B) into two offspring of R04014, and its existence was further substantiated by independent Sanger sequencing (Supplemental Fig. 2).

Most allelic variation is controlled by *Mamu-KIR3DL07*, *-KIR3DL20*, *-KIR3DL01*, and, to a lesser extent, *-KIR2DL04*. The Indian and Burmese populations share four *KIR* alleles, whereas only a single allele was shared between the Indian and Chinese populations (*Mamu-KIR3DS06*016*), the Burmese and Chinese populations (*Mamu-KIR3DL05*007:01*), and all three populations (*Mamu-KIR3DL01*019:03*) (Fig. 4).

Knowledge of the *KIR* cluster in cynomolgus macaques is mainly confined to the artificially introduced Mauritian population, and 49 alleles are documented (31, 33). In the current cohort from different populations, we identified 267 novel alleles that clustered into 55 distinct *KIR* genes (Supplemental Fig. 1, Table II). In addition, 10 of the 46 previously reported *Mafa-KIR* sequences identified in Mauritian cynomolgus macaques were confirmed (31, 33). The highest level of allelic variation was observed for *Mafa-KIR3DL20* and *-KIR1D*, followed by *-KIR2DL04*, *-KIR3DL01*, and *-KIR3DL07*. The different populations seem to have highly unique allelic *KIR* repertoires. A single allele was shared between the northern mainland and the Indonesian/Malaysian populations (*Mafa-KIR3DLW23*001*) and the southern mainland and Mauritian populations (*Mafa-KIR2DL04*002*), whereas two alleles were in common between the Indonesian/Malaysian islands and Mauritian populations (*Mafa-KIR3DLW13*003*, *Mafa-KIR3DLW26*001*), and the southern mainland, the Indonesian/Malaysian islands, and Mauritian populations (*Mafa-KIR1D*030Q*, *Mafa-KIR3DL20*002*) (Fig. 4).

To sum this up, 579 *KIR* alleles were identified in the rhesus and cynomolgus macaque populations studied. Only two alleles were shared between both highly related species: namely, *Mamu-KIR3DLW12*002*/*Mafa-KIR3DLW12*006* and *Mamu-* and *Mafa-KIR3DLW18*001* (Fig. 4). The low number of allele sharing between the macaque species as well as the different populations suggests fast evolution. This is within lineages mainly mediated by point mutations, and contrasts the extensive sharing documented for *MHC* class II and, to a much lesser extent, for *MHC* class I alleles (42).

New KIR *genes in macaques are generated by recombination*

Considering a shared ancestor living 1–3 million years ago, one might expect highly similar repertoires of orthologous *KIR* genes in rhesus and cynomolgus macaques, as is observed for the closely related Bornean and Sumatran orangutan species (15). Apparently, however, this is not the case in both macaque species, as their *KIR* gene repertoires possess species-specific and a differential number of *KIR* gene moieties. Moreover, the 34 rhesus and 55 cynomolgus macaque *KIR* genes that are defined by sequence comparison and phylogenetic analysis indicate a greater expansion of the *KIR* gene repertoire in macaques as compared with humans and other primate species, for which 17 or fewer *KIR* genes were identified (41, 43, 44). The question to be answered, therefore, is how are new *KIR* genes generated. One mechanism that might explain the expanded macaque *KIR* gene repertoire is the occurrence of abundant recombination events, which result in the formation of hybrid genes composed of segments from two different *KIR* genes (Tables III, IV). Along with others, we found evidence of similar events in humans (10, 45), although this mechanism seems to happen more frequently in macaques. In rhesus macaques, hybrid *KIR* genes are named after the allele that contribute the largest segment (41). For example, multiple entities have a large *Mamu-KIR3DL07* segment, which is found in conjunction with a smaller segment of *-KIR3DL05*, *-KIR3DL08*, or *-KIR3DSW08*, but all are named and listed as alleles of *Mamu-KIR3DL07*

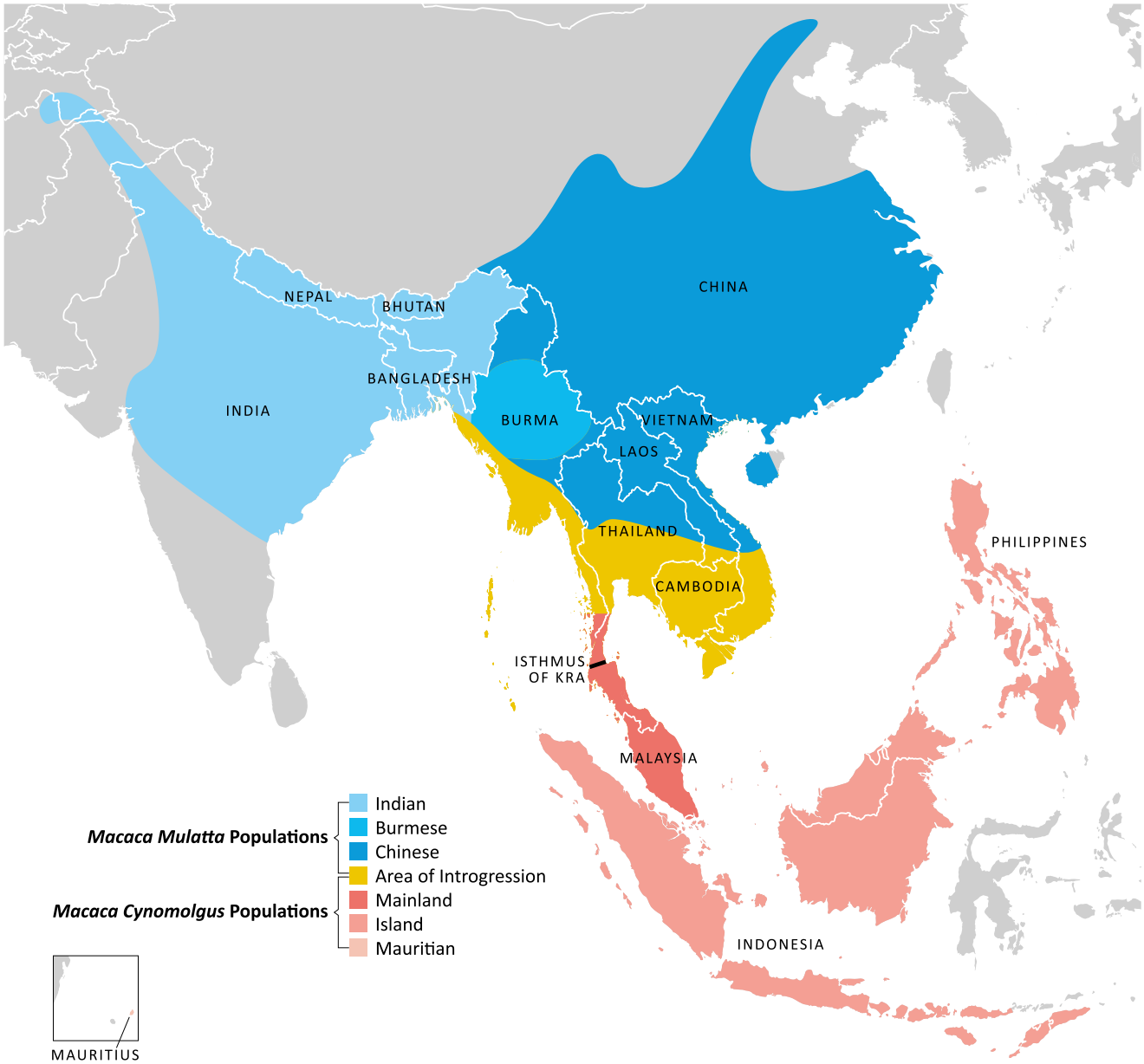

FIGURE 1. A schematic overview of the habitats of different rhesus and cynomolgus macaque populations. Three rhesus macaque populations (Indian, Burmese, and Chinese) are indicated by different blue colors, whereas four cynomolgus macaque populations (mainland, north, and south of the Isthmus of Kra, Malaysian/Indonesian islands, and Mauritius) are highlighted by red colors. The rhesus and cynomolgus macaque habitats include a hybrid zone (illustrated in yellow), in which introgression between the two species occurs. Mauritius is located ~4030 miles out of the South-East African coast and is illustrated in a separate box.

(Table III). Another peculiar recombination event resulted in *Mamu-KIR3DS04*011*, the extracellular domains (exons 1–5) of which originate from *-KIR3DS04*, whereas the cytoplasmic tail is similar to exons 6–9 of *-KIR3DL07*. The name is, therefore, somewhat confusing, as this gene is listed as an allele of *Mamu-KIR3DS04*, although it encodes an inhibitory cytoplasmic tail. In rhesus macaques, at least 19 hybrid *KIR* genes were generated by recombination events (Table III). It would seem that for some of these hybrids the nomenclature is in need of attention (46). From a more general and functional perspective, hybrid gene entities could encode novel genes with potentially distinct functional features, due to differential combinations of ligand-binding domains and signal transduction elements.

