Special Issue Article

Uncovering the transmission dynamics of *Plasmodium vivax* using population genetics

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Population genetic analysis of malaria parasites has the power to reveal key insights into malaria epidemiology and transmission dynamics with the potential to deliver tools to support control and elimination efforts. Analyses of parasite genetic diversity have suggested that *Plasmodium vivax* populations are more genetically diverse and less structured than those of *Plasmodium falciparum* indicating that *P. vivax* may be a more ancient parasite of humans and/or less susceptible to population bottlenecks, as well as more efficient at disseminating its genes. These population genetic insights into *P. vivax* transmission dynamics provide an explanation for its relative resilience to control efforts. Here, we describe current knowledge on *P. vivax* population genetic structure, its relevance to understanding transmission patterns and relapse and how this information can inform malaria control and elimination programmes.

Keywords: Malaria, *Plasmodium vivax*, Transmission, Relapse, Genetics, Genomics, Genetic diversity, Population structure, Linkage disequilibrium, Microsatellites, Single nucleotide polymorphisms, Mitochondrial DNA, Control, Elimination

Introduction

Plasmodium falciparum and *Plasmodium vivax* are responsible for the majority of the malaria burden worldwide. *P. vivax* impacts human populations most significantly in regions outside sub-Saharan Africa; however, the two species are sympatric in many regions. Over the past 15 years, the intensification of malaria control and elimination programmes has halved the burden of malaria globally.[1] In regions where the two species co-exist, control efforts have led to a shift towards *P. vivax* predominance, while *P. falciparum* has been successfully controlled.[2,3] The greater difficulty in controlling *P. vivax* suggests that this parasite has a greater capacity to sustain transmission, even in the context of intensive control efforts.

Since the advancement of sensitive molecular diagnostics, it has been recognised that a significant proportion of the *P. vivax* burden is composed of low-density, asymptomatic infections that remain undetected by standard epidemiological tools (reviewed by Ref. [4]) and that these proportions may increase with decreasing transmission.[5] Genotyping approaches that distinguish between different parasite clones within an infection to estimate the multiplicity of infection (MOI) have provided some insight into the intensity of transmission in different endemic areas. However, proportions of polyclonal infections remain relatively high despite seemingly low transmission.[6,7] Molecular diagnosis and genotyping have thus revealed that the *P. vivax* reservoir is far larger, more silent and more genetically complex than previously thought. To gain a deeper understanding into the complex patterns of transmission over space and time (i.e. transmission dynamics) and in order to provide findings useful to control and elimination programmes, it will be important to define parasite population structure by quantifying genetic relationships among the different parasite clones and populations.[8,9]

The recognition that *P. vivax* has distinct patterns of population genetic structure compared to *P. falciparum* globally[10] and in areas of similar endemicity[11,12] is consistent with higher rates of genetic exchange between *P. vivax* parasites and populations. A major driver of the patterns observed may therefore be the hypnozoite and its capacity to cause relapsing infections several weeks and even months after the primary infection, resulting in a large proportion of infections containing multiple clones.[13] Polyclonal infections increase opportunities for the uptake of genetically distinct clones by the mosquito vector and for the generation of genetic diversity through meiotic recombination. The hypnozoite,

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a dormant and probably metabolically inert stage, may also allow the parasite to disseminate itself and its genetic diversity to distant locations by hitchhiking in the liver of migrating hosts. Within endemic regions, the proportion of new infections compared to those due to relapse will undoubtedly be important in shaping population structure. Molecular studies investigating parasite diversity, multi-locus linkage disequilibrium (LD, i.e. nonrandom associations between alleles at different loci), gene flow and population structure in a range of malaria endemic settings are therefore essential to describe the origins, transmission and spread of *P. vivax* malaria.

