INTRODUCTION

Malaria caused by Plasmodium vivax is often regarded as a benign and self-limiting infection. However, there is increasing evidence that the overall burden, economic impact, and the severity of disease caused by P. vivax have been underestimated. The current knowledge on genetic diversity and population structure of P. vivax lags behind that of P. falciparum, mostly because of lack of appropriate genetic markers for the P. vivax genome and its inability to be propagated continuously in the laboratory except in non-human primates. Sequencing of the P. vivax genome was completed recently and provides a valuable resource adding stimulus to the much-needed study of this neglected species.

Analysis of allelic variation at multiple independent loci provides an effective means to determine population structure. Microsatellites have been estimated to mutate at high rates, and the mechanism of mutation appears to be strand slippage at replication leading to either a lengthening or shortening of the DNA. This variation in length is assumed to give rise to new electrophoretically distinguishable alleles. Thus, genetic diversity and evolutionary history inferred from microsatellite data are based on similarity of electrophoretic mobility, rather than by descent.

The distinct pathologic and epidemiologic nature of P. vivax malaria is caused by several biological characteristics of P. vivax, which makes it differ from P. falciparum. P. vivax forms hypnozoite stages in the liver, which may persist for months or years before initiating relapses (secondary infections), which provide a mechanism for the parasite to hibernate during less optimal transmission periods. Differences in Anopheles mosquito dynamics, which provides P. vivax with a broader temperature tolerance, enables transmission even in temperate climates not tolerated by P. falciparum. Relapses and broader temperature tolerance could be expected to increase gene flow between different geographic regions. However, the distinctive phenotypic features found in parasite populations from different countries suggest barriers to gene flow, or alternatively, strong selection maintaining differentiation at loci underlying these traits.

The ability of P. vivax malaria to re-emerge in regions where malaria eradication or control efforts in the past have been successful is already evident in countries such as Uzbekistan, Azerbaijan, the Republic of Korea and northern Afghanistan. Because of increased international travel and migration, the risk of re-introduction of P. vivax in areas where species of Anopheles with greater vectorial capacity are present is high. The analysis of the parasites genetic make-up would be useful in identifying the geographic origins of these parasites and facilitate meaningful control and preventive measures to be implemented.

In this study, we analyzed the genetic diversity and population structure of 425 P. vivax patient isolates collected in Sri Lanka (2003–2008), Myanmar (2007), and Ethiopia (2006–2008) using 12 highly polymorphic trinucleotide and tetranucleotide microsatellite markers, which have been validated and tested. We considered the geographic and temporal influences on the genetic diversity and population structure of these P. vivax patient isolates. We further evaluated the use of these microsatellite haplotypes as a model for predicting the ancestry of P. vivax isolates using the Bayesian algorithm software STRUCTURE.

MATERIALS AND METHODS

Field isolates. Plasmodium vivax microsatellite typing was conducted on 425 field isolates collected in Sri Lanka (140), Myanmar (167), and Ethiopia (118). A field isolate is defined in this study as a sample of parasites derived from a...
single-infected patient collected on a single occasion. Most patients had fever and were diagnosed as having *P. vivax* malaria by microscopic examination of Giemsa-stained thick and thin blood smears. Transmission levels of malaria differed in the three countries, with the incidence rates in 2006 ranging between 50 and 200, 5 and 49, and 0 and 4 per thousand population in Ethiopia, Myanmar, and Sri Lanka respectively.  

Malaria transmission has decreased steadily in Sri Lanka since 2001 from 55,922 cases to 189 cases of *P. vivax* malaria in 2007 (Annual Report Anti-Malaria Campaign, Ministry of Health, Sri Lanka). Malaria is endemic or hypoendemic in Myanmar. Although morbidity and mortality rates for malaria have decreased gradually in Myanmar, malaria still contributes to a large proportion of deaths in the southeast Asia region.  

Malaria transmission in Ethiopia is unstable and seasonal with occasional devastating epidemics.  

The 140 field isolates of *P. vivax* from Sri Lanka were collected from patients attending medical health clinics in Trincomalee, Batticaloa, Ampara (Eastern Province), Anuradhapura, Polonnaruwa (Northcentral Province), Kurunegala (Northwestern Province), Vavuniya (Northeastern Province), and Colombo. Venous blood samples were collected from these patients in 2003 (SL47, n = 01), 2004 (SLA1–SLH4, n = 38), 2005 (SLA5, SLA7–SLP2E1, n = 61), 2006 (SLP2A2–SLP2H3, n = 18), 2007 (SL24–SL44, n = 21), and 2008 (SL46, n = 01). The 21 isolates collected during 2007 were from an outbreak of malaria that occurred in Trincomalee.  