In cynomolgus macaques, at least seven hybrid *KIR* genes were detected (Tables II, IV). For example, the first six exons of *Mafa-KIR3DSW21* are highly similar (98–99%) to those of *-KIR3DL07*, whereas the transmembrane region and cytoplasmic tail of *-KIR3DSW21* is identical to *-KIR3DSW12*. This suggests that *Mafa-KIR3DSW21* may interact with similar ligands as *-KIR3DL07* but that it transduces activating instead of inhibitory signals. Seven *Mafa-KIR3DSW21* alleles are identified (Table II), suggesting a positive selection for variation on the gene products generated by this recombination event.

The origin of both segments could not be identified for all hybrid *KIR* genes. *Mamu-KIR3DL05*029/*030/*033*, *Mafa-KIR3DLW24, Mafa-KIR3DLW29, Mafa-KIR3DSW18*, and *Mafa-KIR3DSW20* seem to have segments of *Mamu-KIR3DL05, Mafa-KIR3DLW12, Mafa-KIR3DLW13, Mafa-KIR3DSW17*, and *Mafa-KIR3DSW19*, respectively, but it was not possible to trace the donor of the other segment (Tables III, IV). This indicates that when more

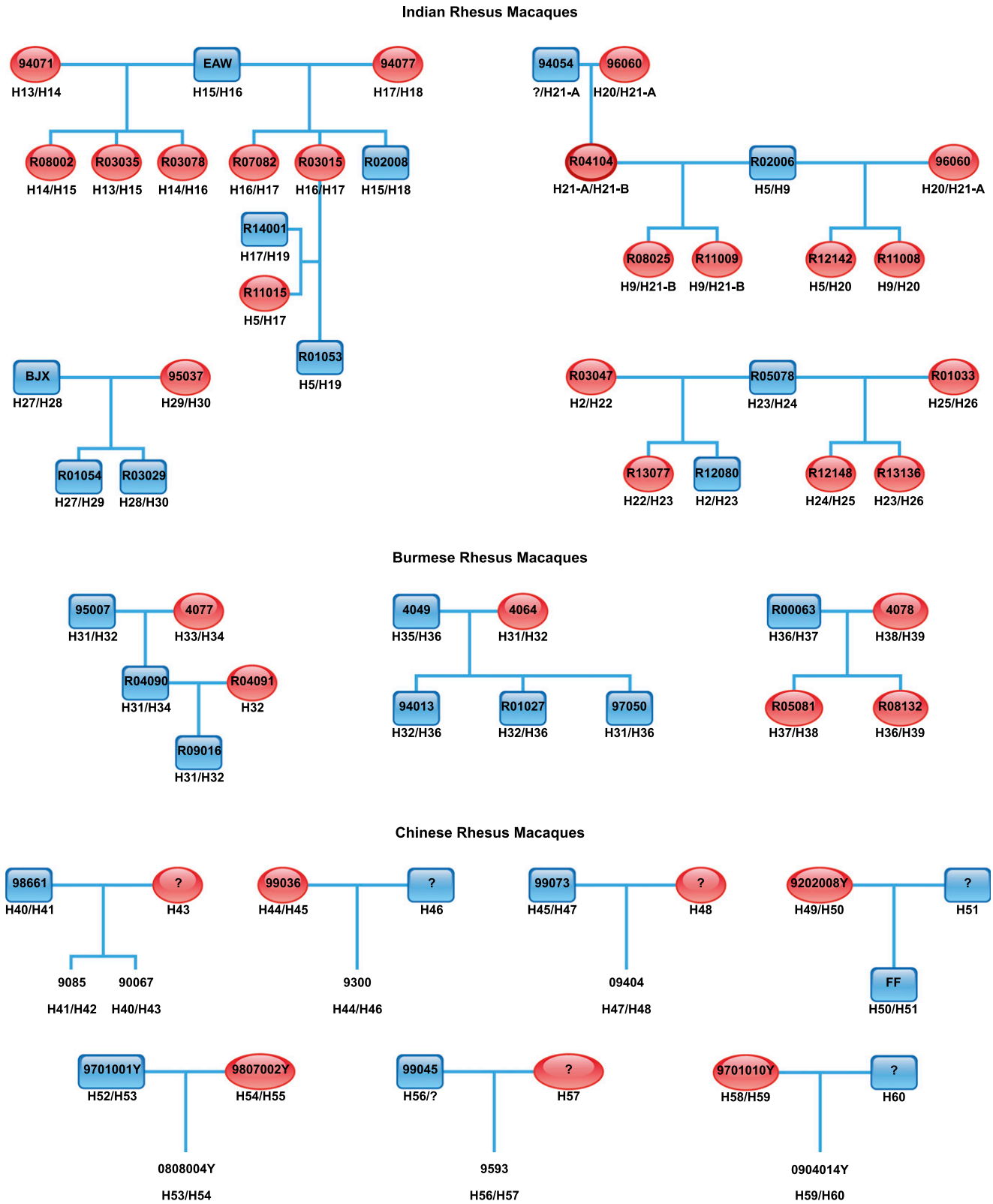

FIGURE 2. Rhesus macaque pedigrees. Fourteen rhesus macaque families are depicted and categorized by origin. Sires are indicated by blue squares, and dames are indicated by red ovals. For some offspring, the sex could not be determined. In six Chinese families, PBMC samples could be obtained from only a single parent, whereas the other parent is indicated with a question mark. Haplotype numbers are given for each animal and correspond to Fig. 5.

sequences become available, additional hybrid *KIR* gene entities and segments are likely to be defined in macaques.

Within the macaque *KIR* repertoire studied, 24 macaque *KIR* genes were highly similar and were considered to be orthologs. These genes most likely represent a single locus in both species, although it is too early to elucidate their exact physical location, as the relevant genomic studies are in progress. One would expect that the number of 24 orthologs shared between two closely related macaque species reflects common ancestry, whereas the relatively high number of species-specific *KIR* genes indicates the

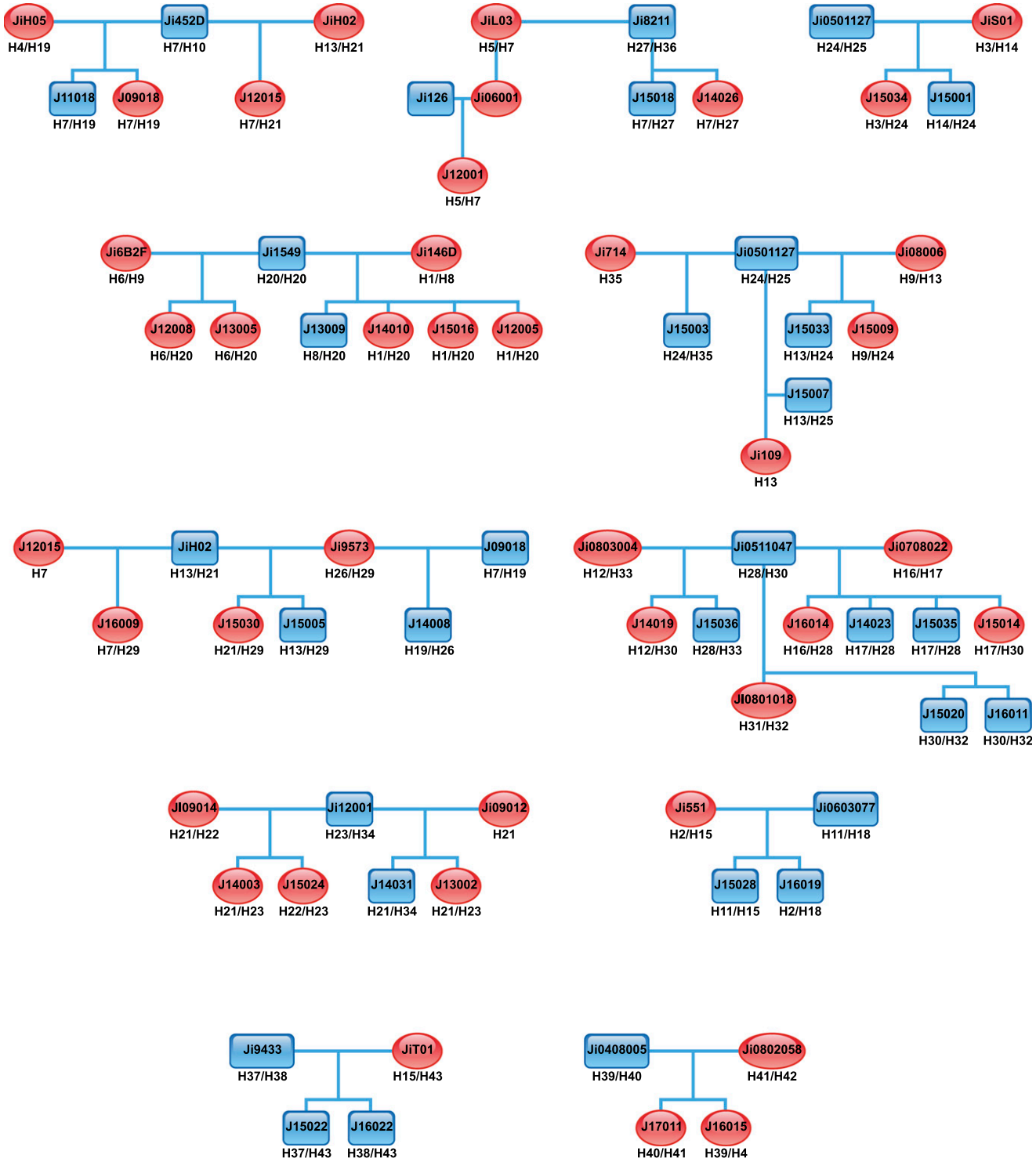

FIGURE 3. Cynomolgus macaque pedigrees. Eleven cynomolgus macaque families are depicted. Sires are indicated by blue squares, and dames are indicated by red ovals. Cynomolgus macaques from different populations are mixed in the families. Haplotype numbers are given for each animal and correspond to Fig. 6.

rapid generation of novel gene entities, which can, in part, be explained by abundant recombination.

