Gene flow and cross-mating in *Plasmodium falciparum* in households in a Tanzanian village

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SUMMARY

The diversity of the genes encoding 2 merozoite surface proteins (MSP-1 and MSP-2) of *Plasmodium falciparum* has been examined in parasites infecting members of 4 households in a village in Tanzania. The polymerase chain reaction (PCR) was used to characterize allelic variants of these genes by the sizes and sequences of regions of tandemly repeated bases in each gene. In each household extensive polymorphism was detected among parasites in the inhabitants and in infected mosquitoes caught in their houses. Similar frequencies of the alleles of these genes were observed in all households. Capture–recapture data indicated that both *Anopheles gambiae* and *A. funestus* freely dispersed among households in the hamlet. The results confirm that cross-mating and gene flow occur extensively among the parasites, and are discussed within the context of spatial clustering of natural populations of *P. falciparum*.

Key words: *Plasmodium falciparum*, merozoite surface proteins, genetic polymorphisms, spatial clustering.

INTRODUCTION

The idea that malaria parasites are 'clustered' in nature has been proposed by several workers. For example, clustering of *Plasmodium malariae* infections in certain houses has been reported in a village in Guinea-Bissau (Snounou *et al.* 1993). Local variations in the intensity and duration of infection and of clinical symptoms among infected individuals in a given community (Molineaux & Gramiccia, 1980) provide circumstantial evidence that such clustering of parasites may occur. Clustering of mosquitoes occurs in nature, and variations in levels of transmission have been observed between different households in a single community (Gamage-Mendis *et al.* 1991). Such variations in transmission could result in local variations in the genetic structure of the parasite populations. Space-time clustering of mini-epidemics of severe *P. falciparum* malaria in a coastal region of Kenya has been attributed to antigenically distinct parasite 'strains' (Snow *et al.* 1993).

Few studies have been carried out to determine whether parasite genotypes are clustered at household levels. It is now possible to examine this subject by studying the distribution of the numerous polymorphic genes which exist in malaria parasite populations, especially in *P. falciparum* and *P. vivax* (Kemp, Cowman & Walliker, 1990; Cheng *et al.* 1993). Isolates of *P. falciparum* from different countries have been found to possess similar alleles of these genes, but often at different frequencies, suggesting that there is a closer relatedness of parasites within rather than between different areas (Creasey *et al.* 1990). Forsyth *et al.* (1989) have produced evidence for variations in the frequency of an S-antigen allele at the community level in different villages in Papua New Guinea. Nevertheless, the diversity of parasites occurring in a single community may be very great; for example, each of 29 isolates of *P. falciparum* examined from a Sudanese village possessed unique genotypes (Babiker *et al.* 1991). In a peri-urban area in The Gambia, the gene pool of *P. falciparum* was smaller in small communities than in larger ones, although there was no evidence of differences in allele frequencies among the communities studied (Conway & McBride, 1991*a*).

In this paper, we examine whether parasite clustering occurs in *P. falciparum* in houses in a village in Tanzania. We make use of the polymerase chain reaction (PCR) to examine polymorphic alleles of 2 antigen genes among these parasites in finger-prick blood samples of the inhabitants, in mosquito bloodmeals, and in oocysts in mosquitoes caught in the houses. We show that there is considerable movement of infected mosquitoes between different households, and that frequent crossing between different clones occurs during mosquito transmission. As a result, extensive genetic diversity of parasites occurs among the parasites in all the households studied.

MATERIALS AND METHODS

Study area

The study was carried out in June 1991 in a small

Table 1. Oocyst rate in mosquitoes in households of Kining'ina, collected by resting catch

Household	Uninfected mosquitoes	Infected mosquitoes (%)	Total
8001/1–2	323	60 (17)	383
8003/1–3	96	11 (10)	107
8004/1–4	347	31 (8)	378
8006/1–4	73	10 (12)	83
Total	839	112 (12)	951

hamlet named Kining'ina, which is 1 km north of Michenga village, near Ifakara, south-east Tanzania. The area is holo-endemic for malaria, *P. falciparum* constituting up to 95% of all malaria cases. Transmission by *Anopheles gambiae* and *A. funestus* occurs throughout the year, with peaks following rains in April/May and November/December (Kilombero Malaria Project, 1992; Smith *et al.* 1993).

