

RESEARCH ARTICLE

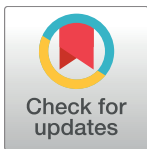
Genomic variation in *Plasmodium vivax* malaria reveals regions under selective pressure

Ernest Diez Benavente¹, Zoe Ward^{1,2}, Wilson Chan^{1,3}, Fady R. Mohareb³, Colin J. Sutherland¹, Cally Roper¹, Susana Campino^{1‡}, Taane G. Clark^{1‡*}

1 London School of Hygiene and Tropical Medicine, Keppel Street, London, United Kingdom, **2** The Bioinformatics Group, School of Water Energy and Environment, Cranfield University, Cranfield, Bedfordshire, United Kingdom, **3** Department of Pathology & Laboratory Medicine, Diagnostic & Scientific Centre, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

‡ These authors are joint last authors on this work.

* taane.clark@lshtm.ac.uk



OPEN ACCESS

Citation: Diez Benavente E, Ward Z, Chan W, Mohareb FR, Sutherland CJ, Roper C, et al. (2017) Genomic variation in *Plasmodium vivax* malaria reveals regions under selective pressure. PLoS ONE 12(5): e0177134. <https://doi.org/10.1371/journal.pone.0177134>

Editor: Georges Snounou, Université Pierre et Marie Curie, FRANCE

Received: January 17, 2017

Accepted: April 21, 2017

Published: May 11, 2017

Copyright: © 2017 Diez Benavente et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: TGC receives funding from the Medical Research Council UK (grant numbers MR/K000551/1, MR/M01360X/1, MR/N010469/1, MC_PC_15103). CR and SC are funded by the Medical Research Council UK (grant no. MR/M01360X/1). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Abstract

Background

Although *Plasmodium vivax* contributes to almost half of all malaria cases outside Africa, it has been relatively neglected compared to the more deadly *P. falciparum*. It is known that *P. vivax* populations possess high genetic diversity, differing geographically potentially due to different vector species, host genetics and environmental factors.

Results

We analysed the high-quality genomic data for 46 *P. vivax* isolates spanning 10 countries across 4 continents. Using population genetic methods we identified hotspots of selection pressure, including the previously reported *MRP1* and *DHPS* genes, both putative drug resistance loci. Extra copies and deletions in the promoter region of another drug resistance candidate, *MDR1* gene, and duplications in the Duffy binding protein gene (*PvDBP*) potentially involved in erythrocyte invasion, were also identified. For surveillance applications, continental-informative markers were found in putative drug resistance loci, and we show that organellar polymorphisms could classify *P. vivax* populations across continents and differentiate between *Plasmodia* spp.

Conclusions

This study has shown that genomic diversity that lies within and between *P. vivax* populations can be used to elucidate potential drug resistance and invasion mechanisms, as well as facilitate the molecular barcoding of the parasite for surveillance applications.

Background

The *Plasmodium vivax* malaria parasite is the second most virulent malaria species after *P. falciparum*. Geographically, it is found throughout Asia, South and Central America, Oceania,

Competing interests: The authors have declared that no competing interests exist.

Middle East and some parts of Africa, with nearly 2.85 billion people at risk of infection [1]. Although *P. vivax* contributes to almost half of all malaria cases outside Africa, as it kills infrequently and is not amenable to continuous *in vitro* culture, it has been relatively neglected compared to the more deadly *P. falciparum* [2]. However, as *P. vivax* drug-resistant strains emerge and spread and fatality rates increase, the need to implement better control and elimination strategies is becoming urgent. Many of the interventions used for controlling *P. falciparum* malaria are not as effective against *P. vivax*. Consequently, *P. vivax* has become the dominant malaria parasite in several countries where *P. falciparum* transmission has been successfully reduced. Hence, control and elimination of *P. vivax* malaria calls for additional interventions, notably against the dormant liver stage of the parasite. However, gaps in our knowledge of *P. vivax* epidemiology and biology may compromise its control. Genomic research can contribute greatly to enhancing our understanding of *P. vivax* basic biology and evolutionary history, supporting the development and surveillance of new interventions.

Since the first characterisation of the *P. vivax* genome sequence (Sal-1, [3]), several population genetic studies, based on microsatellite data and more recently using whole genomes, have shown that this parasite is more polymorphic than *P. falciparum* [4,5,6,7]. *P. vivax* populations harbour high genetic diversity, even on small spatial scales, and can differ extensively between locations due to vector species, host genetics and environmental factors [4,5,6]. Genetic variation enables the parasite to overcome host immune responses and antimalarial drugs to establish persistent infections and increase transmission. Genomic studies in natural populations of *P. vivax* can pinpoint genetic regions that are under selective pressure, including those associated with resistance to antimalarial drugs. Such studies can also contribute to the identification of vaccine targets. Moreover, global genomic studies can assist with identifying sets of polymorphism private to populations, allowing the monitoring of gene flow over space and time, and the tracking of imported infections. By developing a molecular barcode of individual parasites it will also be possible to distinguish recrudescence from re-infections.

Highly polymorphic microsatellites have been the preferred method of genetic analysis, revealing high levels of diversity and highlighting interesting genotypic patterns and geographical clustering across global populations [8, 9, 10, 11, 12]. The advancement of whole genome sequencing technologies has opened up opportunities to obtain a comprehensive picture of the epidemiology and structural variation of *P. vivax*. There is now the ability to perform genome-wide analysis of the various populations without the need for *in vitro* culture and overcoming difficulties with low parasitaemias and high human DNA contamination [13]. Recent studies using genome-wide SNPs highlighted that signals of natural selection suggest that *P. vivax* is evolving in response to antimalarial drugs and is adapting to regional differences in the human host and the mosquito vector [6,7]. Several other whole genome sequence studies have been published [13, 14, 15, 16], covering 10 countries. Using these and other data, we explore the genetic diversity within and between continents, identify signatures of drug pressure and molecular barcodes that could be useful for determining the source of infections and monitoring parasite populations.

Methods

Samples and sequence data

Publicly available whole genome sequence data for 74 *P. vivax* samples were gathered from multiple sources, and included reference strains (India VII, Mauritania X, North Korea II, Brazil I, Sal-1 from El Salvador (see [2])), field and clinical isolates (Cambodia (n = 3) [13], Thailand (n = 39) [13], Madagascar (n = 3) [2,17], Colombia (n = 8) [14] and Peru (n = 11) [7,15]) and clinical samples from travellers (to Papua Indonesia (n = 2) [13], India (n = 2) [13], and

Papua New Guinea (PNG, $n = 6$) [13]). All sequencing data for non-reference strains were generated using Illumina paired end technologies (read lengths ≥ 75 bp). The raw sequence data were mapped to the Sal-1 reference genome (version 10.0) using *bwa-mem* with default parameters. SNPs ($n = 447,232$) were identified using the *samtools* software suite (samtools.sourceforge.net) with high quality scores (*phred* score > 30 , 1 error per 1 kbp). Genotypes were called using ratios of coverage, where the minimal heterozygous calls still present after filtering were converted to the majority genotype if the coverage ratio was 80:20 or greater [18,19]. SNPs were retained if they were biallelic, had low genotype missingness ($< 10\%$) and heterozygous ($< 0.4\%$) calls. SNPs in regions of extreme coverage and very low coverage were excluded, as well as in non-unique regions (using a k-mer approach with length of 54 bp) and highly polymorphic VIR genes. Two samples were found to have *P. vivax* and *P. falciparum* co-infections (ERR020124 and SRR828528), and were excluded from population genetic analysis. Isolates were retained if they had at least average 10-fold genome-wide coverage, and at most 10% missing genotype calls. The final high quality dataset consisted of 46 (62.2%) isolates (Thailand 22, Southeast Asia 24, South America 11; other 11; S1 Table) and 219,288 SNPs, and used as the basis of population genetic analyses. *FreeC* software (<http://bioinfo-out.curie.fr/projects/freec/tutorial.html>) was used to identify regions of the genome with a significant increase or decrease in read coverage identifying potential copy number variants (CNVs) after accounting for GC bias through coverage normalization. Regions identified as CNVs were inspected visually and assessed using *de novo* assembly methods [20].

