Shared Signature of Recent Positive Selection on the *TSBP1–BTNL2–HLA-DRA* Genes in Five Native Populations from North Borneo

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Abstract

North Borneo (NB) is home to more than 40 native populations. These natives are believed to have undergone local adaptation in response to environmental challenges such as the mosquito-abundant tropical rainforest. We attempted to trace the footprints of natural selection from the genomic data of NB native populations using a panel of ~2.2 million genome-wide single nucleotide polymorphisms. As a result, an ~13-kb haplotype in the Major Histocompatibility Complex Class II region encompassing candidate genes *TSBP1–BTNL2–HLA-DRA* was identified to be undergoing natural selection. This putative signature of positive selection is shared among the five NB populations and is estimated to have arisen ~5.5 thousand years (~220 generations) ago, which coincides with the period of Austronesian expansion. Owing to the long history of endemic malaria in NB, the putative signature of positive selection is postulated to be driven by *Plasmodium* parasite infection. The findings of this study imply that despite high levels of genetic differentiation, the NB populations might have experienced similar local genetic adaptation resulting from stresses of the shared environment.

Key words: natural selection, North Borneo, malaria, *BTNL2*, *TSBP1*, *HLA-DRA*.

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Significance

We had identified a putative positive selection signal that spans a 13-kb haplotype in the MHC II region covering genes *TSBP1–BTNL2–HLA-DRA* in North Borneo native populations. The selection signal was estimated to appear 5,500 years ago and coincided with the period of agricultural expansion in Southeast Asia. Considering the long history of endemic malaria in North Borneo, we therefore hypothesized that the putative signature of positive selection is driven by *Plasmodium* parasite infection.

Introduction

The island of Borneo, geographically located in Southeast Asia, is the third-largest island in the world. Malaysia, Brunei, and Indonesia have sovereignty over the island, with the latter having the largest portion of the land area. The Malaysian section of Borneo comprises the states of Sabah and Sarawak. The Sabah state (known as North Borneo [NB]) is home to culturally diverse populations of more than 40 major ethnicities that converse in over 80 local dialects (Combrink et al. 2006). The people are broadly categorized into five major groups based on their linguistic and sociocultural practices, namely Dusunic, Paitanic, Murutic, Ida'anic, and Sama-Bajaw. Their vernacular is part of the Austronesian superfamily of languages. The northern region of Borneo was once linked to the southern Philippines as part of the larger Sundaland before the rise ofsea level ~15 thousand years ago (kya) (Bellwood 2007).

Archaeological evidence suggests that this landmass may have been inhabited by the Australo-Melanesian group and was subsequently replaced by the Austronesian group (Bellwood 2007). Our recent study based on the genome-wide single nucleotide polymorphism (SNP) array suggested that the native populations from NB are closely related to the aborigines from Taiwan and the non-Austro–Melanesian Filipinos (Yew et al. 2018a). Further investigation using whole-genome sequencing technology suggested that the time of divergence of the NB natives predates the Austronesian expansion, implying a possible human habitation in this landmass during the pre-Neolithic period (Yew et al. 2018b).

NB houses part of the world's oldest tropical rainforest. Owing to its unique geological situation and equatorial climate, the tropical rainforest in NB is enriched in biodiversity and natural resources, but this entails enormous environmental stresses to human habitation and survival, such as the hot and humid climate, limited food resources, and pathogen infections, especially *Plasmodium* parasites. This offers a unique and complex environment for selective pressure. Several investigations of positive natural selection of the indigenous populations inhabiting this region have been carried out. Notably, different signals of positive natural selection against malarial infection and other traits have been detected among the indigenous populations (Orang Asli) from Peninsular Malaysia (Deng et al. 2014; Liu et al. 2015), whereas a recent study reported a genetic adaptation signal for breath-holding diving capability among the Sea Bajau people from Borneo (Ilardo et al. 2018).

Historical demographic events such as population expansion or genetic drift will have an influence on the entire genome. In contrast, natural selection typically affects the diversity of local genomic regions and thus is distinguishable from the genome-wide pattern. Population genomics offers an approach that enables us to identify signatures of past events with footprints left in local genomic regions. Therefore, in this study, we investigated the forces of natural selection that could have had impacts on the unique genetic architecture of these populations. We utilized the genotyping array data set that was published earlier (Yew et al. 2018a) comprising five ethnic groups representing three major linguistic groupings in NB: Dusun, Rungus, Sonsogon (all three represent the Dusunic speaking family), Sungai-Lingkabau (representing the Paitanic speaking family), and Murut-Paluan (representing the Murutic speaking family). A strong putative signal of positive selection in the Major Histocompatibility Complex (MHC) Class II region was identified and was estimated to have occurred in the NB populations ~5.5 kya (~220 generations ago), which coincided with the period of Austronesian expansion. Considering the long history and high prevalence of malaria among the NB populations (Copeland, 1935; William et al. 2014), we postulate that the positive selection could have been driven by the endemic *Plasmodium* species.

Results

Genetic Relatedness of the Five NB Populations

Principal component analysis (PCA) revealed that the NB populations formed a closely related but distinct cluster from other Southeast Asian populations (fig. 1*a*, supplementary fig. S1, Supplementary Material online). The phylogenetic tree constructed based on the Fixation index (F_{ST}) of pairwise populations also showed that the NB populations formed a separate clade (fig. 1*b*). The NB populations and the metropolitan Singapore populations (e.g., Malays [MAS] and Han Chinese [CHS]) are in a closer relationship ($F_{ST} = 0.020–0.042$) than any of them with the Orang Asli from Peninsular Malaysia (e.g., Jakun, abbreviated as JKN, an Austronesian speaking indigenous population categorized under Proto-