Kining'ina lies in an area which is farmed. The hamlet consists of 4 families, each of which inhabits a group of 1–4 small huts. The groups of huts are approximately 500 m apart from each other. Each hut contains 1 or 2 beds. At the start of the study, no mosquito nets or other anti-mosquito devices were in use in any of the huts. Households were allocated serial numbers 8001, 8003, 8004 and 8006, and each hut was allocated a subnumber. Thus, for example, 8004/1–4 designates a group of 4 huts, inhabited by members of 1 family.

Isolates of P. falciparum

Fingerprick blood samples (500–700 μl) were collected from all members of the 4 families on 1 day, 21 June 1991. There were 30 inhabitants altogether, of whom 17 were positive for *P. falciparum* by thick blood smear. These 17 blood samples were cryopreserved (Aley *et al.* 1984), stored at −80 °C, and transported to Edinburgh. *P. falciparum* genomic DNA was isolated from the blood samples by the method of Foley, Ranford-Cartwright & Babiker (1992) for subsequent PCR work.

Collection and capture–recapture of mosquitoes

Daily collections of *A. gambiae* s.l. and *A. funestus* were made in each of the huts in the early morning by resting collections (Molineaux *et al.* 1988) between 10 and 23 June 1991. On 15 June, some of the mosquitoes caught in households 8001 and 8004 were dusted with different coloured fluorescent powder (Charlwood, Graves & Birley, 1986) and released from the sites of collection. From 17 June, mosquito nets with four 200 mm diameter holes cut into their sides were placed over 12 of the 15 beds in the hamlet. Mosquitoes were collected from these nets every morning for the next 5 days, and taken immediately to an insectary at the Ifakara Centre for processing. At the end of the experiment new intact nets were given to each family.

Isolation of parasites from mosquitoes

Mosquitoes collected from the huts were maintained in the insectary for 5–7 days to allow oocysts to develop. After dissection and careful microscopical examination, midguts containing only single oocysts were washed twice in drops of fresh RPMI medium on a glass slide, placed individually into Eppendorf tubes containing a PCR lysis buffer/proteinase K mixture, and incubated for 1 h at 55 °C (Ranford-Cartwright *et al.* 1991). After incubation, all preparations were stored at −20 °C, before being transported to Edinburgh. DNA was subsequently isolated from oocysts as described by Ranford-Cartwright *et al.* (1991).

Blood was also prepared from 12 mosquitoes from household 8001. These mosquitoes were dissected on the morning of their collection. Their abdomens with midguts containing blood from the previous night's feeds were placed individually into Eppendorf tubes containing PCR lysis buffer/proteinase K, and then treated in a similar manner to the oocysts, as described above.

Polymerase chain reaction (PCR) of MSP-1 and MSP-2 genes

PCR primers were designed to amplify regions of genes encoding 2 antigen genes denoted MSP-1 and MSP-2. These genes occur as single copies in the genome, MSP-1 being located on chromosome 9 and MSP-2 on chromosome 2 (Triglia, Wellems & Kemp, 1992). Both genes contain regions of tandemly repeated bases. In MSP-1, these repeats occur in block 2 of the gene near its 5′ end (Tanabe *et al.* 1987), while in MSP-2 they occur in a central portion of the gene, also denoted block 2 (Smythe *et al.* 1991). Details of the primers and PCR conditions are given by Ranford-Cartwright *et al.* (1993) and Babiker *et al.* (1994*a*).

Amplified PCR fragments were subjected to electrophoresis on a 1·6% agarose gel. Variations in the number of repeats in different alleles of MSP-1 and MSP-2 could be recognized by differences in size of the amplified products (Kimura *et al.* 1990). Fragments were then Southern blotted on to nylon membranes (Sambrook, Fritsch & Maniatis, 1989).

