MAJOR ARTICLE

Population Structure and Transmission Dynamics of *Plasmodium vivax* in Rural Amazonia

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Understanding the genetic structure of malaria parasites is essential to predict how fast some phenotypes of interest originate and spread in populations. In the present study, we used highly polymorphic microsatellite markers to analyze 74 *Plasmodium vivax* isolates, which we collected in cross-sectional and longitudinal surveys performed in an area of low malaria endemicity in Brazilian Amazonia, and to explore the transmission dynamics of genetically diverse haplotypes or strains. *P. vivax* populations are more diverse and more frequently comprise multiple-clone infections than do sympatric *Plasmodium falciparum* isolates, but these features paradoxically coexist with high levels of inbreeding, leading to significant multilocus linkage disequilibrium. Moreover, the high rates of microsatellite haplotype replacement that we found during 15 months of follow-up most likely do not result from strong diversifying selection. We conclude that the small-area genetic diversity in *P. vivax* populations under low-level transmission is not severely constrained by the low rates of effective meiotic recombination, with clear public health implications.

Plasmodium vivax is associated with 70–80 million clinical cases of malaria reported each year [1], with 2.6 billion people at risk for infection worldwide [2]. Epidemiological trends in Brazil illustrate the importance of *P. vivax* as a reemerging pathogen: the annual incidence of infection with *Plasmodium falciparum* (the predominant malaria parasite species between 1985 and 1990) decreased steadily during the 1990s, whereas that of infection with *P. vivax* maintained an upward trend. *P. vivax* now causes 80% of cases of malaria reported in Brazil [3].

Understanding the genetic structure of malaria parasites is essential to predict how fast phenotypes of interest, such as novel antigenic variants or drug resistance, originate and spread in populations [4]. The extensive polymorphism in antigen-coding loci [5] provides little information on the population structure of *P. vivax,* because it reflects the combined effects of population history and natural selection [6]. The current markers of choice for population genetic studies of eukaryotes are neutrally evolving, short (1–6-bp) tandem repeats known as microsatellites [7], which helped to elucidate the genetic structure and evolutionary history of *P. falciparum* [8, 9]. Although short arrays of microsatellite repeats appear to exhibit little variation in worldwide *P. vivax* populations [10], long arrays of highly polymorphic dinucleotide microsatellites have been described as promising tools for population-level studies of this species [11, 12].

Here, we use newly standardized tri- and tetranucleotide microsatellites [13], which usually yield more-accurate allele scoring than do dinucleotide markers [14], to analyze the population structure of *P. vivax*

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from rural Amazonia. We compare our findings with microsatellite diversity data for a sympatric *P. falciparum* population and explore the transmission dynamics of genetically diverse *P. vivax* haplotypes over 15 months of follow-up. The longitudinal nature of this study enabled us to examine the relative contribution of epidemic and endemic haplotypes to the overall genetic diversity observed in local *P. vivax* populations.

POPULATIONS, MATERIALS, AND METHODS

Study areas and parasite isolates. The 74 *P. vivax* isolates evaluated in the present study were collected in the state of Acre, northwestern Brazil (figure 1). The first sample set ($n = 25$) was collected in July 1999 from febrile patients presenting at malaria clinics in the towns of Plácido de Castro (10°20′ S, 67°11′ W; $n = 11$), Acrelândia (9°43′ S, 66°53′ W; $n = 6$), and Xapuri (10°39′ S, 68°30′ W; $n = 3$), as well as in the city of Rio Branco (9°58′ S, 67°48′ W; $n = 5$), the capital of Acre. *P. vivax* infection was diagnosed by thick-smear microscopy [15] and was confirmed by polymerase chain reaction (PCR), by use of a modification [16] of the method described by Kimura et al. [17]. *P. falciparum* isolates were simultaneously collected during cross-sectional surveys conducted in Plácido de Castro ($n = 20$) and Rio Branco ($n = 14$) [18] and were analyzed with 10 polymorphic trinucleotide microsatellites [19], making between-species comparisons possible.