Impact of *Plasmodium vivax* Biology on Transmission Dynamics and Population Structure

Recent studies comparing the population genetics of *P. vivax* with *P. falciparum* suggest that its unique biological characteristics enhance its transmission potential[11,12] (Table 1) and may explain why this parasite can persist in co-endemic areas despite the near elimination of *P. falciparum*.[2,14] *P. vivax* is more genetically diverse and has less structured populations compared to *P. falciparum*.[10–12] At high transmission, new infections in addition to relapse would increase the rate of recombination between distinct clones and a breakdown in LD compared to that of *P. falciparum*, which does not relapse.[11] Furthermore, the carriage of clones as dormant hypnozoites in mobile human populations would increase rates of gene flow thus limiting population structure.[11] In the context of declining transmission, the livers of previously highly exposed individuals would harbour genetically diverse hypnozoites. Relapses and subsequent recombination between these clones would therefore maintain high diversity even in scenarios of temporarily low vector transmission and low infection rates. In regions of traditionally low transmission where *P. falciparum* diversity is low,[15] high levels of *P. vivax* diversity have been observed together with strong LD[9,12] suggesting that there may be an additional mechanism for generating and maintaining high diversity, such as a higher mutation rate.[12] At low transmission, the strong LD could be explained by relapse because in the absence of a high rate of new infections containing distinct clones, there would be frequent recombination between meiotic siblings (inbreeding) and the maintenance of multiple non-recombining lineages. Furthermore, the longer relapse rates in *P. vivax* strains from temperate versus the shorter relapse rates of strains from tropical climates[4] has likely contributed to the complex patterns observed. The hypnozoite is therefore likely to play a major role in shaping *P. vivax* population structure. However, several other unique features of *P. vivax* biology may also contribute and are summarised in Table 1. Gaining a better understanding of *P. vivax* population structure, alone and in contrast with co-endemic *P. falciparum*, will help define the contribution of this unique biology to *P. vivax* transmission.

Genetic Markers for Investigating *Plasmodium vivax* Population Structure

The *P. vivax* genome is haploid for much of the lifecycle, including in the blood stages from which

Table 1 ***P. vivax*** **characteristics that may increase transmission potential relative to** ***P. falciparum*** **and the putative effect on parasite population structure**

P. vivax characteristic	Transmission potential	Putative effect on population structure
Lower temperature range for mosquito-stage development	Increases geographic distribution and allows dissemination of strains across regions too cold for *P. falciparum* transmission	Widespread dispersion of alleles
Hypnozoite	Chronic blood stage infection and source of transmission-stages (gametocytes)	Maintenance of high diversity and less population structure with decreasing transmission. In the longer term, a lack of new infections would lead to inbreeding (linkage disequilibrium)
	More stable transmission	High diversity even at low transmission
	More distant dissemination of parasite strains	Break down of population structure
	Higher proportion of multiple strain (polyclonal) infections, facilitating recombination between distinct clones	Breakdown of linkage disequilibrium, generation of diversity
Lower density parasitaemia	Lower detectability, infections not treated	High diversity
Continuous production of gametocytes during an infection	Continuous transmission	High diversity
Appearance of gametocytes prior to clinical symptoms	Transmission before infections are treated	High diversity
Earlier development of host immunity	Greater proportion of population asymptomatic, infections not treated	High diversity

most field samples are obtained. This simplifies the establishment of haplotypes from independent loci for population genetics. There are several different classes of markers available for the investigation of *P. vivax* diversity and population structure. An important consideration in determining which markers to use is the number of alleles and the allele frequency distribution, which is strongly influenced by demographic changes and natural selection. Declining transmission due to natural fluctuations or driven by interventions, may lead to a loss of rare variants and thus a decrease in diversity and this reduces the power to distinguish between individual parasite clones. Pilot studies to assess individual marker profiles are therefore recommended before conducting large-scale genotyping studies.[8,9]

The requirements of markers differ for different epidemiological studies. For example, to measure the number of clones present in a sample (known as the MOI), the rate at which new infections are acquired (the Force of Infection, FOI)[16] or to differentiate between relapse and new infections in drug efficacy trials, highly polymorphic markers with many alleles at moderate frequencies, such as antigenic loci and extremely diverse microsatellites, are required. Less polymorphic and/or neutrally evolving loci such as microsatellites with a wide range of allele frequencies and single nucleotide polymorphisms (SNPs) are more susceptible to population size changes and genetic drift and are therefore well suited to measuring changes in the underlying population structure. In addition, the different classes of markers provide information about the population structure on different time scales, and therefore the choice of marker depends upon the resolution required to resolve key questions about parasite evolution and population structure.

