PLASMODIUM FALCIPARUM PARASITES IN FRENCH GUIANA: LIMITED GENETIC DIVERSITY AND HIGH SELFING RATE

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Abstract. The genetic characteristics of Plasmodium falciparum isolates collected in French Guiana, where malaria transmission is low and occurs in isolated foci, were studied. Blood samples were collected from 142 patients with symptomatic malaria and typed using a polymerase chain reaction–based strategy for merozoite surface protein-1 (MSP-1) block 2, the MSP-2 central domain, and glutamate-rich protein (GLURP) repeat domain polymorphism. This showed that the parasite population circulating in French Guiana presented a limited number of allelic forms (4, 2, and 3 for MSP-1 block 2, MSP-1, and GLURP, respectively) and a small number of mixed infections, contrasting with the large genetic diversity of parasite populations and infection complexity reported for Africa, Asia, and other parts of South America. Two groups of isolates displaying identical 3 loci allele combinations were further studied for the Pf332 antigen, histidine-rich protein-1, thrombospondin-related anonymous protein, and Pf60 multigene family polymorphism. Within each group, most isolates were identical for all markers tested. This suggests a high rate of self-fertilization of P. falciparum parasites in French Guiana, resulting in homogenization of the population. The implications of these findings for malaria control in areas of low endemicity are discussed.

Malaria parasites show extensive genetic diversity for a number of markers, including enzymes, antigens, and drug resistance.1,2 The large antigenic polymorphism of Plasmodium falciparum is assumed to be responsible for the slow acquisition of protective immunity.3 In view of facilitating the rationale implementation and/or development of new tools for malaria control, a better appreciation of the nature and extent of the genetic diversity of field isolates and its relationship with the genetic structure of local malaria parasite populations is desirable. Analysis of P. falciparum parasite populations is limited by several technical constraints. In the vast majority of cases, analysis of human infections is restricted to peripheral blood parasites, ignoring the sequestered population, only accessible through biopsies or postmortem autopsies. Furthermore, in many endemic areas, infections by multiple parasite clones are common, with daily fluctuations of parasite genotypes in some cases,4,5 which complicates description of individual infections and calculation of allele frequency. Analysis of parasites in mosquitoes is limited by the low fraction of mosquitoes carrying sporozoites, necessitating collection and analysis of very large numbers of mosquitoes or special field equipment. It is difficult to overcome these limitations unless one studies monoclonal human infections, in which case peripheral blood parasites are representative of the total population.

Numerous techniques have been developed to investigate polymorphism of the blood stages. Each of them has some drawbacks, such as necessity of large number of parasites for isoenzyme typing, possible culture artifacts due to rapid loss of some clones in culture for drug resistance studies, stage restriction, and requirement for in vitro maturation for monoclonal antibody typing. The development of polymerase chain reaction (PCR) typing techniques has helped to overcome some of these problems. Typing by PCR allows one to analyze diversity of numerous loci with a minimal amount of blood, whether or not these loci are expressed by blood stages. This has provided novel information on parasite diversity in several endemic regions,6-11 on the dynamics of infection and on molecular characteristics of infections.5,12,13 The PCR studies have revealed a complex genetic structure for P. falciparum populations and suggest that heterozygous recombination during meiosis is frequent in nature, albeit with varying degree.14-18 The studies conducted so far show that the extent of parasite diversity as well as infection complexity strongly depend on the level of endemicity, so that observations made in holoendemic areas9-11,19,21,22 are not valid for mesoendemic areas.20,23,24 Studies in areas of low endemicity such as Colombia or Sudan have shown a surprisingly high proportion of mixed infections and a substantial parasite diversity.5,7,12,13 Since parasite diversity, mating pattern, and population structure in areas with low and seasonal transmission are likely to be affected by local conditions and are therefore difficult to extrapolate from one area of low endemicity to another, we have conducted an analysis of parasite polymorphism in French Guiana, an unexplored area of low endemicity, where malaria constitutes a serious public health problem.25 This study was undertaken to explore parasite genetic diversity in this region to hopefully derive data of relevance for control guidance and management of malaria. In addition, the methodologic approach used here was similar to that used in previous studies that investigated parasite diversity in different African settings,5,8,11,20,21,26 which provided the opportunity to better understand the impact of transmission intensity on diversity and complexity.