The second sample set ($n = 49$) was obtained during the first 15 months of follow-up (March 2004 through May 2005) of an ongoing, population-based cohort study in Ramal do Granada (9°41′ S to 9°49′ S, 67°05′ W to 67°07′ W), in the eastern part of the state of Acre. In the study area, which is situated 30–50 km northwest of Acrelândia (figure 1), *P. falciparum* and *P. vivax* are transmitted year-round. Cohort recruitment strategies and baseline data are reported elsewhere [20]. In brief, the 114 households identified by a census performed by our field team were visited, and 466 household dwellers of all age groups (98.5% of the residents in the area) were enrolled between March and April 2004; 43 additional subjects (most of whom were newcomers to the area) were enrolled between April and October 2004. These 509 subjects contributed follow-up data (average follow-up, 11.5 months; range, 0.3–14.5 months). Active case detection of symptomatic

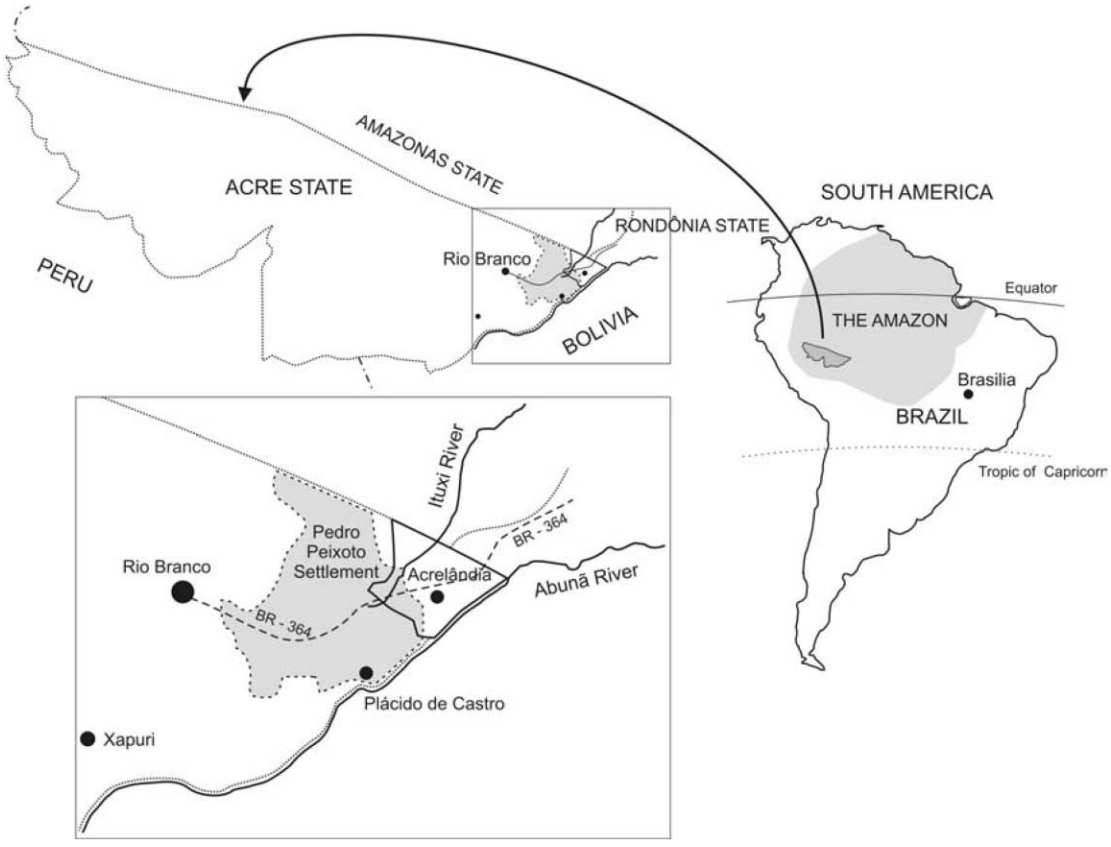

Figure 1. Map of the state of Acre, northwestern Brazil, showing the 4 sites surveyed in 1999 (Plácido de Castro, Acrelândia, Xapuri, and Rio Branco) and the site of the cohort study of 2004–2005 (Ramal do Granada). The most closely located sites, Acrelândia and Plácido de Castro, are 35 km apart, whereas the 2 most distant sites, Acrelândia and Xapuri, are 258 km apart. Ramal do Granada is part of the Pedro Peixoto Agricultural Settlement *(shaded area in the inset),* located 30–50 km northwest of the town of Acrelândia.

malaria started in March 2004 and resulted in the identification of 168 incident episodes of *P. vivax* infection (14 episodes during the first visit to each household and 154 episodes thereafter) and 85 incident episodes of *P. falciparum* infection (7 episodes during the first visit and 78 episodes thereafter). During the first 15 months of follow-up, the average rates of incidence of *P. vivax* and *P. falciparum* malaria were 2.86 cases/100 person-months at risk (95% confidence interval [CI], 2.46–3.31 cases/100 person-months at risk) and 1.44 cases/100 person-months at risk (95% CI, 1.16–1.77 cases/100 person-months at risk), respectively. *P. vivax* infections were treated with chloroquine and primaquine, whereas *P. falciparum* infections were treated with mefloquine or quinine plus doxycycline [21]. Pretreatment blood samples were obtained from 49 subjects (29.2%) with *P. vivax* infections diagnosed during follow-up (2 of the infections were diagnosed by PCR only [16]) (figure 2); 6 subjects contributed >1 blood sample collected during consecutive malaria episodes. Isolates recovered from asymptomatic parasite carriers were not analyzed, because parasite loads were very low (most infections were detected only by PCR), and because sampling was limited to the cross-sectional surveys of the whole population that were conducted at 6-month intervals.