Hybridization of the PCR-amplified fragments with sequence-specific oligonucleotides

Amplified PCR fragments were classified on the basis of their sequence by hybridization of Southern blots with allele-specific probes. All MSP-1 alleles so far examined contain 1 of 3 sequences in block 2, denoted K1, MAD20 and RO33 after the isolates

Table 2. Recapture of marked mosquitoes originally released in houses 8001/1 and 8004/4

Household	*A. gambiae* marked	*A. funestus* marked
8001/1	5	2
8001/2	3	0
8003/1	6	2
8003/2	1	1
8004/1	1	8
8004/2	1	6
8004/3	0	4
8004/4	2	3
8006/1	0	2
8006/2	0	0
Totals	19	28

from which they were originally described (Kimura *et al.* 1990). Similarly, block 3 of the MSP-2 gene has been classified into 2 families distinguishable by specific DNA sequence (Fenton *et al.* 1991; Smythe *et al.* 1991); two oligonucleotide probes denoted IC1 and FC27 were used to identify the two types (Babiker *et al.* 1994*a*). Oligonucleotides for each of these sequences were made, then labelled at their 3′ ends with fluorescein-11-dUTP, using the Amersham 3′ oligolabelling kit. These probes were then hybridized to Southern blots of the PCR-amplified products of each respective gene, and detected using the Enhanced Chemiluminescence (ECL) detection kit (Amersham) (Babiker *et al.* 1994*a*).

RESULTS

Mosquito collections

(*a*) *Resting collections*. Indoor resting catch collections of mosquitoes were made in all households throughout the study period. There were considerable variations between the households in the numbers collected, as well as in the oocyst rates (Table 1).

(*b*) *Collections from nets with holes*. Collections of mosquitoes from nets with holes were made daily between 17 and 22 June. A total of 913 specimens of *A. funestus* and 432 of *A. gambiae* was caught. During the period of study, the populations of both species were relatively stable and old (mostly parous). Mean numbers of *A. gambiae* decreased from 24 to 15/net/night, whereas mean numbers of *A. funestus* increased from 28 to 42. There was no significant difference in the parous rate of the two species, 31 of 38 (82%) *A. funestus* examined and 20 of 26 (77%) *A. gambiae* being parous ($\chi^2 = 0{\cdot}21$, $P = 0{\cdot}65$). Despite these similarities, the oocyst rates were significantly higher in *A. gambiae* than in *A. funestus*; 60 out of 276 (21%) *A. gambiae* dissected were infected, compared to 57 out of 514 (11%) *A. funestus* ($\chi^2 = 16{\cdot}1$, $P < 0{\cdot}0001$).

(*c*) *Mosquito movement and cross-infection of* P. falciparum *between different households*. Mosquitoes collected from nets with holes were examined for

Table 3. MSP-1 and MSP-2 alleles of isolates collected on 21 June 1991 from infected individuals in 4 households in Kining'ina, Tanzania

(Alleles are designated by sequence type and by size of their amplified PCR fragment. For example, for MSP-1, allele $K1^{520}$ contains the K1 sequence and has a fragment size of 520 base pairs (bp); —, allele not detected. N.D. Not done.)

House number	Isolate number	MSP-1 alleles			MSP-2 alleles	
8001/1–2	IfB2	$K1^{520}$	$MAD20^{470}$	$RO33^{470}$	—	$FC27^{600}$
	IfB3	$K1^{520}$	$MAD20^{520}$	—	$IC1^{520}$	$FC27^{480}$
	IfB4	$K1^{540}$	—	$RO33^{470}$	$IC1^{650}$	$FC27^{480}$
8003/1–3	IfB10	$K1^{410}$	$MAD20^{470}$	$RO33^{470}$	$IC1^{600}$	$FC27^{520}$
	IfB11	$K1^{480}$	$MAD20^{560}$	—	$IC1^{520}$	$FC27^{520}$
8004/1–4	IfB13	—	$MAD20^{520}$	$RO33^{470}$	—	$FC27^{580}$
	IfB14	$K1^{480}$	—	$RO33^{470}$	$IC1^{520}$	$FC27^{520}$
	IfB16	—	—	$RO33^{470}$	$IC1^{650}$ $IC1^{520}$	$FC27^{520}$
	IfB17	N.D.	N.D.	N.D.	—	$FC27^{520}$
	IfB18	$K1^{480}$	—	—	$IC1^{520}$	$FC27^{460}$
	IfB20	$K1^{480}$	—	—	$IC1^{520}$	—
	IfB22	$K1^{580}$	$MAD20^{580}$	$RO33^{470}$	$IC1^{520}$	$FC27^{600}$
	IfB23	$K1^{560}$	—	$RO33^{470}$	$IC1^{650}$	$FC27^{520}$
8006/1–4	IfB26	$K1^{480}$	—	—	$IC1^{650}$	$FC27^{520}$
	IfB27	$K1^{540}$ $K1^{470}$	—	—	$IC1^{650}$	$FC27^{480}$
	IfB28	$K1^{580}$	$MAD20^{500}$	$RO33^{470}$	$IC1^{520}$	$FC27^{520}$
	IfB29	—	—	$RO33^{470}$	N.D.	N.D.