Antigen loci

Rapidly evolving genes encoding the highly polymorphic surface antigens Merozoite Surface Protein 1 (MSP1),[17,18] Merozoite Surface Protein 3-alpha (MSP3α),[19] Merozoite Surface Protein 3 beta (MSP3β),[20,21] and the circumsporozite protein (CSP)[22] were developed as markers to measure MOI and parasite diversity, and to track individual clones in the course of natural infections within an individual. These genes contain both SNPs and tandem repeat copy number variations, resulting in size polymorphisms, such that alleles are discernable using either gel or capillary electrophoresis alone, or in combination with restriction enzyme digestion. As a result of their extremely high within population diversity, antigen markers paired with extremely polymorphic microsatellites have identified and tracked individual clones over time within individuals with a high degree of certainty.[23–25] As mentioned above, the high diversity and maintenance of balanced allele frequencies by immune selection makes antigen markers extremely useful for measuring MOI and FOI because they ensure the lowest probability of clones having the same allele, and thus being indistinguishable. On the other hand, the pattern of diversity in antigens limits the detection of divergence between populations; therefore, antigen markers should be used with caution if the aim is to measure underlying patterns of gene flow between populations.[26,27]

Microsatellites

More than 240 microsatellite markers have been developed and utilised as markers to estimate multiplicity of infection and/or to characterise the population structure of *P. vivax*. A comprehensive review of these markers and empirical evidence has been gathered to support their use in different applications including the determination of MOI, genetic diversity and population structure.[27] The meta-analysis of 42 microsatellites provides a detailed characterisation of known allele frequency distributions in different endemic areas and recommendations for their use in population genetic applications.[27]

Essentially, microsatellite markers are appropriate for different applications depending on the degree of polymorphism and mode of evolution.[27] Out of several different multi-locus panels previously developed as putatively neutral markers,[28–30] two panels and combinations thereof have been used in many different studies (reviewed below) for the characterisation of parasite population structure. The use of identical marker panels in different transmission settings facilitates direct comparison of results, as differences in diversity reflect differences in the underlying parasite population.

Single nucleotide polymorphisms

Single nucleotide polymorphisms have slower mutation rates than microsatellites and analysis methods are less prone to artefacts. Single nucleotide polymorphisms are therefore more desirable markers for population genetic analyses on broad geographical scales. SNPs on 100-kb segment of a single chromosome[31] proved useful to type samples from Asia and South America.[32,33] Genome wide SNPs are also being developed for *P. vivax* based on sequence data from at least five geographically disparate strains.[10] This topic is covered in more detail by a review included in this special issue.[34]

Mitochondrial DNA

The 6-kb mitochondrial genome (mtDNA) of *P. vivax* is another well-studied source of variation. Sequencing of mtDNA showed separated clusters of strains from Latin America[35] as well as highly diverse populations in Asia and the South Pacific.[6,36] Because mtDNA is uniparentally inherited, reconstructing connections

between individual parasite isolates is more straightforward than using loci from the nuclear genome, which recombines during sexual replication in the mosquito. It is also important to recognise that the mtDNA evolves much more slowly than microsatellites and nuclear SNPs and therefore diversification of mtDNA haplotypes has been mostly useful to dissect the global spread of malaria many thousands of years ago (see below).[35–37]

Genomics

The first draft *P. vivax* genome sequence, based on the *Sal-1* reference strain, was published in 2008[38] with the current version and annotation available via PlasmoDB.[39] More recently, a small number of whole genome sequences have been analysed and confirm the high diversity of this species.[10,40,41] Presently, approximately 10 high quality full genome *P. vivax* sequences are publically available, yet this number is expected to increase in the near future thus allowing population genomic studies to be conducted on whole genomes. In comparison to *P. falciparum*, *P. vivax* has approximately two-fold more SNPs and significantly higher microsatellite diversity.[10] The deeper population-level analyses soon to be published will provide more accurate estimates and a framework to identify population-specific signatures that may be utilised to track the origins and spread of infections at more recent timescales.