MATERIALS AND METHODS

Study site and blood collection. French Guiana is located in South America in the equatorial zone of the Northern hemisphere. It is bordered on the north by the Atlantic ocean, on the west by the Maroni River, and on the east by the Oyapock River. Both of these rivers form natural frontiers with Suriname and Brazil (Figure 1). The annual global
incidence of malaria in French Guiana has continuously increased from 1,000 to 8,000 cases in the last 20 years, in an overall population of approximately 115,000 inhabitants. Malaria transmission is heterogeneous, and is restricted to small foci disseminated along the Oyapock and Maroni Rivers, whereas the coastal plain remains essentially free of malaria transmission. The emergence of drug resistance has not contributed significantly to increased incidence since 1970, probably because of the discovery of new mining possibilities favoring the settlement of migrants in rural and forest areas. The prevalence of malaria in the Maroni region is less than 5%, with *P. falciparum* being responsible for 95% of the positive blood smears. Eighty percent of the cases reported annually originate from this area. There has been substantial immigration into the Maroni region during recent years due to increasing gold-mining activities. The size and turnover rate of these migratory flows is difficult to evaluate. The settlement of new migrants carrying gametocytes probably introduce new *P. falciparum* alleles, which may modify the pool of parasites in the region. The other site of transmission is located along the Oyapock River at the Brazilian border of eastern French Guiana. The incidence there is much lower, approximately 8%, and due mostly to *P. vivax* infections. Here again, transmission is restricted to small rural areas and associated with the presence of gold-mining activities.

All patients provided informed consent before donating blood samples to be used in this study. This study did not interfere with routine clinical management and was part of chemotherapy evaluations approved by the Centre de Référence de la Chimioresistance de Paludisme de l'Institut Pasteur. Blood samples (1–5 ml) were collected from each patient (*n* = 142) in anticoagulant-coated vacutainers (Becton Dickinson, Rutherford, NJ) as a part of larger volumes obtained for studies on drug resistance. Parasitized blood was stored at −80°C until DNA extraction.

**Extraction of DNA and PCR.** Parasitized erythrocytes were lysed by addition of distilled water and thawing. Free parasites were recovered by centrifugation at 10,000 rpm, washed once with distilled water, and resuspended in 4 volumes of TNE buffer (10 mM Tris-HCl, 1 mM EDTA, 0.15 M NaCl, pH 8) containing 0.5% Triton X-100, 0.5% sodium dodecyl sulfate, and 5 mg/ml of proteinase K. After incubation for 1 hr at 37°C, DNA was extracted with phenol and chloroform, and further purified by precipitation with ethanol. The pellet was suspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) and kept at 4°C.

The analysis was based on the amplification of DNA sequences corresponding to highly polymorphic regions of the genes coding for the merozoite surface proteins 1 and 2 (MSP-1 and MSP-2), the glutamate-rich protein (GLURP), the thrombospondin-related anonymous protein (TRAP), the histidine-rich protein-1 (HRP-1), the Pf332 antigen (Pf332), and the Pf60 multigene family. The sequence of the primers used were as follows: MSP-1-P1 CACATGAAAAGTTATCAAG-AACTTGGTC, MSP-1-P2 CGTACGTCTAATTCATTTGC-ACG, MSP-2–1 ATGAAGGTAATTAAAACATTGTCTAT-TATA, MSP-2–4 ATATGGCAAAAGGATAAAACAAGTGTTG, GLURP-E ATGAATTTGAAGATGTTCACACTGA-C, GLURP-F AAATATTACTATATCTTGTCTATTCC, TRAP-3 GAACTTTGTATGCTGTATTGCATGG, TRAP-4 TCACTATTAGGTAGCTGCTATTTTCC, HRP-1-A CG-GGGATCCACCCATGGTCAGGCTATT, HRP-1-D AG-AATTCCATTGTGCTTTATTTGTTGGCGC, Pf332-A G-TATCAGTTACTGTGAAATAGTAGGAGG, Pf332-B GCTGATACTTTTTCACCACATGGTCAGGCTATT, Pf60–1 TG-GTACTAGAACCTAGTGGTGAC, and Pf60–2 GGATAAT-TATATTCTTCTCAC.