Definition of macaque KIR haplotypes

The family-based study design resulted in the thorough characterization of 49 rhesus and 43 cynomolgus macaque *KIR* haplotypes (Figs. 5, 6), which are categorized on the basis of geographical origin of the analyzed animals (Fig. 1). The rhesus macaque *KIR* haplotypes are referred to as Rh-H13 to Rh-H60, consecutively to the 12 previously reported haplotypes (Fig. 5) (10). Cynomolgus macaque *KIR* haplotypes are referred to as Cy-H1 to Cy-H43, whereas the previously reported Mauritian chromosomal KIR configurations are listed as K1–K8 (Fig. 6) (33). All these haplotypes display extensive CNV. The rhesus macaque haplotypes encoded 4–17 *KIR* transcripts, whereas the cynomolgus macaque equivalents encoded 3–13 *KIR* transcripts. *KIR3DL20* was identified on most macaque haplotypes, except for haplotype Cy-H39, and seems to be absent on the haplotypes of

Table I. Overview of known and novel *KIR* alleles in rhesus macaques, indicated per *KIR* gene and population

Gene	Known Alleles	Novel Alleles				Total
		Indian	Burmese	Chinese	Total Novel	
KIR1D	5	2	3	3	8	13
KIR2DL04	36	0	3	10	13	49
KIR3DL20	30	10	8	15	33	63
KIR3DL01	42	3	7	13	23	65
KIR3DL02	15	0	2	4	6	21
KIR3DLW03	9	1	3	13	17	26
KIR3DL04	4	0	0	0	0	4
KIR3DL05	28	3	3	5	11	39
KIR3DL06	2	0	4	2	6	8
KIR3DL07	34	11	11	17	39	73
KIR3DL08	19	5	3	4	12	31
KIR3DL10	10	1	1	1	3	13
KIR3DL11	13	1	0	5	6	19
KIR3DLW12	0	0	0	2	2	2
KIR3DLW14	0	1	1	2	4	4
KIR3DLW17	0	0	1	2	3	3
KIR3DLW18	0	0	1	0	1	1
KIR3DLW25	0	0	1	0	1	1
KIR3DS01	8	1	0	1	2	10
KIR3DS02	21	2	8	4	14	35
KIR3DS03	5	1	0	0	1	6
KIR3DS04	11	1	2	2	5	16
KIR3DS05	10	0	1	0	1	11
KIR3DS06	14	2	1	4	7	21
KIR3DSW07	4	0	0	1	1	5
KIR3DSW08	13	2	2	3	7	20
KIR3DSW09	9	1	0	1	2	11
KIR3DSW10	0	0	1	0	1	1
KIR3DSW16	0	0	1	0	1	1
KIR3DSW18	0	0	1	0	1	1
KIR3DSW20	0	0	1	0	1	1
KIR3DSW21	0	0	2	2	4	4
KIR3DSW32	0	0	0	1	1	1
KIR3DSW34	0	0	1	0	1	1
Total	342	48	73	117	238	580

Known alleles were mainly reported in the Indian population (10, 25, 28, 29).

the Mauritian animals. However, we assume that *KIR3DL20* should be considered a framework gene in macaques and that a few transcripts were missed because of primer inconsistencies. For the Mauritian cynomolgus macaque, this assumption is confirmed by haplotype Cy-H9, which is identical to K3, defined by another research team, except for the presence of *Mafa-KIR3DL20*002*. Haplotype Cy-H9/K3 is identified in three populations and may indicate an ancestral origin. *KIR2DL04* is observed on 70 and 94% of the rhesus and cynomolgus macaque *KIR* haplotypes, respectively, and represent the only reported macaque *KIR* gene that shares an apparent ortholog with humans. In humans, this gene is considered a framework gene, and there is support that this might also be the case for its cynomolgus macaque ortholog (47).

Recombination influences haplotype architecture and drives CNV

Chromosomal recombinations such as unequal crossing over, gene fusion, and gene duplications can expand or contract a *KIR* haplotype, thereby affecting the genetic content. Two or more apparent allelic copies of a given *KIR* gene were identified on 23 of the 49 rhesus and 11 of the 43 cynomolgus macaque *KIR* haplotypes. It is likely that such genes were once orthologs, but owing to complex

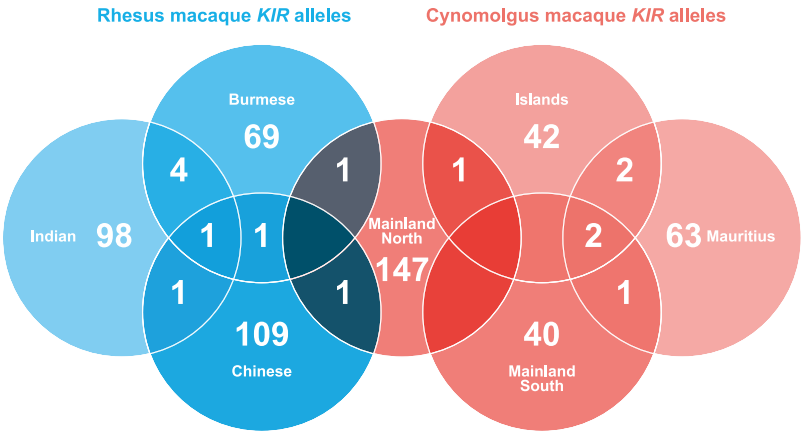

FIGURE 4. KIR allele distribution in rhesus and cynomolgus macaque populations. The three rhesus macaque populations are indicated in blue circles, and the four cynomolgus macaque populations are depicted in red circles. The total number of uniquely identified *KIR* alleles is provided per population, and the number of shared *KIR* alleles is indicated for the involved populations.

Table II. Overview of known and novel *KIR* alleles in cynomolgus macaques, indicated per *KIR* gene

Gene	Known Alleles	Novel Alleles	Total
KIR1D	4	30	34
KIR2DL04	3	38	41
3DL20	1	33	34
3DL01	2	14	16
3DLW03	0	4	4
3DL05	0	5	5
3DL06	0	1	1
3DL07	3	15	18
3DL11	2	16	18
3DLW12	3	11	14
3DLW13	3	6	9
3DLW14	2	6	8
3DLW15	1	3	4
3DLW16	2	2	4
3DLW17	0	3	3
3DLW18	0	1	1
3DLW19	1	1	2
3DLW21	0	1	1
3DLW22	0	1	1
3DLW23	0	1	1
3DLW24	1	0	1
3DLW25	2	2	4
3DLW26	1	0	1
3DLW27	0	1	1
3DLW28	1	0	1
3DLW29	0	1	1
3DLW30	0	1	1
3DS02	0	2	2
3DS04	0	1	1
3DS06	0	3	3
3DSW07	0	3	3
3DSW08	0	3	3
3DSW10	1	3	4
3DSW11	0	4	4
3DSW12	1	9	10
3DSW13	2	0	2
3DSW14	0	2	2
3DSW15	0	9	9
3DSW16	0	3	3
3DSW17	3	1	4
3DSW18	0	2	2
3DSW19	0	2	2
3DSW20	1	3	4
3DSW21	2	5	7
3DSW22	1	2	3
3DSW23	0	1	1
3DSW24	2	3	5
3DSW25	1	0	1
3DSW26	0	1	1
3DSW27	1	1	2
3DSW28	1	0	1
3DSW29	1	1	2
3DSW30	0	3	3
3DSW31	0	2	2
3DSW33	0	1	1
Total	49	267	316

recombination events, they might end up as paralogs. These duplications involved mainly lineage II inhibitory *KIR* genes, such as *KIR3DL01*, *KIR3DL07*, and *KIR3DL11*, but also *Mamu-KIR3DL20* (Rh-H27), *Mafa-KIR1D* (Cy-H11), and *Mafa-KIR3DSW12* (Cy-H1). In total, 15 different *KIR* genes are duplicated on the listed haplotypes, 11 of which are considered orthologs. This suggests that ancestral genes are more often subject to duplication as compared with more recently generated species-specific *KIR* genes. The most extensive CNV is witnessed for *Mamu-KIR3DL01* on haplotype Rh-H26 (Fig. 5), on which four allelic copies exist.