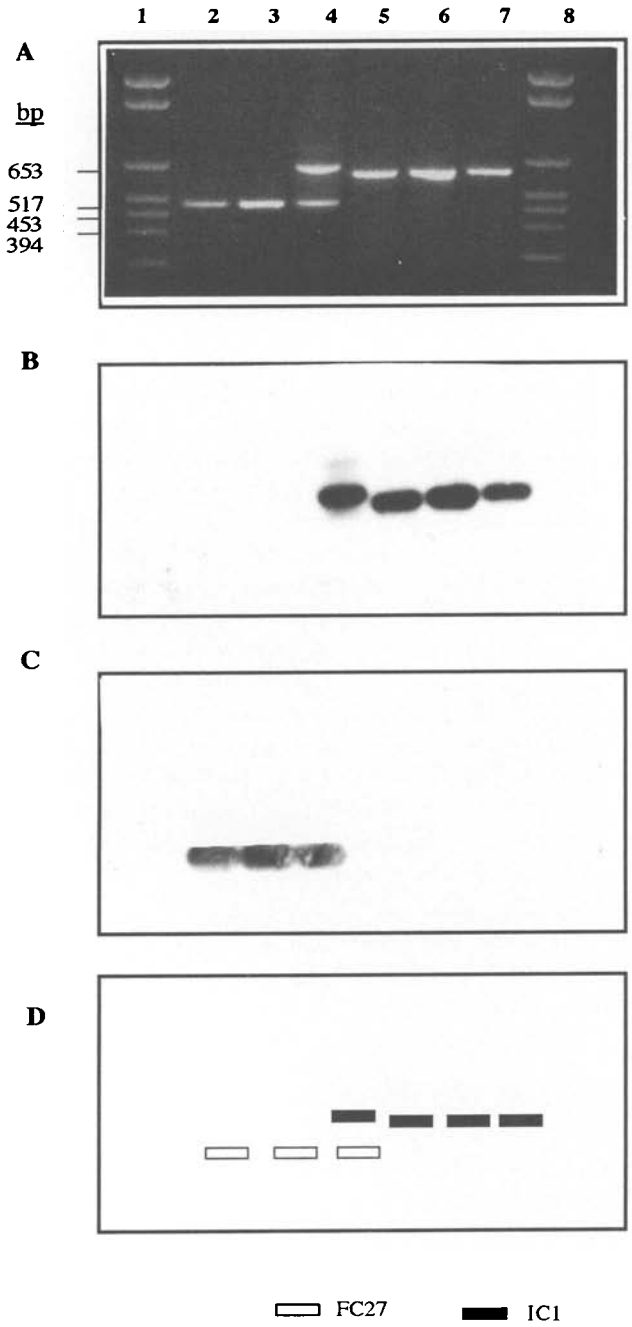

Fig. 1. Alleles of the MSP-2 gene of *Plasmodium falciparum* extracted from parasites from bloodmeals acquired by fed mosquitoes (*Anopheles gambiae* and *A. funestus*). Following electrophoresis on 1·6% agarose gel and ethidium bromide staining of PCR-amplified DNA fragments (A), blots were hybridized with allele-specific probes IC1 (B) and FC27 (C). (D) Schematic interpretation of the results. Lanes 1 and 8: size markers. lanes 2–7: bloodmeals from individual mosquitoes. Note that in the mosquito in lane 4, two alleles were detected, indicating that the bloodmeal contained parasites of mixed genotypes.

fluorescent markings 2 days, i.e. 1 gonotrophic cycle, after release in houses 8001/1 and 8004/1. All but 1 net, from house 8006/2, contained at least 1 marked mosquito (Table 2). It is clear that many of these mosquitoes had moved from the house of release (either 8001/1 or 8004/4) to different houses. Household-specific markings provided some limited evidence of a difference in the recapture pattern between the two species. For example, the majority of the recaptured *A. funestus* originally released in house 8004/4 (21/28) were found in huts of the same household (8004); conversely, 11 out of 19 recaptured *A. gambiae* originally released in household 8001 had moved between different households.

