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Bonobos Maintain Immune System Diversity with Three Functional Types of MHC-B

Emily E. Wroblewski,*,† Lisbeth A. Guethlein,*,† Paul J. Norman,*,† Yingying Li,‡,§ Christiana M. Shaw,‡,§ Alex S. Han,¶ Jean-Bosco N. Ndjango,‖ Steve Ahuka-Mundeke,#,,†† Alexander V. Georgiev,‡‡,1 Martine Peeters,†† Beatrice H. Hahn,‡,§ and Peter Parham*,†**

Fast-evolving MHC class I polymorphism serves to diversify NK cell and CD8 T cell responses in individuals, families, and populations. Because only chimpanzee and bonobo have strict orthologs of all *HLA class I*, their study gives unique perspectives on the human condition. We defined polymorphism of *Papa-B*, the bonobo ortholog of *HLA-B*, for six wild bonobo populations. Sequences for *Papa-B* exon 2 and 3 were determined from the genomic DNA in 255 fecal samples, minimally representing 110 individuals. Twenty-two *Papa-B* alleles were defined, each encoding a different Papa-B protein. No Papa-B is identical to any chimpanzee Patr-B, human HLA-B, or gorilla Gogo-B. Phylogenetic analysis identified a clade of MHC-B, defined by residues 45–74 of the α_1 domain, which is broadly conserved among bonobo, chimpanzee, and gorilla. Bonobo populations have 3–14 Papa-B allotypes. Three Papa-B are in all populations, and they are each of a different functional type: allotypes having the Bw4 epitope recognized by killer cell Ig-like receptors of NK cells, allotypes having the C1 epitope also recognized by killer cell Ig-like receptors, and allotypes having neither epitope. For population Malebo, these three Papa-B are the only Papa-B allotypes. Although small in number, their sequence divergence is such that the nucleotide diversity (mean proportional distance) of *Papa-B* in Malebo is greater than in the other populations and is also greater than expected for random combinations of three *Papa-B*. Overall, *Papa-B* has substantially less diversity than *Patr-B* in chimpanzee subspecies and *HLA-B* in indigenous human populations, consistent with bonobo having experienced narrower population bottlenecks. ***The Journal of Immunology*, 2017, 198: 3480–3493.**

In vertebrates, the *MHC* is a genomic region containing numerous immune system genes. Of these, the *MHC class I* and *II* genes are distinguished from all other vertebrate genes by the depth and breadth of their allelic polymorphism (1). *MHC class I* and *II* genes encode cell surface glycoproteins that bind endogenous and pathogen-derived peptide Ags and present them to various families of lymphocyte receptors. Dedicated to adaptive immunity, MHC class II present Ags of extracellular pathogens to CD4 T cells (2, 3). In contrast, MHC class I function in innate immunity, adaptive immunity, and formation of the placenta during reproduction. During infection, MHC class I present peptide Ags of intracellular pathogens, notably viruses, to the receptors of NK cells of innate immunity (4) and CD8 T cells of adaptive immunity (5). During reproduction, MHC class I on fetal trophoblast cells present peptides of paternal origin to receptors of maternal uterine NK cells (6). Because of these distinctive functions, MHC class I has a more diverse and rapidly evolving polymorphism than MHC class II (7–9).

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All sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Immuno Polymorphism (https://www.ebi.ac.uk/ipd/mhc/). Allele names (with the exception of *Papa-B*21:01*) were assigned by Immuno Polymorphism. The following sequences were submitted to GenBank under accession numbers: *Papa-B*17:01* [KX786188], *Papa-B*11:01* [KX786189], *Papa-B*19:01* [KX786190], *Papa-B*09:01* [KX786191], *Papa-B*12:01* [KX786192], *Papa-B*13:01* [KX786193], *Papa-B*10:01* [KX786194], *Papa-B*21:01* [KX786195], *Papa-B*17:02* [KX786196], *Papa-B*19:02* [KX786197], *Papa-B*02:02* [KX786198], *Papa-B*08:01* [KX786199], *Papa-B*18:01* [KX786200], *Papa-B*20:01* [KX786201], *Papa-B*15:01* [KX786202], *Papa-B*12:02* [KX786203], *Papa-B*14:01* [KX786204], *Papa-B*08:02* [KX786205], and *Papa-B*16:01* [KX786206].

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The online version of this article contains supplemental material.

Abbreviations used in this article: BJ, Bayandjo; BN, Balanga; BPRC, Biomedical Primate Research Center; DRC, Democratic Republic of Congo; IK, Ikela; KIR, killer cell Ig-like receptor; KR, Kokolopori; LK, LuiKotale; β_2-m, β_2 microglobulin; ML, Malebo; p-distance, proportional distance.

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As a consequence of the more rapid evolution of MHC class I, the only living species that have strict orthologs of all of the polymorphic human *MHC class I* genes, *HLA-A*, *HLA-B*, and *HLA-C*, are the great apes: chimpanzee, bonobo, gorilla, and orangutan (10). Coevolving with MHC-A, MHC-B, and MHC-C is the family of killer cell Ig-like receptors (KIRs) (6, 11). Members of this family are inhibitory and activating receptors that recognize a set of alternative epitopes specified by sequence motifs at residues 76–83 in the α_1 domain of MHC class I (12–16). These epitopes make up the Bw4 epitope carried by subsets of MHC-A and MHC-B allotypes, the C1 epitope carried by subsets of MHC-B and MHC-C allotypes, and the C2 epitope carried by the subset of MHC-C allotypes that lack the C1 epitope (4, 6, 11, 14, 16, 17). The interactions between KIRs and their MHC class I ligands are further diversified by sequence variation in the peptide bound by MHC class I, polymorphism at residues in MHC class I other than 76–83, and the high polymorphism of KIR, which rivals that of MHC class I (18–22). These interactions serve to modulate the development and function of NK cells (11). The diversity of MHC–KIR interactions individualizes NK cell responses, as is evident from the broad range of human diseases that correlate with HLA class I and KIR polymorphisms (23–26).

Comparative studies of the genetics and function of chimpanzee MHC class I (27–32) and KIRs (33–35) have provided a unique and valuable perspective that has increased knowledge and understanding of the human immune system. Providing equal opportunity for this approach is the bonobo (*Pan paniscus*), the sibling species to chimpanzee (*Pan troglodytes*) that is as closely related to the human species as are chimpanzees (36). Our previous study of captive bonobos indicated that the bonobo *KIR* locus had undergone a process of gene loss and attenuation of KIR avidity for MHC class I similar to humans (37). However, limiting the interpretation of those results was an almost complete lack of knowledge of the bonobo *MHC*. Studies of captive bonobos identified only eight alleles each for *MHC-A* and *MHC-B* and five alleles for *MHC-C* (10, 38–42). In a recent study of the wild chimpanzee populations of Gombe National Park, Tanzania, we defined the polymorphism of *Patr-B* (*Pan troglodytes*) (32), the ortholog of *HLA-B*, the most polymorphic human *MHC class I* gene (10, 27, 32, 43, 44). That investigation required development of a method for isolating *Patr-B* from chimpanzee feces. Because of the close phylogenetic relationship of chimpanzee and bonobo, that method was directly applicable to the analysis of *Papa-B* (*Pan paniscus*), the bonobo ortholog of *Patr-B* and *HLA-B*. In this article, we define *Papa-B* of wild bonobos resident at sites throughout the bonobo range in the Democratic Republic of Congo (DRC) (45).

Materials and Methods

This project is not classified as animal research by the Stanford Administrative Panel on Laboratory Animal Care, according to National Institutes of Health guidelines. Fecal samples from wild-living bonobos were collected noninvasively. Permission to collect samples in the DRC was granted by its Ministry of Scientific Research and Technology, Department of Ecology and Management of Plant and Animal Resources of the University of Kisangani, Ministries of Health and Environment, and National Ethics committee. Use of the Yerkes National Primate Research Center bonobo samples was approved under Stanford Administrative Panel on Laboratory Animal Care 9057.

Study sites, sample collection, and sample typing

As described by Li et al. (45), teams of local trackers collected 255 fecal samples from nonhabituated bonobos at six sites distributed throughout the bonobo range in the DRC: Malebo (ML), LuiKotale (LK), Ikela (IK), Balanga (BN), Kokolopori (KR), and Bayandjo (BJ) (Figs. 1, 2). Samples were obtained opportunistically, placed into an equal volume of RNAlater (Life Technologies), and labeled with a number, field site code, and GPS coordinates, whenever possible. Because the field sites lack refrigeration,

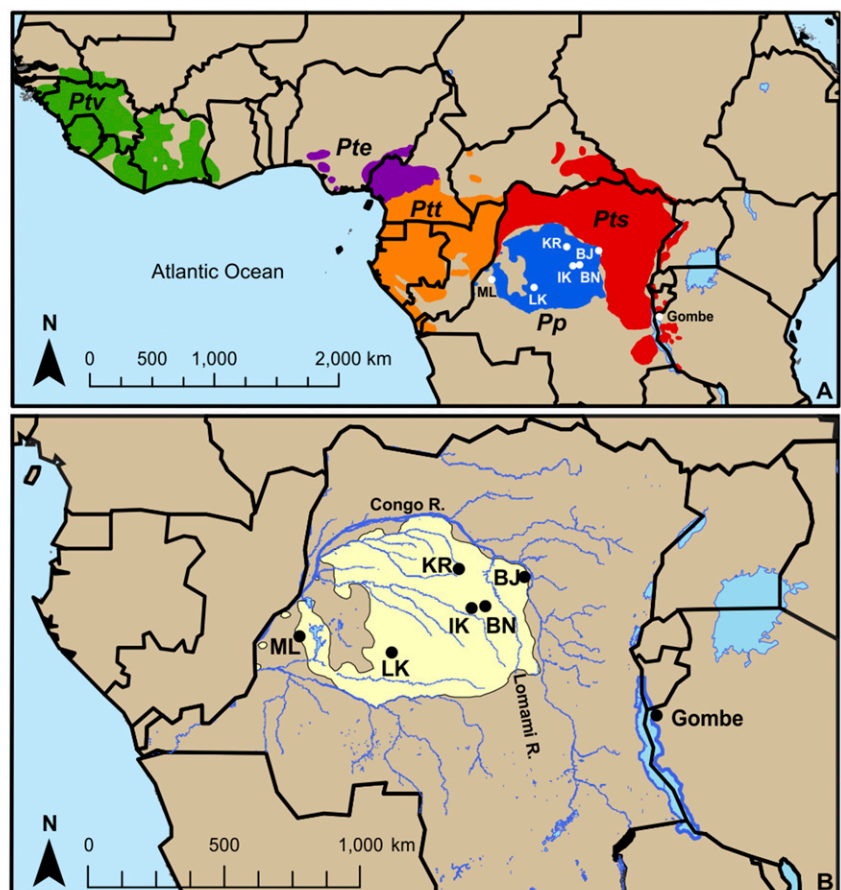

FIGURE 1. The bonobo range and locations of the populations studied. (**A**) Map of Africa showing the range of bonobos (*Pan paniscus* [*Pp*]) and the four chimpanzee subspecies (*P. troglodytes verus* [*Ptv*; western], *P. troglodytes ellioti* [*Pte*; Nigeria-Cameroon], *P. troglodytes troglodytes* [*Ptt*; central], and *P. troglodytes schweinfurthii* [*Pts*; eastern]). The locations of the six bonobo study sites within the DRC are shown as white circles and are labeled with their two-letter codes. The sites are separated by distances of ~30–1000 km. Also marked is the location of the wild *P. troglodytes schweinfurthii* chimpanzee population in Gombe National Park, Tanzania. (**B**) Enlarged view of the six bonobo study sites (black circles; labeled with their two-letter codes) and the site of the Gombe *P. troglodytes schweinfurthii* chimpanzee population. The bonobo range is highlighted in yellow, and blue lines represent rivers in the DRC. The Lomami River separates BJ from the other five bonobo sites.