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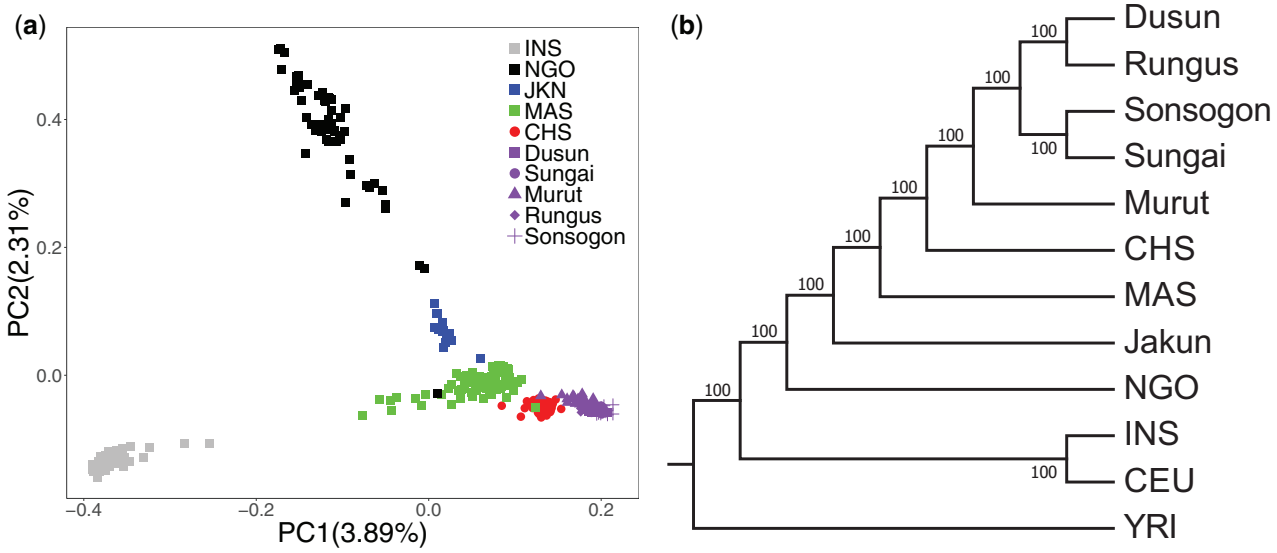

Fig. 1.—Genetic relatedness of the five native populations from NB. (*a*) PCA of Southeast Asian populations; (*b*) An unrooted population phylogenetic tree constructed under a neighbor-joining framework. The branch scores were obtained by 100 replication analyses. MAS, Metropolitan Malays from Singapore; CHS, Southern Han Chinese from Singapore; INS, Southern India from Singapore; NGO, Negrito from Peninsular Malaysia including Negrito Bateq, Negrito Mendriq, and Negrito Jahai; JKN, Proto-Malay Jakun from Peninsular Malaysia; CEU, Northern & Western European; YRI, Yoruba Nigeria.

Malay, and Negrito, abbreviated as NGO, an anthropologically defined Austro-asiatic speaking hunter-gather population from Peninsular Malaysia, also locally known as Semang) (F_{ST} = 0.029–0.048 between the Singapore populations and the Orang Asli; F_{ST} = 0.054–0.089 between the NB populations and the Orang Asli; fig. 1*b*, supplementary table S1, Supplementary Material online). These findings are in agreement with those of Yew et al. (2018a). However, the genetic differentiation among the five NB populations, ranging from 0.014 (Dusun vs. Rungus) to 0.044 (Murut-Paluan vs. Sonsogon), was surprisingly higher when compared with that between the populations from Singapore (F_{ST} = 0.011 between MAS and CHS), indicating nontrivial genetic diversity among the NB populations (supplementary table S1, Supplementary Material online).

Identification of Signatures of Positive Selection

We first estimated the site-specific F_{ST} (Weir and Hill 2002) to scan for putative signals of positive natural selection and subsequently corroborated the results using haplotype-based selection metrics, including the integrated haplotype score (iHS) (Voight et al. 2006) and the cross-population extended haplotype homozygosity (XP-EHH) (Sabeti et al. 2007) (see Materials and Methods for more details). Signals of positive natural selection for each of these metrics are tabulated in supplementary tables S2–S4, Supplementary Material online.

We identified a genomic region spanning ~4.5 Mb of the MHC Class II region (chr6:29,545,208–34,083,564) that was highly differentiated between the NB populations and the surrounding neighbor populations, including MAS, CHS, NGO, and JKN. This putative signal consistently presented in the top 0.1% of the genome-wide F_{ST} in the comparisons between each NB population and each of the three reference populations (except for Sonsogon vs. MAS), but not between any two NB groups (supplementary fig. S2 and table S2, Supplementary Material online). Considering the geographical relatedness of the five NB populations as well as the shared signature of local adaptation, we reasoned that the NB populations might have collectively experienced similar forces of local adaptation in the tropical rainforest. Therefore, we pooled these populations as a single group (denoted in general as NB) in subsequent analyses to locate shared signatures for positive natural selection resulting from local adaptation. We repeated the pairwise F_{ST} analysis using the pooled data set and confirmed that this signal was consistent across pairwise population analyses between NB with MAS, CHS, NGO, and JKN, and was not affected by the Hardy–Weinberg equilibrium filtration of the data (fig. 2*a–d*; supplementary fig. S3*a*, Supplementary Material online). This putative selection signal harboring the MHC Class II region covered 193 genes. As expected, functional enrichment analysis performed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang et al. 2009) showed that these genes were significantly enriched with associations to autoimmune diseases and viral infections (supplementary table S5, Supplementary Material online). We found ten SNPs in this region showing most significant and consistent signals of F_{ST} across population pairs (supplementary table S2, Supplementary Material online).