Population genetics

Genetic diversity was estimated using the average pairwise nucleotide diversity (π) with the R package "*pegas*". An in-house R script was used to compute the allele frequency-based Tajima's *D* test [21] to identify genes under balancing selection in the individual populations (values > 2.5 ; [18]), this method was chosen over the dN/dS approach given the latter being not fit for analysis on individual populations [22]. To detect signals of directional selection, the integrated Haplotype Score (*iHS*) approach [23] was applied to individual populations supported by a principal component analysis (PCA). This approach used the most frequent allele where mixed calls were found so the haplotype analysis will be based on the most abundant strain in each sample [7]. *P*-values for *iHS* were computed from standardised values based on a 2-tailed conversion from a Gaussian distribution [19]. The Salvador-I being the reference and oldest sample was used as ancestral haplotype. Multiplicity of infection was estimated using a novel method of counting the unique haplotypes formed by polymorphism on paired sequencing reads (*estMOI*, [24]). For comparisons between populations, we first applied PCA and neighbourhood joining tree clustering based on a matrix of pairwise identity by state values. These analyses were followed by applying the cross population long-range haplotype method (*XP-EHH* [25], *Rsb* implementation [19]) and the population differentiation metric *F_{ST}* [26]. *P*-values for the *Rsb* estimates were calculated using a Gaussian approximation [19]. A significance threshold of $P < 0.001$ was established for both *iHS* and *Rsb* using bootstrap- and permutation-based simulation approaches [18,19]. We used the ranked *F_{ST}* statistics to identify the informative polymorphism for the barcoding of populations and driving the clustering observed in the PCA. Linkage disequilibrium (LD) was assessed in the two populations with the largest sample sizes (Thailand and South America) using the r^2 and *D'* metrics [27], calculated for pairs of SNPs with different physical separation up to 2 kbp using a sliding window approach. The SNPs were annotated and effects of variants on genes (such as amino acid changes) were predicted using *snpEFF* software [28]. The R statistical package was used to analyse SNP data, including implementation of selection analyses using the "*rehh*" library.

Results

Genetic polymorphisms

The genomic coverage in the nuclear genome was high (median 103-fold, range (30-5973-fold), and in keeping with multiple organellar copies, the mitochondria and apicoplast coverage was 30-fold and 1.8 fold greater than the nuclear coverage. The density of SNPs in the nuclear genome (219,288 SNPs, 1 every 99 bp) was greater than in the mitochondrial (23 SNPs, 1 every 165 bp) and apicoplast genomes (176 SNPs, 1 every 165 bp) (S2 Table). Although 60% of the annotated reference genome is coding (chromosomal range: 54%-64%), approximately half the SNPs in the isolates were found in genic regions (mean 48% per chromosome, range 43% to 52%) (Fig 1A). The proportion of non-synonymous sites is consistent with those found in other *Plasmodium* species, with 52% of coding SNP sites being non-synonymous in the nuclear genome, 36% in the mitochondrion and 56% in the apicoplast. The differences in these genomes suggest they may be subject to differential selective pressure [29]. The majority of SNPs are rare, with nearly half of the mutations (45%) being observed in single samples (Fig 1B) as seen in other *Plasmodium* populations [18]. There was some evidence of polyclonality in 22 samples (Cambodia 1/2, Colombia 5/5, Madagascar 2/2, PNG 2/5, Thailand 11/22).

Analysis of structural variants and copy number variants was limited to Thai, Cambodian and Madagascan isolates, which had high and uniform genomic coverage. CNVs were located

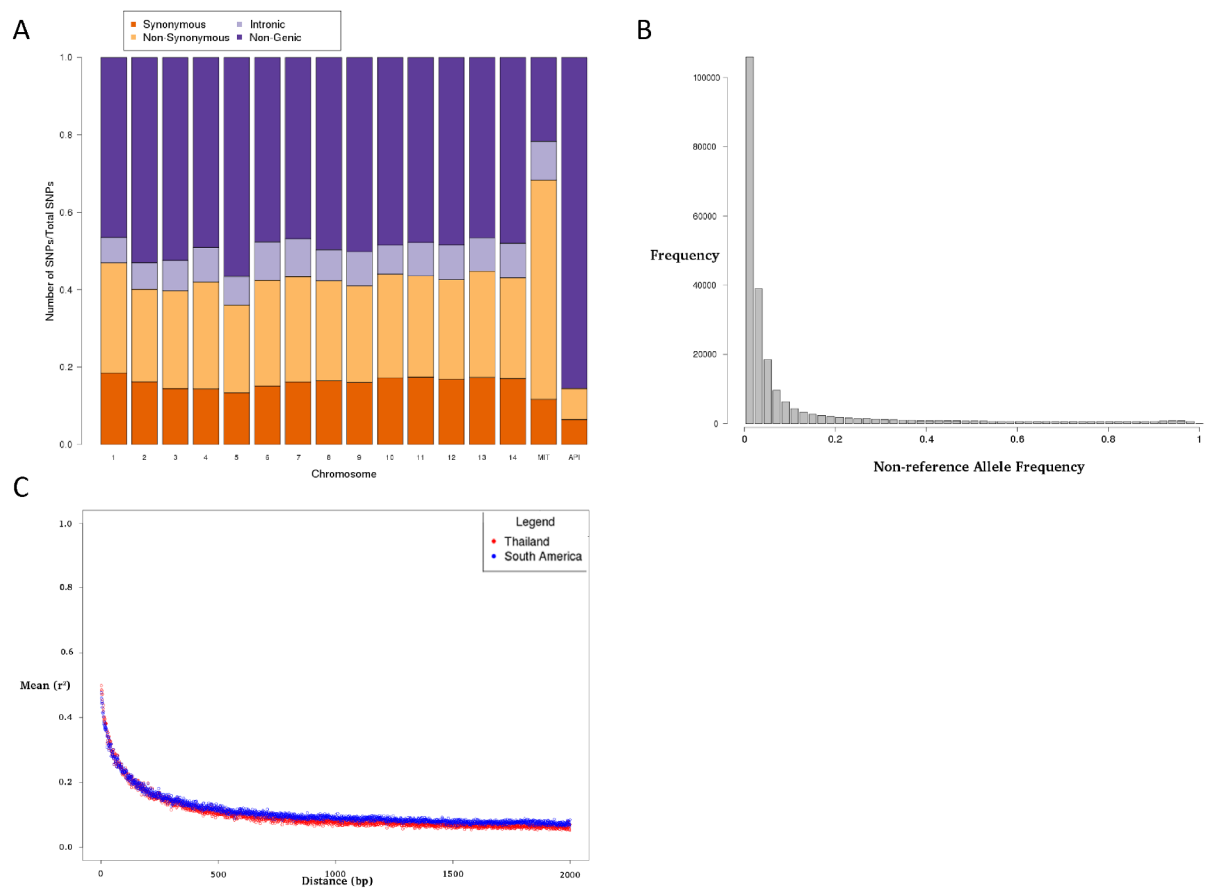


Fig 1. (a) SNP locations by annotation*. (b) Minor allele frequency spectrum indicates a predominance of rare alleles. (c) Linkage disequilibrium (r^2) decays rapidly with physical genetic distance. * established using *snpEFF* software.