Emergence, Global Spread and Evolutionary History

P. vivax is a common cause of human malaria in tropical regions of Asia, the Pacific, South and Central America and some parts of East Africa, but is uncommon throughout Central Africa.[42] The reason for the lack of *P. vivax* transmission in Africa is the fixation of a mutation that prevents the expression of the Duffy antigen, the receptor for *P. vivax* on human erythrocytes.[43] The finding of *P. vivax*-like parasites in African apes and gorillas, and that mtDNA sequences of worldwide strains of *P. vivax* radiate from more diverse sequences of the non-human primate parasites suggests that *P. vivax* originated in Africa and selected for Duffy-negativity. Previous analyses suggested an Asian origin based on high mtDNA diversity in this region, but are likely to have been influenced by the lack of African parasites in the sample and by demographic processes such as population expansion, which increases diversity.[44] There have been some attempts to understand how *P. vivax* subsequently spread around the world using mtDNA[34–36,45]; however, the population structure is complex and requires further deep sampling of strains from all major endemic regions. Nevertheless, some important insights have been gained into the evolutionary history of *P. vivax* in different geographic regions. For example, in South America, there is low local diversity but high divergence consistent with multiple independent introductions[35] possibly from a European source with origins in Africa.[45] Whereas in the Asia Pacific, high local diversity is observed, consistent with ancient population expansion and complex migration patterns.[35,36,46] The reanalysis of new sequences from key geographic areas together with the available dataset of mtDNA sequences (currently more than 700 sequences) will undoubtedly shed further light on the complex past of *P. vivax*.

Global Patterns of Diversity and Population Structure

The endemicity of *P. vivax* is extremely variable worldwide, ranging from hypoendemic in parts of East Africa and South-East Asia, moderate in India and the fringes of the Amazon to hyperendemic in central parts of the Amazon and Papua New Guinea (PNG, parasite rates $>40\%$).[42] A large body of literature has used microsatellites to assess the diversity and structure of *P. vivax* populations from small-scale village level[7,47] to regional[48–50] and intercontinental scale.[29,51,52] Likewise, the dynamics of populations over time was studied using microsatellite genotyping.[9,53,54] Interestingly, while *P. falciparum* diversity is tightly associated with regional levels of endemicity, *P. vivax* harbours high genetic diversity across all endemicities (Fig. 1).[6,7,11,29,47,51,52,55] Most microsatellite studies have demonstrated large numbers of alleles (more than 10) in different *P. vivax* populations around the world (reviewed in Ref. [27]) and the same haplotype is rarely detected in different hosts.[48,50,54,55] Paradoxically, significant multi-locus LD has been observed in some moderate to high transmission settings,[52] which might be explained by serial co-transmission of meiotic recombinants, while in other moderate transmission settings in South-East Asia, no LD was observed.[51] The high diversity of the parasite population even in regions where transmission intensity has declined substantially reflects a large underlying parasite population.[6,49]

P. vivax has higher genetic diversity than *P. falciparum*[10–12] suggesting that it has deeper evolutionary roots and that past control efforts have had less impact on *P. vivax* parasite populations.[10,44,56] Indeed, persistent *P. vivax* transmission in regions where *P. falciparum* is near to or has been eliminated[2] suggests that *P. vivax* populations are not as susceptible as *P. falciparum* to demographic changes as prevalence decreases. The greater diversity of *P. vivax* may contribute to a greater potential for adaptation to different environments and vectors,[10] host immunity and the development of resistance to drugs and vaccines.[57,58] The high diversity also makes