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A

	West	Central				East
Number of:	ML	LK	IK	BN	KR	BJ
Papa-B alleles	3	10	11	13	14	2
Individuals (Min)	14	17	18	22	37	2
Samples	46	37	36	67	67	2

B

Papa-B		Number of individuals with allele					
		ML	LK	IK	BN	KR	BJ
All sites	*07:01	14	8	7	6	22	
	*09:01	4	2	3	2	2	2
	*15:01	2	3	1	3	1	
Some sites	*01:01			2	3	3	
	*17:01			6	7	17	
	*11:01			2	2	2	
	*19:01			3	4	4	
	*12:01		1		1	6	
	*13:01		1	6	7		
	*02:02		1		1	1	
	*18:01		2	2	2		
	*20:01			1	2		
	*12:02			1	2		
One site	*04:01						2
	*10:01					2	
	*21:01					1	
	*17:02		1				
	*19:02					1^2	
	*08:01					3	
	*14:01		11				
	*08:02					1	
	*16:01		2				

C

Papa-B		Number of individuals with allele	
Allele	Frequency	Heterozygote	Homozygote
*01:01	0.036	8	
*02:02	0.014	3	
*04:01	0.009	2	
*07:01	0.327#	42	15
*08:01	0.014	3	
*08:02	0.005	1	
*09:01	0.068	15	
*10:01	0.014	1	1
*11:01	0.027	6	
*12:01	0.036	8	
*12:02	0.014	3	
*13:01	0.082	10	4
*14:01	0.055	10	1
*15:01	0.046	10	
*16:01	0.009	2	
*17:01	0.141	29	1
*17:02	0.005	1	
*18:01	0.027	6	
*19:01	0.050	11	
*19:02	0.005	1	
*20:01	0.014	3	
*21:01	0.005	1	

FIGURE 2. Summary of fecal sampling and *Papa-B* genotyping results. (**A**) The number of *Papa-B* alleles; the minimum estimate of the number of individuals sampled, as determined by the combination of mitochondrial haplotypes and microsatellite and *Papa-B* genotypes (Individuals [Min]); and the number of fecal samples genotyped (Samples) are given for each population. West, Central, and East denote the three regional bonobo populations, as defined by F_{ST} distances based on mitochondrial haplotype (46). (**B**) Distribution of *Papa-B* alleles among individuals in the six study sites. The alleles are grouped according to their presence in all sites (top), two or three sites (middle), or one site (bottom). Alleles found in a single individual at one site were detected from one sample (1) or two (1^2). Previously identified *Papa-B* alleles are highlighted in gray. (**C**) *Papa-B* allele frequencies in the total study population of 110 bonobos are presented in order of ascending allele number, along with the number of bonobos that possess the allele as heterozygotes or homozygotes. *Papa-B*07:01* appears to have an excess of homozygotes, but this is because of the large number of homozygotes expected and observed in the ML population, which only has three *Papa-B* alleles. #*Papa-B*07:01* is more frequent than the other alleles ($p < 0.0001$, Fisher exact test).

samples were kept at ambient temperature before they were frozen (typically several weeks, but up to several months in some cases). Samples from ML were frozen at −80°C at the Institut National de Recherche Biomédicale in Kinshasa before being sent directly to the University of Montpellier. Samples from the five other sites were frozen at −20°C in the central laboratory at Kisangani, from which they were sent to the United States. DNA was extracted from the samples and analyzed for mitochondrial hypervariable D-loop haplotype and by genotyping for four to eight microsatellite loci [Li et al. (45): LK, IK, and KR; this study: ML, BN, and BJ]. For the fecal samples from ML, BN, and BJ, the sex of sample donors was determined using the PCR-based method described by Sullivan et al. (47).

DNA extraction and Papa-B *PCR and sequencing*

DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN) and the protocol that we described earlier (32). Fecal DNA was amplified in three separate PCR reactions to yield exons 2 and 3 of *Papa-B* [as described for chimpanzees by Wroblewski et al. (32)]. Exons 2 (270 bp) and 3 (276 bp) were targeted because they encode, respectively, the α_1 and α_2 domains that form the peptide binding site of MHC-B. The α_1 and α_2 domains are the most variable and functionally engaged part of MHC-B. Each exon was amplified separately using primers designed from the intron sequences that flank the exons. These are conserved between chimpanzee *Patr-B* and bonobo *Papa-B*. The two standard PCR reactions produced amplicons of 425 bp (exon 2) and 411 bp (exon 3), similar sizes to the 137–328-bp amplicons of the bonobo microsatellite typing system. The third reaction was designed specifically to amplify exon 2 of alleles of the chimpanzee *Patr-B*17* lineage (429-bp amplicon), which the standard exon 2 primers cannot amplify. This amplification was applied to all samples that appeared homozygous for exon 2 using the standard primers. However, no bonobo had an allele related to the *Patr-B*17* lineage for exon 2 or exon 3.

We used the forward primer to sequence all PCR products. When this sequence indicated the presence of a novel allele or when the sequence was ambiguous, the PCR products were cloned and sequenced. On detecting a candidate novel allele of *Papa-B*, its identity was confirmed with a second amplification, either from another individual or an independent amplification from the same individual. Before being applied to fecal DNA from wild bonobos, the standard pairs of amplification primers were validated by their capacity to amplify and sequence exons 2 and 3 of *Papa-B* from DNA extracted from PBMCs of two captive bonobos (Lorel [*Papa-B*01:01*, **07:01*] and Matata [homozygous *Papa-B*07:01*]) obtained from the Yerkes Regional Primate Research Center (Atlanta, GA). Bonobo PBMCs were isolated from samples of peripheral blood by Ficoll gradient separation. DNA was extracted from PBMCs using the QIAamp DNA Blood Kit (QIAGEN).

Papa-B *allele inference*

Because exons 2 and 3 were amplified separately, we had to infer the phase of the exons to define the alleles. When exon 2 and 3 sequences were identical to those of a previously characterized allele (e.g., *Papa-B*07:01*), they were assumed to signify that allele and were paired together (Supplemental Fig. 1A). For heterozygous individuals containing an exon 2 and 3 pair from a previously identified allele, the other exon 2 and 3 pair was then inferred to define the second allele (Supplemental Fig. 1A). Most alleles (exon 2 and 3 pairs) were amplified more than once from different fecal DNA samples (from different individuals) and in different heterozygous combinations. Therefore, the repeated exon pair was inferred to make up an allele, identifying the remaining pair as the second allele (Supplemental Fig. 1B). For all alleles observed in only one or two individuals at a particular site, all but one were observed in heterozygous genotypes (both for exons 2 and 3) with alleles commonly observed in other individuals and/or at other sites (Fig. 2, Supplemental Table Ia–f). This facilitated the identification of alleles according to the above criteria. In one exception, a novel exon 3 sequence was obtained from just one KR sample that was heterozygous for the exon (Fig. 2B, Supplemental Table Ie). However, it was unclear whether both exon 3 sequences shared the same, single exon 2 sequence obtained from the sample. Therefore, conservatively,

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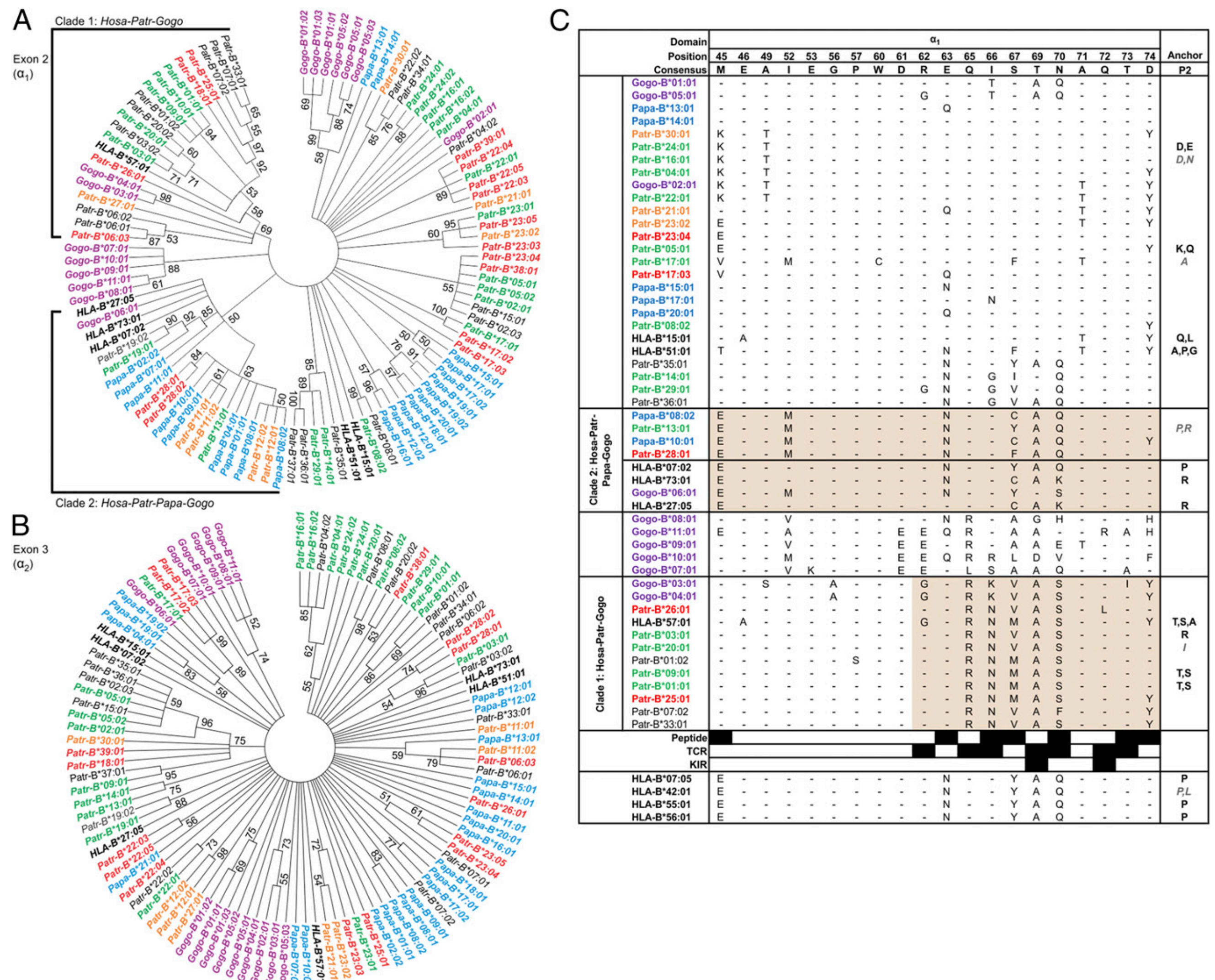