Hybrid *KIR* genes (Tables III, IV) are associated with chromosomal recombination events and might mark expanded and contracted *KIR* haplotypes. For example, two hybrid *Mamu-KIR3DL20* genes, composed of exons encoding the extracellular domains of *Mamu-KIR3DL20* (exons 1–7), and the cytoplasmic tail of *Mamu-KIR1D* (Rh-H38) or *Mamu-KIR2DL04* (Rh-H14, -H18), seem to coincide with a centromeric haplotype contraction. Also, haplotype Rh-H27 carries an example of a gene that consists of the first four exons of *Mamu-KIR3DL01* and the last five exons of *Mamu-KIR3DL08* (Fig. 5, Table III). The formation of this gene probably resulted in another contracted haplotype, as only four *KIR* genes are present at the telomeric end. In the cynomolgus macaque, haplotypes Cy-H6 and -H21 seem to be expanded, marked by the presence of the recombinant genes *Mafa-KIR3DLW27* and *Mafa-KIR3DSW20*, whereas the relatively short haplotype Cy-H38 contains another hybrid gene *Mafa-KIR3DSW21*, the emergence of which might have resulted in a contraction (Fig. 6, Table IV).

This in-frame fusion mechanism occurs rather frequently, as 21 rhesus macaque and 21 cynomolgus macaque haplotypes contain a recombinant gene (Figs. 5, 6), although not each hybrid gene seems to mark contraction or expansion. Thus, the *KIR* gene cluster in both macaque species seems to be subjected to frequent gene duplications and chromosomal recombination events, which not only generate novel gene entities, but also result in a differential *KIR* haplotype architecture.

KIR *gene frequencies differ between species*

The occurrence of at least 24 orthologs in both macaque species is most likely due to the sharing of a common ancestor, but introgression between the two species may also have an impact. The frequency of these orthologs, however, differs considerably between both macaque species (Fig. 7). The orthologous genes that are encountered more frequently on rhesus than on cynomolgus macaque haplotypes are *KIR3DL01*, *KIR3DLW03*, *KIR3DL05*, *KIR3DL06, KIR3DL07*, *KIR3DS02*, *KIR3DS06*, and *KIR3DSW08*. It is noted that for these genes the allelic variation is higher in rhesus macaques than in cynomolgus macaques (Tables I, II).

Other orthologs are more often present in cynomolgus macaques, such as *KIR3DL11*, *KIR3DLW12*, *KIR3DLW14*, *KIR3DLW25*, *KIR3DSW10*, *KIR3DSW20*, and *KIR3DSW21*, that, with the exception of *KIR3DL11*, display greater allelic variation compared with rhesus macaques (Tables I, II). An exceptional example is formed by *Mafa-KIR1D*, which is present on 82% of the haplotypes in cynomolgus monkeys but only on 22% of the haplotypes in rhesus macaques. Moreover, the allelic variation of *Mafa-KIR1D* exceeds that of *Mamu-KIR1D*, despite the difference in the number of animals studied per species (Tables I, II). These differences may indicate that *KIR1D* in cynomolgus macaques executes a more essential role.

On average, one more inhibitory *KIR* gene was present on haplotypes of rhesus macaques, whereas an additional activating KIR was encoded on cynomolgus macaque haplotypes. The differential gene and allele frequencies are indicators for species-specific selection and might involve different infectious pathogen encounters due to varying habitats.

Differential KIR *gene content and frequency in populations*

The populations of rhesus and cynomolgus macaques (Fig. 1) parade differences in *KIR* gene content and gene frequency. Rhesus macaques from the Burmese population encoded, on average, one and two additional KIR3DL and KIR3DS receptors, respectively, as compared with the haplotypes that stem from the Indian and Chinese populations. Approximately 70% of the haplotypes contained at least one *Mamu-KIR3DL01* and/or *Mamu-KIR3DL07* copy, regardless of the origin, whereas multiple other

Table III. Novel gene entities that are generated by chromosomal recombination events in rhesus macaques

Rhesus Macaques				
	Gene Segment 1		Gene Segment 2	
Novel Entity (Allele Name)	Gene Donor 1	Segment	Gene Donor 2	Segment
*Mamu-KIR3DL01*054*	*Mamu-KIR3DL05*	Exons 1–3	*Mamu-KIR3DL01*	Exons 4–9
*Mamu-KIR3DL02*005*, **011*	*Mamu-KIR3DL02*	Exons 1–6	*Mamu-KIR3DL01*	Exons 7–9
*Mamu-KIR3DL02*006*, **010*	*Mamu-KIR3DL02*	Exons 1–7	*Mamu-KIR3DL01*	Exons 8–9
*Mamu-KIR3DL02*016*	*Mamu-KIR3DL10*	Exons 1–4	*Mamu-KIR3DL02*	Exons 5–9
*Mamu-KIR3DLW03*023*	Unknown donor	Exons 1–3	*Mamu-KIR3DLW03*	Exons 4–9
*Mamu-KIR3DL05*029*, **030*, **033*	*Mamu-KIR3DL05*	Exons 1–7	Unknown donor	Exons 8–9
*Mamu-KIR3DL07*042*	*Mamu-KIR3DL07*	Exons 1–5	*Mamu-KIR3DL08*	Exons 6–9
*Mamu-KIR3DL07*045*	*Mamu-KIR3DL05*	Exons 1–3	*Mamu-KIR3DL07*	Exons 4–9
*Mamu-KIR3DL07*056*	*Mamu-KIR3DSW08*	Exons 1–3	*Mamu-KIR3DL07*	Exons 4–9
*Mamu-KIR3DL07*064*	*Mamu-KIR3DL05*	Exons 1–4	*Mamu-KIR3DL07*	Exons 5–9
*Mamu-KIR3DL07*065*	Unknown donor	Exons 1–3	*Mamu-KIR3DL07*	Exons 4–9
*Mamu-KIR3DL08*018*, **019*, **020*	*Mamu-KIR3DL02*	Exons 1–3	*Mamu-KIR3DL08*	Exons 4–9
*Mamu-KIR3DL08*021*	*Mamu-KIR3DL01*	Exons 1–4	*Mamu-KIR3DL08*	Exons 5–9
*Mamu-KIR3DL20*030*	*Mamu-KIR3DL20*	Exons 1–7	*Mamu-KIR2DL04*	Exons 8–9
*Mamu-KIR3DL20*044*	*Mamu-KIR3DL20*	Exons 1–7	*Mamu-KIR1D*	Exons 8–9
*Mamu-KIR3DS02*012*, **029*	*Mamu-KIR3DS02*	Exons 1–4	*Mamu-KIR3DSW09*	Exons 5–9
*Mamu-KIR3DS04*011*	*Mamu-KIR3DS04*	Exons 1–5	*Mamu-KIR3DL07*	Exons 6–9
*Mamu-KIR3DS06*019*	*Mamu-KIR3DSW07*	Exons 1–3	*Mamu-KIR3DS06*	Exons 4–9
*Mamu-KIR3DSW09*005*, **011*	*Mamu-KIR3DSW08*	Exons 1–3	Unknown donor	Exons 4–9

The gene donors and corresponding donated segments are indicated. For some novel entities, only a single donor could be identified. The novel entities are named as an allele of the gene that contributed the largest segment.

KIR genes were differently distributed over the rhesus macaque populations (Fig. 8). For example, *Mamu-KIR1D* is located on 56% of the haplotypes from Burmese animals, whereas it is present on only 16 and 14% of the Indian and Chinese rhesus macaque *KIR* haplotypes, respectively. Eleven *KIR* genes were identified in a single rhesus macaque population, including newly defined activating *KIR3DS* genes that were only encountered in the Burmese cohort studied.

In cynomolgus macaques, animals that originate from the mainland populations seem to have, on average, one additional inhibitory KIR receptor (KIR3DL) per haplotype as compared with the subjects that inhabit the Indonesian/Malaysian islands. Differential gene distribution trends are observed for several *KIR* genes (Fig. 9). For example, *Mafa-KIR3DL01* and *-KIR3DLW12* were more frequently identified in the northern-mainland population, whereas *-KIR3DSW13* and *-KIR3DLW28* were found present only in the Mauritian population. Activating *KIR* genes with orthologs in Indian rhesus macaques were mainly identified in the northern-mainland population, including *KIR3DS02* and *KIR3DSW07*, whereas the other cynomolgus macaque populations have species-specific activating *KIR* genes.

Genes that were identified in either three rhesus or four cynomolgus macaque populations mainly encode inhibitory receptors (Figs. 8, 9). The activating receptor are more often observed in two or one populations (Figs. 8, 9). Overall, the observed variable gene content and gene frequency in the different macaque populations support evidence pointing to rapid evolution of the *KIR* genes at the population level.