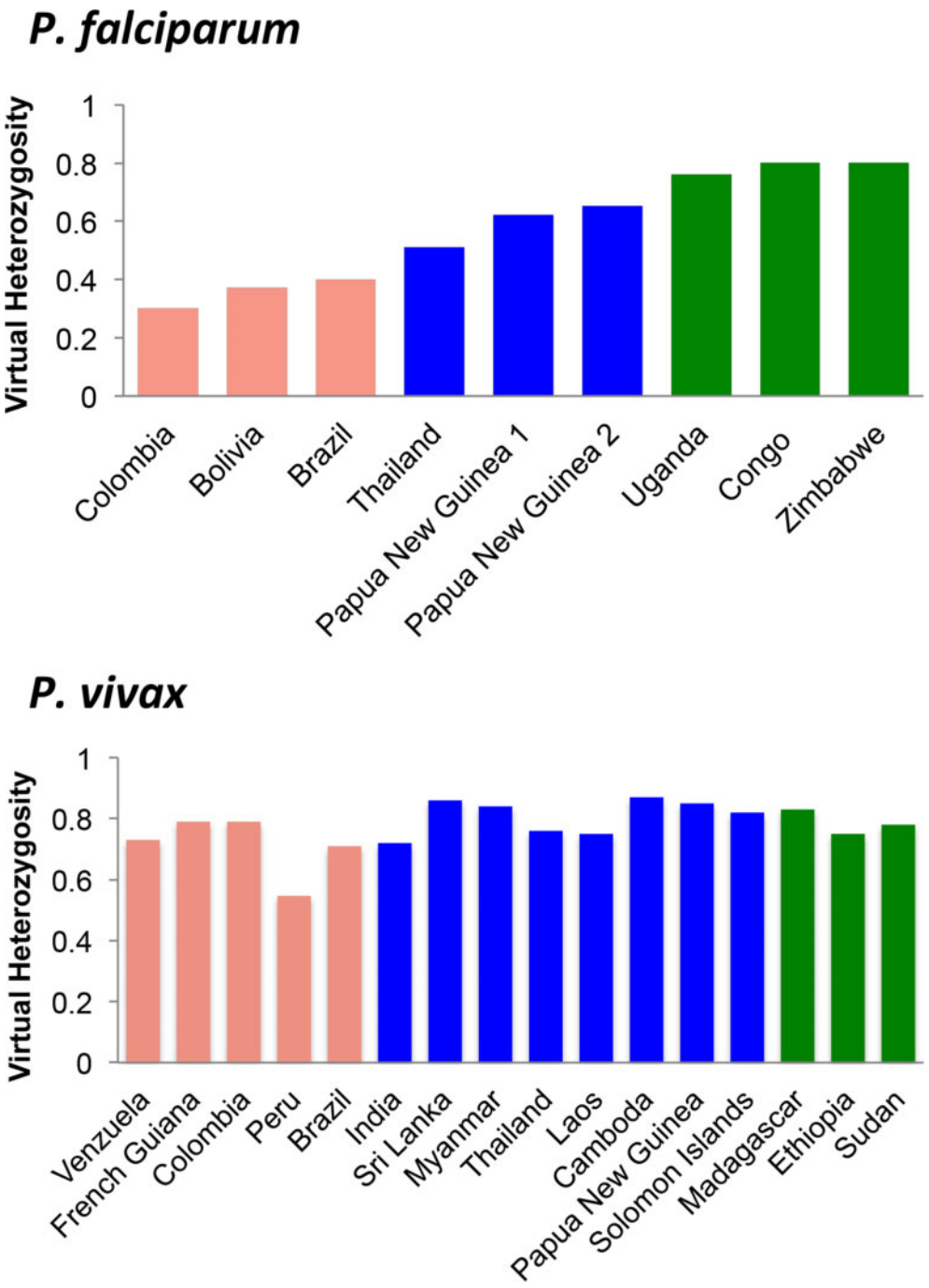

Figure 1 Comparative diversity of *Plasmodium falciparum* and *Plasmodium vivax* populations in a range of epidemiological settings around the world. The mean virtual heterozygosity (H_e) based on panels of microsatellite markers for the two species (obtained from published data) was plotted from low to high endemicity for *P. falciparum*. The data for *P. vivax* were plotted based on the analogous geographic origins, but is not ordered by endemicity. Note that the highest endemicity for *P. vivax* occurs in Papua New Guinea and Brazil and lowest in Sri Lanka and African countries[41] but diversity was similar across these locations. Colours indicate continent of origin: pink, Americas; blue, Asia Pacific; green, Africa. Data adapted from Ref. [6,9,12,15,29,47,48,51,52,55,62].

inferring population structure more challenging, as a higher number of isolates might be required to detect subtle population differentiation. Nevertheless, based on microsatellites, clear geographic population structure between sites from different countries has been observed.[51,52]

In analyses of microsatellite haplotypes, isolates from Latin America were clearly separated from all other populations, and the differentiation between parasites from different locations within Colombia,[51] Brazil[55] and the Peruvian Amazon[47] was as high as that between South American and Asian parasites.[51] As mentioned above, high levels of population structure among South American *P. falciparum* populations have been proposed to be the result of multiple independent introductions from Africa.[15,59] In contrast to *P. vivax*, *P. falciparum* has shown near-clonal population expansion since its near elimination in

the 1960s.[60] It is not known whether the pattern of *P. vivax* population structure in Latin America is due to populations introduced from multiple independent regions (e.g. Europe and South-East Asia), adaptation to locally available vectors or other unknown factors.