FIGURE 3. Two trans-species clades of *MHC-B* alleles. Neighbor-joining phylogenetic trees constructed from the sequences of exon 2 (**A**), which encodes the α_1 domain, and exon 3 (**B**), which encodes the α_2 domain. Included are all bonobo *Papa-B*, chimpanzee *Patr-B*, and gorilla *Gogo-B* alleles, as well as representative human *HLA-B* alleles. Allele names are colored blue for *Papa-B*, and *Patr-B* are colored according to subspecies when known: green for western *P. troglodytes verus*, orange for central *P. troglodytes troglodytes*, and red for eastern *P. troglodytes schweinfurthii*. Representative *HLA-B* alleles are in bold black, and gorilla *Gogo-B* alleles are in purple. Nodal bootstrap values are based on 1000 replications. Nodes with <50% support were collapsed (full trees are shown in Supplemental Fig. 2). The brackets in (A) show two trans-species clades of alleles. Clade 1 contains *HLA-B*, *Patr-B*, and *Gogo-B* alleles (*Hosa-Patr-Gogo*). Clade 1 was identified previously (28, 32) and contains alleles associated with control of HIV-1 progression (*HLA-B*57:01*) and SIVcpz (*Patr-B*06:03*) infection (31, 32, 58–63). Clade 2, defined in this study, includes *HLA-B*, *Patr-B*, *Papa-B*, and *Gogo-B* alleles (*Hosa-Patr-Papa-Gogo*). *HLA-B*27:05*, in Clade 2, also associates with control of HIV-1 progression (61); however, its inclusion in the clade has weak support. HLA-B*27:05 differs from the clade Papa-B and Patr-B allotypes at key functional positions (63, 70), which contribute to differences in their position 2 (P2) peptide-binding motif (**C**). Because arginine is the P2 residue of the HIV Gag KK10 epitope targeted by HLA-B*27:05 (48, 49), the associated protective effects of HLA-B*27:05 are unlikely to be preserved by Clade 2 bonobo and chimpanzee alleles, which are likely to bind peptides with P2 proline. (C) Table of amino acid sequence differences in residues 45–74 of the MHC-B α_1 domain. Representatives of each sequence motif within this region for the alleles of tree (A) are included in the upper part. (The full allele set is given in Supplemental Table Ih). Allotype names are colored according to species or subspecies, as in (A). Identity to the consensus is denoted by a dash. The regions containing motifs that define Clade 1, positions 62–74 (28, 32), and Clade 2, positions 45–74 (Supplemental Fig. 3), are highlighted in tan. Additional HLA-B sequences with the Clade 2 motif are provided in the lower portion. Black-filled boxes between the two sets of sequences show the positions that contribute to binding sites for peptide, TCR, and KIR. Positions 45, 63, 66, 67, and 70 contribute to the B pocket of the MHC-B molecule, which binds the anchor residue, typically at P2, of nonamer peptides. The P2 residues for each MHC-B allotype [compiled from the SYFPEITHI database of MHC ligands and peptide motifs, http://www.syfpeithi.de/ (50) and from de Groot et al. (31)] are listed in the "Anchor" column. MHC-B P2 residues that were inferred from known ligands are italicized and in gray type.

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we did not assign the unique exon 3 sequence a companion exon 2 sequence, and the exon 3 sequence was given the provisional allele name *Papa-B*21:01* (Fig. 4, see Supplemental Fig. 1C for more details).

Hardy–Weinberg equilibrium and allele frequency differences

We used GENEPOP v4.2 (Hardy–Weinberg exact test, using probability test) to test for deviations between the observed genotype frequencies and those expected under Hardy–Weinberg equilibrium for all 110 bonobos, as well as the five well-sampled populations. GENEPOP uses the Markov chain method to estimate *p* values (51, 52). Differences in allele frequencies were tested with the Fisher exact test using GraphPad QuickCalcs.

Phylogeny, pairwise differences, and amino acid variability

Neighbor-joining trees of *MHC-B* nucleotide sequences were created using the Tamura–Nei model in MEGA6 (53), with pairwise deletion and 1000 bootstrapped replications. MEGA6 was also used to calculate pairwise

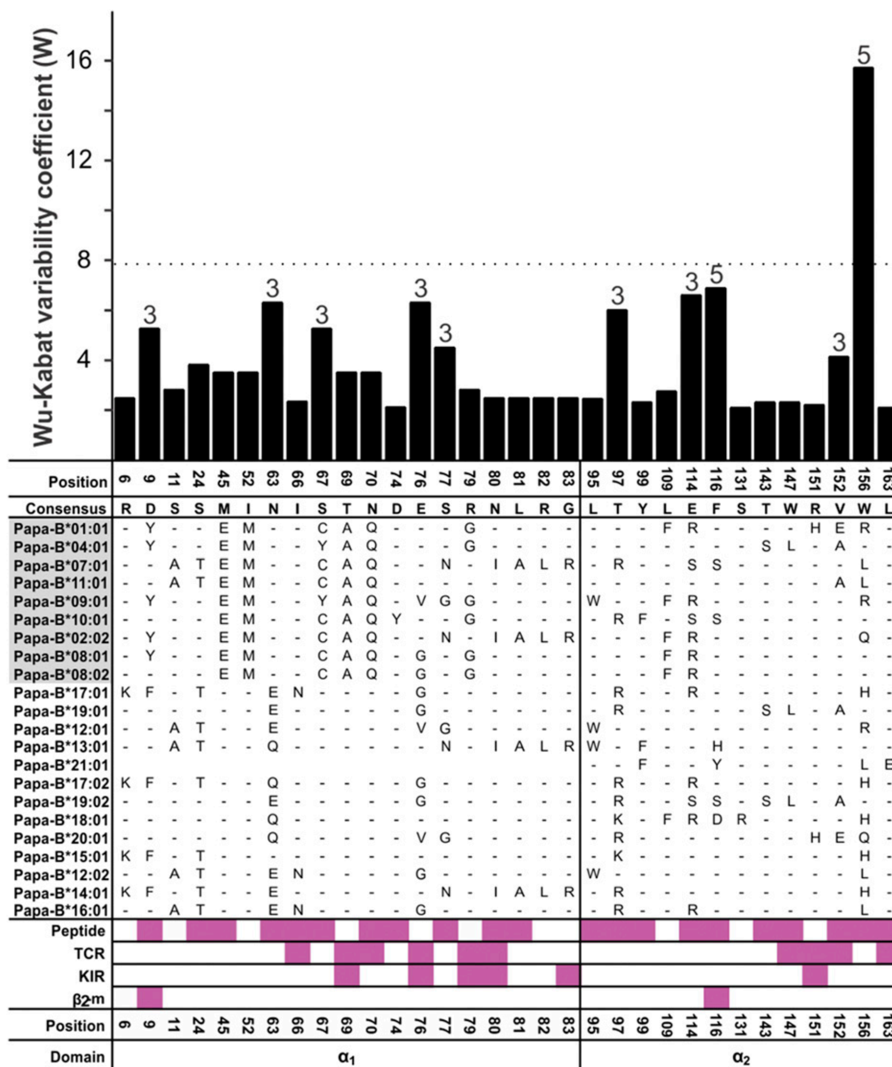

FIGURE 4. High amino acid sequence variability in Papa-B focuses on position 156 in the α_2 domain. The coefficient of amino acid sequence variability (W) for Papa-B allotypes is plotted in the bar graph. Only polymorphic positions are shown. The dotted line marks the value of W that is twice the mean value for W at all polymorphic positions. For positions that are not dimorphisms, the observed number of alternative amino acids is given above the bar. Amino acid sequence differences that distinguish the Papa-B allotypes are shown in the table. Identity to the consensus is denoted by a dash. Based on the criteria for phasing exon 2 and exon 3 sequences, Papa-B*21:01 could not be assigned an α_1 (exon 2) sequence (see *Materials and Methods* for details) (Supplemental Fig. 1). Clade 2 allotypes (Hosa-Patr-Papa-Gogo) are highlighted in gray. Pink boxes denote positions that contribute to binding sites for peptide, TCR, KIR, and β_2-m, the invariant subunit of MHC class I.

distances between nucleotide sequences, using pairwise deletion and proportional distance (p-distance). The difference between the mean p-distances was tested with unpaired *t* tests using GraphPad QuickCalcs. The Wu–Kabat variability coefficient was used to assess the amino acid diversity at each position in the α_1 and α_2 domains of MHC-B (54, 55). For each position in the sequence, the coefficient was calculated as $(N*k)/n$, where N is the total number of sequences, k is the number of different amino acid residues occurring at that position, and n is the number of sequences in which the most common amino acid at that position is observed.