The data obtained in this study can be used to estimate the proportion of infected mosquitoes caught in the nets with holes which carry oocysts from infections in other households. This proportion is estimated by EPI, where E is the proportion of mosquitoes caught in different houses from those of their release, P is the parous rate of these mosquitoes, and I is the chance of a mosquito taking up gametocytes giving rise to oocysts. Details of the calculations involved are given in the Appendix. EPI is calculated from the data as 0·056 for *A. gambiae* and 0·0132 for *A. funestus*.

Genotypes of parasites in infected people

Allele typing of MSP-1 and MSP-2 was carried out on parasite DNA prepared from fingerprick samples of the 17 individuals found to be positive for *P. falciparum* by blood smear examination (Table 3). Twelve alleles of MSP-1, and 8 of MSP-2, distinguishable by size and/or sequence were detected. Many samples contained mixed infections, in which 2 or more alleles of 1 or both genes could be detected. Each infected individual in households 8001 and 8003, 6 of the 8 in household 8004 and 3 of the 4 in household 8006 had such mixed infections. In our previous study, mixed infections were found in approximately 85% of the inhabitants of Michenga village (Babiker *et al.* 1994*b*).

The majority of isolates contained parasites with different combinations of alleles of each gene. Exceptions were IfB18 and IfB20 in household 8004 which both contained genotypes possessing the K1/480 and IC1/520 alleles of MSP-1 and MSP-2 respectively (Table 3). This combination could also have been present in IfB11 and IfB14; however, since these isolates contained more than 1 allele at both loci, it was not possible to define the precise genotypes present without cloning. For the same reason, it is possible but not certain that other mixed isolates could have contained parasites with the same genotypes.

Genotypes of parasites in blood samples from mosquito midguts

Bloodmeals from 10 of the 12 fed mosquitoes collected in house 8001/1 gave positive PCR products for MSP-1 and/or MSP-2. Results are shown in Fig. 1 and Table 4.

Table 4. MSP-1 and MSP-2 alleles of parasites extracted from bloodmeals of mosquitoes collected in house 8001/1 in Kining'ina

(See Table 3 legend for explanation of symbols. *, Sequence not determined. N.D., Not done.)

Mosquito number	Date collected	MSP-1 alleles	MSP-2 alleles
F1	24.6.91	*470	N.D.
F2	24.6.91	*470, 540	N.D.
F3	24.6.91	*470	N.D.
F4	24.6.91	$K1^{540}$, $RO33^{470}$	$FC27^{480}$
F5	24.6.91	$K1^{560}$, $MAD20^{560}$	$FC27^{480}$
F6	24.6.91	*470	$IC1^{650}$, $FC27^{480}$
F9	25.6.91	N.D.	$IC1^{630}$
F10	25.6.91	N.D.	$IC1^{630}$
F11	25.6.91	*470, 520	N.D.
F12	25.6.91	$K1^{540}$, $RO33^{470}$	$IC1^{630}$

Table 5. Two-locus genotypes of oocysts collected from households in Kining'ina

(For household 8004, oocysts nos. If22, 23, 32, 39 and 40 were from a pooled collection of mosquitoes caught in the 4 huts of this household. See Table 3 legend for explanation of symbols. N.D., Not determined.)