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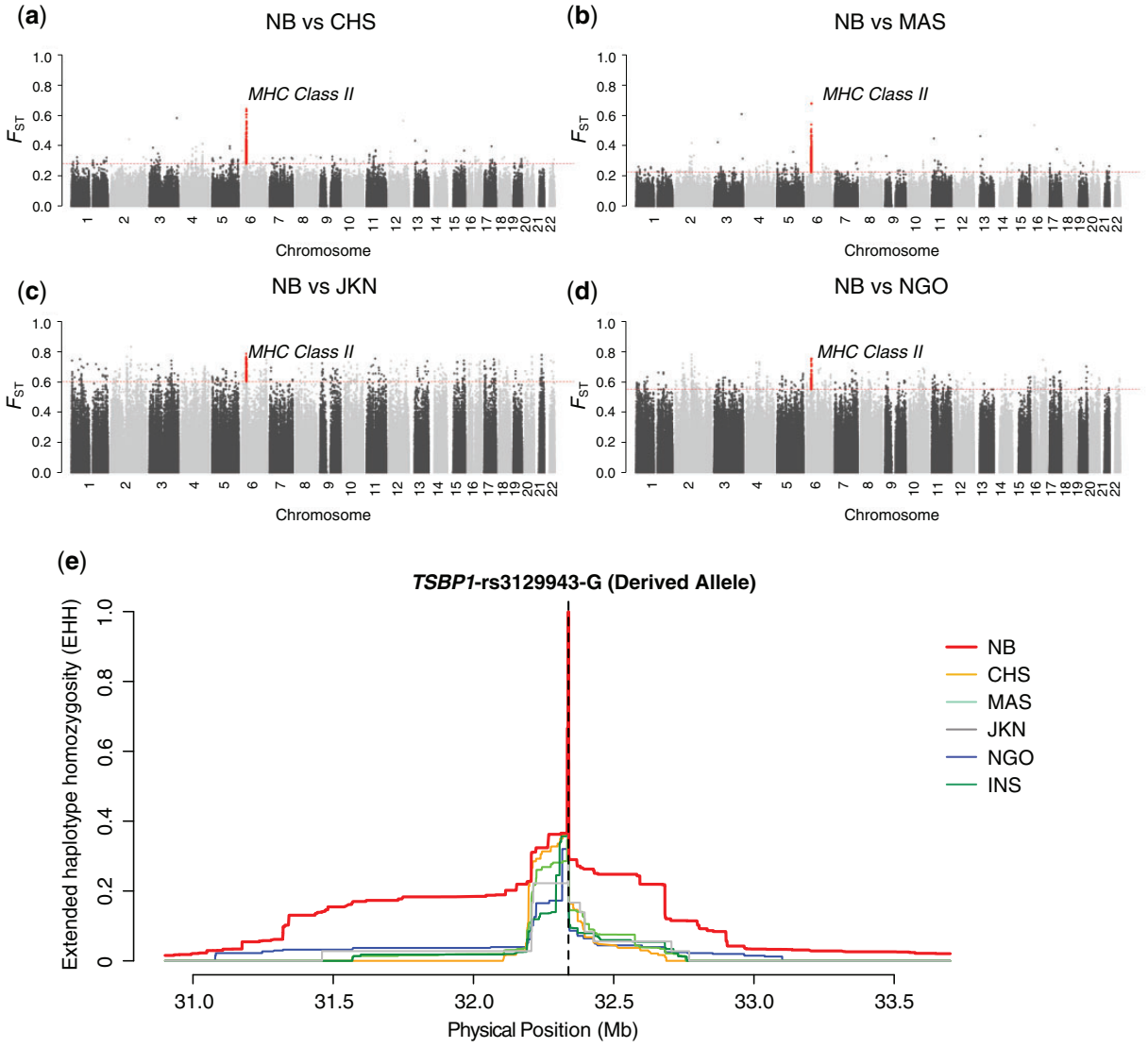

Fig. 2.—A putative signal of positive selection on Chromosome 6 in the North Borneo (NB) populations. Manhattan plot of F_{ST} showing profound differentiation on Chromosome 6 between NB and (*a*) Southern Chinese from Singapore (CHS); (*b*) Metropolitan Malays from Singapore (MAS); (*c*) Proto Malay Jakun from Peninsular Malaysia (JKN); (*d*) Negrito from Peninsular Malaysia (NGO). In each plot, the red-dashed line indicates the top 0.1% cutoff of the genome-wide F_{ST} in each population pair, and the red dots indicate signals in the MHC Class II region. (e) Haplotype decay around *TSBP1*-rs3129943 in NB and non-NB populations.

The putative signal at the MHC Class II region was further confirmed with iHS and XP-EHH analyses (supplementary tables S3–S4, Supplementary Material online). We found a 500-kb region (chr6:32,200,001–32,700,001) showing very high density of iHS signals (in the top 5% of the whole genome). Especially, the proportion of iHS signals in chr6:32,300,001–32,400,001 reaches the top 1% of the whole genome. Notable candidates in this region include rs9268605 with the top iHS value (|iHS| = 4.62), and rs3129943 and rs984778 amongst the ten candidate SNPs identified by the F_{ST} test. These variants could possibly mediate the gene expression changes according to the Genotype-Tissue Expression (GTEx) database (https://gtexportal.org/home/, last accessed October 17, 2020): rs984778 was reported as a splicing quantitative locus (sQTL) for *HLA-DRA* in multiple tissues; rs3129943 is an sQTL for *TSBP1* predominantly in testis and is an eQTL for *BTNL2* in nerve. The variant rs9268605 was only 255 bp upstream to rs984778, and 61.1 kb downstream to rs3129943. We observed an apparent extension of haplotypes with higher frequencies in this region in NB compared with the other populations (fig. 2e; supplementary figs. S3b, S4, and S5, Supplementary Material online). In addition, we found a region (chr6:26,300,001-26,500,001) upstream to the MHC Class

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II also showed outstanding iHS signal (in the top 1% of the whole genome; top |iHS| score = 4.97 at rs9467750). Since we aimed to locate the adaptive sites shared across the NB populations but highly differentiated between NB and other populations, we did not focus on this region in the subsequent analyses as it was not captured by cross-population analyses (F_{ST} or XP-EHH).

We observed an overall reduction in genome-wide heterozygosity in the five NB populations compared with other South and Southeast Asian populations, including the pooled NGO populations. Interestingly, the heterozygosity for the putative signal region of MHC Class II was slightly lower than at the genome-wide scale in the NB populations; this was unexpected assuming balancing selection could have occurred in the region (supplementary fig. S6 and table S6, Supplementary Material online). In contrast, pairwise F_{ST} exhibited an overall higher level of genetic differentiation in the MHC region than at the genome-wide scale in the NB populations (supplementary fig. S7, Supplementary Material online). These collective findings are consistent with the hallmark characteristics of positive natural selection (Meyer et al. 2018).

The three selected SNPs of interest (rs3129943-G, derived allele; rs9268605-G, ancestral allele; rs984778-G, derived allele) showed higher allele frequencies in the NB populations than in the non-NB populations ($P = 6.85 \times 10^{-5}$, 8.08×10^{-5}, and 6.89×10^{-5}, respectively; one-sided Wilcoxon rank-sum test; table 1; supplementary table S7, Supplementary Material online). The linkage between rs3129943 and rs984778 was moderately strong in NB ($r^2 = 0.491$ in the pooled NB population; highest in Sungai-Lingkabau, $r^2 = 0.648$) and JKN ($r^2 = 0.359$) but was almost nil in others (MAS, CHS, INS, and NGO) (table 2). All three variants were homozygous in Sonsogon. Interestingly, rs9268605 and rs984778 exhibited strong linkage disequilibrium (LD) in the NB populations but not in NGO or JKN. We were not able to assess the LD between rs9268605 and rs984778 in MAS, CHS, and INS, as the rs9268605 genotype was not successfully captured in these data. Genes affected by these three SNPs were *TSBP1*, *BTNL2*, and *HLA-DRA*, in which *TSBP1* and *BTNL2* were in strong LD (supplementary fig. S8, Supplementary Material online).