Additional MHC-B *data sets*

Several *MHC-B* data sets were used to examine bonobo *Papa-B* variation in context. More than 4600 human *HLA-B* alleles have been identified in human populations worldwide, through the typing of prospective donors of hematopoietic stem cells for clinical transplantation (43). For our analyses, the *HLA-B* dataset was reduced to a set of 20 *HLA-B* alleles that represent all human variation (Supplemental Table Ig) (J. Robinson, L.A. Guethlein, N. Cereb, S.Y. Yang, P.J. Norman, S.G.E. Marsh, and P. Parham, manuscript in revision). Fifteen *Gogo-B* alleles from Western gorilla (*Gorilla gorilla*) and 64 *Patr-B* alleles from chimpanzee were also included. *Patr-B* alleles were also identified as being specific to chimpanzee subspecies (Fig. 1A): western *P. troglodytes verus* (21 alleles), central *P. troglodytes troglodytes* (8 alleles), and eastern *P. troglodytes schweinfurthii* (16 alleles) (32). *Patr-B* in the fourth chimpanzee subspecies, *P. troglodytes ellioti*, has yet to be studied.

Data sets of *Patr-B* from two chimpanzee populations were compared with *Papa-B* in bonobo populations. One population consists of the 125 wild *P. troglodytes schweinfurthii* chimpanzees of Gombe National Park in Tanzania (32). The other includes 32 wild-born *P. troglodytes verus* chimpanzees from Sierra Leone that were used to found the captive population formerly housed at the Biomedical Primate Research Center (BPRC) in the Netherlands (31). *HLA-B* allele frequencies for six human populations were included in the comparisons. Four are indigenous populations: the Hadza from Tanzania (56), the Tao from Taiwan (57, 58), the Asaro from Papua New Guinea (59), and the Yucpa from Venezuela (60). The other two human populations are admixed urban populations: Africans from Kampala, Uganda (61) and Europeans from Bergamo, Italy (57, 58).

Results

Study of six separated bonobo populations identified 22 Papa-B *alleles*

We studied MHC variation in bonobos resident at six sites in the DRC: ML, LK, IK, BN, KR, and BJ. These sites are between 30 and 1000 km apart and, collectively, they represent much of the bonobo range (Fig. 1). A total of 255 samples of bonobo feces was used to study *Papa-B*, the ortholog of chimpanzee *Patr-B* and human *HLA-B*. Only two fecal samples were obtained from site BJ, whereas 36–67 samples were collected from each of the other sites (Fig. 2A). Because almost all amino acid sequence diversity and the sites of functional interaction localize to the α_1 and α_2 domains of MHC class I (6, 11), we targeted these domains of Papa-B. The methods used were those developed in our study of *Patr-B* polymorphism in the wild chimpanzee population of Gombe National Park in Tanzania (32).

Exon 2, encoding the α_1 domain, and exon 3, encoding the α_2 domain, were amplified separately by PCR from DNA isolated from bonobo feces and sequenced. By comparing the exon 2 and 3 sequences obtained from all 255 fecal samples, we could define,

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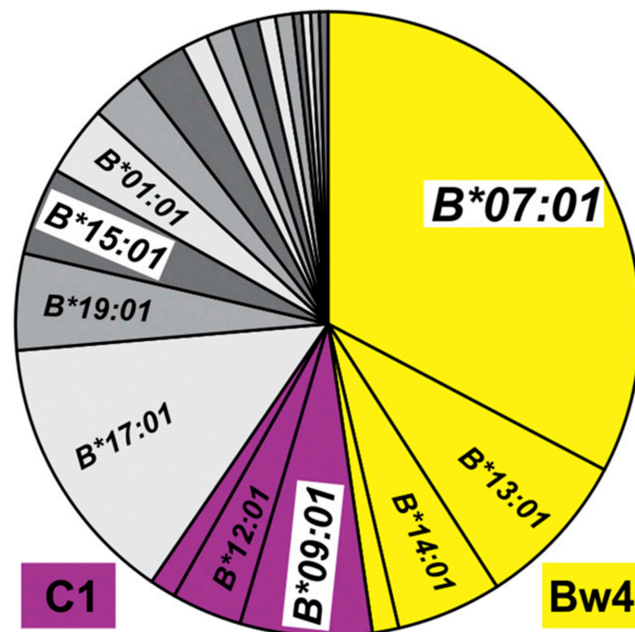

FIGURE 5. Pie chart of *Papa-B* allele frequencies in the study population of 110 bonobos. Alleles encoding the Bw4 KIR ligand are colored yellow, and those encoding the C1 KIR ligand are colored purple. Alleles that do not encode a KIR ligand are in shades of gray. Alleles present at >3% in the population are labeled. *Papa-B*07:01*, *B*09:01*, and *B*15:01*, highlighted by white boxes, are present in all five well-sampled bonobo populations.

unambiguously, which combination of exon 2 and 3 is present in each *Papa-B* allele (Supplemental Fig. 1). This approach defined 22 different *Papa-B* alleles (Fig. 2). Three of these, *Papa-B*01:01*, *Papa-B*04:01*, and *Papa-B*07:01*, were known from earlier studies of very small numbers of captive bonobos (39, 41). Thus, 19 of the *Papa-B* alleles identified in this study in wild bonobos are novel.

Because the identity of the individual bonobo providing each fecal sample is unknown, we cannot discern precisely how many bonobos from each site contributed to our study. However, a minimum size for each population was obtained from the number of combined microsatellite genotypes, mitochondrial haplotypes, and *Papa-B* genotypes detected in the population (Fig. 2A, Supplemental Table Ia–f). In total, we studied a minimum of 110 bonobos, a comparable number to the 125 Gombe chimpanzees studied for *Patr-B* (32). Between 14 and 37 bonobos were studied for each of the five well-represented sites, and the two BJ samples came from different individuals (Fig. 2A, Supplemental Table Ia–f). Although both BJ samples typed identically as heterozygous for *Papa-B*04:01* and *Papa-B*09:01*, they differ in microsatellite genotype (Supplemental Table If). In subsequent comparative analyses we will use the word "populations" to refer to the five well-represented sites.

Between 3 and 14 *Papa-B* alleles were identified in each of five bonobo populations (Fig. 2A). Eight of the *Papa-B* alleles were found in only one of the five populations (Fig. 2B). In addition, a ninth allele, *Papa-B*04:01* was found only in the two BJ individuals (Fig. 2B). Ten of the *Papa-B* alleles were observed in two or three populations. In contrast, the *Papa-B*07:01*, *Papa-B*09:01*, and *Papa-B*15:01* alleles were present in all five populations (Fig. 2B), and they were also among the most frequent *Papa-B* alleles (Fig. 2C), accounting for 44.1% of all *Papa-B*. With a frequency of 32.7%, *Papa-B*07:01* has a significantly higher frequency than any other *Papa-B* allele ($p < 0.0001$, Fisher exact test). The genotype frequencies, for each population and for their combination, conform to Hardy–Weinberg equilibrium (GENEPOP v4.2, Hardy–Weinberg exact test, using probability test) (Fig. 2C, Supplemental Table Ia–f). This result indicates that we have defined the common *Papa-B* alleles in the five populations and that any undetected allele has relatively low frequency. That *Papa-B*04:01* was found only in BJ samples indicates that this eastern population of bonobos likely harbors additional novel *Papa-B* alleles.

Bonobo and chimpanzee share a distinctive clade of MHC-B *alleles*

Phylogenetic trees constructed from hominid *MHC-B* sequences typically show shallow branches and little evidence for long-lived alleles or allelic lineages (27, 32) [(Fig. 3A) (exon 2) and (Fig. 3B) (exon 3)] (Supplemental Fig. 2). An exception is a deeper branch formed by the exon 2 sequences of a trans-species clade of chimpanzee *Patr-B*, western gorilla *Gogo-B*, and human (*Homo sapiens*, *Hosa*, *HLA-B*) *MHC-B* alleles (28, 31, 32) (Fig. 3A, Supplemental Fig. 2). Defining this *Hosa-Patr-Gogo* clade (Clade 1) is a sequence motif in codons 62–74 of exon 2 (28, 32) (Fig. 3C, Supplemental Table Ih). Included in Clade 1 are human *HLA-B*57:01* and chimpanzee *Patr-B*06:03*, alleles associated with control of the progression of HIV-1 and SIVcpz infection, respectively (31, 32, 62–67). Remarkably, no bonobo *Papa-B* allele clusters in this trans-species clade (Fig. 3A, Supplemental Fig. 2). Because this clade is predicted to have been present in the common ancestor of chimpanzee and bonobo, it appears to have been subsequently lost by the bonobo lineage. A possible factor contributing to this loss is that bonobos, unlike chimpanzees and humans, are not endemically infected with a primate lentivirus corresponding to chimpanzee SIVcpz and human HIV-1 (45, 68–71).

Including *Papa-B* alleles in phylogenetic analysis of *MHC-B* revealed a second deep branch, Clade 2, which includes subsets of human, gorilla, chimpanzee, and bonobo *MHC-B* alleles (Fig. 3A, Supplemental Fig. 2). Defining Clade 2 is a sequence motif in codons 45–74 of exon 2 (Fig. 3C, Supplemental Fig. 3, Supplemental Table Ih). The key clade-defining residues are E45, M52, N63, A69, and Q70, as well as three alternative residues at position 67 (C, F, or Y) (Fig. 3C, Supplemental Table Ih). HLA-B allotypes in this clade underwent species-specific divergence, changing M52 to I52. Several of the clade-defining residues contribute to the B pocket, which has a crucial role in binding peptides to MHC class I (72, 73). Comparing residues 45–74 of Clades 1 and 2 identifies eight positions of difference. The degree and pattern of sequence diversity within this region strongly suggest that Clade 1 and Clade 2 Papa-B bind distinct sets of peptide Ags, which also differ from those bound by other Papa-B (Fig. 3C, Supplemental Table Ih).