All 167 field isolates of *P. vivax* from Myanmar were collected between July and November 2007 from patients attending medical health clinics at Kayin State, which is situated along the Myanmar–Thailand border (MYY3–MYY229, n = 96), Kachin State (MY KC19–MYKC1478, n = 56), and Rakhine State, along the Myanmar-Bangladesh border (MYY148–MYY1264, n = 15). Capillary blood from a fingertip was collected from each patient on a filter paper.  

The 118 *P. vivax* field isolates from Assendabo, Ethiopia, were collected from fever patients attending health clinics during 2006 (E1–E61, n = 59), 2007 (E84V–E2400V, n = 52), and 2008 (E434–E488V, n = 07). Capillary blood from a fingertip was collected from each patient on a filter paper.  

Either a venous or capillary blood sample was collected from each infected patient after informed consent was obtained. Filter paper samples from Ethiopia were de-linked from patient identifiers and provided as discarded samples from the clinic. DNA templates for polymerase chain reaction (PCR) amplification were isolated from venous blood by using the Nucleon genomic DNA extraction kit (Tepnel Life Sciences, Manchester, United Kingdom) and from filter papers by using either the fast methanol-based protocol or the QIAamp DNA purification Mini kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Extracted DNA was dissolved in sterile distilled water and stored at −20°C. Because of limited availability of genomic DNA in these samples, the extracted DNA was further subjected to whole genome amplification (WGA) using a REPLI-g Mini-kit (Qiagen). The study protocol was reviewed and approved by the Human Subjects Committee of the Harvard School of Public Health (#P10299-111/0209GENE) and the Ethical Review Committee of the Faculty of Medicine, University of Colombo (EC/08/092).  

**Microsatellite typing of *P. vivax***. Twelve validated highly polymorphic microsatellite markers (MS1, MS2, MS3, MS4, MS5, MS7, MS8, MS10, MS12, MS15, MS16, and MS20) were used for typing all isolates. Apart from MS2, which has a tetrancleotide repeat array, all other markers consist of trinucleotide tandem repeat motifs. These 12 markers are situated on different chromosomes (Table 1). The 12 markers were PCR-amplified using oligonucleotide primers with the forward primer labeled with fluorescent dyes (6-FAM, VIC, NED, and PET) as shown in Table 1. The PCRs with all primer pairs (6.4 pmol working solution of each) were performed on a PTC-200 thermo cycler (MJ Research, Waltham, MA) with 1.5 µL of genomic DNA template, 1 unit of Platinum Taq polymerase, 0.7 µL of 50 mM MgCl₂, 2.0 µL of 10× reaction buffer, and 1 mM of each dNTP (all supplied by Invitrogen, Carlsbad, CA) in a final reaction volume of 20 µL. Cycling parameters were the same for all primer pairs: 1 cycle at 94°C for 2 minutes; 40 cycles at 94°C for 30 seconds, 58°C for 40 seconds, and 72°C for 30 seconds; and a final cycle at 72°C for 5 minutes. Length variation of labeled PCR products was measured on an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) by using ABI GS500LIZ internal size standards and the GENESCAN, GENOTYPER, and GENEMAPPER software (Applied Biosystems).  

**Data analysis.** The single or predominant allele at each locus was considered for computing allele frequencies. All 12 markers are single-copy loci. Because blood-stage malaria parasites are haploid, the presence of one or more additional alleles at a particular locus was interpreted as a co-infection with two or more genetically distinct clones (multiple-clone infections) in the same isolate. An additional allele was scored if the peak was at least one-third the height of the predominant allele (highest peak) as shown by the electropherogram traces, a method that has been validated. Only the predominant allele was used to define haplotypes in multiple-clone infections.  

The genetic diversity of the parasite populations from Sri Lanka, Myanmar, and Ethiopia was determined by calculating the virtual heterozygosity (Hv) at each locus in each separate population. Virtual heterozygosity is the average probability that a pair of alleles randomly obtained from the population is different and was defined as Hv = n[(n−1)](1−Σp²), with n being the number of isolates analyzed and pi the frequency of the ith allele in the population. Virtual heterozygosity ranges between 0 and 1, with values close to 1 reflecting high genetic diversity levels in a population. The eBURST version 3 software was used to search for nearly identical multilocus haplotypes (those differing by a single locus).  