Dating Natural Selection

We next speculated as to the specific driving force(s) of positive natural selection in this region. To address this question, we first estimated the time since the natural selection occurred based on the extended haplotypes. Analysis on the haplotype diversity extended from the selection signal revealed a region ~13 kb in length with exceedingly reduced haplotype diversity in the NB population. This region consisted of 71 SNPs, yet only seven haplotypes were observed, and they were further divided into three highly divergent haplotype groups: Haplotype group "A" (Hap 1–3) and "C" (Hap 5–7) consisting three haplotypes, respectively; Haplotype group "B" consisting one haplotype (Hap 4) (fig. 3; supplementary fig. S9, Supplementary Material online). The three haplotype groups could be differentiated using 59 of the 71 SNPs. The adaptive variants at rs9268605 (G) and rs984778 (C) were found in haplotype group "A." Then we assigned samples from the 1000 Genomes Project Phase III dataset (1KGP; http://www.internationalgenome.org/, last accessed October 17, 2020) into the three haplotype groups (we allowed for variations up to three SNPs in the haplotype group,otherwise the haplotype would be was assigned to "others"), and this revealed that the three major groups explained most of the haplotypes of worldwide populations. It was noted that the frequency of the Haplotype group A reached 0.75–1 in the NB populations, largely attributed to Hap1 (0.4–0.61) (table 3; supplementary table S8, Supplementary Material online). However, the Haplotype group A frequency is much lower in other worldwide populations (0.18–0.73) (**table 3**). Assuming that the haplotype decay followed a Poisson process, our estimation revealed that the selection of Hap1 arose ~5.5 kya (~220 generations; 4.6–3.1 kya for each NB population) (table 4). The estimated onset of the positive natural selection matched the the emergence of agricultural society, which may have led to malarial expansion (Volkman 2001; Joy 2003).

We then asked which of the three candidate genes (*TSBP1*, *BTNL2*, and *HLA-DRA*) could possibly be the key target of natural selection. We found that all these three genes, particularly *TSBP1*, in the NB populations showed greater genetic differentiation as measured by a higher pairwise F_{ST} yet with reduced heterozygosity when compared with the entire MHC region, or with genomic regions showing comparable length and SNP density with them (fig. 4; supplementary fig. S10 and table S9, Supplementary Material online). We suspect that *TSBP1* was possibly the targeted candidate gene under positive natural selection in NB. Nonetheless, we do not rule out the possibility that *BTNL2* and *HLA-DRA* may also have been under positive selection as epistatic effects might exist (Traherne 2008; Meyer et al. 2018), and they were functionally associated to *TSBP1* according to the STRING network (https://string-db.org/network/9606.ENSP00000415517, last accessed October 17, 2020) (supplementary fig. S11, Supplementary Material online).

Discussion

The MHC region has been a classical landmark for balancing selection, likely under the pressure from pathogen diversity (Meyer et al. 2006; Traherne et al. 2006; Yasukochi and Satta 2013; Field et al. 2016). In this study, we have identified a region within the MHC Class II that was under positive natural selection among the NB populations. This putative signal was

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Table 1

Derived Allele Frequencies of the Four SNPs of Interest in the Five Native Populations from NB

Population	rs9467750-C[a]	rs3129943-G[a]	rs9268605-G[b]	rs984778-C[a]
NB	0.964	0.934	0.913	0.893
Dusun	0.975	0.875	0.875	0.875
Murut-Paluan	0.975	0.875	0.875	0.750
Rungus	0.975	0.975	0.925	0.925
Sonsogon	1.000	1.000	1.000	1.000
Sungai	1.000	0.947	0.947	0.921
Malay (MAS) - SGVP	NA	0.534	NA	0.365
Han Chinese (CHS) - SGVP	NA	0.354	NA	0.240
Southern Indian (INS) - SGVP	NA	0.313	NA	0.301
Negrito (NGO)	0.982	0.264	0.482	0.218
Jakun (JKN)	0.967	0.300	0.333	0.133
African Caribbean (ACB)	0.370	0.313	0.729	0.594
African American (ASW)	0.410	0.328	0.664	0.484
Bengali Bangladesh (BEB)	0.791	0.390	0.616	0.413
Chinese Dai (CDX)	0.941	0.398	0.527	0.446
Northern & Western European (CEU)	0.874	0.258	0.596	0.349
Northern Han Chinese Beijing (CHB)	0.913	0.354	0.408	0.243
Southern Han Chinese (CHS)—1kgp	0.957	0.305	0.338	0.238
Colombians Medellin (CLM)	0.819	0.181	0.644	0.356
Esan Nigeria (ESN)	0.328	0.379	0.808	0.732
Finnish Finland (FIN)	0.869	0.273	0.717	0.354
British England (GBR)	0.885	0.225	0.632	0.357
Gujarati Indian (GIH)	0.786	0.354	0.524	0.384
Gambian West Division of Gambia (GWD)	0.336	0.155	0.553	0.319
Iberian Spain (IBS)	0.855	0.248	0.603	0.332
Indian Telugu (ITU)	0.878	0.451	0.583	0.343
Japanese Tokyo (JPT)	0.933	0.313	0.615	0.389
Kinh Vietnam (KHV)	0.904	0.485	0.525	0.222
Luhya Kenya (LWK)	0.288	0.424	0.727	0.551
Mende Sierra Leone (MSL)	0.318	0.288	0.435	0.359
Mexican (MXL)	0.703	0.211	0.594	0.359
Peruvian Lima (PEL)	0.800	0.112	0.365	0.182
Punjabi Pakistan (PJL)	0.828	0.260	0.474	0.365
Puerto Ricans (PUR)	0.760	0.188	0.567	0.389
Sri Lankan Tamil (STU)	0.809	0.368	0.456	0.304
Toscani Italy (TSI)	0.836	0.262	0.556	0.336
Yoruba Nigeria (YRI)	0.407	0.361	0.662	0.523

NOTE.—The derived allele frequencies for rs3129943 (G) and rs984778 (C) and the ancestral allele frequency for rs9268605 (G) are much higher in the NB populations than in other worldwide populations.

NA, not available in the data set.

[a]Derived allele.

[b]Ancestral allele.

highly robust as it was consistently identified despite that different approaches were used.

We showed that although the genetic differentiation among the five NB populations was quite high, similar driving force(s) of natural selection could have affected their genetic structure, as supported by profoundly lower pairwise F_{ST} values in the MHC II region among the NB populations compared with that between NB and non-NB populations (supplementary table S6, Supplementary Material online). We reasoned that if selection favored similar sets of alleles or haplotypes within a broadly defined geographical region (in this case, the interior region of NB), reduced genetic differentiation (as measured by F_{ST}) would have been expected (Meyer et al. 2006).

The three candidate genes *TSBP1*, *BTNL2*, and *HLA-DRA* in the NB populations exhibited typical characteristics of positive selection, including the increased genetic differentiation, reduced heterozygosity relative to the genome-wide scale, and a strong LD block (Schierup et al. 2000). We reasoned that selection favors different alleles in distinct populations, thus driving locally adaptive MHC alleles to higher frequencies and resulting in increased population differentiation (Meyer et al.