The Clade 2 *Papa-B* and *Patr-B* alleles represent a lineage of *Pan-B* alleles that was present in the common ancestor of bonobos and chimpanzees. Consistent with this hypothesis, *Papa-B*07:01* and *Papa-B*09:01*, both Clade 2 members, are present in the five bonobo populations (Fig. 2). Likewise for the chimpanzee, Clade 2 alleles were identified in three chimpanzee subspecies: two in western *P. troglodytes verus*, four in central *P. troglodytes troglodytes*, and two in eastern *P. troglodytes schweinfurthii* (Fig. 3A). (Nothing is known of Patr-B in the fourth chimpanzee subspecies, *P. troglodytes ellioti*.) Further supporting our hypothesis, some Clade 2 bonobo and chimpanzee alleles have identical exon 2 sequences. *Papa-B*08:01* shares exon 2 with *Patr-B*12:02*, and *Papa-B*09:01* shares exon 2 with *Patr-B*11:01* and *Patr-B*11:02*. It is most likely that the shared exons were inherited from the common ancestor of bonobo and chimpanzee. Of note, the exon 2 sequences of chimpanzee *Patr-B*11:01* (28, 39), *Patr-B*11:02* (27), and *Patr-B*12:02* (27, 28), which are shared with bonobo, have been found only in *P. troglodytes troglodytes*, a chimpanzee subspecies whose range borders that of bonobo (Fig. 1).

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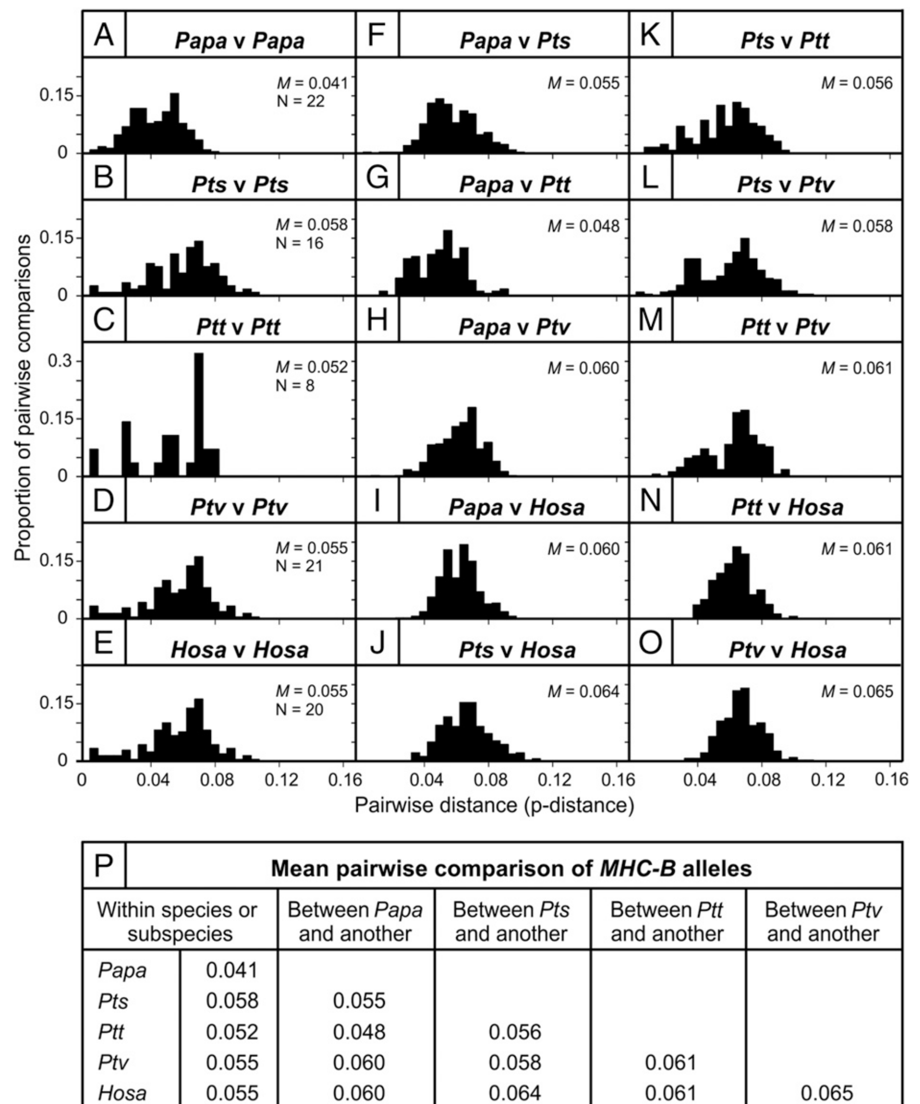

P Mean pairwise comparison of *MHC-B* alleles

Within species or subspecies		Between *Papa* and another	Between *Pts* and another	Between *Ptt* and another	Between *Ptv* and another
Papa	0.041				
Pts	0.058	0.055			
Ptt	0.052	0.048	0.056		
Ptv	0.055	0.060	0.058	0.061	
Hosa	0.055	0.060	0.064	0.061	0.065

FIGURE 6. Bonobo *Papa-B* has less nucleotide sequence diversity than chimpanzee *Patr-B* and human *HLA-B*. (**A**–**E**) Within-group comparisons of p-distances for *MHC-B* alleles (exons 2 and 3) are plotted as histograms. (**F**–**O**) Between-group comparisons. Mean p-distances (*M*) are given in the individual panels and are summarized in (**P**). N refers to the number of alleles. See Supplemental Table Ii for statistical results. *Hosa*, *H. sapiens*; *Papa*, *P. paniscus*; *Pts*, *P. troglodytes schweinfurthii*; *Ptt*, *P. troglodytes troglodytes*; *Ptv*, *P. troglodytes verus*.

Common and widespread Papa-B *allotypes carry the Bw4 and C1 epitopes*

The peptide-binding domains of the 22 Papa-B allotypes differ at 32 positions of amino acid substitution (Fig. 4). This compares with 57 positions for Patr-B and 178 for HLA-B. Almost all of the variable positions in Papa-B are associated with functional interactions of the α_1 and α_2 domains with peptide, TCR, KIR, and β_2-microglobulin (β_2-m). The Papa-B α_1 domain has more variable positions ($n = 19$) than the α_2 domain ($n = 13$). Of the 32 variable positions, 22 are dimorphisms, 8 are trimorphisms, and 2 display five alternative amino acid residues (Fig. 4). The 10 positions exhibiting more than two residues are evenly distributed between the two domains, but the α_2 domain contains positions 116 and 156 that exhibit five alternative residues. Calculation of the Wu–Kabat coefficient of variability (54, 55) shows that position 156 in Papa-B has a much higher variability than all of the other positions (Fig. 4, upper portion).

The Bw4 epitope recognized by KIR is defined by a sequence motif at positions 76–83 in the α_1 domain (6, 11). Four bonobo allotypes have this sequence motif: Papa-B*07:01, Papa-B*02:02, Papa-B*13:01, and Papa-B*14:01 (Fig. 4). Together, they account for 47.7% of the Papa-B in the bonobo population (Figs. 2C, 5). The C1 epitope recognized by KIR is defined by a sequence motif at positions 76 and 80 in the α_1 domain (6, 11). Three bonobo allotypes have this motif: Papa-B*09:01, Papa-B*12:01, and Papa-B*20:01. These C1$^+$ allotypes account for 11.8% of the Papa-B in the bonobo population. Papa-B*07:01 and Papa-B*09:01, both Clade 2 molecules and the most common Bw4$^+$ and C1$^+$ allotypes, respectively, are present in all five bonobo populations (Fig. 2, Supplemental Table Ia–f). Thus, we see a balance in bonobo populations between three types of Papa-B allotypes: one having the Bw4 epitope, one having the C1 epitope, and one having neither epitope.

Bonobo Papa-B *is less diverse than chimpanzee* Patr-B *and human* HLA-B

Among the 546 nucleotides of exons 2 and 3, 58 exhibit nucleotide variation among the 22 *Papa-B* alleles. This number is comparable to 59 positions in eight *P. troglodytes troglodytes Patr-B* alleles but is lower than the 86 positions present in 16 *P. troglodytes schweinfurthii Patr-B* alleles, the 91 positions present in 21 *P. troglodytes verus Patr-B* alleles, and the 88 positions present in 20 *HLA-B* alleles. These comparisons provided a first insight that *Papa-B* has more limited diversity than its orthologs in chimpanzee subspecies and humans.

MHC-B diversity was further compared by analysis of the nucleotide differences (p-distance) between pairs of alleles from within each species or subspecies (Fig. 6). The mean p-distance of 0.041 for *Papa-B* (Fig. 6A) is significantly less than the mean p-distances for *P. troglodytes schweinfurthii Patr-B* (mean = 0.058, $p < 0.0001$,

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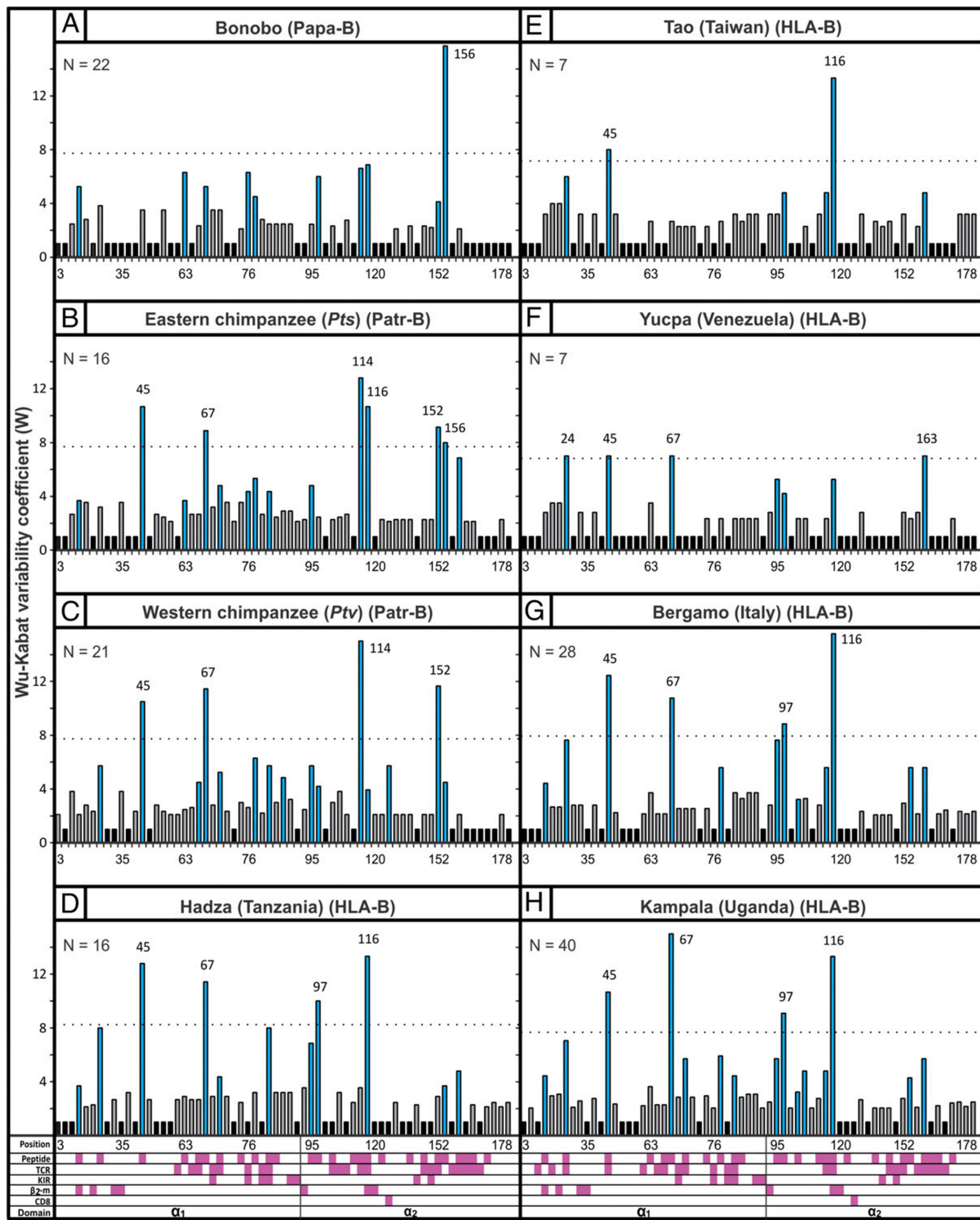