Amplifications were performed in 50 μl of reaction buffer (Promega, Madison, WI) containing the DNA template, 1.5 mM MgCl2, 2 μM of each primer, 200 μM of each dNTP, and 5–10 units of *Taq* polymerase (Promega). Samples were subjected to 32–37 cycles of amplification using the following scheme: denaturation at 95°C for 10 sec, annealing at 57°C (MSP-1 and MSP-2), 51°C (GLURP), 58°C (TRAP, HRP-1, and Pf60), or 59°C (Pf332) for 90 sec, and synthesis at 72°C for 120 sec using a PHC-2 thermocycler (Techne, Cambridge, United Kingdom). The products of amplification were analyzed for size polymorphism by agarose gel electrophoresis in the presence of 0.5 μg/ml of ethidium bromide.

The MSP-1 and MSP-2 genes contain variable domains.
showing considerable diversity, and are grouped into 3 and 2 allelic families, respectively. Assignment of the PCR fragments to 1 allelic family was based on hybridization to allelic-specific probes. Briefly, 5 μl of each PCR mixture was spotted onto Hybond N nylon membranes (Amersham, Paris, France), denatured, and fixed as recommended by the manufacturer. Membranes were prehybridized at 37°C for 1 hr and hybridized overnight at 37°C in 6× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2) containing 2.5% nonfat milk and 100 mg/ml of herring sperm DNA with 1 of the various allele-specific probes: Mad20 (ACAAGTGGAAACAGCTGTACAA) or Ro33 (GTTGTGCAATGAGCCTGCAAGGTGCT) for MSP-1 sequences, and Fc27 (GCTGATACTATTGCTAGTGGAAG) or Indo/3D7 (GAGAAATTTAAGTACTCGCGTAC) for MSP-2 sequences. Hybridization with the MSP-1 K1-specific probe, which consisted of a recombinant plasmid carrying the entire polymorphic block 2 of the K1 strain, was performed at 65°C. The probes were radiolabeled either by kination (nucleotides) or by nick-translation (plasmid) according to manufacturer’s recommendation (Boehringer Mannheim, Mannheim, Germany). All filters were successively washed at the hybridization temperature in a series of solutions of increasing stringency (2×, 0.5×, 0.1× SSC) to check the specificity of positive signals. After each wash, membranes were exposed to X-Omat films (Eastman Kodak, Rochester, NY).

Alletic frequency analysis and estimations of number of clones in blood samples. To estimate the mean number of clones per host and the allele frequencies in blood samples, we used the method developed by Hill and Babiker. Assuming a Poisson distribution of the number of distinct genotypes per infected host, formulas were derived for the frequency of different classes of blood samples, and maximum likelihood methods were used to estimate the mean number of clone/isolate and allele frequencies. The results were then compared with those obtained when considering the blood form reflects the parasite zygote (see Discussion).

RESULTS

Blood samples. A total of 142 isolates were collected from July 1994 to September 1996 from clinically ill patients infected with *P. falciparum*. The geographic origin of these parasitized blood samples was consistent with distribution of malaria in French Guiana: 73% were from patients living in rural areas close to the Maroni River, a major communication route between small villages and St. Laurent du Maroni, the nearest main city, whereas 3% of the isolates included in the study originated from the Oyapock River area. The site of infection was unknown for 24% of the blood samples examined.

Parasite density varied considerably, ranging from 0.01% to 30%. Thirty-two percent of the isolates were from patients with parasitemias <0.1%, and 46.2% and 18.5% of the samples were from patients with parasitemias of 0.1–1% or 1–5%, respectively. The few remaining (3.3%) had parasitemia >5%.