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Table 2
LD Estimation between the SNPs of Interest

Population	rs3129943 vs. rs9467750	rs3129943 vs. rs9268605	rs9268605 vs. rs984778	rs984778 vs. rs3129943	rs984778 vs. rs9467750	rs9467750 vs. rs9268605
NB	0.025	0.625	0.791	0.491	0.042	0.059
Dusun	0.004	0.587	1	0.587	0.004	0.004
Murut-Paluan	0	0.673	0.636	0.429	0	0.036
Rungus	1	0.316	1	0.316	0.316	0.316
Sonsogon	NA	NA	NA	NA	NA	NA
Sungai	1	1	0.648	0.648	1	NA
Malay (MAS)	NA	NA	NA	0.068	NA	NA
Han Chinese (CHS)	NA	NA	NA	0.026	NA	NA
Southern Indian (INS)	NA	NA	NA	0.109	NA	NA
Negrito (NGO)	0	0.207	0.3	0.09	0.001	0.017
Jakun (JKN)	0.025	0.585	0.308	0.359	0.005	0.017

Note.—rs984778 and rs3129943 showed a moderately strong LD among the NB compared with the non-NB populations, except Jakun (JKN). The four SNPs are fixed in Sonsogon; Two SNPs, rs9467750 and rs9268605, are missing in MAS, CHS, and INS. rs9467750 was only captured by iHS and thus was not considered in subsequent analysis; it was used as a control.

NA, not available in the data set.

2018). This pattern is particularly prominent in *TSBP1*. Essentially, there is no straightforward strategy for locating a single gene contribution to a trait of interest in which multiple linked interacting genes are at work. Although less likely, we also caution that the selection signal may be confounded by demographic history and genetic drift forces that would lead to sudden expansion of the haplotype frequency of interest. Further laboratory investigations may be required to rule out these possibilities.

We believe that this selection signal is likely a product of the local adaptation process for survival in the tropical rainforest—one of the toughest environments for human habitation characterized by unusually high protozoa and pathogen diversity (Fan et al. 2016) that may influence the reproductive success of the affected population. In this regard, we note that the parasitic protozoan *Plasmodium* species that causes malaria, resulting in significant mortality rates in tropical countries, likely exerts the strongest selection pressure (Kwiatkowski, 2005).

NB has been persistently recognized as a malaria-endemic region since the past several centuries (Copeland, 1935; William et al. 2013, 2014). Studies over the years have recorded a number of *Plasmodium sp.* in NB, including simian *Plasmodium knowlesi* (William et al. 2013, 2014). Notably, a high prevalence of malaria infection was recorded in Murut-Paluan and Dusun nearly a century ago (Copeland 1935). That study suggested a close attribution between malaria and the low juvenile populations and low birth rates in Murut-Paluan and Dusun in Ranau. Interestingly, the Ranau and Kudat districts, where the Rungus and Dusun samples were collected (Yew et al. 2018a), respectively, showed the highest density of knowlesi malaria (Barber et al. 2011; William et al. 2014).

Some evidence supported our postulation. First, gene expression of *HLA-DRA* was significantly upregulated in placental malaria (Muehlenbachs et al. 2007), whereas a selection signal in *HLA-DRA* was found in the low-altitude Ethiopians known to be affected by malaria and schistosomiasis (Alkorta-Aranburu et al. 2012). Second, the chimeric mice with BTNL2$^{-/-}$ had significantly decreased cerebral malaria survival rate (Subramaniam et al. 2015). In addition, the top candidate SNP rs3129943 located in *TSBP1* showed substantially higher derived allele frequency (0.934) in the NB populations than in the other populations (0.11–0.53, see table 1). Although the ancestral allele A at this locus was reported to be associated with asthma (Hirota et al. 2011), we do not think that the selection signal was driven by asthma, owing to its lower prevalence in NB Malaysia (Lin and Kasim 1997). Analysis from the STRING database version 11.0 suggested plausible interactions of *TSBP1*, *BTNL2*, and *HLA-DRA* (the interaction score = 0.673–0.725; supplementary fig. S11, Supplementary Material online). Therefore, it is plausible to postulate that these neighboring genes may have demonstrated an epistatic effect, that is, these genes are tuned to work together as a set of alleles on a particular haplotype, hence the lack of detectable recombination due to the preference of favorable immunological function through selection (Traherne et al. 2006).

Owing to their natural habitat being similar to those of the NB populations, indigenous populations from Peninsular Malaysia (the Orang Asli) have been routinely exposed to malaria infection. Indeed, our earlier study had reported several putative signals of natural selection in the Orang Asli (Liu et al. 2015). We found that within a small geographical region in the tropical rainforest in Peninsular Malaysia, the Orang Asli exhibited differential evidence of positive selection against malaria. We found that none of the putative selection signals identified in the Orang Asli presented in the NB native populations, suggesting plausible attribution to their different population histories; presumably the adaptations occurred after the population diverged. In addition, recent reports

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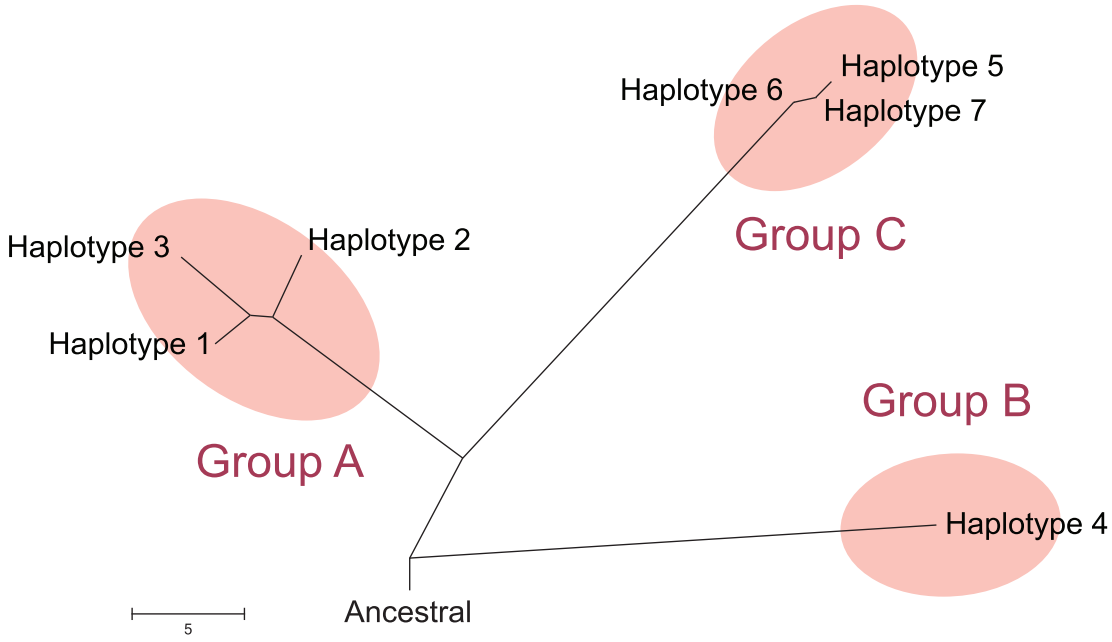