FIGURE 7. Comparison of MHC-B amino acid sequence variability in bonobo, chimpanzee, and humans. Plots of the coefficient of amino acid sequence variability (W) for MHC-B allotypes within bonobo (**A**), *P. troglodytes schweinfurthii* (*Pts*) chimpanzees (**B**), *P. troglodytes verus* (*Ptv*) chimpanzees (**C**), three indigenous human populations (**D**–**F**), and two urban human populations (**G** and **H**). N gives the number of allotypes for each population. Black bars represent positions with no sequence variability, gray bars represent dimorphic positions, and blue bars represent polymorphic positions. The dotted line is set at twice the mean value of W for all variable positions within a population. The pink boxes show the positions that contribute to human binding sites for peptide, TCR, KIR, the invariant subunit β_2-m, and the CD8 T cell coreceptor (CD8). The Hadza (56), Tao (57, 58), and Yucpa (60) are indigenous human populations from Africa (Tanzania), Asia (Taiwan), and South America (Venezuela), respectively; Bergamo (57, 58) and Kampala (61) are admixed urban populations from Europe (Italy) and Africa (Uganda), respectively.

Fig. 6B), *P. troglodytes troglodytes Patr-B* (mean = 0.052, $p = 0.0015$, Fig. 6C), *P. troglodytes verus Patr-B* (mean = 0.055, $p < 0.0001$, Fig. 6D), and *HLA-B* (mean = 0.055, $p < 0.0001$, Fig. 6E) (The statistical comparisons are also summarized in Supplemental Table Ii). Of note are the similar mean *p*-distances for humans and each chimpanzee subspecies.

The p-distances were also calculated for comparisons between one *Papa-B* allele and one chimpanzee or human *MHC-B* allele. Comparison with *P. troglodytes schweinfurthii Patr-B* gave a mean p-distance of 0.055 (Fig. 6F) compared with 0.048 with *P. troglodytes troglodytes Patr-B* (Fig. 6G), 0.060 with *P. troglodytes verus Patr-B* (Fig. 6H), and 0.060 with *HLA-B* (Fig. 6I)

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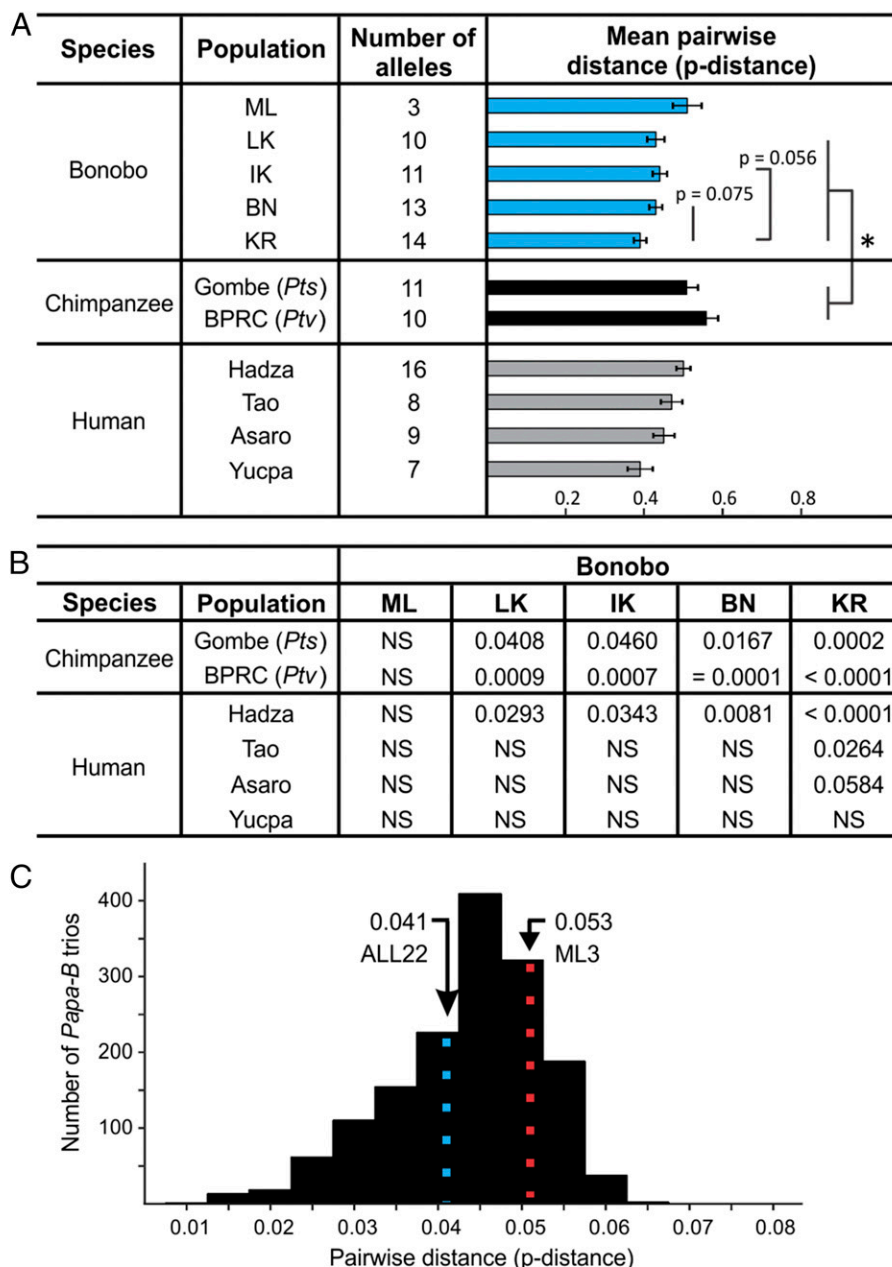

Species	Population	Bonobo ML	LK	IK	BN	KR
Chimpanzee	Gombe (*Pts*)	NS	0.0408	0.0460	0.0167	0.0002
	BPRC (*Ptv*)	NS	0.0009	0.0007	= 0.0001	< 0.0001
Human	Hadza	NS	0.0293	0.0343	0.0081	< 0.0001
	Tao	NS	NS	NS	NS	0.0264
	Asaro	NS	NS	NS	NS	0.0584
	Yucpa	NS	NS	NS	NS	NS

FIGURE 8. Comparison of *Papa-B* diversity in the five bonobo populations. (**A**) Number of alleles and mean p-distances (± SEM) for *MHC-B* in the five bonobo populations and their comparison with chimpanzee and human populations. Representing indigenous chimpanzees are the wild Gombe *P. troglodytes schweinfurthii* (*Pts*) (32) and captive BPRC *P. troglodytes verus* (*Ptv*) (31) populations. The *p* values shown were calculated using an unpaired *t* test. *Statistical results of comparisons between bonobo populations and those of chimpanzees and humans are given in (**B**). The indigenous human populations represent Africa (Hadza, Tanzania) (56), East Asia (Tao, Taiwan) (57, 58), Melanesia (Asaro, Papua New Guinea) (59), and South America (Yucpa, Venezuela) (60). (B) The *p* values from comparisons of the p-distances of populations in (A) (unpaired *t* tests). (**C**) Distribution of mean p-distances for all possible trios of *Papa-B* alleles that can be permuted from the set of 22 *Papa-B* alleles (n = 1540). The dotted lines show the means for all possible *Papa-B* trios (ALL22, blue dots) and for the three *Papa-B* alleles of the ML population (ML3, red dots).

(p < 0.0001 for both *Papa-Pts-Patr* and *Papa-Ptt-Patr* compared with *Papa-Ptv-Patr*, Supplemental Table Ii). This result shows that *Papa-B* is more similar to *P. troglodytes schweinfurthii Patr-B* and *P. troglodytes troglodytes Patr-B*, the two chimpanzee subspecies whose range borders that of bonobos, than to the more distant *P. troglodytes verus* chimpanzees. For interspecies comparisons involving one *Papa-B* allele, the range of mean p-distances is 0.048–0.60 (Fig. 6F–I), whereas that for interspecies comparisons not involving a *Papa-B* allele is 0. 056–0.065 (Fig. 6J–O). A summary of the mean *p*-distances is given in Fig. 6P (statistical comparisons are also summarized in Supplemental Table Ii). Thus, the nucleotide diversity of *MHC-B* in bonobo is significantly less than that in humans and each of the three chimpanzee subspecies, and it is significantly more similar to that in *P. troglodytes troglodytes* and *P. troglodytes schweinfurthii* chimpanzees than in *P. troglodytes verus* chimpanzees.