Discussion

An essential step in the evolution of the primate *KIR* cluster started with the initial expansion of a lineage II *KIR* gene progenitor. Subsequently, other *KIR* lineages seem to have emerged through deletion and recombination events. In macaques, lineage II *KIR* genes (KIR3D) were subjected to substantial expansion (10, 17, 32), which coincides with an extended *MHC* class I gene repertoire (16, 24). The present study involves the comparative analysis of rhesus and cynomolgus macaque populations from distinct geographic areas. The *KIR* gene repertoires were found to reflect rapid evolution. Our data illustrate that not only within these closely related species, but even within their populations, new *KIR* gene entities are generated by complex recombination processes resulting in the formation of hybrid genes. In addition, a high level of allelic polymorphism was encountered in both macaque species, but the sharing of alleles was virtually absent. Moreover, recombination resulted in marked differences in the *KIR* haplotype

Table IV. Novel gene entities that are generated by chromosomal recombination events in cynomolgus macaques

Cynomolgus Macaques				
	Gene Segment 1		Gene Segment 2	
Novel Entity (Gene Name)	Gene Donor 1	Segment	Gene Donor 2	Segment
Mafa-KIR3DLW24	Unknown donor	Exons 1–3	*Mafa-KIR3DLW12*	Exons 4–9
Mafa-KIR3DLW26	*Mafa-KIR3DSW15*	Exons 1–3	*Mafa-KIR3DLW27*001*	Exons 4–9
Mafa-KIR3DLW27	*Mafa-KIR3DSW22*001*	Exons 1–4	*Mafa-KIR3DLW26*001*	Exons 5–9
Mafa-KIR3DLW29	Unknown donor	Exons 1–4	*Mafa-KIR3DLW13*	Exons 5–9
Mafa-KIR3DSW18	*Mafa-KIR3DSW17*	Exons 1–4	Unknown donor	Exons 5–9
Mafa-KIR3DSW20	*Mafa-KIR3DSW19*	Exons 1–4	Unknown donor	Exons 5–9
Mafa-KIR3DSW21	*Mafa-KIR3DL07*	Exons 1–6	*Mafa-KIR3DSW12*	Exons 7–9

The gene donors and corresponding donated segments are indicated. For some novel entities, only a single donor could be identified. The novel entities received a gene workshop number.

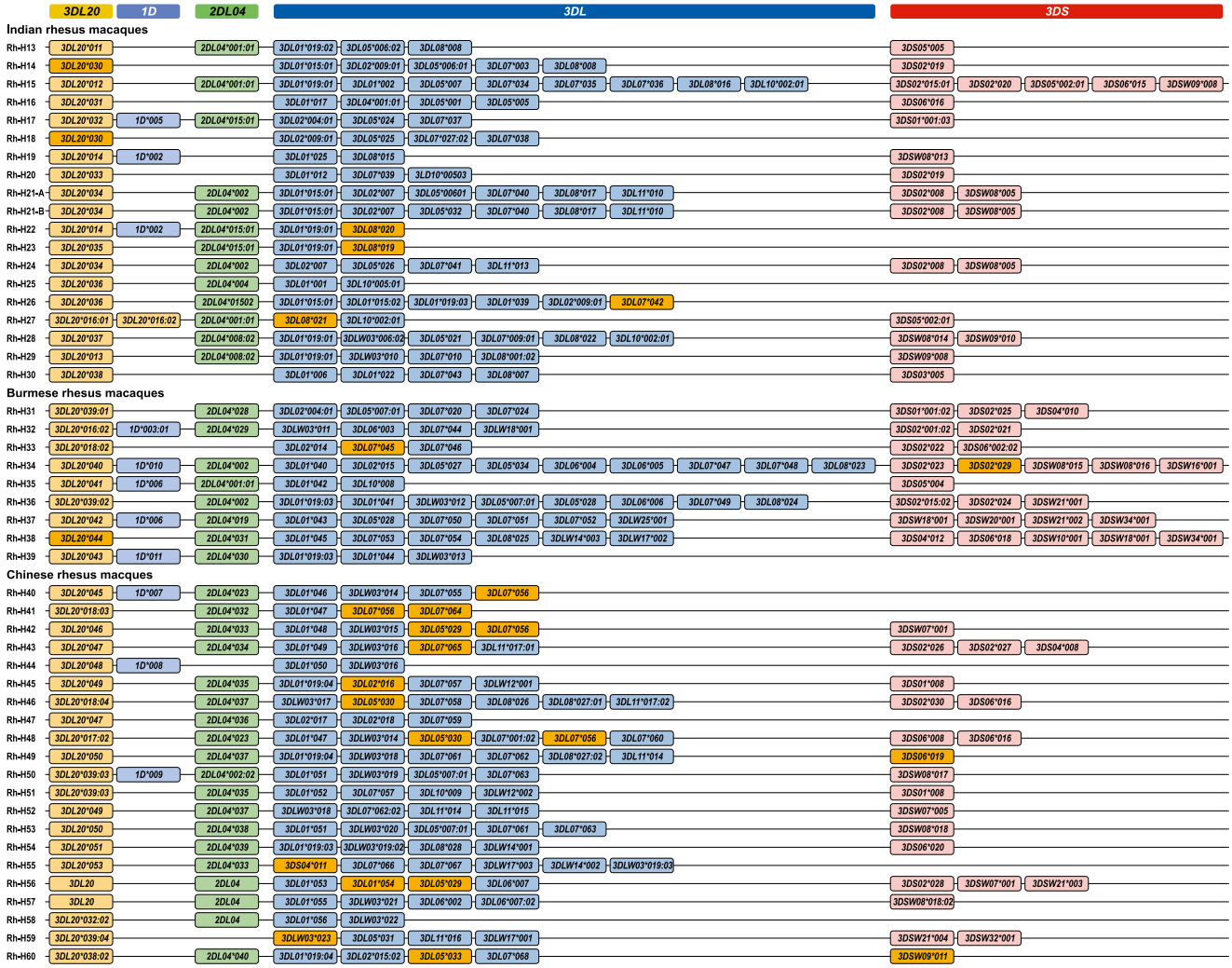

FIGURE 5. Rhesus macaque *KIR* haplotypes at the transcription level. A schematic overview of 49 defined rhesus macaque *KIR* haplotypes, categorized by population. Most of the haplotypes expressed *Mamu-KIR3DL20* (yellow boxes). Expression of *Mamu-KIR1D* and *Mamu-KIR2DL04* is indicated in light blue and green boxes, respectively. Inhibitory lineage II *KIR* genes are illustrated by blue boxes, whereas activating *KIR* genes of the same lineage are depicted by red boxes. Recombinant genes, for which there is evidence that they are hybrids consisting of segments of two different genes, are indicated by orange boxes. For several haplotypes, the presence of a gene was determined but could not be distinguished at the allele level. The lineage II *KIR* genes are depicted in random order, whereas the physical locations of *Mamu-KIR3DL20*, *Mamu-KIR1D*, and *Mamu-KIR2DL04* are deduced from a genomically sequenced macaque haplotype (28). Haplotypes H21-A and H21-B are similar, except for the de novo *Mamu-KIR3DL05*032* allele.

architecture of both species, again testifying the rapid evolution of the macaque *KIR* genes, which has not been described in other NHP species.

In humans, the *KIR* gene cluster mainly diversifies at the allelic level, whereas gene expansion is modest and mainly confined to lineage III genes (43). Two major haplotype configurations are recognized, for which a trade-off has been suggested based on differential haplotype frequencies in human populations (47). The A haplotype configurations standardly express seven receptors and have an inhibitory profile, whereas the B haplotypes show moderate gene content variability, including multiple activating *KIR* genes (7–13 *KIR* genes) (Fig. 10) (44). Chimpanzees (*Pan troglodytes*) and humans diverged from a common ancestor ~5 million years ago, and, although the complexity of the *KIR* clusters is, to some extent, comparable, species-specific diversification is observed in receptor structure, haplotype architecture, *MHC* class I recognition potential, and gene content (48). The chimpanzee *KIR* region mainly comprises inhibitory genes and resemble human A haplotypes. Several chimpanzee *KIR* genes are actually recombinant genes (48). The repertoire, however, is limited to 13 *KIR* genes, four of which are orthologous framework genes that are shared with humans (Fig. 10) (41, 48). Although little is known about the allelic variation in chimpanzees, the limited *KIR* gene repertoire might suggest that the ancient selective sweep, which targeted the ancestral chimpanzee *MHC* class I region and was likely caused by a retroviral infection (49, 50), may also have had an indirect impact on its ligands within the *KIR* gene region. Bonobos (*Pan paniscus*) and chimpanzees shared a common ancestor ~2.3 million years ago. In this species, only seven *KIR* genes are reported, five and three genes of which are orthologs, shared with common chimpanzees and humans, respectively (51). The short bonobo *KIR* haplotypes, the limited *KIR* gene repertoire, and the reduced bonobo *MHC* class I content (52–55) may result from subsequent selective sweeps (56). The expansion of lineage III *KIR* genes, which in macaques are represented by *KIR1D*, correlates with the emergence of *HLA*-C–like genes in orangutans (57–59). In orangutans, 13 *KIR* genes are identified, of which the framework genes share orthologs with humans (15, 41, 57, 58). Two sibling orangutan species inhabit Sumatra (*Pongo abelii*) and Borneo (*Pongo pygmaeus*) (60). Only one gene is species-specific