<https://doi.org/10.1371/journal.pone.0177134.g001>

less than 1 kbp distance from the *MDR1* gene (chromosome 10, *PVX_080100*) in Cambodian and Thai isolates (S1 Fig). Several *MDR1* variants have previously been reported, some considered putative chloroquine- and mefloquine-resistance alleles [14,30–35]. At the *MDR1* locus, we observed either a duplication of ~35kb (position 351kbp to 389kbp, $n = 1$, Thailand), a major deletion in the promoter region of the gene ($n = 7$, Thailand; $n = 1$ Cambodia), or a combination of both structural variants, including two copies, one with the deletion in the promoter and another copy with a complete promoter ($n = 4$, Thailand); as confirmed by the increase or decrease in coverage and accumulation of split reads in the regions where a break in the coverage occurs. The known duplication in the Duffy binding protein *PvDBP* (chr. 6: 974,000–982,000, *PVX_110810*) in Malagasy [36] was confirmed in one of the two Madagascar isolates (SRR828416). The *PvDBP* gene is potentially involved in erythrocyte invasion, and the duplication was also observed in thirteen Thai isolates. A further duplication was observed in *Pv-fam-e* (a RAD gene, chr. 5: 895,000–900,000, $n = 8$, Thailand), a gene linked to *P. vivax* selectivity for young erythrocytes and/or immune evasion [36].

Assessing genetic diversity, LD and positive directional selection

The average polymorphism (pair-wise mismatches measured by nucleotide diversity π) was calculated by gene and chromosome. There was little difference across the chromosomes with mean 11.1×10^{-4} (range 6.0×10^{-4} to 19.0×10^{-4}), which is consistent with other studies with similar sample size [14] as well as larger datasets when restricted to high quality SNPs (1.5×10^{-3}) [6, 7]. LD decays rapidly for non-rare polymorphism (minor allele frequency $\geq 5\%$) within a few hundred base pairs, and reaches a baseline within 500bp in South American and Thai nuclear genomes (Fig 1C). Like *P. falciparum*, there is a high correlation between non-rare SNPs (median D' 0.918, range 0.425–1) in the mitochondrial and apicoplast genomes supporting the inference that the organelles are co-inherited and supporting the view that these SNPs have potential utility for barcoding [29].

To examine evidence for signatures of positive natural selection we calculated the *iHS* metric in the Thailand and South America populations, informed by the population structure reported in S3 Fig. Five contiguous loci of strong positive directional selection were identified, including the *MRP1* gene (*PVX_097025*) and its promoter region in Thailand, and a region surrounding the *MRP2* gene (*PVX_097025*, *P. falciparum* homologue associated with primaquine and antifolate drug sensitivity [14]). Several surface proteins were identified in both populations, including the *MSP7* and *MSP3.1*, which are thought to be under selection pressure due to their role in erythrocyte invasion and strong vaccine candidates and have been identified before by other studies using sanger sequencing [29] (Table 1, Table 2, S2 Fig). In addition, some helicases showed strong signals of selection (*PVX_088190* and *PVX_111220*) which were also detected in the same study [36] reinforcing the method used. Furthermore, we identified in South America a proximal region of selection (chr14: 1,414,164–1,479,586) described elsewhere [7].

Allele frequency spectrum and balancing selection

The allele frequency spectrum of different classes of nucleotide sites all show an excess of rare alleles, with coding, non-synonymous, synonymous and intergenic sites more skewed than expected under a Wright-Fisher model of constant population size [18]. This observation could indicate a population expansion in the recent past, where as a population grows in size, the frequency of rare alleles also increases [18]. The Tajima's *D* method was applied to genes with at least five SNPs in the two main populations (Thailand 4,673 (91.0%) and South America 3,549 (70.0%) genes). The majority of Tajima's *D* values were negative (Thailand 90.2%;

Table 1. Regions under directional selective pressure in Thailand *.

Chr	Position / Range	Max <i>iHS</i>	Gene	Annotation
1	284000	5.083	<i>PVX_087910</i>	E3 ubiquitin-protein ligase, putative
1	379430	3.511	.	.
2	148413	4.319	.	Promoter region MRP1
2	158122	3.452	<i>PVX_097025</i>	multidrug resistance-associated protein 1, MRP1
4	576773	4.289	.	.
4	629852	3.483	<i>PVX_003770</i>	merozoite surface protein 5 (MSP5)
5	673939	3.566	<i>PVX_089575</i>	trafficking protein particle complex protein, TRAPPC2L
7	778719	3.986	.	.
7	1397181	5.829	<i>PVX_086903</i>	Plasmodium exported protein, unknown function
8	766604	3.709	<i>PVX_095055</i>	Rh5 interacting protein, putative (RIPR)
8	921104	3.451	<i>PVX_095235</i>	protein phosphatase inhibitor 2, putative
8	927191	3.489	<i>PVX_095245</i>	hypothetical protein, conserved
8	985454	3.778	<i>PVX_095305</i>	hypothetical protein, conserved
9	107123	6.186	<i>PVX_090925</i>	protein kinase domain containing protein
9	311594	3.590	.	.
9	526557	4.115	<i>PVX_091440</i>	hypothetical protein, conserved
10	1222646	5.499	<i>PVX_097715</i>	hypothetical protein
10	1225529	4.109	.	.
10	1261650	9.180	.	Promotor region MSP3.1
10	1261852	3.982	<i>PVX_097670</i>	merozoite surface protein 3 (MSP3.1)
11	926166	4.034	.	.
12	732115	3.531	<i>PVX_082735</i>	thrombospondin-related anonymous protein (TRAP)
12	734223–745860	4.901	<i>PVX_082730</i>	hypothetical protein, conserved
12	746536	4.319	.	.
12	751773	5.473	<i>PVX_082710</i>	hypothetical protein
12	752332	3.558	.	.
12	765929	6.849	.	.
12	766784	6.170	<i>PVX_082675</i>	merozoite surface protein 7 (MSP7)
12	864218	3.561	<i>PVX_082510</i>	hypothetical protein
12	865780	3.930	<i>PVX_082505</i>	CPW-WPC family protein, putative
12	1020235	5.042	.	.
12	2475528	4.740	.	.
12	2540841	3.633	<i>PVX_118270</i>	serine/threonine protein kinase, putative
12	2622092	3.446	<i>PVX_118345</i>	protein transport protein SEC7, (SEC7)
12	2638874	4.900	.	.
12	2671299	3.501	<i>PVX_118380</i>	GTP-binding protein, putative
12	2732268	3.486	<i>PVX_118460</i>	hypothetical protein, conserved
14	3028986	5.168	.	.

* $|iHS| > 3$.

<https://doi.org/10.1371/journal.pone.0177134.t001>

South America 64.4%), reinforcing the presence of an excess of low frequency and singleton polymorphisms, potentially due to population expansion in the recent past or purifying selection. For Thailand, we identified 398 (8.5%) genes with positive Tajima's *D* values, of which 14 were in excess of 2.5 and potentially under balancing selection (Table 3). Similarly, for South America, of the 1,260 (35.5%) values that were positive, 12 were in excess of 2.5 (Table 3). The loci under potential balancing selection in both populations encode proteins with predominantly roles surface proteins (e.g. MSPs) and antigens. The majority of the 26 genes identified

Table 2. Regions under directional selective pressure in South America *.