Hut number	Oocyst number	Date collected	MSP-1 alleles			MSP-2 alleles	
8001/1	If14	11.6.91	$K1^{460}$	—	—	$IC^{1600.520}$	—
	If15	11.6.91	—	$MAD20^{500}$	—	—	$FC27^{600}$
	If25	11.6.91	—	$MAD20^{520}$	—	N.D.	N.D.
	If34	12.6.91	—	$MAD20^{520}$	—	N.D.	N.D.
	If42	13.6.91	$K1^{520}$	—	—	$IC1^{480}$	$FC27^{620}$
	If44	12.6.91	$K1^{540}$	—	—	$IC1^{620}$	$FC27^{480}$
	If50	18.6.91	—	$MAD20^{500}$	$RO33^{470}$	$IC1^{560}$	—
	If57	19.6.91	$K1^{520}$	—	—	$IC1^{580}$	—
	If58	19.6.91	$K1^{480}$	$MAD20^{480}$	—	$IC1^{560}$	$FC27^{520}$
	If59	19.6.91	$K1^{520}$	—	—	—	$FC27^{480}$
	If68	20.6.91	$K1^{540}$	$MAD20^{520}$	—	$IC1^{460}$	—
8003/1	If9	11.6.91	—	—	$RO33^{470}$	N.D.	N.D.
	If10	11.6.91	$K1^{520}$	—	$RO33^{470}$	—	$FC27^{480}$
	If11	11.6.91	$K1^{540}$	—	—	N.D.	N.D.
	If31	13.6.91	—	$MAD20^{520}$	—	$IC1^{680}$	—
	If73	20.6.91	$K1^{560}$	$MAD20^{520}$	—	$IC1^{600}$	—
8004/1–4	If22	12.6.91	$K1^{540}$	—	$RO33^{470}$	N.D.	N.D.
8004/1–4	If23	12.6.91	$K1^{540}$	—	—	$IC1^{680}$	—
8004/1–4	If32	13.6.91	$K1^{480}$	—	—	N.D.	N.D.
8004/1–4	If39	12.6.91	$K1^{520}$	$MAD20^{520}$	—	—	$FC27^{500}$
8004/1–4	If40	12.6.91	$K1^{500}$	—	—	$IC1^{480}$	$FC27^{620}$
8004/1–3	If48	17.6.91	$K1^{500}$	—	—	$IC1^{580;540}$	—
8004/1–4	If66	20.6.91	$K1^{520}$	$MAD20^{520}$	—	$IC1^{540}$	$FC27^{540}$
8004/1–3	If70	20.6.91	$K1^{540}$	$MAD20^{520}$	—	—	$FC27^{540}$
8004/1–2	If71	20.6.91	—	$MAD20^{520}$	$RO33^{470}$	$IC1^{650;540}$	—
8004/1–2	If72	20.6.91	$K1^{470}$	—	$RO33^{470}$	$IC1^{620;560}$	—
8004/1–2	If74	21.6.91	$K1^{540}$	—	$RO33^{470}$	$IC1^{600}$	$FC27^{450}$
8004/1–4	If77	21.6.91	$K1^{480}$	—	—	—	$FC27^{500}$
8006/1	If47	17.6.91	$K1^{520}$	—	$RO33^{470}$	$IC1^{600;540}$	—
	If51	18.6.91	$K1^{480}$	—	$RO33^{470}$	$IC1^{580;540}$	—
	If61	19.6.91	$K1^{520}$	—	$RO33^{470}$	$IC1^{580}$	$FC27^{520}$
	If62	19.6.91	$K1^{520}$	—	—	N.D.	N.D.
	If63	19.6.91	$K1^{540}$	—	$RO33^{470}$	$IC1^{620;500}$	—
	If69	20.6.91	$K1^{560}$	—	$RO33^{470}$	—	$FC27^{520}$
	If75	21.6.91	$K1^{520}$	—	$RO33^{470}$	$IC1^{600}$	—
	If76	21.6.91	—	—	$RO33^{470}$	$IC1^{600}$	—

Table 6. Comparison of allele frequencies of the MSP-1 gene between Kining'ina hamlet and Michenga village, and between individual households in Kining'ina and Michenga village

(Data for Michenga village are from Babiker *et al.* (1994*b*).)

	K1 (%)	MAD20 (%)	RO33 (%)	χ^2	*P* value
Michenga (all houses)	30 (46)	18 (27)	18 (27)		
Kining'ina (all houses)	42 (55·2)	17 (22·2)	17 (22·2)	1·36	0·71
8001/1	12 (54·5)	9 (41)	1 (4·5)	6·51	0·039
8003/1	4 (40)	3 (30)	3 (30)	0·11	0·95
8004/1–4	15 (62·5)	4 (16·7)	5 (20·8)	2·15	0·34
8006/1	11 (55)	1 (5)	8 (40)	5·66	0·059

* Likelihood ratio chi-squared (2 D.F.), to test whether the distribution of alleles in households in Kining'ina differs from that in Michenga.