From each subject, a 5-mL sample of venous blood was collected for parasite DNA typing, after written, informed consent was provided. DNA templates for PCR amplification, isolated from 200 μL of whole blood by use of GFX genomic blood DNA purification kits (Amersham Pharmacia Biotech), were dissolved in 100 μL of sterile distilled water and were stored at −20°C. The study protocol was approved by the Human Subjects Committee of the Harvard School of Public Health (2005) and by the ethics review board of the Institute of Biomedical Sciences, University of São Paulo (2002).

Typing* P. vivax *microsatellites. Fourteen highly polymorphic microsatellites (MS1, MS2, MS3, MS4, MS5, MS6, MS7, MS8, MS9, MS10, MS12, MS15, MS16, and MS20) were analyzed [13]. MS2 has tetranucleotide repeat arrays; all other markers consist of tandem repeats of trinucleotide motifs. These markers could not be assigned to individual chromosomes, on the basis of the publicly available *P. vivax* genome draft [22, 23], but most of them were situated in different contigs (relatively long segments of contiguous DNA sequence assembled during genome analysis). The markers in the same contig are (1) MS4 and MS5 (0.15 Mb apart in contig 423), (2) MS7 and MS8 (1.14 Mb apart in contig 428), and (3) MS12 and MS15 (1.04 Mb apart in contig 397). Ten markers (MS3, MS4, MS5, MS6, MS7, MS8, MS10, MS15, MS16, and MS20) are located in sequences coding for hypothetical proteins, whereas 2 markers (MS9 and MS12) are located in predicted introns of hypothetical protein-coding genes [13].

The 14 markers were PCR amplified exactly as described elsewhere [13]; fluorescent dyes (6-FAM, VIC, NED, and PET) were used to label forward primers. Length variation of labeled PCR products was measured using an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems) with the use of ABI GS500 LIZ internal size standards and GeneScan and Genotyper software (Applied Biosystems).

Data analysis. The single or predominant allele at each locus was counted to calculate allele frequencies [8]. The finding of $\geqslant$1 additional alleles was interpreted as a coinfection with $\geqslant$2 genetically distinct clones in the same isolate (i.e., multiple-clone infection) [14], because all markers are single-copy loci [13], and because blood-stage malaria parasites are haploid. We scored additional alleles giving peaks in electropherogram traces that were at least one-third the height of the predominant allele (the one giving the highest peak), but only the predominant allele was used to define haplotypes (unique combinations of alleles at each locus) in mixed-clone infections [14].

The within-population diversity was examined by calculating the virtual heterozygosity (H_E) of each locus, defined as $H_E = [n/(n-1)][1-\Sigma p_i^2]$, where n is the number of isolates analyzed and p_i is the frequency of the ith allele in the population. H_E (range, 0–1) estimates the average probability of scoring different alleles, at a given locus, in any pair of haplotypes randomly drawn from the population. The genetic relatedness among haplotypes was examined by calculating pairwise proportions of shared alleles between haplotypes [8] and by applying the eBURST algorithm [24] to identify clusters of closely related haplotypes. eBURST applies a simple evolutionary model that assumes that the founding haplotype propagates in the population and diversifies to generate a cluster of closely related haplotypes. Here, we sought to identify clusters of hap-

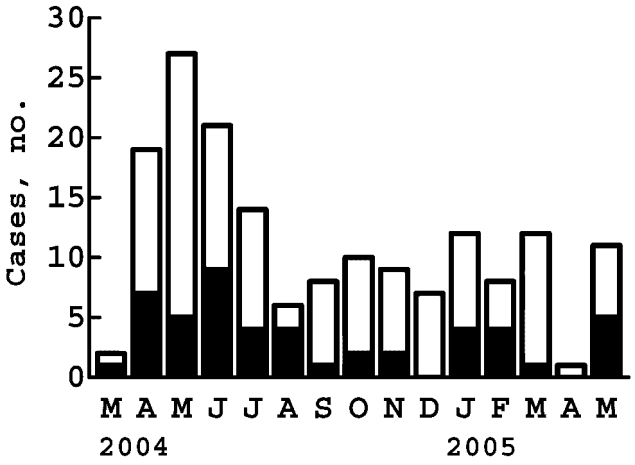

Figure 2. Monthly distribution of incident symptomatic *Plasmodium vivax* infections (n = 168) diagnosed in the cohort of 509 subjects followed between March 2004 (denoted by the first "M" on the *X*-axis) and May 2005 (denoted by the last "M" on the *X*-axis) in rural Amazonia. Black shading denotes the no. of isolates that were typed with 14 polymorphic microsatellites (n = 49); they represent 29.2% of all infections observed in the cohort.