A standardized index of association (Ia) was used to test for evidence of overall multilocus linkage disequilibrium in each parasite population from Sri Lanka, Myanmar, and Ethiopia. This test compares the variance (V_{ij}) 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_{ij}) and is defined as Ia = (V_{ij}/N_i)(r−1), with r being the number of loci analyzed. V_{ij} is derived from 10,000 simulated data sets in which alleles were randomly reshuffled among haplotypes. Significant linkage disequilibrium is detected if V_{ij} is > 95% of the values derived from the reshuffled data sets. Data was analyzed using LIAN software version 3.5.  

The Bayesian clustering model to assign isolates to K populations according to allele frequencies at each locus was applied using STRUCTURE 2.2 software to test whether microsatellite haplotypes clustered according to the geographic
Table 1
Characterization of the 12 polymorphic *Plasmodium vivax* microsatellite loci (n = 425)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosome</th>
<th>Core repeat sequence in the Salvador-1 strain</th>
<th>Primers (5'-3')</th>
<th>Size range, basepairs</th>
<th>No. alleles</th>
<th>Hs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS1</td>
<td>3 (CM000444)</td>
<td>(GAA)$_{11}$</td>
<td>F:6-FAMTCACA CTGTTGGAAGGGCAAT R:ctgtctt TGGGGAAGAGCTCCTTTCTC</td>
<td>201–249</td>
<td>16 17 13</td>
<td>0.8997 0.9022 0.7692</td>
</tr>
<tr>
<td>MS2</td>
<td>6 (CM000447)</td>
<td>(TAAA)$<em>{2}$TATA(TAAA)$</em>{6}$TATA (TAAA)$_{10}$</td>
<td>F:VICGAGCTAGCCAAGGTTTCAACA R:ctgtctt TGGGGAAGAGCTCCTTTCTC</td>
<td>182–286</td>
<td>16 21 13</td>
<td>0.8514 0.8929 0.8014</td>
</tr>
<tr>
<td>MS3</td>
<td>4 (CM000445)</td>
<td>(GAA)$_{11}$</td>
<td>F:NEDGAAGATCTCTTGGAGGAGCA R:ctgtctt CTCCCTCGCTCCTTTCTT</td>
<td>152–200</td>
<td>15 16 14</td>
<td>0.8835 0.8445 0.8617</td>
</tr>
<tr>
<td>MS4</td>
<td>6 (CM000447)</td>
<td>(AGT)$_{18}$</td>
<td>F:PETGATTTATGTCTGGAAGCTGAA R:ctgtctt TGGGGAGGAGAGCCTTG</td>
<td>171–228</td>
<td>11 18 14</td>
<td>0.7848 0.8116 0.7850</td>
</tr>
<tr>
<td>MS5</td>
<td>6 (CM000447)</td>
<td>CCTCTT(CCT)$_{11}$</td>
<td>F:VICGAGCTAGCCAAGGTTTCAACA R:ctgtctt AAGAGGAGAGAGCTAACCA</td>
<td>139–232</td>
<td>17 16 6</td>
<td>0.8068 0.7933 0.6577</td>
</tr>
<tr>
<td>MS7</td>
<td>12 (CM000453)</td>
<td>(GAA)$_{8}$</td>
<td>F:VICGAGCTAGCCAAGGTTTCAACA R:ctgtctt CTCCCTCGCTCCTTTCTT</td>
<td>124–178</td>
<td>17 17 9</td>
<td>0.8526 0.9004 0.6523</td>
</tr>
<tr>
<td>MS8</td>
<td>12 (CM000453)</td>
<td>(CAG)$<em>{1}$ (CAG)$</em>{1}$</td>
<td>F:VICGAGCTAGCCAAGGTTTCAACA R:ctgtctt CTCCCTCGCTCCTTTCTT</td>
<td>165–345</td>
<td>42 44 37</td>
<td>0.9701 0.9666 0.9534</td>
</tr>
<tr>
<td>MS10</td>
<td>13 (CM000454)</td>
<td>GAA(GGA)$<em>{2}$,AGA(GGA)$</em>{2}$AGA(GGA)$<em>{2}$ GAGA(GGA)$</em>{2}$ GAGA(GGA)$_{2}$</td>
<td>F:PETTTATCCCTGCTGGATTGAA R:ctgtctt TTCCCTGAGGGACTGTGGT</td>
<td>174–249</td>
<td>19 23 18</td>
<td>0.8973 0.9095 0.9035</td>
</tr>
<tr>
<td>MS12</td>
<td>5 (CM000446)</td>
<td>(TTC)$<em>{10}$ (TGC)$</em>{2}$</td>
<td>F:FAMAATGGGATTCATGCTTGCTTGCTTGC</td>
<td>191–263</td>
<td>20 24 19</td>
<td>0.9305 0.9397 0.8983</td>
</tr>
<tr>
<td>MS15</td>
<td>5 (CM000446)</td>
<td>(TCT)$_{10}$</td>
<td>F:FAMAATGGGATTCATGCTTGCTTGCTTGC</td>
<td>236–263</td>
<td>10 9 8</td>
<td>0.8502 0.8570 0.7536</td>
</tr>
<tr>
<td>MS16</td>
<td>9 (CM000450)</td>
<td>(ACA)$<em>{1}$,GCA(ACA)$</em>{1}$,GCA(ACA)$<em>{1}$,GCA(ACA)$</em>{1}$,ACC(ACA)$<em>{1}$,ACC(ACA)$</em>{1}$</td>
<td>F:PETTTATCCCTGCTGGATTGAA R:ctgtctt TTCCCTGAGGGACTGTGGT</td>
<td>132–330</td>
<td>21 10 3</td>
<td>0.6893 0.3682 0.1995</td>
</tr>
<tr>
<td>MS20</td>
<td>10 (CM000451)</td>
<td>(GAA)$<em>{11}$,GAG(GAA)$</em>{11}$,GAA(GAA)$_{11}$</td>
<td>F:VICGAGCTAGCCAAGGTTTCAACA R:ctgtctt GTGCGATGGCTCATCTTCT</td>
<td>150–246</td>
<td>31 30 20</td>
<td>0.9164 0.9540 0.7843</td>
</tr>
</tbody>
</table>
RESULTS