Group	Haplotypes
Ancestral	GAATGGGGCATTAAGGCGCAGCCCAGTGTCGACGGCACAGATCGGTTCACCCAGTGGGCACCGGCGACACC
Group A	GAACGAAGGGTTA-G-CGCAGC-C-GTGC-GACG-CGCGCATC-GTAGAC---ATGGTAAAC-GC-ACGCC
Haplotype 1	GAACGAAGGGTTAAGGCGCAGCCCAGTGCAGACGGCGCGCATCAGTAGACGCAATGGTAAACGGCGACGCC
Haplotype 2	GAACGAAGGGTTAAGACGCAGCCCGGTGCCGACGGCGCGCATCGGTAGACCCCATGGTAAACGGCGACGCC
Haplotype 3	GAACGAAGGGTTAGGGCGCAGCCCAGTGCCGACGACGCGCATCGGTAGACGAAATGGTAAACAGCGACGCC
Group B	GGATGGGTGATTC-G-CATGGT-G-GCGT-AACC-TGTAGATC-GGTCGT---GGTCGCACG-GT-TCAAC
Haplotype 4	GGATGGGTGATTCAGGCATGGTCGAGCGTCAACCGTGTAGATCGGGTCGTCCAGGTCGCACGGGTGTCAAC
Group C	TAGCAAAGCGGGA-A-AGCAAC-C-ATAT-GGTG-TACAGGCT-ATTCAC---GTGGGCCCC-AC-AAGCG
Haplotype 5	TAGCAAAGCGGGAAAGAGCAACACAATATCGGTGGTACAGGCTGATTCACCCAGTGGGCCCCGACAAAGCG
Haplotype 6	TAGCAAAGCGGGAAAGAGCAACCCAATATCGGTGGTACAGGCTGATTCACCCAGTGGGCCCCGACGAAGCG
Haplotype 7	TAGCAAAGCGGGAAAGAGCAACACAATATCGGTGGTACAGGCTGATTCACCCAGTGGGCCCCGACGAAGCG

Fig. 3.—Haplotypes of the ~13 kb region in the NB populations. All 71 SNPs in this region are shown. The ancestral state information for each SNP is provided by dbSNP. For the three different haplotype groups, the dash "–" symbol represents a polymorphic nucleotide within all haplotypes. Sequences with one different nucleotide variation were defined as different haplotypes. The maximum-parsimony tree was constructed using the SPR algorithm implemented in MEGA7 (Kumar et al. 2016) with search level set to be 0, and the initial trees were obtained by the random addition of sequences (ten replicates). The branch length was calculated using the average pathway method (Nei and Kumar 2000) based on the number of nucleotide changes over the whole sequence.

suggested that the predisposition of *Plasmodium* parasites differed between NB and Peninsular Malaysia populations (Yap et al. 2018; Hussin et al. 2020). These factors may contribute to the different selection signals between the Orang Asli and native NB populations.

We acknowledge that different analyses of positive selection approaches differ in their power to detect a selection signal, depending on how long ago selection began, how close the selected allele is to fixation, and how different allele frequencies are in different populations. In this study, we selected the putative signals that showed a profound population differentiation. Although there were genomic regions that did not fit this criterion, the possibility of these regions being true selection signals should not be ruled out and warrants further investigation.

We note that all supporting evidence attributed to malaria so far has been indirect. However, we wish to reiterate that: 1) Malaria is believed to be the strongest selection pressure on human populations identified to date (Kwiatkowski 2005). There have been numerous records of malaria endemicity in NB populations since the last century. On a separate note, prevalence data for other parasitic infections among the NB populations are lacking. In the absence of other recorded evidence of parasitic or immune-related diseases among these native NB populations, malaria appears as the most likely driving force of the identified selection signal. 2) Functional studies in relation to malaria pathogenesis have been carried out

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Table 3
Haplotype Frequencies in the NB and Global Populations from the 1000 Genomes Project

Population	Region	Number of Haplotypes	Haplotype Group Frequency (%)			
			A	B	C	Others
Dusun	NB	40	87.5	0.0	12.5	0.0
Murut-Paluan	NB	40	75.0	7.5	17.5	0.0
Rungus	NB	40	92.5	0.0	7.5	0.0
Sungai	NB	38	92.1	2.6	5.3	0.0
Sonsogon	NB	38	100.0	0.0	0.0	0.0
Sri Lankan Tamil (STU)	South Asia	204	30.4	15.2	49.0	5.4
Gujarati Indian (GIH)	South Asia	206	38.4	14.1	47.1	0.5
Indian Telugu (ITU)	South Asia	204	34.3	24.0	40.2	1.5
Punjabi Pakistan (PJL)	South Asia	192	36.5	10.9	49.5	3.1
Bengali Bangladesh (BEB)	South Asia	172	41.3	20.4	38.4	0.0
Southern Han Chinese (CHS)	East Asia	210	23.8	10.0	54.8	11.4
Japanese Tokya (JPT)	East Asia	208	38.9	22.6	32.7	5.8
Northern Han Chinese Beijing (CHB)	East Asia	206	24.3	16.5	51.0	8.3
Kinh Vietnam (KHV)	East Asia	198	22.2	30.3	41.4	6.1
Chinese Dai (CDX)	East Asia	186	44.6	8.1	46.2	1.1
Gambian West Division of Gambia (GWD)	Africa	226	31.9	23.5	41.6	3.1
Yoruba Nigeria (YRI)	Africa	216	51.9	13.9	24.1	10.2
Esan Nigeria (ESN)	Africa	198	73.2	7.6	16.2	3.0
Luhya Kenya (LWK)	Africa	198	55.1	17.7	20.2	7.1
Mende Sierra Leone (MSL)	Africa	170	35.9	7.7	37.1	19.4
African Caribbean (ACB)	America	192	59.4	13.5	20.8	6.3
African American (ASW)	America	122	48.4	18.0	29.5	4.1
Puerto Rican (PUR)	America	208	38.5	18.3	42.3	1.0
Colombian Medellin (CLM)	America	188	35.6	28.7	34.6	1.1
Peruvian from Lima (PEL)	America	170	18.2	18.2	63.5	0.0
Mexican (MXL)	America	128	35.9	23.4	40.6	0.0
Iberian in Spain (IBS)	Europe	214	33.2	27.1	38.3	1.4
Toscani Italy (TSI)	Europe	214	33.6	22.0	43.9	0.5
Northern & Western European (CEU)	Europe	198	34.9	24.8	40.4	0.0
Finnish Finland (FIN)	Europe	198	35.4	36.4	28.3	0.0
British England (GBR)	Europe	182	35.7	27.5	36.8	0.0

NOTE.—A total of 59 SNPs were included in the haplotype group assignment. Haplotypes with more than three nucleotide differences with the three groups were assigned to "Others." The NB populations were significantly enriched with haplotype group A.