Polymorphism. The genetic diversity was first studied by PCR amplification of highly polymorphic regions of the MSP-1, MSP-2, and GLURP genes. Twenty-one samples did not yield a product in any of the 3 reactions, whereas 77 samples were amplified in the 3 typing reactions. For MSP-1 and MSP-2 markers, the failure of amplification was correlated with low parasite counts. The MSP-1 block 2 and MSP-2 sequences were amplified at higher efficiency (106 of 142 and 102 of 142, respectively) and at better yields than those derived from GLURP (77 of 142).

The various allelic forms at each locus were identified by size polymorphism and for the MSP-1 block 2 and MSP-2 loci by hybridization with family-specific probes. The relative distribution of the various allelic forms is summarized in Table 1. For each isolate, amplification of the polymorphic MSP-1 block 2 generated a fragment of either 540 or 620 basepairs (coded A and B, respectively). Hybridization with allele-specific probes allowed identification of 4 distinct MSP-1 block 2 allelic forms. There were 2 distinct K1 alleles, A-K1 and B-K1, which accounted for approximately 70% of the alleles, whereas there was a single Ro33 and Mad20 allelic form of 540 basepairs each, which represented 23% and 7% of the MSP-1 block 2 alleles, respectively. The frequency of individual alleles, calculated using the method of Hill and Babiker, differed markedly as indicated in Table 1. The predominant allele was B-K1 with a frequency of 0.409. The A-K1 and A-Ro33 alleles were fairly abundant (allelic frequencies = 0.293 and 0.233, respectively), whereas A-Mad20 was rare (allelic frequency = 0.065).

Similarly, MSP-2 typing generated 2 fragments of 760 and 870 basepairs (coded C and D, respectively), which were assigned by hybridization to the Fc27 and Indo/3D7 allelic family, respectively. The 870-basepair Indo/3D7-type allele was observed in 76% of the isolates, and the 760-basepair Fc27-type allele was found in the remaining 24%. The calculated allelic frequencies were 0.763 and 0.237 for D-3D7 and C-Fc27, respectively (Table 1).

Amplification of the GLURP repeat region yielded 3 fragments of 1,160, 1,200, and 1,360 basepairs labeled E, F, and G, respectively. They were present in 31%, 15%, and 54% of the samples successfully typed, respectively. The calculated individual frequencies were 0.311, 0.147, and 0.542, respectively.

Figure 2 shows the comparison of the prevalence of the various MSP-1 block 2 and MSP-2 alleles at 3-month intervals from July 1994 to September 1996. The MSP-1 and MSP-2 allelic frequencies fluctuated markedly over the study period. The MSP-1 K1 alleles consistently predominated during the study, with a maximum frequency occurring during the main dry season, but the relative proportion of A-K1 to B-K1 was reversed in July–September 1994 compared with July–September 1996. Interestingly, parasites carrying the MAD20 sequence were first detected in blood samples collected in February 1995; the prevalence of this allele remained stable for about 1 year, but from February 1996 on, it was no longer observed.

For the MSP-2 locus (lower panel of Figure 2), the Indo1/3D7 allele predominated over the entire sampling period, with a frequency fluctuating between 62% and 87%.

Mixed infections and clonality. A small proportion of isolates (15 of 142) contained more than 1 allele for these single copy loci (i.e., reflected mixed infections), as visualized on agarose gels as a double band and/or as a complex hybridization profile on blots. Using the methods described
DIVERSITY OF *P. FALCIPARUM* IN FRENCH GUIANA