Table 1. Proportions of mixed-clone infections, mean number of microsatellite alleles per locus, genetic diversity (virtual heterozygosity [H_E]), and linkage disequilibrium (standardized index of association [I_A^S]) in malaria parasite populations from Acre, Brazil.

Year, parasite population (no. of isolates analyzed)	Multiple-clone infection, %	Alleles per locus, mean ± SE (range)	H_E, mean ± SE (range)	I_A^S,[a] by infection type: All	I_A^S,[a] by infection type: Those with unique haplotypes
1999					
Plasmodium vivax ($n = 25$)	48.0	7.2 ± 0.7 (3–12)	0.80 ± 0.03 (0.54–0.92)	0.101	0.069[b]
Plasmodium falciparum ($n = 34$)[c]	17.6	4.4 ± 0.7 (2–9)	0.51 ± 0.07 (0.06–0.79)	0.113	0.124[d]
2004–2005, *P. vivax* ($n = 49$)	49.0	6.9 ± 0.8 (3–14)	0.71 ± 0.04 (0.34–0.88)	0.202	0.119[e]

[a] I_A^S significantly larger than zero ($P = .001$), denoting significant multilocus linkage disequilibrium.
[b] $n = 23$.
[c] Data from Machado et al. [19] are based on 10 trinucleotide microsatellites.
[d] $n = 29$.
[e] $n = 39$.

lotypes that are identical to each other at 13 of the 14 loci analyzed, using eBURST (version 3) [25]. To test whether the proportion of alleles shared by haplotypes decreased with increasing distances between dates of isolate collection, we used PopTools (version 2.7.1) [26], to run a Mantel matrix correlation test with 10,000 permutations.

A standardized index of association (I_A^S) was used to test for overall multilocus linkage disequilibrium in each parasite population. This test compares the variance (V_D) of the number of alleles shared between all pairs of haplotypes observed in the population (D) with the variance expected under random association of alleles (V_E) as follows: $I_A^S = (V_D/V_E - 1)(r - 1)$, where r is the number of loci analyzed [27]. V_E is derived from 10,000 simulated data sets in which alleles were randomly reshuffled among haplotypes. Significant linkage disequilibrium is detected if V_D is >95% of the values derived from the reshuffled data sets. Separate analyses considered all haplotypes or only unique haplotypes (i.e., haplotypes found in >1 isolate were only counted once in the analysis), to remove the effect of epidemic expansion of particular haplotypes on linkage disequilibrium in randomly mating populations [28]. Data were analyzed with LIAN software (version 3.1) [29, 30].

To test for linkage disequilibrium between every pair of markers, observed data were arranged in contingency tables, and Markov chains were used to explore all contingency tables obtained with 10,000 simulated data sets in which alleles were randomly reshuffled among haplotypes. Significant linkage disequilibrium is defined if the level of association detected in a given contingency table, containing real data, is found in <5% of the contingency tables derived from the reshuffled data sets. Arlequin software (version 3.01) [31], was used in this analysis.

RESULTS

***Microsatellite diversity and linkage disequilibrium in* P. vivax.** Comparable levels of genetic diversity and similar proportions of mixed-clone infections were found in *P. vivax* samples collected in 1999 (at 4 sites; $n = 25$) and in 2004–2005 (at 1 site; $n = 49$) (table 1). Twenty-three unique haplotypes were found in 1999 (2 haplotypes shared by 2 isolates each), and 39 were found in 2004–2005 (4 haplotypes shared by ⩾2 isolates). Interestingly, shared haplotypes in 1999 involved isolates collected in different towns (Acrelândia and Plácido de Castro, and Rio Branco and Xapuri), suggesting a relatively intense gene flow among study sites. No haplotype was shared by parasites collected in 1999 and in 2004–2005; in fact, parasites collected 5–6 years apart shared no more than 6 of 14 alleles. The average proportion (± SD) of alleles shared between haplotypes recovered at different time points (i.e., 1999 and 2004–2005) was 0.175 ± 0.04.