Comparison of Wu–Kabat variability coefficients (54, 55) shows that Papa-B also has the lowest variability in amino acid sequence (Fig. 7). In Papa-B, only position 156 has high variability, having more than twice the mean (Fig. 4, upper portion, Fig. 7A). This contrasts with six positions of high variability in *P. troglodytes schweinfurthii* Patr-B (Fig. 7B) and four in *P. troglodytes verus* Patr-B (Fig. 7C). Comparable differences are seen for human populations. The Hadza, hunter-gatherers from Tanzania (56) (Fig. 7D), Yucpa Amerindians (60) (Fig. 7F), and urban populations from Italy (57, 58) (Fig. 7G) and Uganda (61) (Fig. 7H) all have four highly variable positions, whereas the indigenous Taiwanese Tao population has only two (57, 58) (Fig. 7E). Like bonobos, position 156 is variable in *P. troglodytes schweinfurthii* chimpanzees and is dominated by nonpolar residues (Supplemental Table Ij). However, position 156 is approximately half as variable in *P. troglodytes schweinfurthii* chimpanzees as it is in bonobos (Fig. 7A, 7B). This difference is because Papa-B has a balance between leucine (22.7%) and tryptophan (31.8%), whereas Patr-B is biased toward leucine (66.7% in *P. troglodytes verus* Patr-B and 50% in *P. troglodytes schweinfurthii* Patr-B) (Supplemental Table Ij).

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ML bonobos maintain considerable diversity with only three Papa-B *alleles*

Based on their mitochondrial DNA sequences, it has been suggested that bonobos subdivide into three geographically separate groups corresponding to the western, central, and eastern areas of their range (46) (Figs. 1, 2A). Of the sites that we studied, ML is western; LK, IK, BN, and KR are central; and BJ is eastern. Of the five well-represented sites, ML has only 3 *Papa-B* alleles compared with 10–14 alleles in the four central sites (Figs. 2A, 8A). The three Papa-B of ML bonobos represent distinct functional allotypes. Papa-B*07:01 has the Bw4 epitope, Papa-B*09:01 has the C1 epitope, and Papa-B*15:01 has neither epitope (Figs. 4, 5).

ML bonobos maintain considerable nucleotide diversity, as assessed by mean pairwise difference (p-distance). *Papa-B* diversity is highest in ML, of intermediate value in LK, IK and BN, and lowest in KR (Fig. 8A). *Papa-B* diversity in central bonobo populations is significantly less than Patr-B diversity in wild *P. troglodytes schweinfurthii* Gombe chimpanzees (32) and a captive *P. troglodytes verus* population (31) but is comparable to *HLA-B* diversity in four indigenous human populations (Fig. 8A, 8B). In contrast, *Papa-B* diversity in ML is comparable to that of chimpanzee *Patr-B* and human *HLA-B* (Fig. 8A, 8B) and is higher than the mean diversity for all possible combinations of three *Papa-B* alleles (Fig. 8C). Differences in amino acid sequence diversity are also seen among the five bonobo populations. In the LK, IK, BN, and KR populations, position 156 stands out as the one highly variable position (Fig. 9), as also observed in the total bonobo population (Fig. 4, upper portion, Fig. 7A). Although ML has only three Papa-B allotypes, each has a different residue at position 156 (Fig. 9).

The bonobo populations differ in the frequency distribution of their *Papa-B* alleles (Fig. 10). Most similar are the IK and BN bonobos (Fig. 10) that live at closely located sites in the central range (Fig. 1). The frequency of Papa-B allotypes that have the Bw4 epitope is similar (36.4–41.7%) in the three most central sites (IK, BN, and KR), but it is higher in the more western populations (67.6% in LK and 78.6% in ML) (Fig. 10A). The frequency of Papa-B allotypes having the C1 epitope is similar (8.8–11.4%) in four of the populations but is noticeably higher in ML (14.3%). In all five populations, the *Papa-B*07:01* allele, encoding the Bw4 epitope, is the most frequent or the second most frequent *Papa-B* allele (Fig. 10B, 10C). In ML, the *Papa-B*07:01* allele is particularly dominant (Fig. 10C). A less extreme dominance by one *MHC-B* allele is seen in the captive *P. troglodytes verus* chimpanzee population, as well as in the Hadza and Tao human populations (32) (Fig. 10C). Like the Gombe chimpanzees, the LK and KR bonobo populations have two high-frequency alleles and a majority of low-frequency alleles (32). In the IK and BN populations, there is a less skewed distribution of allelic frequencies, more similar to the urban human populations of Kampala and Bergamo (32).

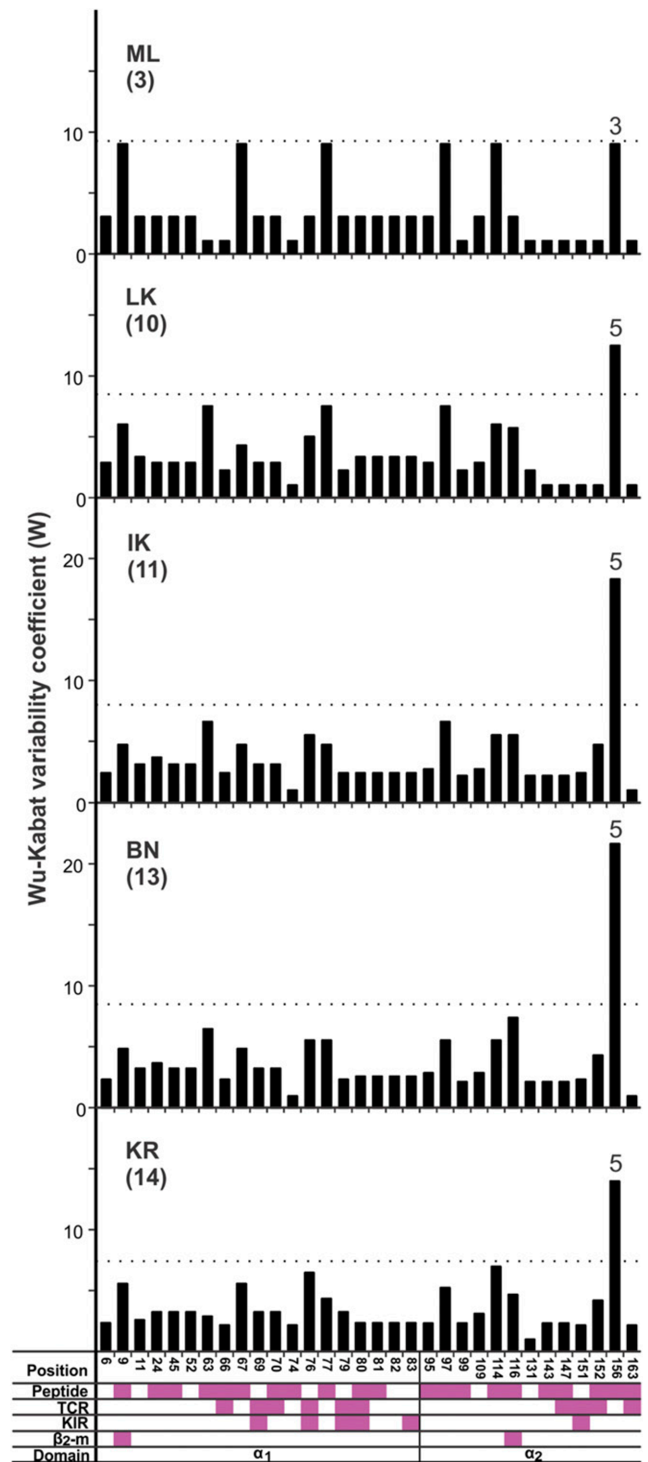

FIGURE 9. Comparison of Papa-B amino acid sequence variability in five bonobo populations. Plots of the coefficient of amino-acid sequence variability (W) for the Papa-B allotypes of the five bonobo populations (numbers of allotypes are given in parentheses). The dotted line marks the value for W that is twice the mean W value for the polymorphic positions in each population. The number of alternative amino acid residues occurring at position 156 is shown above the bar. The pink boxes denote positions that contribute to human binding sites for peptide, TCR, KIR, and the invariant subunit β_2-m.

Discussion

Bonobos and chimpanzees are sibling species and humans' closest relatives (36). These great apes are invaluable for understanding all aspects of human evolution. This is particularly true for the highly polymorphic *MHC class I* genes that function in innate immunity, adaptive immunity, and reproduction (6, 11). Strict orthologs of all of the classical human *MHC class I* genes (*HLA-A*, *HLA-B*, and *HLA-C*) are present only in great apes (10). Moreover, it is in chimpanzee and bonobo that the organization of the *MHC class I* gene family is most like that of humans (10, 11, 38). Although there has been steady acquisition of knowledge of the chimpanzee *MHC* since the late 1980s (10, 27, 28, 74, 75), next to nothing is known about the bonobo *MHC* (10, 38–42). Emphasizing this paucity, the numbers of *MHC-B* sequences currently deposited in *MHC* databases are 8 for bonobo, 64 for chimpanzee, and 4647 for human (43, 44). The eight *Papa-B* sequences were derived from captive bonobos of unknown provenance (39, 41).