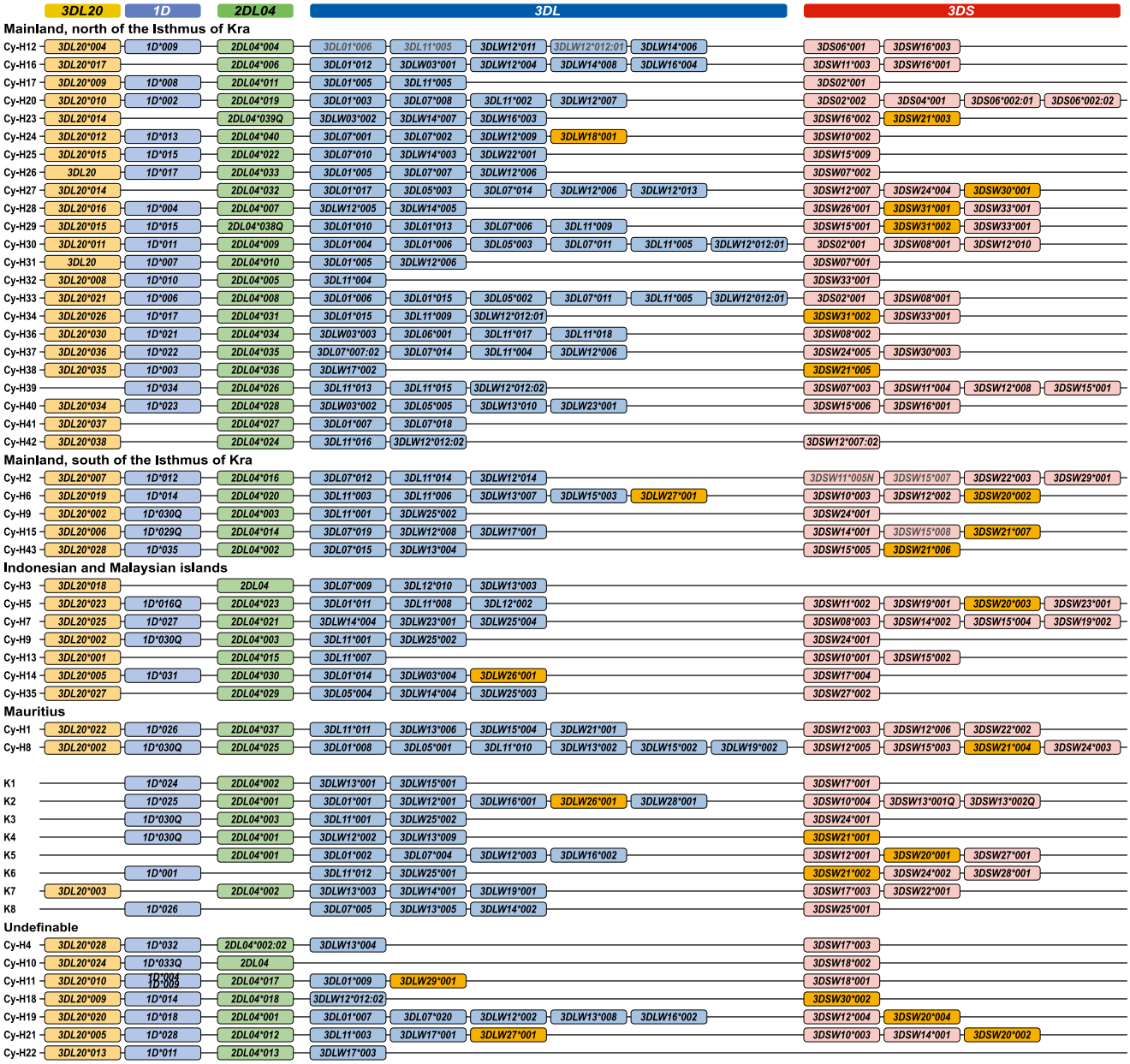

FIGURE 6. Cynomolgus macaque *KIR* haplotypes at the transcription level. A schematic overview of 43 defined cynomolgus macaque *KIR* haplotypes, categorized by population. Eight haplotypes previously reported in Mauritian animals are also illustrated (K1–K8). Expression of *Mafa-KIR3DL20*, *Mafa-KIR1D*, and *Mafa-KIR2DL04* is indicated by yellow, light blue, and green boxes, respectively. Inhibitory lineage II *KIR* genes are illustrated by blue boxes, whereas activating *KIR* genes of the same linage are depicted by red boxes. Recombinant genes, which consist of segments of two different genes, are indicated by orange boxes. Some alleles were confirmed in two animals but were observed in a low number of reads (<3 reads), which are indicated by gray text. The lineage II *KIR* genes are depicted in random order, whereas the physical location of the remaining genes is deduced from a previously reported complete macaque haplotype (28).

(*KIR2DS15* in Bornean orangutans), whereas all other genes are orthologs. Ten of the 130 *KIR* alleles that were identified are shared between both sister species. For the human and great ape species discussed above, rapid evolution is mainly reflected by the gain in allelic variation, whereas the generation of novel gene entities and the formation of complex haplotype architectures seem to be relatively limited (Fig. 10).

The present communication sheds light on the evolution of the *KIR* region in two highly related Old-World monkey species, which share an introgression zone. In rhesus and cynomolgus macaques, the massive expansion of the lineage II *KIR* genes exceeds the modest lineage III expansion in great apes and humans. The rapid evolution of the macaque *KIR* region is reflected not only by allelic polymorphism, but even more prominently by the large number of species-specific recombinant genes and haplotypes with a complex architecture in different macaque populations. In humans and other hominids, these events seem to be less abundant. Moreover, the allelic variation in macaques seems to exceed the numbers that are encountered in humans and higher primates. We recorded a total number of 579 *KIR* alleles, and it is important to realize that the number of samples that were analyzed is relatively small as compared with the human situation (43). The reason for the extensive expansion of the *KIR* gene cluster in macaques is of interest. Whereas in humans, the less variable *KIR* gene content and haplotype configurations seem to be the result of a trade-off, such an indication appears to be absent in macaques.

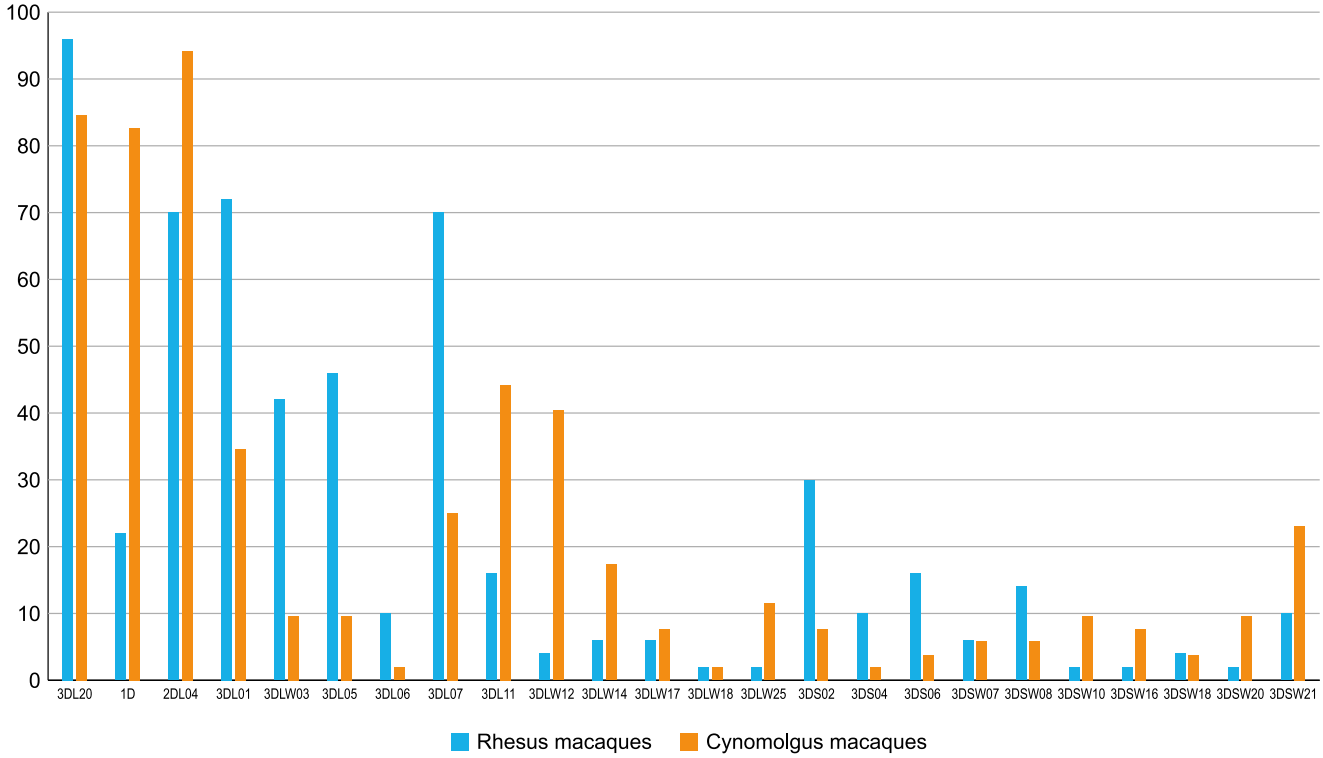

FIGURE 7. Gene frequencies of orthologous *KIR* genes in rhesus and cynomolgus macaques. The gene frequencies are given for orthologous *KIR* genes in rhesus (blue bars) and cynomolgus (orange bars) macaques.