In the South Pacific, geographic population differentiation was low despite a distance of up to 1500 km and open sea between sampling locations in PNG and Solomon Islands.[11,48,50] In the highlands of PNG, transmission is lower and mostly restricted to local outbreaks, yet *P. vivax* isolates collected during outbreaks still display high diversity, while co-endemic *P. falciparum* shows a clonal population structure suggesting that low level endemic transmission continues to occur in these regions.[61]

In South-East Asia over a billion people are at risk of *P. vivax* infection.[42] Transmission levels range from intense to moderate and focal. Here, diversity was very high, with higher numbers of alleles found than on other continents[51,52] Population structure between South-East Asian countries and regions is moderate[51,52] yet higher than in the South Pacific indicating the existence of some barriers to gene flow.

Limited information on *P. vivax* structure is available from Africa. In Madagascar and the Horn of Africa (Ethiopia and Sudan), transmission is stable and consequently high parasite diversity was observed.[52,62] The relationship of *P. vivax* in Sub-Saharan Africa to populations in Madagascar, East Africa and elsewhere is not known. Likewise, it is unknown whether a single clone, able to infect Duffy-negative individuals has recently spread through Africa, or whether in the past *P. vivax* in Africa has been misdiagnosed as other species.

Local Patterns of Genetic Diversity and Population Structure

Even within highly endemic areas, parasite populations are not evenly distributed. Heterogeneity in the microepidemiology of malaria is related to overall regional levels of transmission,[15,47,55] host factors[63,64] and environmental factors such as biogeography, topography[65] and climate.[66] From an operational point of view, malaria control is carried out on a subnational level (e.g. district or province) and therefore more fine-scale population genetic surveys than those described above are needed to identify local patterns of transmission and guide the implementation of targeted, more efficient interventions. Such surveys should ideally include multiple geographically and epidemiologically distinct populations to gain insight into the variables influencing population structure within a region.

By examining parasite genetic diversity in multiple sites within endemic regions, it is theoretically possible to map the movement of strains among catchment areas, villages and even households and this could help identify drivers of heterogeneity at different spatial scales. A few recent studies have begun to explore genetic diversity and population structure relevant to control programmes by surveying large numbers of samples from multiple sites within a region. In PNG, prior to the widespread distribution of insecticide treated bednets, extremely high levels of genetic diversity (relative to other worldwide populations) were observed despite one area having only half the prevalence of infections. This indicates that the difference in prevalence was not significant enough to impact the parasite population diversity.[67] Furthermore, the lack of population structure in these regions was consistent with high levels of gene flow and a very large effective population size.[3,48] Since these initial surveys however, routine surveillance by the control programme has indicated a 50% reduction in prevalence (I. Mueller, unpublished data). It will be interesting to assess whether this has impacted the parasite population by comparing the population structure at different time points.

Evidence of reduced diversity and limited gene flow between local areas indicates that control efforts have been successful in reducing the spread of infections among regions. In Malaysian Borneo, *P. vivax* infections were predominantly monoclonal, but population diversity was high indicating that substantial outcrossing continues to occur.[49] However, this diversity was lower and population structure more evident than in other *P. vivax* populations in the Asia Pacific[48,51,52] suggesting that intensified control efforts have begun to interrupt transmission and exert pressure on the parasite population. In Peru, four sites clustered around Iquitos (within 10 km of each other) had moderate levels of diversity but parasite populations were strongly differentiated from each other.[47] Indeed, most South American isolates show clear population structure on a small scale with different clusters detected even in neighbouring villages.[9,47] Nevertheless, when South American isolates collected over several years from the same villages were analysed, a complex pattern of predominance of different clusters at different time points and replacement of haplotypes was observed indicating a highly dynamic parasite population.[9,55,68] In Temotu, a Solomon Islands province targetted for malaria elimination, *P. vivax* diversity remained high and there was low to moderate genetic differentiation between islands. This suggests relatively free gene flow or alternatively, insufficient time for genetic drift to impact allele frequencies since their isolation.[50] In Vanuatu, where malaria is hypoendemic, very low diversity was observed in an outbreak on Aneytium after several years of undetected *P. vivax*, suggesting cases were the result of an introduced epidemic.