The linkage disequilibrium found in 1999 and in 2004–2005 remained significant when each haplotype was counted only once (table 1), indicating that recent epidemic expansions of particular strains in these populations [28] do not account for these results. We next sought to examine whether physical linkage between markers located in the same contig (MS4–MS5, MS7–MS8, and MS12–MS15) might have biased our results. First, we recalculated the overall association index (I_A^S) after excluding from the data set 1 member of each pair of such markers (MS4, MS7, and MS12 were excluded). Significant linkage disequilibrium ($P = .001$) was still detected in both data sets ($I_A^S = 0.1085$ for all haplotypes and $I_A^S = 0.0665$ for unique haplotypes in 1999; $I_A^S = 0.1827$ for all haplotypes and $I_A^S = 0.0997$ for unique haplotypes in 2004–2005). Next, we examined, in both populations, patterns of linkage disequilibrium between every pair of markers. Although MS4–MS5 and MS7–MS8 were in linkage disequilibrium in both populations, MS12–MS15 is one of the few pairs for which linkage disequilibrium was not found in the 2004–2005 sample (figure 3). Because significant linkage disequilibrium was often observed between markers in different contigs, especially in the 2004–2005 sample, we conclude that inbreeding, instead of physical linkage between particular markers, is the most likely

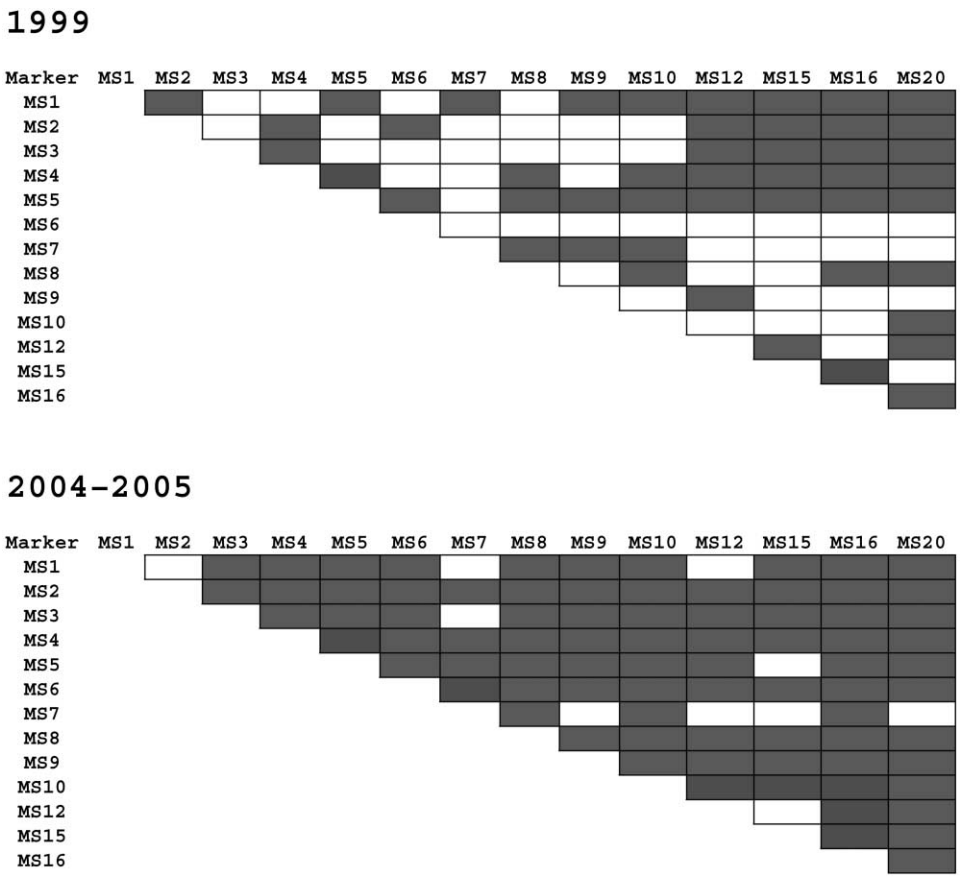

Figure 3. Patterns of linkage disequilibrium between pairs of microsatellite markers in 2 *Plasmodium vivax* populations from rural Amazonia. Gray shading denotes significant linkage disequilibrium, at the 5% level. Data for parasites collected in 1999 ($n = 25$) *(top)* and in 2004–2005 ($n = 49$) *(bottom)* are shown separately.

explanation for the overall linkage disequilibrium observed. Alternatively, natural selection might have affected, at least partially, the patterns of linkage disequilibrium observed (see Discussion).