The most plausible driving forces of the rapid *KIR* cluster evolution in macaques might involve coevolution with the extended *MHC* class I region, differential infectious pathogen encounters, a discontinuous habitat, and susceptibility to chromosomal recombination. Nonetheless, we cannot rule out that the extensive expansion of the macaque *KIR* gene system may have evolved due to the lack of evolutionary pressure on this system. The ligand of only a few receptors have been identified and, therefore, the functional impact of the expanded macaque KIR repertoire remains largely unclear. However, diversification of ligand interactions is suggested by overlapping, but nonredundant, MHC class I specificity of multiple KIR (61–66). The extensive diversity of the macaque MHC and KIR clusters might facilitate interactions with allele-level specificity, differential affinity, and peptide dependency and may contribute to rapid adaption driven by environmental conditions.

The general high levels of allelic polymorphism detected in the *KIR* region in primates might indicate that it is more prone to generate mutations than other regions of the genome. Mutation rates are elevated in CpG islands, which are genomic regions that are enriched for CpG sites with an observed-to-expected ratio >60%. All *KIR* genes indeed carry CpG islands (67). CpG site mutations, however, mainly involve cytosine to thymine transitions, whereas mutations in the generation of the novel *Mamu-KIR3DL05* allele involved T > C and G > T transitions, which are not commonly observed transition events. The two-point mutations are separated by only two nucleotides, which suggests that one mutation initiated the other and, perhaps, was caused by the recruitment of error-prone repair mechanisms (68). In addition to CpG islands and error-prone repair, other factors that might enhance the regional mutation rate may include recombination events, deletion and insertion events, chromatin configurations, distance to the telomere, and replication time (69, 70). Furthermore, relatively more single-nucleotide polymorphisms were observed in regions that were homologous in humans, chimpanzees, and macaques, which substantiates the extensive variation of the *KIR* gene region (69). The birth of novel *KIR* alleles has been described previously in human families (71), and together with the event recorded in macaques, this might suggest that point mutations substantially contribute to the extensive allelic *KIR* variation. Of course, it is clear that the generation of mutations is only one side of the coin, and that selection determines which polymorphisms will be enriched in the populations or are eventually rooted out.

In humans, genetic *KIR* variation is documented for over 250 populations and mainly records allelic variation and differential haplotype distribution in relation to gene frequencies (47, 72). Similar observations were made for the different macaque populations. Genes that are shared in all three rhesus or four cynomolgus macaque populations mainly involved inhibitory *KIR* genes (Figs. 8, 9). The conserved nature of these genes suggests an impact on essential functions, such as NK cell education, a process for which the involvement of inhibitory KIR is well established in humans (73–75). The role of activating KIR in humans is less understood, but associations with disease progression or protection are described, and in vitro studies demonstrate specific binding to certain peptide–MHC class I complexes (76–81). In macaques, activating *KIR* genes were mainly identified in one or two populations and may substantiate more specialized functions, like pathogen recognition (Figs. 8, 9). For instance, *Mamu-KIR3DSW18* is encountered only in Burmese rhesus macaques, and a similar observation was made for *Mafa-KIR3DSW08* in the northern-mainland cynomolgus macaques. In addition, the majority of the *KIR* alleles appear not to be shared at the population level (Fig. 4). Again, this hints at a speedy generation of allelic polymorphism. For the Indian rhesus macaque population, a genetic bottleneck is evident (82), but it did not result in a reduced *KIR* gene content, which might indicate that the rapid evolution of the *KIR* repertoire erased the genetic footprint of a bottleneck.

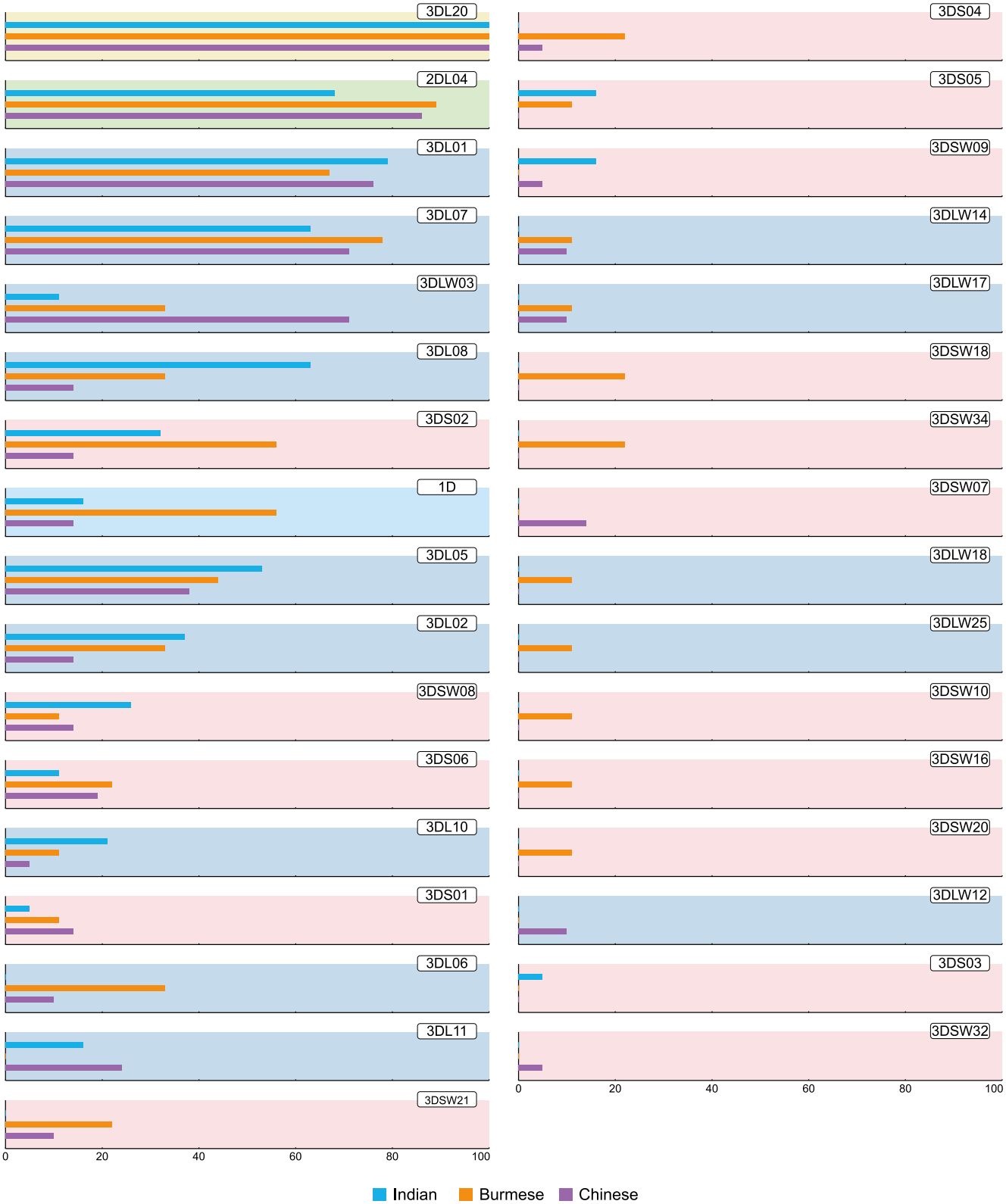

FIGURE 8. Gene distributions between the different rhesus macaque populations. Gene frequencies are listed for rhesus macaque *KIR* genes that were identified in the Indian (blue bars), Burmese (orange bars), and Chinese (purple bars) populations. The genes are listed from the most frequent genes in all populations to the least frequent species-specific genes. The background color indicates *Mamu-KIR3DL20* (yellow), *-KIR2DL04* (green), *-KIR1D* (light blue), and the inhibitory (blue) and activating (red) lineage II *KIR* genes. The frequencies are based on the presence on a haplotype of known origin rather than the presence in an individual.