Microsatellite diversity in natural \textit{P. vivax} isolates from Sri Lanka. All parasite isolates from Sri Lanka were highly polymorphic and showed unique haplotypes, with the number of alleles per locus ranging from 10 to 42 (mean = 19.6; Table 1 and Supplemental Tables 1 and 2, available at www.ajtmh.org). No single haplotype was shared between isolates. Adjacent haplotypes (differing at a single locus) were seen between two pairs of isolates from Sri Lanka (SL32-SL40 and SL28-34), which were from one location in Sri Lanka (Trincomalee) collected during an outbreak in 2007. The average genetic distance (1 − proportion of microsatellite alleles shared by pairs of haplotypes) of isolates from Sri Lanka collected between the 2007 outbreak in Trincomalee (outbreak isolates = 0.7845) was significantly lower than that calculated for pairwise comparisons involving outbreak isolates and the rest of the isolates collected from Sri Lanka (non-outbreak isolates = 0.854; \( P < 0.0001 \), by two-sample randomization test performed with Poptools 2.5 software).32 No identical haplotypes occurred in either outbreak or non-outbreak isolates.3.4\% (\( N = 57 \)) alleles were private (present only in the Sri Lankan population) and not shared between populations. 55.0\% (\( N = 77 \)) of isolates were mixed-clone infections.

The virtual heterozygosity (\( H_v \)) values for the entire parasite population from Sri Lanka ranged from 0.6893 to 0.9701 (mean \( = 0.8610 \pm 0.0216 \); Table 1). High diversity values were present even when considered according to the year of collection of the isolates (Table 2). Significant multilocus linkage disequilibrium was found in the isolates (\( n = 140 \)) from Sri Lanka (\( F_{st} = 0.0236, P < 0.001 \); Table 2). \( F_{st} \) was also calculated separately for the isolates considering their time of collection, and significant linkage disequilibrium was seen for subpopulations from 2004, 2005, and 2007: \( F_{st} = 0.0120, 0.0275, \) and \( 0.2709 \), respectively; \( P < 0.05 \). The association was extremely high in the 2007 subpopulation (all collected from a single site) in comparison to the other subpopulations. Further analysis of \( F_{st} \) conducted according to geographic location of isolates and times of collection showed significant associations at individual sites: Batticaloa (2005, \( n = 17; F_{st} = 0.0669 \)), Trincomalee (2007, \( n = 21; F_{st} = 0.2709 \)), and Ampara (2005, \( n = 4; F_{st} = 0.2104 \)); the \( F_{st} \) in the 2005 collection from Trincomalee (\( n = 20; F_{st} = 0.0127 \)) was not significant (Table 2).