**TABLE 1** Distribution of the merozoite surface protein-1 (MSP-1) block 2, MSP-2, and glutamate-rich protein (GLURP) genotypes in French Guiana*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of samples (n = 108)</th>
<th>Genotypes</th>
<th>Number of samples (n = 101)</th>
<th>Genotypes</th>
<th>Number of samples (n = 77)</th>
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<tr>
<td>A-K1</td>
<td>32</td>
<td>C-Fc27</td>
<td>21</td>
<td>E</td>
<td>23</td>
</tr>
<tr>
<td>A-Mad20</td>
<td>4</td>
<td>D-3D7</td>
<td>74</td>
<td>F</td>
<td>10</td>
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<tr>
<td>A-Ro33</td>
<td>20</td>
<td>C-Fc27 and D-3D7</td>
<td>6</td>
<td>G</td>
<td>41</td>
</tr>
<tr>
<td>B-K1</td>
<td>41</td>
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<td>1</td>
<td>E and F</td>
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<tr>
<td>A-K1 and A-Mad20</td>
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<td>E and G</td>
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<tr>
<td>A-K1 and A-Ro33</td>
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<td>1</td>
<td>F and G</td>
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</tr>
<tr>
<td>A-K1 and B-K1</td>
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<td>1</td>
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<td></td>
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<tr>
<td>A-Ro33 and B-K1</td>
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<table>
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<tr>
<th>Allelic frequencies</th>
<th>Allelic frequencies</th>
<th>Allelic frequencies</th>
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<tbody>
<tr>
<td>A-K1</td>
<td>0.293</td>
<td>C-Fc27</td>
</tr>
<tr>
<td>A-Mad20</td>
<td>0.065</td>
<td>D-3D7</td>
</tr>
<tr>
<td>A-Ro33</td>
<td>0.233</td>
<td></td>
</tr>
<tr>
<td>B-K1</td>
<td>0.409</td>
<td></td>
</tr>
</tbody>
</table>

Deduced number of clones per infection: 1.15, 1.17, 1.07

*The number of samples successfully typed for each locus is indicated in parentheses. The allelic frequency at each locus and deduced number of clones per isolate were calculated according to the methods developed by Hill and Babiker based on blood data. Note that no more than 2 alleles have been found in the isolates studied.

by Hill and Babiker, the mean number of clones per blood infection was estimated as approximately 1.1 (1.15 for MSP-1, 1.17 for MSP-2, and 1.07 for GLURP, as indicated in Table 1). In fact, the vast majority of the samples generated a single band for the 3 reactions, strongly suggesting that they contained a single parasite type (i.e., reflecting single clone infections).

For the numerous isolates generating a single band for each reaction, genotype associations could be derived. As shown in Table 2, 14 distinct multi-locus associations were detected. Importantly, about half of the single infections were ascribed to one of 3 multi-locus genotypes: A-K1/D/G (cluster A), B-K1/D/G (cluster B), and A-Ro33/D/G (cluster C), observed in 20%, 14%, and 13% of the isolates, respectively.

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**FIGURE 2.** Quarterly frequency of K1, Ro 33, and Mad 20 allelic forms of the merozoite surface protein-1 (MSP-1) polymorphic block 2 (arrows indicate appearance and loss of the MSP-1 A-MAD 20 allele) (top panel) and Indo/3D7 and Fc27 allelic families of MSP-2 in isolates collected from July 1994 to October 1996 (bottom panel).
We next investigated whether the isolates presenting the same 3-locus genotype were genetically identical for the other loci. A group of isolates was randomly selected within each cluster and was further studied by PCR using additional single copy polymorphic markers: Pf332, TRAP, HRP-1, and the Pf60 multigene family. Results are shown in Figure 3. Seven of the 12 isolates from cluster A generated identical amplification profiles for all characters analyzed. Similarly, all but 1 isolate belonging to cluster B were genetically indistinguishable. In contrast, no 2 strains from cluster C were identical to each other, indicating that this cluster contained distinguishable. In contrast, no 2 strains from cluster C were genetically in-

TABLE 2

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>MSP-1</th>
<th>MSP-2</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-K1</td>
<td>D</td>
<td>G</td>
<td>15 (20)</td>
</tr>
<tr>
<td>B-K1</td>
<td>D</td>
<td>G</td>
<td>11 (14)</td>
</tr>
<tr>
<td>A-Ro33</td>
<td>D</td>
<td>G</td>
<td>9 (12)</td>
</tr>
<tr>
<td>A-Ro33</td>
<td>D</td>
<td>E</td>
<td>5 (6)</td>
</tr>
<tr>
<td>B-K1</td>
<td>D</td>
<td>E</td>
<td>4 (5)</td>
</tr>
<tr>
<td>B-K1</td>
<td>C</td>
<td>F</td>
<td>4 (5)</td>
</tr>
<tr>
<td>A-Ro33</td>
<td>C</td>
<td>E</td>
<td>4 (5)</td>
</tr>
<tr>
<td>A-K1</td>
<td>D</td>
<td>E</td>
<td>3 (4)</td>
</tr>
<tr>
<td>B-K1</td>
<td>D</td>
<td>F</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>B-K1</td>
<td>C</td>
<td>E</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>A-Mad20</td>
<td>C</td>
<td>G</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>B-K1</td>
<td>C</td>
<td>G</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>A-Mad20</td>
<td>C</td>
<td>E</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>

* MSP = merozoite surface protein; GLURP = glutamate-rich protein.