***Comparing sympatric isolates of* P. vivax *and* P. falciparum.** Table 1 also provides estimates of microsatellite diversity and linkage disequilibrium for *P. falciparum* isolates ($n = 34$) collected in 1999 [19]. *Plasmodium vivax* and *P. falciparum* circulating essentially in the same areas of Acre in July 1999 differ according to the prevalence of mixed-clone infections (48.0% vs. 17.6%) and the overall levels of genetic diversity (average H_E, 0.80 vs. 0.51). Nevertheless, both species exhibit significant linkage disequilibrium that cannot be attributed to recent epidemic expansions of some haplotypes in this area of relatively low malaria transmission.

Exploring the transmission dynamics of* P. vivax *haplotypes. We next examined the propagation and persistence of microsatellite haplotypes in the 2004–2005 population. Given the high levels of inbreeding inferred for this population, one could expect that some clonal haplotypes would persist unchanged for several generations [32]. Relapses might represent an additional factor promoting the persistence of haplotypes in *P. vivax* populations [33]. However, haplotype 19, found at the beginning and the end of the follow-up (figure 4, *top*), was the only clear example of haplotype persistence in 2004–2005. The absence of haplotypes shared by the 1999 and 2004–2005 populations provides further support for a high turnover rate of haplotypes in this area.

Genetic drift, migration, and variant-specific acquired immunity in the host population are all factors that could lead to the high rate of haplotype replacement observed in our cohort [34]. Alternatively, one can hypothesize that parasite lineages might rapidly mutate and recombine, while propagating in populations, generating groups of closely related, but not identical, multilocus haplotypes. This hypothesis is strengthened by the high nonmeiotic recombination rates usually seen in microsatellite-type sequences [7]. In fact, eBURST analysis identified 2 clusters of such haplotypes in the 2004–2005 population (figure 4, *bottom*). Cluster A comprises haplotype 38 (found in 19 April 2004) and its single-locus variants (haplotypes that differ from haplotype 38 at a single locus) subsequently found in the population: haplotypes 32, 34, 36

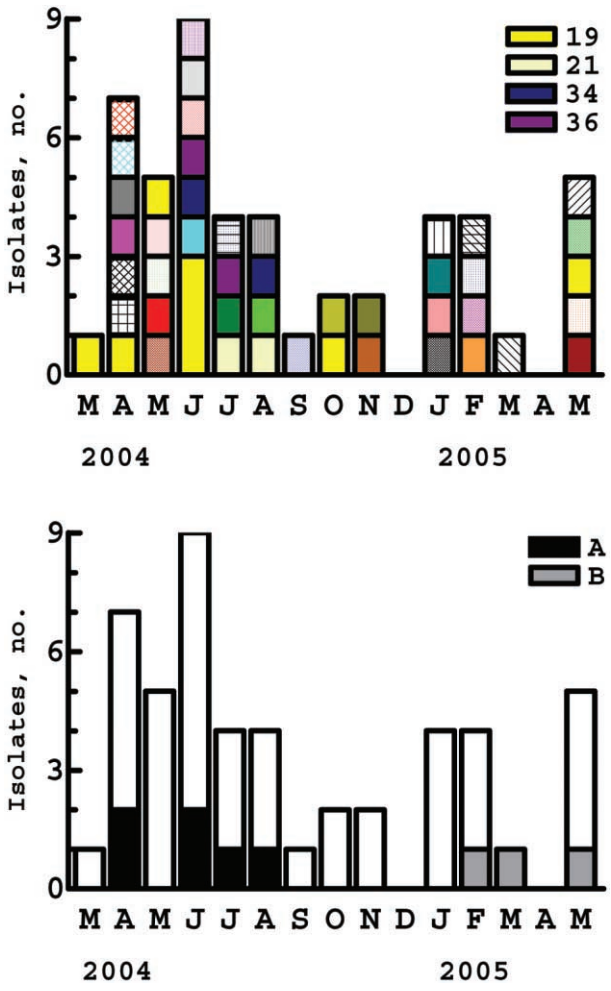

Figure 4. Transmission dynamics of genetically diverse *Plasmodium vivax* microsatellite haplotypes in rural Amazonia. *Top,* Monthly distribution of *P. vivax* haplotypes found in 49 symptomatic infections diagnosed in the cohort between March 2004 (denoted by the first "M" on the *X*-axis) and May 2005 (denoted by the last "M" on the *X*-axis). Haplotypes are defined as unique combinations of alleles at each of the 14 loci analyzed. The 39 unique haplotypes found in this cohort are represented by different color and shading patterns; haplotypes shared by ⩾2 isolates (haplotypes 19, 21, 34, and 36) are identified in the key. *Bottom,* Monthly distribution of haplotypes belonging to 2 clusters (clusters A and B) of closely related haplotypes. These clusters, identified by eBURST analysis [25], contain haplotypes that are identical to ⩾1 member of the same group at 13 of the 14 loci analyzed.