Table 4
Selection Time Estimation of the NB Populations

Population	Time of selection in KYA (generations)		
	Haplotype 1	Haplotype 2	Haplotype 3
NB	5.51 (*220.4*)	5.93 (*237.2*)	3.98 (*159.2*)
Dusun	4.64 (*185.6*)	—	3.87 (*154.8*)
Lingkabau	3.46 (*138.4*)	—	—
Murut-Paluan	3.29 (*131.6*)	—	—
Rungus	3.06 (*122.4*)	—	3.02 (*120.8*)
Sonsogon	4.29 (*171.6*)	—	2.30 (*92*)

NOTE.—Selection time was estimated based on the extended haplotypes. NB, combination of the five NB populations. To minimize potential bias introduced by sample size, haplotypes with less than ten counts were excluded from the time estimation. Number of generations is shown in the brackets.

KYA, thousand years ago.

on two of the candidate genes, namely *HLA-DRA* and *BTNL2* (Muehlenbachs et al. 2007; Subramaniam et al. 2015). 3) It is generally accepted that the expansion of malaria infection in human populations is attributed to the expansion of agricultural technology. The estimated time of the selection signal identified in this study is in agreement with the period when agricultural expansion occurred in Southeast Asia, that is, between 4,000 and 6,000 years ago (Bellwood 2007).

In summary, an MHC Class II haplotype encompassing candidate genes *TSBP1—BTNL2—HLA-DRA* was identified as the putative signature of positive selection among the NB populations. This signal of selection is likely to have occurred during the period of agricultural expansion. With the supporting evidence from earlier studies, it is conceivable to hypothesize that the selection event was driven by *Plasmodium* parasite

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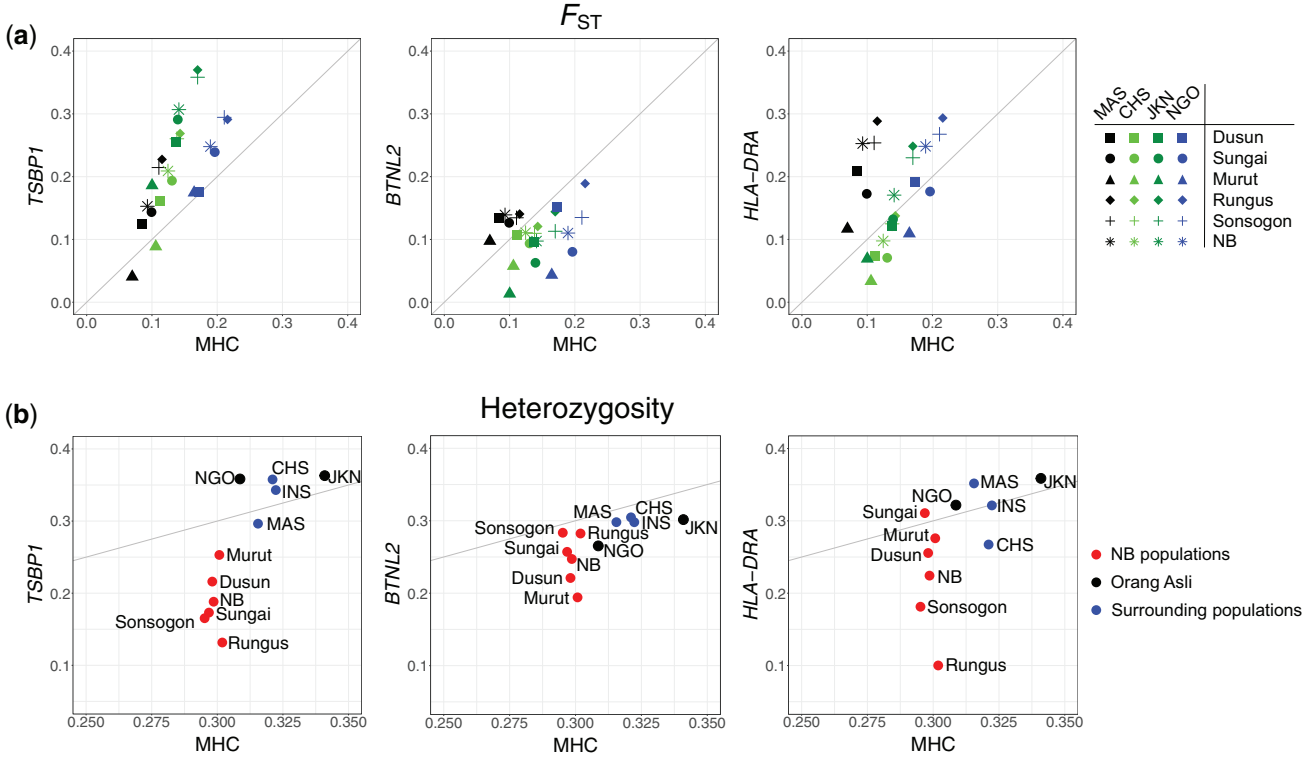

Fig. 4.—Genetic diversity of *BTNL2*, *TSBP1*, and *HLA-DRA* relative to the MHC Class II region. (*a*) F_{ST} for pairs of populations, averaging across 100 bootstrap replications. Each dot depicts the average F_{ST} of all sites in the MHC Class II region (*x* axis) and that in a candidate gene (*y* axis). The F_{ST} was calculated between the collective NB population and the non-NB populations (asterisk dots) and between each single NB population and non-NB populations (black dots): Metropolitan Malays from Singapore (MAS); red dots: Negrito (NGO); light green dots: Southern Han Chinese from Singapore (CHS); dark green dots: Proto-Malay Jakun (JKN). The dots above the grey line indicate candidate genes with higher average F_{ST} than that of the whole genome; (*b*) Heterozygosity of the candidate genes of interest relative to the MHC Class II heterozygosity. Heterozygosity of the candidate genes was lower than that of the MHC Class II region.

infection. Considering the prominent role of these candidate genes in the regulation of the autoimmune system, their plausibility in affecting the susceptibility to pathogen infection points to a fine balance between a strong and appropriate immune response to challenge by a pathogen and an excessive and inappropriate response leading to autoimmune disease (Hollox and Hoh 2014). However, further laboratory validation is required to explore this hypothesis and demonstrate the function(s) of the candidate genes in the response to Plasmodium infection. We also suggest that future studies expand the population range and involve full MHC sequences to assess the differential contributions of selection and recombination in shaping the contrasting evolutionary history of ancestral haplotypes.