DISCUSSION

We have analyzed the genetic diversity of P. falciparum isolates collected in French Guiana. To our knowledge, no such study had been undertaken so far in this part of South America, where malaria transmission is so low that indigenous populations have limited if any immunity and most infections are symptomatic. The genetic analysis was based on amplification of polymorphic genes located on different chromosomes and coding for antigens. Some isolates, although collected from patients with detectable peripheral parasitemia, could not be analyzed because no PCR product was obtained. In a previous study, we estimated the minimum amount of DNA template required to obtain a product directly detectable by agarose gel electrophoresis and staining with ethidium bromide using a simple PCR to be 0.2–25 pg.26 To increase the amplification efficiency, which also allows minor parasite populations to be detected, we carried out a series of nested PCRs performed on 15 randomly selected isolates. However, this did not identify any additional product, indicating that failure of amplification was not due to lack of sensitivity but to some as yet unidentified factor. Whether this was due to DNA degradation or to the presence of some PCR inhibitors is unclear. These samples were excluded from the analysis.

The first conclusion of our study is that the P. falciparum populations in French Guiana have a very limited genetic diversity. Four distinct MSP-1 alleles were detected in the French Guiana samples and polymorphism of the MSP-2 locus was minimal, since only 2 different allelic forms were detected. This contrasts with observations made in Senegal,8,11,20,21,26 Tanzania,10,19,22 and Papua New Guinea,9,17 or in low endemicity areas such as Columbia,6 Sudan,7,12,13,19 or Honduras.28 The limited genetic polymorphism observed in French Guiana indicates that the P. falciparum population is small enough and the transmission rate low enough to result in local reduction in the genetic diversity due to a strong genetic drift effect, resulting in loss of alleles. A probable illustration of such an effect was the loss of the MAD20 MSP-1 allele during the study period, shown in the upper panel of Figure 2. The geographic isolation of the endemic area in French Guiana reduces the introduction of novel alleles by immigration, and thus genetic drift is not compensated by import.

Another striking characteristic of the French Guiana parasite population was the unusually low proportion of mixed infections, probably the lowest score of mixed infections ever reported for P. falciparum (only 10% of the isolates contained more than 1 parasite clone). This observation is of importance because complex parasite genotypes are frequently observed in individuals living in endemic areas. For instance, the fraction of isolates containing mixed infections is about 50% in mesoendemic regions of Africa,20,23,24 and reaches 83–100% in holoendemic areas.2,11,19,22 In Colombia, Brazil, or Honduras, areas where endemicity is lower and asymptomatic carriage is rare, an incidence of mixed infections of approximately 30% has been reported.6,28,29 In Sudanese villages where the entomologic inoculation rate is less than 1 infective bite/person/year, 20% of the blood samples collected from symptomatic cases contained more than 1 P. falciparum clone.19 Mixed infections are a prerequisite for cross-fertilization, and a source for novel genotype combinations, and novel alleles. As indicated above, in all settings studied so far, the number of distinct alleles for the loci investigated here is much larger, including areas of low endemicity such as Sudan.19 The limited genetic heterogeneity of P. falciparum in French Guiana may result from a low cross-fertilization rate due to the low proportion of human infections with more than 1 clone.