Chr	Position / Range	Max <i>iHS</i>	Gene	Annotation
1	490369	3.020	PVX_088190	helicase, putative
1	662401	3.050	PVX_093585	SF-assemblin, putative
2	244790–1	5.316	.	Promoter region PVX_081315
3	247791	3.813	PVX_000860	hypothetical protein, conserved
3	372679	4.109	PVX_000695	hypothetical protein, conserved
4	574831	3.029	PVX_003830	serine-repeat antigen 5 (SERA)
5	560637	3.649	PVX_089445	RAD protein (Pv-fam-e)
5	1046482	3.358	PVX_090020	hypothetical protein, conserved
6	627960–1	3.605	PVX_111230	hypothetical protein, conserved
7	437942–60	4.773	PVX_099005	cysteine repeat modular protein 1, CRMP1
7	527651	3.182	PVX_099125	pseudouridylate synthase, putative
7	1116251–2	5.712	PVX_099915	RNA-binding protein, putative
7	1214179	6.840	PVX_087145	nucleolar protein Nop52, putative
8	219359	3.180	PVX_094405	hypothetical protein, conserved
9	730034	3.611	PVX_091700	circumsporozoite-related antigen, EXP1
9	751857	3.149	.	Promoter region PVX_091715
9	829890	3.161	PVX_091770	calcium-dependent protein kinase 7, CDPK7
9	1042906–7	5.519	PVX_092035	6-phosphofructokinase, putative
9	1136873	3.110	PVX_092160	hypothetical protein, conserved
10	380535	3.108	PVX_080110	G10 protein, putative
10	1063432	3.592	PVX_097895	TBC domain containing protein
10	1257251	3.291	.	Promoter region MSP3.2
10	1260441–2	5.201	.	1 Kb from MSP3.2
11	822715	4.026	PVX_114575	transmembrane amino acid transporter protein
11	1973708	6.074	.	.
12	955488	3.652	PVX_082400	myosin C, putative
12	1317195	3.435	PVX_116815	hypothetical protein, conserved
13	215135	3.283	PVX_084350	hypothetical protein, conserved
13	611668	5.708	PVX_084755	hypothetical protein, conserved
13	856226–30	4.519	PVX_085030	aspartyl protease, putative
14	1275835	3.114	PVX_123250	aquaporin, putative (AQP2)
14	1665191	3.572	PVX_123685	histone-lysine N-methyltransferase, SET10
14	1875833	4.986	PVX_123890	hypothetical protein, conserved

* |*iHS*| > 3.

<https://doi.org/10.1371/journal.pone.0177134.t002>

in this study have had positive indices of balancing selection in previous studies [37, 38], or have orthologues in *P. falciparum* [18].

Population structure and evidence of differing directional selection in populations

Both a principal component and a neighbourhood joining tree analysis (Fig 2, S4 Fig) revealed clustering by continent, in keeping with similar *P. falciparum* analyses [18, 19]. The across population long-range haplotype method (*Rsb* implementation) was applied to compare Thailand to the South American population, to identify regions potentially under recent directional selection (Table 4). We detected several loci including at multidrug resistance-associated protein *MRP1* (PVX_097025), and the CCR4-associated factor 1 (*CAF1*, PVX_123230) located

within 20kb of *DHPS* (associated with resistance to sulfadoxine [14]). Five non-synonymous mutations were identified in the *DHPS* gene (M616T, P553A, P383A, P382R, P382A), with evidence that the P383A has driven toward fixation across all geographical regions. Except for mutation in codon 616, all the others have been previously reported [39–42]. The *DHFR* gene, associated with resistance to pyrimethamine (part of the SP drug combination), exhibited elevated *Rsb* (>3). Seven non-synonymous mutations were identified, including the previously described S58R and S117N [42–44] that were fixed across populations, and F57I/L and T61M [44–46] that were absent from South America (S3 Table). No evidence was observed of a hard sweep around the *MDR1* copy number gene. However, nine non-synonymous SNP mutations were identified, five of which have been reported previously. These included the fixed alleles T958M and M908L, F1076L at high frequency across populations, and G698S and S513R absent from South America [41–44]. There was no evidence of a sweep around the *P. vivax* orthologues of the *falciparum* chloroquine related *CRT* (*pvcr1-o*, *PVX_087980*) or GTP cyclohydrolase I folate pathway (*GTPCH*, *PVX_123830*) genes. No common non-synonymous mutations were identified within the *CRT* gene, while 7 low frequency non-synonymous SNPs were identified in the *GTPCH* locus.

The *Rsb* analysis also revealed loci associated with the diversity of vectors, including the *P28* (*PVX_111180*) gene expressed in the surface of the ookinete stage during the mosquito part of the life cycle, *pv47* (*PVX_083240*) and *pv48/45* (*PVX_083235*) involved in the transmission of the parasite. There are continental-specific *pv47* and *pv48/45* SNPs (and haplotypes) as previously found [47, 48], consistent with the presence of different species of mosquito in each the regions [49], resembling a similar pattern found in *P. falciparum* [50].

Table 3. Genetic regions under potential balancing selection pressure in South America (SA) and Thailand (T)*.

Chr.	Gene Start	Gene End	Tajima's <i>D</i>	Gene **	Annotation	Population
1	521978	527387	3.265	<i>PVX_088235</i>	ferlin, putative	SA
3	19187	30715	14.166, 7.134	<i>PVX_001080</i>	hypothetical protein, conserved	SA, T
4	265018	267216	2.928	<i>PVX_002785</i>	ATP-dependent acyl-CoA synthetase	T
4	562755	566374	4.871, 6.085	<i>PVX_003840</i>	serine-repeat antigen 3 (SERA)	SA, T
4	567313	571093	4.944	<i>PVX_003835</i>	serine-repeat antigen 1 (SERA)	T
4	572172	575852	3.36	<i>PVX_003830</i>	serine-repeat antigen 5 (SERA)	T
4	596283	600192	4.224	<i>PVX_003805</i>	serine-repeat antigen (SERA), putative	SA
5	1297808	1301010	3.279	<i>PVX_090285</i>	Pvstp1, putative	T
5	1345372	1354047	15.907	<i>PVX_090325</i>	reticulocyte binding protein 2c (RBP2c)	T
5	1358748	1360820	5.762	<i>PVX_090330</i>	reticulocyte binding protein 2 (PvRBP-2)	T
7	1157742	1162997	5.593, 4.124	<i>PVX_099980</i>	merozoite surface protein 1 (MSP1)	SA, T
9	6424	7811	4.195	<i>PVX_090835</i>	hypothetical protein	T
10	22046	23460	2.925	<i>PVX_079700</i>	hypothetical protein, conserved	T
10	65101	69250	2.793	<i>PVX_079750</i>	hypothetical protein, conserved	T
10	1187639	1188909	5.206	<i>PVX_097760</i>	60S ribosomal protein L31, RPL31	SA
10	1218512	1221845	2.939, 5.585	<i>PVX_097720</i>	merozoite surface protein 3 (MSP3.10)	SA, T
10	1272354	1274193	3.869	<i>PVX_097660</i>	4-diphosphocytidyl-2-C-methyl- kinase, IspE	SA
10	1306384	1308153	5.991	<i>PVX_097600</i>	hypothetical protein, conserved	SA
12	751041	752204	5.578	<i>PVX_082710</i>	hypothetical protein	SA
13	37121	59181	3.876	<i>PVX_084160</i>	dynein heavy chain, putative	SA
13	128618	131751	4.099	<i>PVX_084260</i>	hypothetical protein, conserved	SA
14	3044644	3046339	2.773	<i>PVX_101575</i>	hypothetical protein, conserved	T

* Tajima's *D* > 2.5

** at least 5 SNPs per gene.

<https://doi.org/10.1371/journal.pone.0177134.t003>

Towards molecular barcoding of *P. vivax*

The development of molecular barcode for *P. vivax* could ultimately assist with surveillance and disease control. Previous work [51] has described a 42 SNP barcode to classify geographically *P. vivax* across 7 countries. Across the 46 isolates analysed here, we found 3 SNPs in the barcode to be either non-segregating or not passing quality control filtering. Use of the remaining 39 SNPs led to imperfect clustering by continent (S4 Table, S5 Fig). Application of the F_{ST} population differentiation metric identified SNPs driving the observed differences between Thailand, South America and other populations (S5 Table). These SNPs occurred in drug resistance loci, including *MRP1* (PVX_097025), *DHPS* (PVX_123230) and *UBP1* (PVX_081540) (all $F_{ST} > 0.72$), and in close proximity (e.g. PVX_089960 within 8kb of *DHFR*). Population differentiation due to genetic diversity in drug resistant loci is also observed in *P. falciparum* [18,19].