Rhesus and cynomolgus macaques are widely used as preclinical models in translational biomedical research to further a better understanding of human diseases and the development of vaccines and therapies (34, 35). The genetic makeup of the different macaque species, however, can vary considerably and might potentially influence the outcome of studies. Even at the population

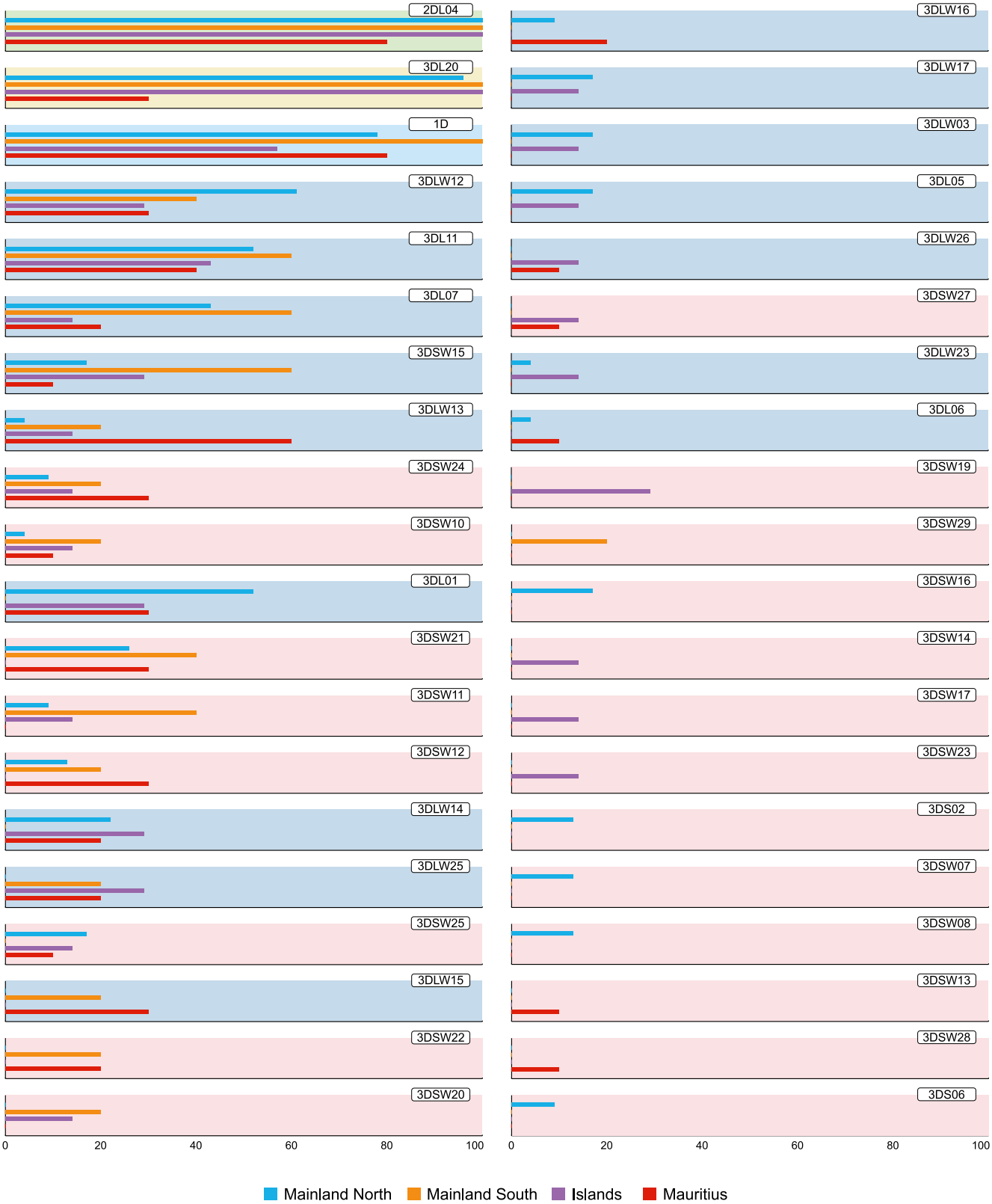

FIGURE 9. Gene distributions between the different cynomolgus macaque populations. Frequencies are provided for *KIR* genes identified in cynomolgus macaque populations: mainland, north (blue bars) or south (orange bars) of the Isthmus of Kra, the Malaysian/Indonesian islands (purple bars), or Mauritius (red bars). The background color indicates *Mamu-KIR3DL20* (yellow), *-KIR2DL04* (green), *-KIR1D* (light blue), and the inhibitory (blue) and activating (red) lineage II *KIR* genes. The frequencies are based on the presence of a gene on haplotype of known origin rather than on the presence in an individual.

level, a differential disease susceptibility has been reported: for example, in SIV/AIDS-related experiments in rhesus macaques of Indian and Chinese origin (36, 37, 83). It is possible that the *KIR* repertoire may be one of the factors that have an impact on disease outcome, as correlations between *KIR* gene content and disease phenotypes in humans (6) and macaques are documented (38, 39).

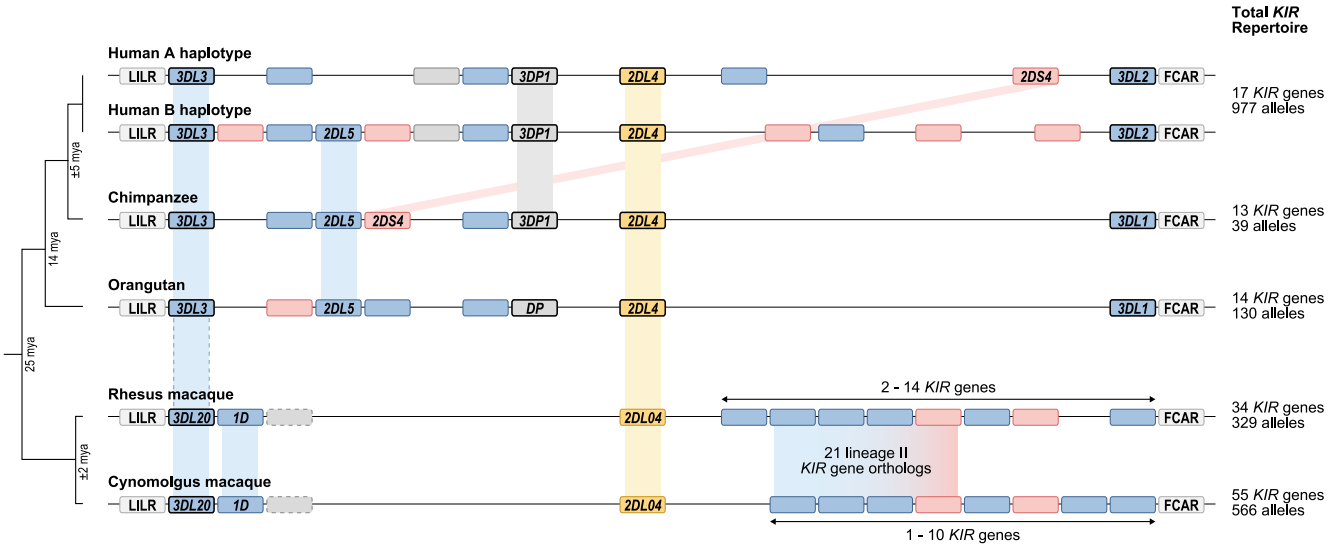

FIGURE 10. KIR haplotypes in different primate species. The KIR haplotype configurations of humans (A and B haplotypes), chimpanzees, orangutans, and rhesus and cynomolgus macaques are schematically illustrated. The evolutionary distance between the different species is depicted on the left, whereas the numbers of the documented *KIR* repertoires are provided on the right. The inhibitory and activating *KIR* genes are illustrated with blue and red boxes, whereas pseudogenes are in gray. The yellow boxes indicate the conserved *KIR2DL4/KIR2DL04* genes. Framework genes are indicated with black outlining, and the orthologous and homologous genes shared in the depicted primate species are connected to each other. In total, 21 lineage II *KIR* genes are identified as orthologs in rhesus and cynomolgus macaques. *KIR3DL20*, identified as a framework gene in both macaque species, is a potential ortholog of the other primate *KIR3DL3* genes. A putative pseudogene on the centromeric macaque KIR haplotype is indicated by a gray box with a dashed outlining. The *KIR* region is flanked by the *LILR* and *FCAR* genes in all primate species depicted.

This study design, including rhesus and cynomolgus macaque families from different geographical origin, allowed the transcriptomic characterization of the complex *KIR* cluster. The high level of allelic polymorphism, the number of novel gene entities, the plastic haplotype architecture, and the diversification at the species and population levels illustrate the unparalleled rapid evolution of the *KIR* gene region in macaques. This communication paves the way to study the impact of *KIR* genes in NHP models for human health and disease, but also may help in selecting animals with particular genetic markers for studies in the area of personalized medicine.

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Disclosures

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