Table 7. Comparison of allele frequencies of MSP-2 within households of Kining'ina

(Likelihood ratio chi-squared (3 D.F.), to test whether the distribution of alleles differs between households in Kining'ina. χ^2, 1·47; $P = 0·69$.)

House number	IC1 (%)	FC27 (%)	Total
Michenga (all houses)	33 (53)	29 (47)	62
Kining'ina (all houses)	36 (60)	24 (40)	60
House 8001/1	11 (61)	7 (39)	18
House 8003/1–3	4 (67)	2 (33)	6
House 8004/1–4	10 (50)	10 (50)	20
House 8006/1–4	11 (69)	5 (31)	16

The parasites in these mosquitoes were most probably derived from the inhabitants of the hut in which they were caught. However, it cannot be entirely excluded that they might have come into the huts with a bloodmeal taken elsewhere, or that they might have contained oocysts from previous feeds. Only parous mosquitoes could have had developing oocysts from previous feeds, and only immigrant mosquitoes would have been infected elsewhere.

Oocyst genotypes

A total of 951 mosquitoes were examined for oocysts 5–7 days after collection (Table 1). Of these, 110 contained developing oocysts, 36 of which contained only single oocysts. These were examined for MSP-1 and MSP-2 alleles (Table 5). The remaining 74 infected mosquitoes continued 2 or more oocysts, and these were not studied further.

Fourteen alleles of MSP-1 and 20 of MSP-2 were detected. In 7 oocysts (If15, If23, If31, If57, If59, If77 and If76), only 1 type of allele of each gene was detected. These oocysts had most probably derived from homozygous zygotes, resulting from self-fertilization events between identical gametes, although it cannot be entirely excluded that they were heterozygous at other loci not examined here. Oocysts with different alleles of one or both genes (e.g. If42, If44, etc.) had clearly originated from heterozygotes, derived from crossing between unlike gametes (Ranford-Cartwright *et al.* 1993; Babiker *et al.* 1994*b*). Twenty-three oocysts were in this category. Six oocysts which produced positive results only for MSP-1 were homozygous for this gene, but in the absence of MSP-2 data it is not known whether they were derived from zygotes homozygous at both loci.

The oocysts found in the mosquitoes kept in the insectary 5 days had almost certainly derived from the inhabitants of the hut in which they were caught, although it cannot be entirely ruled out that they had developed from gametocytes in a blood meal taken elsewhere. It is also possible that sporozoites resulting from earlier feeds by the mosquitoes could have been present around the midguts which could have been the source of some PCR products; however, we consider this to be unlikely because of the washing of each midgut sample before preparation for PCR.

Comparisons of MSP-1 and MSP-2 allele frequencies of oocysts from different households

To investigate the possibility of spatial clustering of the parasites, frequencies of the alleles of each gene in oocysts found in Kining'ina were calculated and compared to those of Michenga village as a whole, using the likelihood chi-squared test. In view of the small number of samples involved, the alleles were 'binned' into groups classified by sequence only (Babiker *et al.* 1994*b*). Table 6 shows the results for MSP-1. No significant differences were seen in these frequencies ($\chi^2 = 1·36$; $P = 0·71$). The frequencies of the MSP-1 alleles found in each separate house-

hold were also compared to those of the whole village. Those of households 8003, 8004 and 8006 were not significantly different; however, in household 8001 the χ^2 test indicated a border value ($\chi^2 = 5{\cdot}66$; $P = 0{\cdot}059$) (Table 6).

The frequencies of MSP-2 alleles showed no significant differences between the households (Table 7) ($\chi^2 = 1{\cdot}47$; $P = 0{\cdot}69$), or between Kining'ina as a whole and Michenga ($P > 0{\cdot}5$).