(shared by 2 isolates), and 37. This cluster includes 6 of 29 isolates typed between April and August 2004. Cluster B comprises haplotype 62 and its single-locus variants, haplotypes 42 and 43, all found between February and May 2005, corresponding to 3 of 10 isolates typed during this interval. In conclusion, we were able to detect short-term, but not long-term, propagation of closely related haplotypes during the 15-month follow-up. The significant negative correlation between the proportion of pairwise allele sharing and the temporal distance between dates of collection of the respective isolates ($r = -0.082$; $P = .02$) might be interpreted as further supporting the model of gradual haplotype diversification over time, because of mutations and recombination. Nevertheless, we suggest that this weak correlation should not be overinterpreted.

We next examined the role of relapses in maintaining particular haplotypes in the population, under the assumption that relapsing parasites are genetically identical to those found in the primary infection [35, 36]. Of the 4 haplotypes recovered from ⩾2 isolates (figure 4, *top*), haplotype 21 was found in 2 unrelated subjects who had samples obtained 12 days apart (July and August 2004), and haplotype 36 was found in 2 unrelated subjects who had samples obtained 23 days apart (June and July 2004). Haplotype 34, however, was recovered during consecutive infections of the same subject (subject 316) (figure 5), as would be expected for parasites causing relapses of infection. Haplotype 19, the most prevalent haplotype, was recovered from 8 infections (figure 4, *top*); 2 infections were consecutive infections in the same subject (subject 42) (figure 5), again suggesting a relapse. Three subjects carrying haplotype 19 between May and June 2004 had prior episodes of *P. vivax* infection confirmed by microscopy ~2 months previously. This temporal pattern is consistent with relapses, but parasites from putative primary infections are not available for typing, because infections predated enrollment of the subjects. No evidence for relapse is available for the 3 remaining infections with haplotype 19 (detected in April and October 2004 and in May 2005) (figure 4, *top*, and figure 5). Because the evidence that relapsing parasites are similar to those in the primary infection has been derived from a low number of cases analyzed with low-resolution genotyping technology [35, 36], it is possible that some relapses involve parasites of different haplotypes. Therefore, we found some evidence that relapses can favor the short-term persistence of some haplotypes in natural *P. vivax* populations.

DISCUSSION

In the present study, we showed that *P. vivax* isolates obtained during cross-sectional and longitudinal surveys in rural Amazonia display extensive genetic diversity and frequent multiple-clone infections coexisting with strong multilocus linkage disequilibrium. Moreover, with the single exception of a multilocus haplotype recovered from isolates collected 15 months apart, we found no evidence for long-term propagation of clonal lineages of parasites. No comparable longitudinal analysis of microsatellite variation is available for sympatric *P. falciparum* populations.

The high rate of turnover of antigenic serotypes of *P. falciparum,* observed over 24 months in a small village in Papua New Guinea, has been interpreted as an example of immune-mediated frequency-dependent selection [37]. This situation is

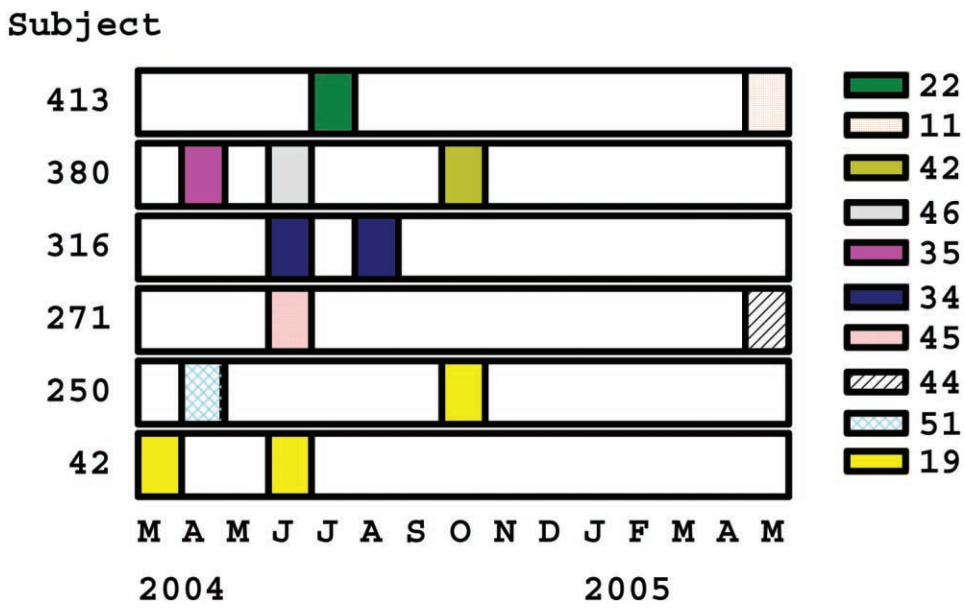

Figure 5. Haplotypes in consecutive *Plasmodium vivax* infections diagnosed in the same subjects in rural Amazonia. The same color and shading patterns are used to represent haplotypes in figure 4, *top,* and figure 5. Note that subjects 42 and 316 had parasites with an identical haplotype recovered at different time points, a pattern consistent with relapsing infections.