Microsatellite diversity in natural \textit{P. vivax} isolates from Myanmar. The parasite isolates from Myanmar were also highly polymorphic, with the number of alleles per locus ranging from 9 to 44 (mean = 20.4; Table 1 and Supplemental Tables 1 and 2). All isolates showed unique haplotypes with 5.6\% (\( n = 112 \)) of the alleles being private to Myanmar. A total of 73.7\% (\( n = 112 \)) were mixed-clone infections. The virtual heterozygosity (\( H_v \)) values for the parasite population from Myanmar (\( n = 167 \)) ranged from 0.5682 to 0.9466 (mean \( = 0.8450 \pm 0.0460 \); Table 1) and showed significant multilocus linkage disequilibrium with \( F_{st} = 0.0149, P < 0.001 \) (Table 2). Significant \( F_{st} \) was encountered in collections from Kayin State (2007, \( n = 96; F_{st} = 0.0130, P < 0.001 \)) and Kachin State (2007, \( n = 56; F_{st} = 0.0313, P < 0.001 \)), but not from Rakhae State (2007, \( n = 15; F_{st} = 0.0164, P = 0.139 \)).

Microsatellite diversity in natural \textit{P. vivax} isolates from Ethiopia. Parasite populations from Ethiopia were also highly polymorphic, having between 3 and 37 alleles per locus (mean = 14.5; Table 1 and Supplemental Tables 1 and 2). All isolates had unique haplotypes with only 0.07\% (\( n = 1 \)) of the alleles being private to Ethiopia. A total of 73.7\% (\( n = 87 \)) were mixed-clone infections. The virtual heterozygosity (\( H_v \)) values for the entire parasite population from Ethiopia ranged from 0.1995 to 0.9534 (mean \( = 0.7517 \pm 0.0568 \); Table 1). Comparable levels of genetic diversity were found for isolates collected in 2006 (\( n = 59; H_v = 0.7432 \pm 0.0551 \)), 2007 (\( n = 52; H_v = 0.7314 \pm 0.0695 \)), and 2008 (\( n = 7; H_v = 0.6190 \pm 0.0839 \)). Significant multilocus linkage disequilibrium was also seen among the isolates from Ethiopia (\( n = 118 \)), with \( F_{st} = 0.0137 \).
$P < 0.001$ (Table 2) remaining significant even when considered according to the time of collection of the isolates: $F_A = 0.0199$, $P < 0.001$ for 2006, $F_A = 0.0180$, $P = 0.006$ for 2007, and $F_A = 0.0697$, $P = 0.044$ for 2008 ($n = 7$).

**Geographic structure of *P. vivax***. Analysis of 425 isolates from Sri Lanka, Myanmar (Asia) and Ethiopia (Africa) identified haplotypes that were all unique. Private alleles for Asia and Africa were 13.4% and 0.07% respectively. Multiple-clone infections were 64.9% ($n = 276$).

The clustering patterns obtained with $K = 2$ shown in Figure 1 showed clustering of *P. vivax* isolates into Asian and African origins with much admixture in both regions. Adding

![Figure 1](http://www.ajtmh.org)

on a third population ($K = 3$, $P < 0.001$) did not help to further differentiate between populations from Sri Lanka and Myanmar (Figure 2). However, the first 21 (Figure 2) isolates from Sri Lanka collected from one site (Trincomalee) during a $P$. vivax malaria outbreak in 2007 and isolates from Assendabo, Ethiopia (308–425; Figure 2) collected during seasonal outbreaks in 2006, 2007, and 2008 are shown clearly in different clusters. The population structure of $P$. vivax according to ancestry at $K = 2$ is shown in Figure 3. The red cluster shows 62.7% (n = 74) of isolates with predominant (> 70%) ancestry from Africa, and the green cluster shows only a 48.8% (n = 150) membership fraction from Asia.

**Predictive power of ancestry of $P$. vivax isolates.** We further assessed the predictive power of ancestry for $P$. vivax isolates using two-thirds of the isolates (n = 283) as a model. Of the 142 isolates that were tested in this model, 47 were from Sri Lanka, 56 from Myanmar, and 39 from Ethiopia. The percentages of test isolates that were correctly identified to ancestry at $K = 2$ is shown in Figure 3. The red cluster shows predominantly in blue) and Ethiopia (cluster 3, predominantly in red) was observed, which is consistent with epidemic transmission of clones. The significantly lower average genetic distance between the outbreak and non-outbreak isolates from Sri Lanka and Myanmar (Figure 2), a strong clustering of the outbreak isolates from Sri Lanka (first portion of cluster 1 in Figure 2 shown predominantly in blue) and Ethiopia (cluster 3, predominantly in red) was observed, which is consistent with epidemic transmission of clones. The significantly lower average genetic distance between the outbreak and non-outbreak isolates from Sri Lanka (0.7845 versus 0.854; $P < 0.0001$) also substantiates this result. Unfortunately, comparisons between outbreak and non-outbreak isolates from Africa could not be made because non-outbreak isolates were not available.