In contrast, there was high diversity for other neighbouring islands, consistent with ongoing endemic transmission.[14] Although *P. vivax* genetic diversity is not as susceptible to changes in transmission as that for *P. falciparum*, differences in the patterns of population structure exist and could inform control programmes. However, the relationship between local population structure and transmission in different epidemiological settings and with decreasing transmission needs to be better defined.

Using Population Genetics to Understand Relapses

One of the most fascinating aspects of *P. vivax* biology is its ability to remain dormant in the liver of infected individuals for weeks, months or even years. These dormant forms can reactivate to cause new blood stage infection when environmental conditions are optimal for the parasite life cycle.[69] Relapses significantly contribute to the burden of disease. In PNG children, it is estimated that relapse is responsible for up to 80% of blood stage infections.[70] In French Guiana, approximately 45.5% of new cases of *P. vivax* malaria were estimated to be relapses on the basis of their occurrence within 90 days of the primary infection.[71]

Genetic analysis of sequential infections is essential to study *P. vivax* where a new blood infection can either be caused by a relapse or a new infection and can help understand several aspects of hypnozoite biology including: (i) the patterns and triggers of relapses, (ii) the assessment of anti-hypnozoite drug efficacy and (iii) ultimately how to improve malaria control measures. However, it is challenging to distinguish relapses from new infections. Exposure to *P. vivax* infections leads to the formation of a pool of heterologous hypnozoites in the liver.[72] Individuals with limited history of exposure such as returning soldiers,[73] travellers[22] and Thai infants[74] are likely to present homologous relapses. Fully homologous recurrent infections were observed in eight patients in Borneo, where transmission had been reduced substantially.[49] On the other hand, individuals with history of multiple exposures are likely to present genetically distinct relapses.[23,54,72] While these results may be confounded by inbreeding and the resolution of the markers used (see below), the relatedness of relapses to primary infection appears to be dependent on previous exposure, since under higher transmission, the liver might already contain distinct parasite clones from a previous mosquito inoculum.

Consecutive infections resulting from highly related sibling clones present in relapsed infections results in difficulties in interpreting genotyping results when using a limited number of markers[74] with the degree of difficulty inversely associated with previous exposure. Recently, a study characterised consecutive relapses by deep genome wide sequencing in a Canadian *P. vivax* patient returning from Sudan who presented with a first episode and subsequently suffered two additional relapses. Despite microsatellite genotyping suggesting that all infections were clonal, the deep sequencing approach found that relapses shared large fragments of identical sequence consistent with the primary infection and relapses being the products of the same meiotic recombination.[75] New molecular approaches such as high-resolution SNP genotyping and genome wide sequencing will bring a higher level of resolution to study relapses.

Dynamics of Population Structure with Declining Transmission

As malaria transmission decreases, infections become more infrequent and clustered within residual pockets of transmission thought to sustain local transmission and serve as a source of transmission to other areas.[76] As discussed above, reductions in local malaria transmission have also been shown to lead to a reduction in diversity and an increase in LD.[49,55] In areas where malaria transmission has been interrupted, substantial population structure can be observed.[49]

With a decrease in transmission, strong LD can be advantageous for the adaptive evolution of the parasite population. For example, when mutations at more than one locus are needed to acquire resistance against a drug, limited outcrossing helps maintain the resistant haplotype.[77] As has been observed for *P. falciparum*, very low transmission, leading to an increase in multi-locus LD between previously unlinked loci might provide the perfect conditions for emergence of drug resistance due to the maintenance of resistance mutations in linked loci.[78] However, the high diversity of *P. vivax* populations even at low transmission[6,51,52,55] may make this parasite less susceptible to these influences, providing one possible explanation as to why *P. vivax* has been slower to develop resistance to antimalarials.