The epidemiologic conditions in French Guiana are peculiar, with a low transmission rate associated with the absence of asymptomatic carriers. Thus, most if not all blood infections likely result from a single infectious bite. It is a reasonable assumption for the isolates with monomorphic genotyping profiles, namely for the majority of the samples analyzed here. It may also be the case for the isolates classified as mixed infections, in which the number of single-locus genotypes per isolate never exceeded 2. In this context, mixed infections may result from the inoculation by a single mosquito of 2 distinct haplotypes, originating either from a single heterozygous zygote or from 2 zygotes carried by one mosquito rather than sequential infection by different mosquitoes. If we consider the hypothesis of inoculation by a single mosquito, where single infections originate from an homozygote oocyst and mixed infections originate from an heterozygote oocyst, we are in a position to calculate the actual allele frequency for each locus, since the blood-stage forms directly reflect the zygote genotype. The method de-
Table 3 shows that under this assumption, the inbreeding coefficient of the parasites circulating in French Guiana was greater than 0.84 ($f_{MSP-1} = 0.85$, $f_{MSP-2} = 0.84$, $f_{GLURP} = 0.93$), indicating a significant deviation from random mating. The calculated effective number of clones per host is similar to that obtained without using the hypothesis of blood stage/oocyst equivalence (1.18 versus 1.15 for MSP-1, 1.20 versus 1.17 for MSP-2, and 1.07 versus 1.07 for GLURP). Such a similarity tends to validate our hypothesis. We can therefore conclude with confidence that an unusually high selfing rate of approximately 90% is predicted for French Guiana. This is consistent with the observation of a high frequency of similar multilocus genotypes (clusters A and B). However, as reported for Papua New Guinea, no linkage disequilibrium was observed for any loci investigated, indicating that cross-fertilization does occur, even if it is a rare event. Lack of linkage disequilibrium is indeed easy to attain, since the loci here investigated are located on distinct chromosomes.

In French Guiana, the low prevalence of malaria infection (reinforced by an efficient medical coverage providing rapid and efficient drug treatment) results in a strong genetic drift and in a high selfing rate. In addition, the geographic isolation of the endemic areas reduces import of new alleles. The combination of these 2 effects results in a reduced polymorphism and a limited complexity in the local parasite infections. Such a parasite population in an isolated area of low endemicity constitutes a particularly propitious situation for analyzing the factors that structure populations and the consequences of interventions. Furthermore, the circulation of genetic material between areas of high and low endemicity may also contribute to the spread of resistance to antimalarial drugs.

**Table 3.** Inbreeding coefficients ($f$) and effective numbers of clones per host ($N_{e}$) for the parasites circulating in French Guiana, calculated using the hypothesis of blood stage/oocyst equivalence for MSP-1, MSP-2, and GLURP.

**Figure 3.** Agarose gel electrophoretic analysis of the fragments amplified by the polymerase chain reaction from the Pf332 antigen, thrombospondin-related anonymous protein (TRAP), histidine-rich protein-1 (HRP-1), and Pf60 multigene family polymorphic markers (top panel) and deduced multilocus genotypes from isolates 1–12 (cluster A), 13–18 (cluster B), and 19–23 (cluster C) (bottom panel). Amplification of Pf332 sequences did not allow the isolates to be differentiated because 3 products of 1,450, 1,150, and 430 basepairs (allele a), were amplified in all DNA preparations, except for sample 21 in which no product larger than 1,000 basepairs was detected (allele b). The 430-basepair (bp) fragment observed in all samples is not visible in the gel region shown. Two TRAP alleles were visualized: a 720-bp fragment (coded c) was amplified in 17 of 23 isolates and an 840-bp fragment (coded d) was detected in isolates 8, 10, 12, and 13. Both alleles were coamplified in sample 2, indicating that it contained a mixture of at least 2 parasite populations. No product was detected in isolates 21 and 22. Amplification of the HRP-1-3' gene region generated 2 distinct fragments. A 640-bp product (coded e) was amplified in 21 of 23 isolates examined, whereas the 700-bp fragment (coded f) was detected once (sample 20). No amplification product was obtained from sample 2. The Pf60 typing reaction, which amplifies multiple sequences in the parasite genome, generated complex banding patterns. Several fragments from 270 to 350 basepairs were visualized and arranged in various arrays to give 7 distinct patterns (patterns g to m).
of parasites with similar genotypes opens the possibility of investigating the clinical impact of a particular parasite genotype, a crucial question in understanding *P. falciparum* pathogenesis that cannot be addressed in regions where parasite diversity is large and human populations are immune.

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