Previous work has proposed the mitochondria and apicoplast organellar genomes as candidate regions for a barcode [29]. Genotyping of organellar markers would benefit from greater copy number and coverage as well as highly conserved sequences [29]. Eight markers across five apicoplast genes could differentiate Thai and Southeast Asian samples from the other isolates, and two non-genic markers were found to be exclusive to South America (all $F_{ST} > 0.7$, S6 Table). No informative mitochondrial markers were identified (all $F_{ST} < 0.7$). Further, as the

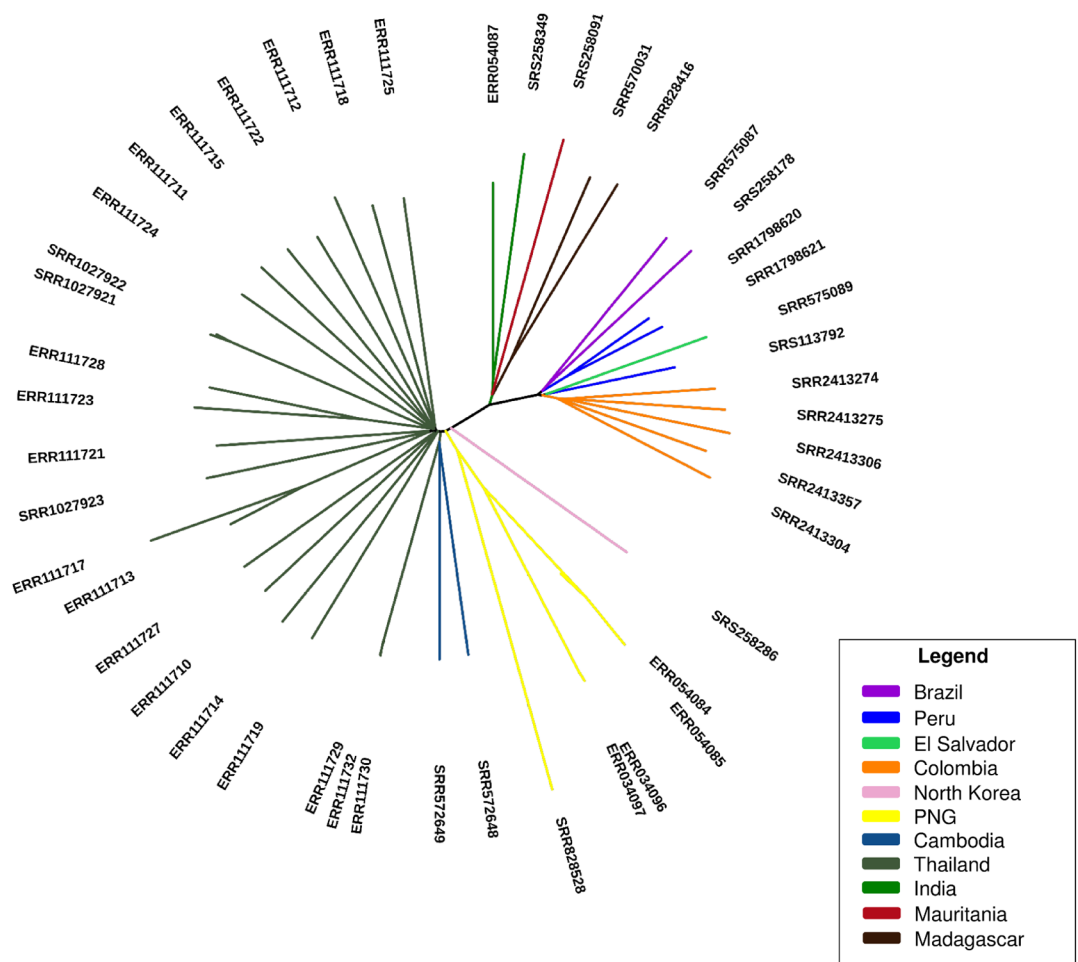


Fig 2. Population structure analysis based on 219,288 SNPs shows clustering by continent.

<https://doi.org/10.1371/journal.pone.0177134.g002>

Table 4. Regions under directional selective pressure between Thailand and South America *.

Chr	Position/Range	Rsb	Gene	Annotation
2	145708–151606	11.80	.	Promoter region MRP1
2	154067–158122	5.230	PVX_097025	multidrug resistance-associated protein 1, MRP1
2	175191–176803	3.717	PVX_081215	hypothetical protein, conserved
4	91457	5.879	PVX_002550	hypothetical protein, conserved
4	607568–607837	4.457	PVX_003795	serine-repeat antigen (SERA)
4	629831–630120	5.205	PVX_003770	merozoite surface protein 5
5	1132736	10.20	PVX_090105	holo-[acyl-carrier-protein] synthase, putative (ACPS)
5	964771	3.624	PVX_089950	bifunctional dihydrofolate reductase-thymidylate synthase, DHFR-TS
6	199049–199165	4.703	PVX_001850	hypothetical protein
6	605656–608119	3.788	PVX_111260	hypothetical protein, conserved
6	635433–635539	4.406	PVX_111220	RNA helicase, putative
6	661816	6.048	PVX_111180	28 kDa ookinete surface protein, (P28)
7	1396929–1396961	4.708	.	Promoter region PVX_086903
7	1397181	4.700	PVX_086903	Plasmodium exported protein, unknown function
8	219359–220251	5.257	PVX_094405	hypothetical protein, conserved
8	1417014–1417038	4.406	PVX_119515	hypothetical protein, conserved
8	1533222	3.214	PVX_119360	hypothetical protein
9	419318–419619	4.971	.	Promoter region PVX_091307
9	920056–920166	4.676	PVX_091880	hypothetical protein, conserved
9	1048990	3.304	PVX_092040	geranylgeranyl pyrophosphate synthase (GGPPS)
9	1229833	3.296	PVX_092275	apical membrane antigen 1 (AMA1)
10	1251585–1257251	7.094	.	Promoter region MSP3.2
10	1257754–1257815	6.617	PVX_097675	merozoite surface protein 3 (MSP3.2)
11	1517269	3.234	PVX_113775	6-cysteine protein (P12)
11	1223546–1223790	3.816	.	Promoter region PVX_114125
11	1383108–1383155	6.010	PVX_113925	hypothetical protein, conserved
12	286960	3.227	PVX_083240	6-cysteine protein (P47)
13	141889–142286	5.680	PVX_084280	hypothetical protein, conserved
13	620154–620261	5.922	.	Promoter region PVX_084770
13	731328–792522	5.375	PVX_084860	hypothetical protein, conserved
13	1034635–1034718	4.101	PVX_085235	hypothetical protein
13	1042774	5.406	PVX_085245	hypothetical protein, conserved
13	1553113	4.126	PVX_085835	hypothetical protein, conserved
14	1231525–1231528	6.056	PVX_123205	CCR4-associated factor 1, (CAF1)
14	1429874	3.903	PVX_123415	adrenodoxin-type ferredoxin, putative

* Rsb > 3; genes in bold refer to loci related with mosquito life stages of the parasite or drug-resistance.