Materials and Methods

Genotyping Data, Data Assemblage, and Quality Control (QC)

Genotyping data (comprising ~2.2 million autosomal SNPs) of 98 unrelated samples representing Dusun, Rungus, Sonsogon, Sungai-Lingkabau, and Murut-Paluan from NB were included in this study as described in Yew et al. (2018a). Briefly, this study was approved by the Medical Research Ethics Committee of Universiti Malaysia Sabah (ref.no: JKEtika 4/10(3)), and the District Officers of Ranau, Pitas, Kota Marudu, Nabawan, and the respective village chiefs and chairpersons of the Committee for Village Development and Security, and complies with the Helsinki Declaration 1975 as revised in 2000. Genomic DNA was extracted from whole blood or buffy coat using the DNeasy Blood and Tissue kit (Qiagen, Germany). The DNA samples were genotyped with Illumina's Human Omni2.5 bead chip array following the manufacturer's protocol. Calling of SNP genotypes was performed in Genome Studio (Illumina) with the default GenCall score of 0.15.

Data assemblage and QC were carried out using PLINK version 1.07 (Purcell et al. 2007). Criteria for exclusion included: 1) individuals with missing rate >10%; 2) SNPs with missing rate >10%; 3) SNPs with minor allele frequency <0.01; 4) SNPs deviating from Hardy–Weinberg equilibrium ($P < 0.0001$). A total of 98 NB samples (84%) containing >1.2 million bi-allelic SNPs remained for subsequent analyses. Haplotype phasing for the final data sets was carried out using

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Shapeit2 without any reference population (Delaneau et al. 2011). SNPs were annotated using the human reference genome GRCh37. The coordinates of genes were provided by the UCSC hg19 RefSeq annotation.

Additional data sets analyzed in this study include metropolitan Malays (MAS) and Chinese from Singapore (CHS) provided by the Singapore Genome Variation Project (SGVP) (Teo et al. 2009), the Orang Asli from Peninsular Malaysia including Negrito (Bateq, Mendriq, and Jehai) and Proto-Malay (Jakun) (Aghakhanian et al. 2015; Liu et al. 2015), and the global populations from the 1000 Genomes Project Phase III dataset (1KGP; http://www.internationalgenome.org/, last accessed October 17, 2020). Data filtration was carried out independently for each population, using the same criteria as described above.

Analysis of Population Relatedness

PCA was performed using flashPCA version 2.0 (Abraham et al. 2017). Unbiased estimation of F_{ST} was computed according to Weir and Hill (2002) with 100 times bootstrap replications. A Neighbor-Joining tree was then generated based on F_{ST} using Phylip version 3.695 (http://evolution.genetics.washington.edu/phylip.html, last accessed October 17, 2020).

Estimation of Heterozygosity

Observed heterozygosity (H_o) of an SNP was calculated by the ratio of the number of heterozygous individuals to all genotyped individuals. Expected heterozygosity (H_e) was calculated following Nei (1973). The heterozygosity of one region was calculated by averaging across all the sites within this region.

Identifying Signatures of Positive Selection

We identified alleles or regions that were highly differentiated from other populations using pairwise F_{ST}. SNPs with the top 0.1% of the most extreme differentiation were considered as putative signals of positive selection. iHS and XP-EHH (Voight et al. 2006; Sabeti et al. 2007) were computed using the *Selscan* version 1.2.0 (Szpiech and Hernandez 2014). Default settings were used, and normalization was set at 100 bins with 100-kb nonoverlapping windows using the "norm" feature available in *Selscan*. Genomic regions within the top 1% in each calculation were considered as putative signals for positive selection.

Estimating the Time of Positive Natural Selection

An evolutionary history was inferred using the Maximum Parsimony method implemented in MEGA7 (Kumar et al. 2016). The tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level set to be 0, and the initial trees were obtained by the random addition of sequences (10 replicates) (Nei and Kumar 2000).

The time since selection of Hap1 in NB populations was estimated based on the extended haplotype homozygosity (EHH). We assumed that the decay of haplotype homozygosity followed a Poisson process:

$$\Pr(\text{Homozygosity}) = e^{-2rg}$$

where Pr(Homozygosity) is the probability that two haplotypes are homozygous at a distance r to the selected haplotype, and g is the number of generations. Given a threshold of Pr(Homozygosity) to be 0.25 and a generation time of 25 years as previously reported (Voight et al. 2006; Tishkoff et al. 2007), g could be estimated.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Ethical Approval and Consent to Participate

This study was approved by the Research and Ethics Committee of Universiti Teknologi MARA [Ref no: 600-RMI (5/1/6)], the Department of Orang Asli Development (Jabatan Kemajuan Orang Asli Malaysia, JAKOA) [JHEOA.PP.30.052.Jld 5(17)], and the Universiti Malaysia Sabah Medical Research Ethics Committee [code: JKEtika 4/10(3)] as well as the district offices, village chief, and the chairperson of the Committee of Village Development and Security. It was also approved by the Biomedical Research Ethics Committee of Shanghai Institutes for Biological Sciences (ER-SIBS-261903). Informed written consent was obtained from the volunteers aged 18 years and above. Their family history, pedigree, and self-reported ethnicity were recorded via an interview using local dialect.

Data Availability

The genotyping data have been deposited in the National Omics Data Encyclopedia (NODE) (http://www.biosino.org, last accessed October 17, 2020) with accession number: OEP000154.

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Authors Contributions

B.P.H., V.K.S., and S.X. conceived the study; S.X. and B.P.H. designed and supervised the project; B.P.H., V.K.S., X.Z., and L.D. prepared the manuscript; B.P.H., X.Z., L.D., K.Y., C.W.Y., and W.Y.S. performed the data analysis; C.W.Y., M.Z.H., F.A., M.E.P., and V.K.S. involved in sample collection. All authors have read and approved the submission of the manuscript.

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