Figure 3 shows the $P$. vivax populations sorted according to Asian or African ancestry, with much admixture in both fractions, especially the Asian fraction. Almost 63% of isolates from Ethiopia had a predominant ancestry (> 70%) from Africa, and only 45% from Myanmar and 54% from Sri Lanka were shown to be from Asia. Many alleles were shown to be private for Asia (13.4%; Supplemental Table 2) than Africa.
(0.07%), which corroborates the historical origins of *P. vivax* in Asia. Microsatellites are known to be a valuable resource for the study of genetic diversity because they are generally hyper-variable, codominant, locus-specific, and not directly subject to host immunity. However, they have high mutation rates and significant mutation-rate heterogeneity among loci, which means that it might be difficult to translate estimates of genetic distance into absolute timescales. More importantly, alleles generated by microsatellite data represent similarities in electrophoretic mobility rather than by descent. Therefore, use of microsatellites in population genetics and evolution has its limitations. However, the population structure generated using these microsatellites show promise for microsatellite data being a good tool to identify parasite differentiation occurring over a short period, such as for mapping outbreaks.

The predictive power of ancestry using two-thirds of isolates as a model gave good results for isolates from all three populations. Predominant ancestry (> 70%) for African or Asian origins were 85% African and 3% Asian for isolates from Ethiopia, 79% Asian and 5% African for isolates from Myanmar, and 72% Asian and 11% African for isolates from Sri Lanka. Thus, microsatellite data as a model for predicting the origin of *P. vivax* parasites holds promise and would provide useful information regarding genetic structure of parasites, in context to the resurgence of *P. vivax* malaria in many regions worldwide.

High levels of genetic diversity in *P. vivax* have been demonstrated in Papua New Guinea, Thailand, India, Colombia, and Laos by use of dinucleotide microsatellites. High diversity levels have also been observed in India and Myanmar with the use of genetic markers. Previous studies with *P. vivax* parasites from Sri Lanka, Brazil, and Myanmar demonstrated high genetic diversity and multiple clone infections despite low levels of transmission in these areas. The co-occurrence of significant linkage disequilibrium in the presence of high diversity and multiple clone infections was also demonstrated in parasites from both Sri Lanka and Brazil. Findings of the present study involving three geographic sites add further strength to the above observations. Linkage disequilibrium in parasite populations may arise as an artifact of the admixture of two or more subpopulations with different allele frequencies. Therefore, isolates from each population were analyzed individually according to their times and sites of collection. Apart from some sites in Sri Lanka (Trincomalee, 2005, n = 20, Anuradhapura 2004, n = 20, Polonnaruwa 2004 and 2005, n = 16 and 4, respectively), Vavuniya (2005, n = 7), and Kurunegala (2006, n = 16) and a single site in Myanmar (Rakhine State, 2007, n = 12), significant linkage disequilibrium was consistently present among the remaining sites (Table 2).

The predominance of inbreeding in *P. vivax* with high proportions of multiple-clone infections and genetic diversity is unusual. High rates of selling may occur in the presence of multiple clone infections if only a small proportion of the co-infecting genetically distinct clones produce viable gametocytes that are taken up by mosquitoes or when strand slippage events occur so frequently during mitotic replication of parasites that new haplotypes would be generated without affecting the overall patterns of association between loci.

In conclusion, microsatellite-based analysis of *P. vivax* parasites from Sri Lanka, Myanmar, and Ethiopia showed extensive genetic diversity co-occurring with significant multilocus linkage disequilibrium. These parasite populations were seen to cluster according to their geographic locations and ancestry. The predictive power of ancestry with this microsatellite data as a model is promising and could be useful in identifying the origin of *P. vivax* malaria cases and enabling meaningful control and preventive measures to be implemented. Although microsatellite data may have some limitations in its use in population genetics and evolution of malaria parasites, it appears to serve as an excellent tool for mapping short-term outbreaks of malaria.

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