<https://doi.org/10.1371/journal.pone.0177134.t004>

organelle genomes are known to be highly conserved between *Plasmodia* species, when comparing a set of *P. falciparum* geographical markers [26] to *P. vivax* sequences, we found evidence of positions close in the sequence. Two of the samples (ERR020124 and SRR828528) had a high density of mixed calls in the organellar genomes, in this case, a signature of *P. falciparum* overlaying onto *P. vivax* (S6 Fig). In general, this density signature is indicative of a co-infection of *P. vivax* with another *Plasmodium* spp. By comparing the sequencing reads to the *Plasmodium knowlesi* reference genome [52], there was no evidence of any *vivax* and *knowlesi* co-infections. However, the presence of a unique triallelic SNP reinforces the potential for an organellar inter-plasmodia species barcode (S6 Fig).

Discussion

Several studies have previously described the genomic diversity of *P. vivax* populations using whole genome data, but with low sample sizes. Recently, two papers using a combined collection of over 400 isolates from 17 countries described major genomic diversity in *Plasmodium vivax* [6, 7]. Here we analysed a complementary collection of 46 high quality isolates spanning 10 countries across 4 continents in order to position them within the context of this new work. As expected we confirmed that *P. vivax* genomic diversity is greater compared to *P. falciparum*, and even at a relatively low sample size, the samples clustered geographically. We reveal a wider genomic distance between South American and Southeast Asian continents than observed between *P. falciparum* African and Southeast Asian populations [6, 18, 19], highlighted by the greater and more uniform distribution of SNPs with a high F_{ST} across the genome. Hot-spots of selection pressure were identified, including the previously reported *MRP1*, *DHPS* [14] and other putative drug resistance genes, as well as several loci related with the mosquito stage of the parasite life cycle. The latter observation is consistent with recent work [6, 7] and the presence of different *Anopheles* species across continents. We identified structural variants, including extra copies and deletions in the promoter region of the *MDR1* gene, a locus associated with multiple antimalarial drugs [14]. We also confirmed the duplication in the Duffy binding protein gene (*PvDBP*) in a Madagascan sample, and detected it in Thai isolates. This duplication has been found in parasites from several regions in Africa, South America and Asia [6, 37]. Many of these locations are areas where Duffy-negative individuals make up >45% of the population. However other regions like Cambodia do not present Duffy-negative individuals [53]. It has been theorized that the duplication allows the parasite to infect Duffy negative individuals [53], however more research is needed in this area.

Microsatellite genotyping has been used previously to cluster geographically *P. vivax* isolates, and together with antigen genotyping identify mixed infections and extent of transmission, used as the basis of genetic epidemiology. In comparison, whole genome sequencing provides a higher specificity in the application of geographical clustering [51]. While other studies have focused on creating a barcode using the nuclear genome [51], we also considered organelle genomes (mitochondrion and apicoplast), which are more stable over time, do not undergo recombination and are co-inherited [29]. The analysis revealed organellar markers that are potentially Southeast Asian and South American specific, and others that highlighted the presence of multi-species mixed infections. The sequencing of large numbers of isolates, beyond currently published samples sizes, will be required to establish robust intra- and inter-species organellar-based barcode. Such large-scale datasets across multiple regions will also serve to identify the high genomic diversity that lies within and between *P. vivax* populations, which could be exploited for biological insights, including elucidating drug resistance and invasion mechanisms, and ultimately measures of disease control.

Conclusion

This study has shown that genomic diversity that lies within and between *P. vivax* populations can be used to elucidate potential drug resistance and invasion mechanisms, as well as facilitate the molecular barcoding of the parasite for surveillance applications.

Supporting information

S1 Fig. Structural variants located around the *MDR1* gene (chromosome 10) in the Thai-land population; (i) a sample without a copy number variant or deletion (even coverage), (ii) a major deletion in the promoter region of the gene ($n = 7$); (iii) duplication of ~35kb (position 351kbp to 389kbp, $n = 1$); and (iv) a combination of both structural variants (ii) and (iii),

including two copies, one with the deletion in the promoter and another copy with a complete promoter ($n = 4$, Thailand). The horizontal dashed line is average chromosomal coverage and the red outline encloses the promoter region of the *MDR1* gene.

(TIFF)

S2 Fig. Intra-population evidence of directional selective pressure (*iHS)** a) Thailand b) South America. * *iHS* integrated haplotype score; see [Table 1](#) and [Table 2](#) for a summary of the hits.

(TIFF)

S3 Fig. Principal component analysis based on 225k SNPs reveals strong clustering of isolates by continent.

(PNG)

S4 Fig. Identifying regions under directional selective pressure between Thailand and South America. Blue line: $|Rsb| > 3$ ($P < 0.003$); Red line represents a human GWAS cut-off; see [Table 4](#) for a summary of the hits.

(PNG)

S5 Fig. Principal component analysis based on the previously characterised 42 barcoding SNPs* does not reveal strong population clustering. * SNPs and genotypes are shown in [S4 Table](#)

(PNG)

S6 Fig. Signatures of a mixed species infection based on heterozygous calls in mitochondrial markers (positions: 3,736–3,935bp).

(PNG)

S1 Table. The 46 study isolates.

(DOCX)

S2 Table. The SNPs.

(DOCX)

S3 Table. Non-synonymous mutations in candidate genes.

(DOCX)

S4 Table. Previously characterised 42 barcoding SNPs* in the 46 study isolates.

(DOCX)

S5 Table. Sites of population differentiation between Thailand and South America.

(DOCX)

S6 Table. Population informative apicoplast variants.

(DOCX)

Acknowledgments

We thank Samuel Assefa, Francesc Coll, and Mark Preston for providing computing support. Sequence data were analysed using the MRC UK funded eMedlab computing resource.

Author Contributions

Conceptualization: EDB CJS CR SC TGC.

Data curation: EDB.

Formal analysis: EDB ZW WC SC TGC.

Funding acquisition: TGC CR.

Supervision: FRM SC TGC.

Writing – original draft: EDB SC TGC.

Writing – review & editing: EDB ZW WC FRM CJS CR SC TGC.