reminiscent of that described for *Streptococcus pneumoniae* serotypes in countries where multivalent pneumococcal vaccines are widely used [38]. The high rates of microsatellite haplotype replacement in our longitudinal analysis of *P. vivax* suggest that strong natural selection on antigenic determinants is not necessarily the only explanation for such dramatic variations over time. Although variant-specific immunity may determine temporal fluctuations [34] and the probability of persistence of particular serotypes in malaria parasite populations [39], here we provide an example of how neutral mechanisms, such as random genetic drift, migration [40], and frequent nonmeiotic recombination in microsatellite repeat arrays [7], may account for epidemiologic patterns that are usually interpreted as resulting from natural selection. The first 2 mechanisms are more effective in relatively small parasite populations [40], such as those from areas of low malaria endemicity.

Although linkage disequilibrium had not been previously reported in natural populations of *P. vivax,* this finding is not surprising. *P. falciparum* populations from areas of low transmission, such as the Amazon Basin, often display low effective recombination rates and significant linkage disequilibrium [8, 19, 41]. *P. falciparum* from such areas, however, exhibits low genetic diversity and infrequent multiple-clone infections (14%–31% of all *P. falciparum* infections), as detected by multilocus microsatellite typing [8, 19]. The predominance of inbreeding in *P. vivax* populations with high proportions of multiple-clone infections is somewhat puzzling, because genetically unrelated gametocytes are likely to co-occur in the same mosquito blood meal, favoring outbreeding during the meiotic reproduction of parasites in the vector.

One possible explanation for this puzzle is that the microsatellite markers used might not be strictly neutral, because 10 of 14 markers map to loci encoding either hypothetical or annotated proteins that may be under purifying or diversifying selection. Although none of them map to known surface antigens putatively under strong immune-mediated diversifying selection, we cannot assume that they are free of any selective constraints; functional constraints, for example, might affect proteins where a stretch of amino acids can vary in length between parasite genotypes. This same limitation applies to some (5 of 12) microsatellite markers standardized for use in epidemiological studies of *P. falciparum* [14], which map to hypothetical or annotated proteins and cannot be considered to be strictly neutral. Therefore, natural selection may have affected, at least in part, the analysis of linkage disequilibrium patterns in populations of both species of malaria parasites.

Between-species comparisons must be interpreted with caution. In addition, microsatellite typing may have underestimated the prevalence of multiple-clone *P. falciparum* infections in Brazil [8, 19], because ~40% of local isolates harbor ⩾2 variants of a single-copy antigen-coding gene [18, 42], and because, to date, virtually all local isolates cloned by limiting dilution comprise genetically heterogeneous lineages [43]. We conclude that multiple-clone infections are probably highly prevalent among *P. falciparum* and *P. vivax* populations cocir-

culating in the Amazon Basin, a finding that is at odds with the high levels of inbreeding estimated for both species.

The finding of the same multilocus microsatellite haplotype in *P. falciparum* isolates collected in Bolivia 4 years apart [8] implies the persistence of clonal lineages over several generations [32]. Perhaps even more surprisingly, identical alleles of genes encoding surface antigens, which are under strong diversifying selection, are observed among *P. falciparum* isolates collected in the same areas in Brazil nearly 10 years apart [44, 45]. We found no example of such a long-term persistence of *P. vivax* microsatellite haplotypes in Brazil, although relapses could theoretically maintain clonal lineages in the population. Differences in effective population sizes and mutation and migration rates [40] and acquired variant-specific immunity in the host population [34] are potential explanations for contrasting haplotype replacement rates in populations of different human malaria parasites. Again, differences in the resolution of typing strategies based on either microsatellites or antigen-coding genes may affect the validity of such comparisons.

The extensive diversity and short persistence of haplotypes in our cohort are findings with clear implications for malaria control. Microsatellite typing may also be applied to the differentiation between relapses of *P. vivax* infection and new infections [35, 36] and genomewide searches for loci underlying drug resistance and other phenotypes of interest [46].

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