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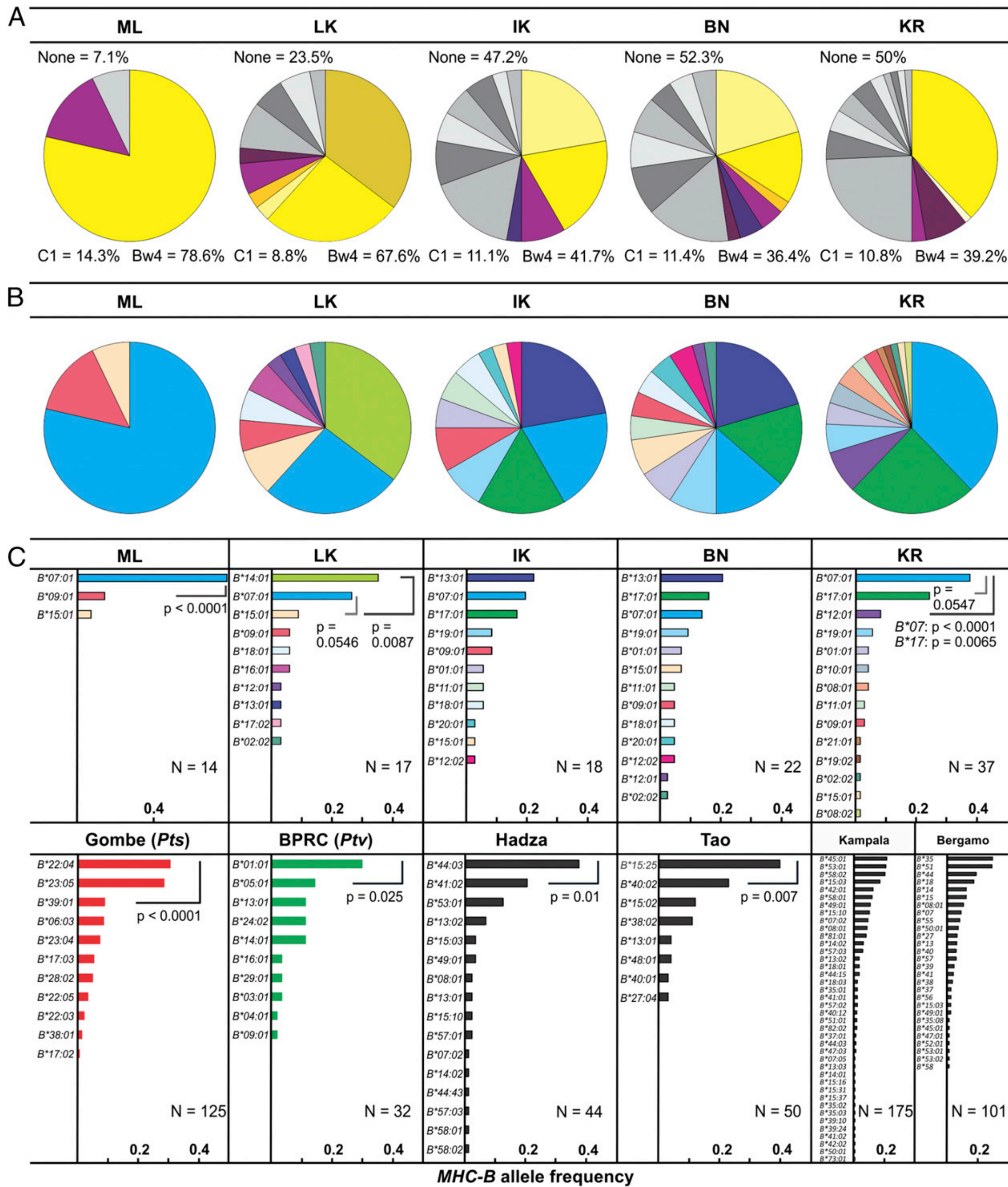

FIGURE 10. *MHC-B* allele distribution in bonobo, chimpanzee, and human populations. (**A**) Pie charts for each population show the proportions of *Papa-B* alleles that encode the Bw4 epitope (shades of yellow) or the C1 epitope (shades of purple). Alleles not encoding Bw4 or C1 are shaded gray. Each pie segment corresponds to a different allele. Segments in different pies that have identical yellow or purple color denote the same allele, but alleles in the same shade of gray are not necessarily the same allele. The frequencies, as percentages, for the alleles encoding an epitope recognized by KIR (Bw4 or C1) and alleles that encode neither epitope (None) are shown for each population. (**B**) Pie charts for each population showing frequencies of the 22 *Papa-B* alleles. Each allele is defined by a different color (same in each pie). Segments are organized in order of decreasing frequency. (**C**) Comparison of the distributions of *MHC-B* allele frequency in five bonobo populations (upper panels), two chimpanzee populations (lower left panels), and four human populations (lower right panels). The bars show the *MHC-B* allele frequencies in descending order. The bar graphs for the bonobo populations use the same color scheme as in (B) to distinguish the *Papa-B* alleles. For the chimpanzee and human, each population is distinguished by bars of a different color, but all alleles are colored identically within a population. The Gombe chimpanzees are the *P. troglodytes schweinfurthii* (*Pts*) subspecies (32), and the BPRC population is the *P. troglodytes verus* (*Ptv*) subspecies (31). *HLA-B* allele frequencies are also shown for the Hadza (56), Tao (57, 58), Kampala (61), and Bergamo (57, 58) human populations. The Hadza and Tao are indigenous populations from Africa (Tanzania) and Asia (Taiwan), respectively; Kampala and Bergamo are admixed urban populations from Africa (Uganda) and Europe (Italy), respectively. N shows the number of individuals analyzed. Allele frequencies were compared using the Fisher exact test. The narrower black brackets (ML, BPRC, Hadza, Tao) denote that the most common allele is significantly more frequent than the second most common allele; the wider black brackets (KR, Gombe) show that the two most common alleles are significantly more frequent than the third most common (*Figure legend continues*)

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In contrast, we studied *Papa-B* in six populations of wild-living bonobos (45). This approach enabled us to study the polymorphism of *Papa-B* and place it in the context of the bonobo's natural population structure. In studying ≥110 individuals, we identified 22 *Papa-B* alleles among the six populations. This number compares with the 11 *Patr-B* alleles present in 125 chimpanzees, forming three communities, in Gombe National Park of Tanzania (32) (Fig. 1). The set of *Papa-B* alleles is less diverse than comparable sets of *HLA-B* and *Patr-B* alleles, and this holds true even when *Patr-B* are limited to those alleles of a single chimpanzee subspecies. Our results are consistent with whole-genome comparisons, which concluded that bonobos experienced a severe population bottleneck and consequently exhibit a strong signature of inbreeding (36).

Phylogenetic analysis identified two trans-species clades of *MHC-B* that are defined by sequence motifs in part of the Ag-recognition site contributed by the α_1 domain. The *Pan* clade (Clade 2) is broadly conserved among African apes, including some *Papa-B* of bonobo and *Patr-B* of the three chimpanzee subspecies studied, as well as some gorilla *Gogo-B*. The other trans-species *MHC-B* clade (Clade 1), which includes some human, chimpanzee, and gorilla *MHC-B* alleles (28, 32), correlates with resistance to disease progression of human HIV-1 (*HLA-B*57:01*) and chimpanzee SIV (*Patr-B*06:03*) infections (31, 32, 62–67). However, none of the 22 *Papa-B* alleles are part of Clade 1. This absence could be due to insufficient sampling of bonobo populations. In this regard, it is unfortunate that we had only two samples from the BJ site. This population is separated from the other populations by the Lomami River (Fig. 1B), which is a known barrier to bonobo gene flow (46, 76). The possibility of BJ bonobos having *Papa-B* alleles that eluded our analysis is likely, because *Papa-B*04:01* was found only in the two BJ bonobos. A relevant and intriguing fact is that SIV has never been detected in bonobos, either in the wild or captivity (45, 77). In contrast, two of the four chimpanzee subspecies (Fig. 1A), *P. troglodytes troglodytes* and *P. troglodytes schweinfurthii*, have endemic SIVcpz infection (69). It is possible that Clade 1 *Papa-B* alleles are present at low frequency within bonobos, in the absence of pressure from SIV infection to drive them to higher frequency. Alternatively, Clade 1 was present in the common ancestor of chimpanzee and bonobo but was subsequently lost on the bonobo branch.

The ML population provides an informative example of a population bottleneck in bonobos. Of similar size to the LK and IK populations, ML has only 3 Papa-B alleles compared with 10 in LK and 11 in IK. For comparison, the South Amerindian Yucpa, a small and bottlenecked human population, has seven *HLA-B* alleles (60). One important characteristic of the three ML Papa-B allotypes (Papa-B*07:01, Papa-B*09:01, and Papa-B*15:01) is that they are the only Papa-B present in all five well-sampled study populations. A second important characteristic is that they represent three functionally distinctive groups of Papa-B. Papa-B*07:01 has the Bw4 epitope recognized by KIR (6, 11). Papa-B*09:01 carries the C1 epitope, also recognized by KIR (6, 11). In contrast, Papa-B*15:01 has neither Bw4 nor C1 and, thus, is dedicated to presenting peptide Ags to the AgRs of CD8 T cells. Their presence in all populations and retention through the bottleneck experienced by ML bonobos indicate that this combination of three Papa-B allotypes has been essential for the survival of bonobo populations. Supporting this hypothesis, the sequence differences among these allotypes give ML bonobos the highest nucleotide diversity of the five populations. Moreover, this diversity is higher than the mean p-distance of all possible combinations of three *Papa-B* alleles.

Conservation of Bw4 and C1 in the five bonobo populations points to the importance that interactions of these epitopes with KIR could have in the education and immune response of bonobo NK cells (6, 11). Comparison of the human and chimpanzee KIR families identified similarities in their component KIR but differences in the organization of the *KIR* locus (11, 35). Chimpanzee *KIR* haplotypes are variations on a theme of multiple strong inhibitory HLA-C receptors (33, 35). In contrast, the human *KIR* locus has two distinctive forms that are present and balanced in all human populations (6, 60). Human *KIR A* haplotypes are similar to chimpanzee *KIR* haplotypes, whereas human *KIR B* haplotypes have genes encoding additional activating KIRs and weaker inhibitory KIRs (6, 22, 35). The *KIR A* and *B* haplotype difference influences NK cell responses and correlates with a wide range of infectious and inflammatory diseases, as well as pregnancy syndromes and outcomes of clinical therapies, notably hematopoietic cell transplantation (25, 26, 78–82). A first analysis of the bonobo *KIR* region showed that it is different again from both the chimpanzee and human *KIR* loci (37).

Bonobo *KIR* haplotypes form two distinctive groups, with even frequencies in the cohort of nine captive bonobos studied (37). One haplotype group resembles chimpanzee *KIR* haplotypes (11, 35, 37). The other group has contracted in size, leaving only the conserved framework genes that define the ends and the center of the *KIR* locus (37). Missing are the genes that, in humans, encode the KIRs that recognize Bw4 and C1. This division of bonobo *KIR* haplotypes into two qualitatively different groups parallels the division of the human *KIR* locus into *A* and *B* haplotypes (11, 37) and contrasts with the chimpanzee *KIR* locus (11, 35). In humans, there is increasing evidence that the *KIR A* and *KIR B* haplotype difference evolved as a compromise between NK cell functions in immunity and reproduction (6, 22). That could also be the case for the bonobo *KIR* haplotype groups. The considerable insight gained from this population study of one bonobo *MHC class I* gene makes targeted capture and next-generation sequencing of entire bonobo *MHC* and *KIR* regions an exciting proposition. A method for such analysis of human *HLA* and *KIR* has proved applicable to chimpanzee and should also apply to bonobo (20, 83).

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Disclosures

The authors have no financial conflicts of interest.

allele but do not differ significantly from each other (p values are given below the bracket; $p < 0.0001$ applies to both high-frequency Gombe alleles). In LK, the most common allele is significantly more frequent than the third most common allele; gray brackets (LK, KR) denote alleles that were of nearly significantly different frequencies ($p < 0.1$).

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