References

1. World Health Organization. World malaria report 2013. http://www.who.int/malaria/publications/world_malaria_report_2013/en/
2. Bright AT, Tewhey R, Abeles S, Chuquiyauri R, Llanos-Cuentas A, Ferreira MU, et al. Whole genome sequencing analysis of *Plasmodium vivax* using whole genome capture. *BMC Genomics* 2012; 13:262. <https://doi.org/10.1186/1471-2164-13-262> PMID: 22721170
3. Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, et al. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature* 2008; 455:757–63. <https://doi.org/10.1038/nature07327> PMID: 18843361
4. Arnott A, Barry AE, Reeder JC. Understanding the population genetics of *Plasmodium vivax* is essential for malaria control and elimination. *Malar J* 2012; 11:14. <https://doi.org/10.1186/1475-2875-11-14> PMID: 22233585
5. Neafsey DE, Galinsky K, Jiang RH, Young L, Sykes SM, Saif S, et al. The malaria parasite *Plasmodium vivax* exhibits greater genetic diversity than *Plasmodium falciparum*. *Nat Genet* 2012; 44:1046–50. <https://doi.org/10.1038/ng.2373> PMID: 22863733
6. Hupalo DN, Luo Z, Melnikov A, Sutton PL, Rogov P, Escalante A, et al. Population genomics studies identify signatures of global dispersal and drug resistance in *Plasmodium vivax*. *Nat Genet* 2016; 48(8), 953–958. <https://doi.org/10.1038/ng.3588> PMID: 27348298
7. Pearson RD, Amato R, Auburn S, Miotto O, Almagro-Garcia J, Amaratunga C, et al. Genomic analysis of local variation and recent evolution in *Plasmodium vivax*. *Nat Genet* 2016; 48(8), 959–964. <https://doi.org/10.1038/ng.3599> PMID: 27348299
8. Imwong M, Nair S, Pukrittayakamee S, Sudimack D, Williams JT, Mayxay M, et al. Contrasting genetic structure in *Plasmodium vivax* populations from Asia and South America. *Int J Parasitol* 2007; 37:1013–22. <https://doi.org/10.1016/j.ijpara.2007.02.010> PMID: 17442318
9. Dharia NV, Bright AT, Westenberger SJ, Barnes SW, Batalov S, Kuhlen K, et al. Whole-genome sequencing and microarray analysis of ex vivo *Plasmodium vivax* reveal selective pressure on putative drug resistance genes. *Proc Natl Acad Sci USA* 2010; 107:20045–50. <https://doi.org/10.1073/pnas.1003776107> PMID: 21037109
10. Gunawardena S, Karunaweera ND, Ferreira MU, Phone-Kyaw M, Pollack RJ, Alifrangis M, et al. Geographic structure of *Plasmodium vivax*: microsatellite analysis of parasite populations from Sri Lanka, Myanmar, and Ethiopia. *Am J Trop Med Hyg* 2010; 82:235–42. <https://doi.org/10.4269/ajtmh.2010.09-0588> PMID: 20133999
11. Koepfli C, Rodrigues PT, Antao T, Orjuela-Sánchez P, Van den Eede P, Gamboa D, et al. *Plasmodium vivax* Diversity and Population Structure across Four Continents. *PLoS Negl Trop Dis* 2015; 9: e0003872. <https://doi.org/10.1371/journal.pntd.0003872> PMID: 26125189
12. Kim JY, Goo YK, Zo YG, Ji SY, Trimarsanto H, To S et al. Further Evidence of Increasing Diversity of *Plasmodium vivax* in the Republic of Korea in Recent Years. *PLoS One* 2016; 11:e0151514. <https://doi.org/10.1371/journal.pone.0151514> PMID: 26990869
13. Auburn S, Marfurt J, Maslen G, Campino S, Ruano Rubio V, Manske M, et al. Effective preparation of *Plasmodium vivax* field isolates for high-throughput whole genome sequencing. *PLoS One* 2013; 8: e53160. <https://doi.org/10.1371/journal.pone.0053160> PMID: 23308154
14. Winter DJ, Pacheco MA, Vallejo AF, Schwartz RS, Arevalo-Herrera M3, Herrera S, et al. Whole Genome Sequencing of Field Isolates Reveals Extensive Genetic Diversity in *Plasmodium vivax* from Colombia. *PLoS Neg Trop Dis* 2015; 9:e0004252.
15. Flannery EL, Wang T, Akbari A, Corey VC, Gunawan F, Bright AT, et al. Next-Generation Sequencing of *Plasmodium vivax* Patient Samples Shows Evidence of Direct Evolution in Drug-Resistance Genes. *ACS Infect Dis* 2015; 1:367–379. <https://doi.org/10.1021/acsinfecdis.5b00049> PMID: 26719854

16. Shen HM, Chen SB, Wang Y, Chen JH. Whole-genome sequencing of a *Plasmodium vivax* isolate from the China-Myanmar border area. *Mem Inst Oswaldo Cruz* 2015; 110:814–6. <https://doi.org/10.1590/0074-02760150216> PMID: 26517664
17. Parobek CM, Bailey JA, Hathaway NJ, Socheat D, Rogers WO, Juliano JJ. Differing Patterns of Selection and Geospatial Genetic Diversity within Two Leading *Plasmodium vivax* Candidate Vaccine Antigens. *PLoS Negl Trop Dis* 2014; 8: e2796. <https://doi.org/10.1371/journal.pntd.0002796> PMID: 24743266
18. Ocholla H, Preston MD, Mipando M, Jensen AT, Campino S, MacInnis B, et al. Whole-genome scans provide evidence of adaptive evolution in Malawian *Plasmodium falciparum* isolates. *J Infect Dis* 2014; 210:1991–2000. <https://doi.org/10.1093/infdis/jiu349> PMID: 24948693
19. Samad H, Coll F, Preston MD, Ocholla H, Fairhurst RM, Clark TG. Imputation-based population genetics analysis of *Plasmodium falciparum* malaria parasites. *PLoS Genet* 2015; 11:e1005131. <https://doi.org/10.1371/journal.pgen.1005131> PMID: 25928499
20. Campino S, Diez Benavente E, Assefa S, Thompson E, Drought LG, Taylor CJ, et al. Genomic variation in two gametocyte non-producing *Plasmodium falciparum* clonal lines. *Malar J* 2016; 15:229. <https://doi.org/10.1186/s12936-016-1254-1> PMID: 27098483
21. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 1989; 123:585–95. PMID: 2513255
22. Kryazhimskiy S, Plotkin JB. The Population Genetics of dN/dS. *PLOS Genetics* 2008; 4(12): e1000304. <https://doi.org/10.1371/journal.pgen.1000304> PMID: 19081788
23. Voight BF, Kudavalli S, Wen X, Pritchard JK. A map of recent positive selection in the human genome. *PLoS Biol.* 2006; 4:e72. <https://doi.org/10.1371/journal.pbio.0040072> PMID: 16494531
24. Assefa S, Preston M, Campino S, Ocholla H, Sutherland CJ, Clark TG. estMOI: Estimating multiplicity of infection using parasite deep sequencing data. *Bioinformatics* 2014; 30:1292–4. <https://doi.org/10.1093/bioinformatics/btu005> PMID: 24443379
25. Sabeti PC, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, et al. Genome-wide detection and characterization of positive selection in human populations. *Nature* 2007; 449:913–8. <https://doi.org/10.1038/nature06250> PMID: 17943131
26. Holsinger KE, Weir BS. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST} . *Nat Rev Genet* 2009; 10:639–50. <https://doi.org/10.1038/nrg2611> PMID: 19687804
27. Hill WG, Robertson A. Linkage disequilibrium in finite populations. *Theor Appl Genet* 1968; 38:226–31. <https://doi.org/10.1007/BF01245622> PMID: 24442307
28. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w(1118); iso-2; iso-3. *Fly* 2012; 6(2), 80–92. <https://doi.org/10.4161/fly.19695> PMID: 22728672
29. Preston MD, Campino S, Assefa SA, Thompson E, Drought LG, Taylor CJ, et al. A barcode of organellar genome polymorphisms identifies the geographic origin of *Plasmodium falciparum* strains. *Nat Commun* 2014; 5:4052. <https://doi.org/10.1038/ncomms5052> PMID: 24923250
30. Brega S, Meslin B, de Monbrison F, Severini C, Gradoni L, Udomsangpetch R, et al. Identification of the *Plasmodium vivax* mdr-like gene (pvmdr1) and analysis of single-nucleotide polymorphisms among isolates from different areas of endemicity. *J Infect Diseases* 2005; 191(2), 272–277.
31. Schousboe ML, Ranjitkar S, Rajakaruna RS, Amerasinghe PH, Morales F, Pearce R, et al. Multiple Origins of Mutations in the mdr1 Gene—A Putative Marker of Chloroquine Resistance in *P. vivax*. *PLoS Negl Trop Dis* 2015; 9(11), e0004196. <https://doi.org/10.1371/journal.pntd.0004196> PMID: 26539821
32. Barnadas C, Ratsimbaoa A, Tichit M, Bouchier C, Jahevitra M, Picot S, et al. *Plasmodium vivax* resistance to chloroquine in Madagascar: clinical efficacy and polymorphisms in pvmdr1 and pvcrt-o genes. *Antimicrobial Agents and Chemo* 2008; 52(12), 4233–4240.
33. Lu F, Lim CS, Nam DH, Kim K, Lin K, Kim TS, et al. Genetic polymorphism in pvmdr1 and pvcrt-o genes in relation to in vitro drug susceptibility of *Plasmodium vivax* isolates from malaria-endemic countries. *Acta Tropica* 2011; 117(2), 69–75. <https://doi.org/10.1016/j.actatropica.2010.08.011> PMID: 20933490
34. Suwanarusk R, Russell B, Chavchich M, Chalfein F, Kenangalem E, Kosaisavee V, et al. Chloroquine Resistant *Plasmodium vivax*: In Vitro Characterisation and Association with Molecular Polymorphisms. *PLoS ONE* 2007; 2(10), e1089. <https://doi.org/10.1371/journal.pone.0001089> PMID: 17971853
35. Imwong M, Pukrittayakamee S, Pongtavornpinyo W, Nakeesathit S, Nair S, Newton P, et al. Gene amplification of the multidrug resistance 1 gene of *Plasmodium vivax* isolates from Thailand, Laos, and Myanmar. *Antimicrobial Agents and Chemotherapy* 2008; 52(7), 2657–2659. <https://doi.org/10.1128/AAC.01459-07> PMID: 18443118