Population Genetics of Re-emerging *Plasmodium vivax*

The high diversity observed at very low transmission may be the result of importation from diverse parasite populations.[6,79] The relationship between *P. vivax* transmission and genetic diversity is ill defined, especially at very low transmission and this needs further exploration. Multiple independent sources of reintroduction provide the potential for genetic recombination between genetically distinct clones and unless reintroductions are identified early, this may

complicate the distinction between local and imported parasites. In elimination settings, early and thorough investigation of suspected imported infections will be necessary to identify the source and provide a baseline upon which to classify cases in the future. Studies in South Korea, which monitored the re-emergent parasite population, suggests that from a small pool of imported parasites, *P. vivax* can establish a high degree of genetic diversity demonstrating that it can rapidly gain a foothold after reintroduction.[80]

Public Health Implications

When implementing malaria control programmes, countries seek to understand whether interventions are making an impact, how much more effort will be needed and how to best utilise limited malaria control resources. In areas where malaria control has succeeded in reducing prevalence to very low levels, reductions in diversity, increasing LD and increasing spatial clustering of infections may indicate that control efforts are interrupting transmission and impacting populations,[81] signalling that a shift to a targeted approach might be more effective than broad-ranging interventions.[76] In addition, this would indicate a low risk of reintroductions after subnational elimination and restricted spread of infections carrying advantageous traits, such as drug resistance, between different areas. In endemic areas, where the overall aim of programmes is to control malaria, the measurement of population structure can define transmission networks and clustering patterns, providing assess whether targeted malaria control may be considered. The great challenge will be in defining the accuracy of these predictions and their spatial resolution, both of which have very important consequences on the utility of this information for control programmes. Furthermore, it is not clear whether interventions should be targeted to the highest transmission areas or fragmented populations in lower transmission areas that would be most vulnerable to elimination. Pilot studies are therefore needed in a region with heterogeneous transmission and comprising a variety of population structures to test the impact of different approaches.

In pre-elimination settings, the classification of clinical cases into local or imported origin is essential in order to quantify the contribution of imported infections to overall transmission[82,83] and thus assesses the feasibility of elimination and direct interventions to the right location.[84] Genetic relatedness gives important insights about transmission patterns over space and time, which traditional indicators such as case incidence and travel history fail to convey.[50] This is particularly relevant to tracking the origin of *P. vivax* infections since dormant parasites can be carried for long periods of time. Genotyping analyses conducted on a broad scale using informative markers could generate a database of population-specific genotypes or 'geographic markers', to identify the origin of imported cases.[50,85,86] Primarily, control programmes will need adequate surveillance to rapidly identify cases for investigation and the capacity to mount a response. In addition, the length of time and the advanced skills needed to conduct genetic analyses is a barrier that needs to be overcome before their implementation in a public health setting.

Gaps in Knowledge and Future Trends

The transmission dynamics of *P. vivax* remain an area of malaria epidemiology with many knowledge gaps that if addressed, will help better understand the biology of *P. vivax* and its contribution to the transmission and spread of the disease, as well as provide critical information for planning malaria control programmes. Specifically, it will be important to gain a better understanding of:

(1) The relationship between transmission and population genetic parameters in the context of declining malaria transmission.
(2) How diversity in *P. vivax* is generated and maintained, especially at low transmission.
(3) The relationship between population structure and the emergence and spread of drug-resistant strains.
(4) How to track the origins of *P. vivax* infections and rapidly classify infections on the basis of genetic information.

Data from a large sequencing effort led by the Broad Institute and New York University and another led by the Sanger Institute are now publically available and offer the potential to reveal important insights into *P. vivax* evolution and population biology. Research addressing these issues, including modelling approaches to dissect the complex patterns observed, will shed light on the clinical and epidemiological relevance of *P. vivax* diversity and population structure and help develop more effective strategies to tackle this malaria parasite.

Key Points

- Available molecular tools for *P. vivax* population genetics; however, more robust, high-resolution markers may be required for use in malaria control and elimination programmes and to understand the biology of relapse.
- *P. vivax* has more genetically diverse and less structured populations than *P. falciparum*, indicating that it has more stable effective population sizes and providing an explanation for its relative resilience against antimalarial interventions.

- *P. vivax* populations are diverse even at low transmission.
- *P. vivax* can rapidly gain a foothold after re-emerging.
- Population genetics is essential to address key knowledge gaps about the transmission of *P. vivax*.

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