36. Cornejo EO, Fisher D, and Escalante AA. Genome-Wide Patterns of Genetic Polymorphism and Signatures of Selection in *Plasmodium vivax*. *Genome Biol Evol* 2015; 7:106–119.
37. Menard D, Chan ER, Benedet C, Ratsimbaoa A, Kim S, Chim P, et al. Whole genome sequencing of field isolates reveals a common duplication of the Duffy binding protein gene in Malagasy *Plasmodium vivax* strains. *PLoS Negl Trop Dis* 2013; 7:e2489. <https://doi.org/10.1371/journal.pntd.0002489> PMID: 24278487
38. Ord R, Polley S, Tami A, Sutherland CJ. High sequence diversity and evidence of balancing selection in the *Pvmsp3α* gene of *Plasmodium vivax* in the Venezuelan Amazon. *Mol Biochem Parasitol* 2005; 144:86–93. <https://doi.org/10.1016/j.molbiopara.2005.08.005> PMID: 16159677
39. Triglia T, Cowman AF. Primary structure and expression of the dihydropteroate synthetase gene of *Plasmodium falciparum*. *Proc National Academy Sci* 1994; 91(15), 7149–7153.
40. Hawkins VN, Suzuki SM, Rungsihirunrat K, Hapuarachchi HC, Maestre A, Na-Bangchang K, Sibley CH. Assessment of the origins and spread of putative resistance-conferring mutations in *Plasmodium vivax* dihydropteroate synthase. *Am J Trop Med Hyg* 2009; 81(2), 348–355. PMID: 19635897
41. Imwong M, Sudimack D, Pukrittayakamee S, Osorio L, Carlton JM, Day NP, et al. Microsatellite variation, repeat array length, and population history of *Plasmodium vivax*. *Mol Biol Evol* 2006; 23(5):1016–8. <https://doi.org/10.1093/molbev/msj116> PMID: 16507919
42. Hawkins VN, Joshi H, Rungsihirunrat K, Na-Bangchang K, Sibley CH. Antifolates can have a role in the treatment of *Plasmodium vivax*. *Trends in Parasitology* 2007; 23(5), 213–222. <https://doi.org/10.1016/j.pt.2007.03.002> PMID: 17368986
43. Hawkins VN, Auliff A, Prajapati SK, Rungsihirunrat K, Hapuarachchi HC, Maestre A, et al. Multiple origins of resistance-conferring mutations in *Plasmodium vivax* dihydrofolate reductase. *Malaria J* 2008; 7, 72. <https://doi.org/10.1186/1475-2875-7-72>
44. Saralamba N, Nakeesathit S, Mayxay M, Newton PN, Osorio L, Kim JR, et al. Geographic distribution of amino acid mutations in DHFR and DHPS in *Plasmodium vivax* isolates from Lao PDR, India and Colombia. *Malaria J* 2016; 15(1), 484.
45. Rungsihirunrat K, Na-Bangchang K, Hawkins VN, Mungthin M, Sibley CH. Sensitivity to antifolates and genetic analysis of *Plasmodium vivax* isolates from Thailand. *Am J Trop. Med Hyg* 2007; 76(6), 1057–1065. PMID: 17556611
46. Lu F, Lim CS, Nam DH, Kim K, Lin K, Kim TS. Mutations in the antifolate-resistance-associated genes dihydrofolate reductase and dihydropteroate synthase in *Plasmodium vivax* isolates from malaria-endemic countries. *Am J Trop Med Hyg* 2010; 83.
47. Tachibana M, Suwanabun N, Kaneko O, Iriko H, Otsuki H, Sattabongkot J, et al. *Plasmodium vivax* gametocyte proteins, Pvs48/45 and Pvs47, induce transmission-reducing antibodies by DNA immunization. *Vaccine* 2015; 33(16), 1901–1908. <https://doi.org/10.1016/j.vaccine.2015.03.008> PMID: 25765968
48. Vallejo AF, Martinez NL, Tobon A, Alger J, Lacerda MV, Kajava AV, et al. Global genetic diversity of the *Plasmodium vivax* transmission-blocking vaccine candidate Pvs48/45. *Malaria J* 2016; 15, 202.
49. Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, et al. The dominant anopheles vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps and bionomic précis. *Parasit Vectors* 2011; 4:89. <https://doi.org/10.1186/1756-3305-4-89> PMID: 21612587
50. Molina-Cruz A, Garver LS, Alabaster A, Bangiolo L, Haile A, Winikor J, et al. The human malaria parasite Pfs47 gene mediates evasion of the mosquito immune system. *Science* 2013; 340:984–7. <https://doi.org/10.1126/science.1235264> PMID: 23661646
51. Baniecki ML, Faust AL, Schaffner SF, Park DJ, Galinsky K, Daniels RF et al. Development of a single nucleotide polymorphism barcode to genotype *Plasmodium vivax* infections. *PLoS Negl Trop Dis* 2015; 9:e0003539. <https://doi.org/10.1371/journal.pntd.0003539> PMID: 25781890
52. Pain A, Böhme U, Berry AE, Mungall K, Finn RD, Jackson AP, et al. The genome of the simian and human malaria parasite *Plasmodium knowlesi*. *Nature* 2008; 455:799–803. <https://doi.org/10.1038/nature07306> PMID: 18843368
53. Hostetler JB, Lo E, Kanjee U, Amaratunga C, Suon S, Sreng S, et al. Independent Origin and Global Distribution of Distinct *Plasmodium vivax* Duffy Binding Protein Gene Duplications. *PLOS Neglected Tropical Diseases* 2016; 10(10), e0005091. <https://doi.org/10.1371/journal.pntd.0005091> PMID: 27798646



DSPACE

<https://dspace.org/>

Genomic variation in Plasmodium vivax malaria reveals regions under selective pressure

Diez Benavente, Ernest; Ward, Zoe; Chan, Wilson; Mohareb, Fady R.; Sutherland, Colin J. ...

2017-05-11

Public Library of Science (PLoS)

<https://doi.org/10.1371/journal.pone.0177134>

Diez Benavente E, Ward Z, Chan W, et al., (2017) Genomic variation in Plasmodium vivax malaria reveals regions under selective pressure. PLoS ONE, Volume 12, Issue 5, May 2017, Article number e0177134