DISCUSSION

The most striking finding of this study is the remarkable degree of genetic diversity which occurs in *P. falciparum* in individual households in the hamlet of Kining'ina. The fingerprick blood samples and the mosquito bloodmeals illustrate clearly the numbers of infections in the inhabitants containing mixtures of clones. The oocysts show that an extensive degree of crossing between clones occurs in this small community. The mosquito capture–recapture data illustrate that considerable gene flow is likely to occur among the parasites of the households. Taken together, the findings illustrate emphatically the inappropriateness of the idea that *P. falciparum* in such communities consists of a collection of distinct 'strains'.

The information obtained from the single hut 8001/1 provides a good example of the complexity of infection in just a single family. Fig. 2 illustrates the MSP-2 alleles of parasites of fingerprick samples of 2 inhabitants of this hut, and in the bloodmeals and oocysts of mosquitoes caught there. The oocysts possessed very diverse alleles of both MSP-1 and MSP-2, in homozygous and heterozygous combinations. These alleles can only have derived from the gametocytes taken up by the mosquitoes, but most were not present in the blood samples examined. While it is possible that some of the oocysts were the result of feeds on inhabitants of other houses in the area, it is more likely that the inhabitants of hut 8001/1 did indeed contain parasites with these alleles at the times when the mosquitoes fed. Studies in Papua New Guinea have shown that marked changes in *P. falciparum* genotypes occur in a single patient over periods as short as 3 days (M. C. Bruce and M. Walmsley, unpublished observations).

Three of the 5 members of household 8001 were positive for *P. falciparum* on the day of sampling. Each of the 3 contained mixed clones, as shown by the presence of 2 alleles of 1 or both genes. Their parasites contained a total of 5 alleles of MSP-1 and 4 of MSP-2. Certain alleles were found in 2 of the 3 people, e.g. FC27/480 of MSP-2 in IfB3 and IfB4, while others were present in only one, e.g. IC1/650. Both these alleles were found in the bloodmeals of mosquitoes caught in this hut 3 and 4 days later, implying that parasites with these alleles were still present in the inhabitants at the time of the feed. The FC27/480 allele was also found in the oocyst in mosquito If59 caught on 19 June, as well as in If44 caught 1 week earlier.

A similar degree of parasite diversity was observed in each of the other Kining'ina households. This was not unexpected in such a highly endemic situation, where each unprotected individual can receive an annual average of 329 infective bites (Smith *et al.* 1993) and where there is free movement by vectors between households. The mosquito sampling showed that 20% of the *A. gambiae* and 10% of the *A. funestus* were infected. Ignoring the mosquitoes that we did not collect, this means that parasites were developing in at least 18 *A. gambiae* and 20 *A. funestus* in the hamlet every day of the study.

A few instances were found of parasites of similar genotypes in different people in the same house, which were most probably due to infection from a common mosquito; a similar explanation was used by Conway & McBride (1991*b*) for parasites containing identical alleles of MSP-1 and MSP-2 in family members of a village in The Gambia.

We have found no evidence of clustering of *P. falciparum* genotypes among the Kining'ina households. The frequencies of alleles found in individual oocysts are more precise than those estimated from those of blood forms alone (Babiker *et al.* 1994*b*). These data showed no significant difference in allele frequencies of either gene among the households of Kining'ina and Michenga village, with the exception of MSP-1 in household 8001. We consider that this result was most probably produced by the small numbers of oocysts examined in the study, rather than by the spatial isolation of this household from the others. When the information obtained from blood samples was taken together with the oocysts, the numbers of alleles of MSP-1 and MSP-2 found were comparable to those in Michenga village as a whole; a total of 17 alleles of MSP-1 and 24 of MSP-2 were detected in 53 blood samples and 71 oocysts from the whole village (Babiker *et al.* 1994*b*), while there were 14 and 18 respectively in the Kining'ina households.

In summary, while the number of samples studied here was small, there was no evidence for spatially structured parasite populations within which mating could be restricted, as suggested in the Papua New Guinea studies by Day *et al.* (1992). The high level of genetic diversity among the parasites studied here is a consequence of the intensity of transmission and sexual reproduction among these parasites. It is possible that a larger survey involving more remote houses in this area might produce evidence of spatial variations. It would also be of considerable interest to compare the findings obtained in this highly endemic area with those in an area of much lower

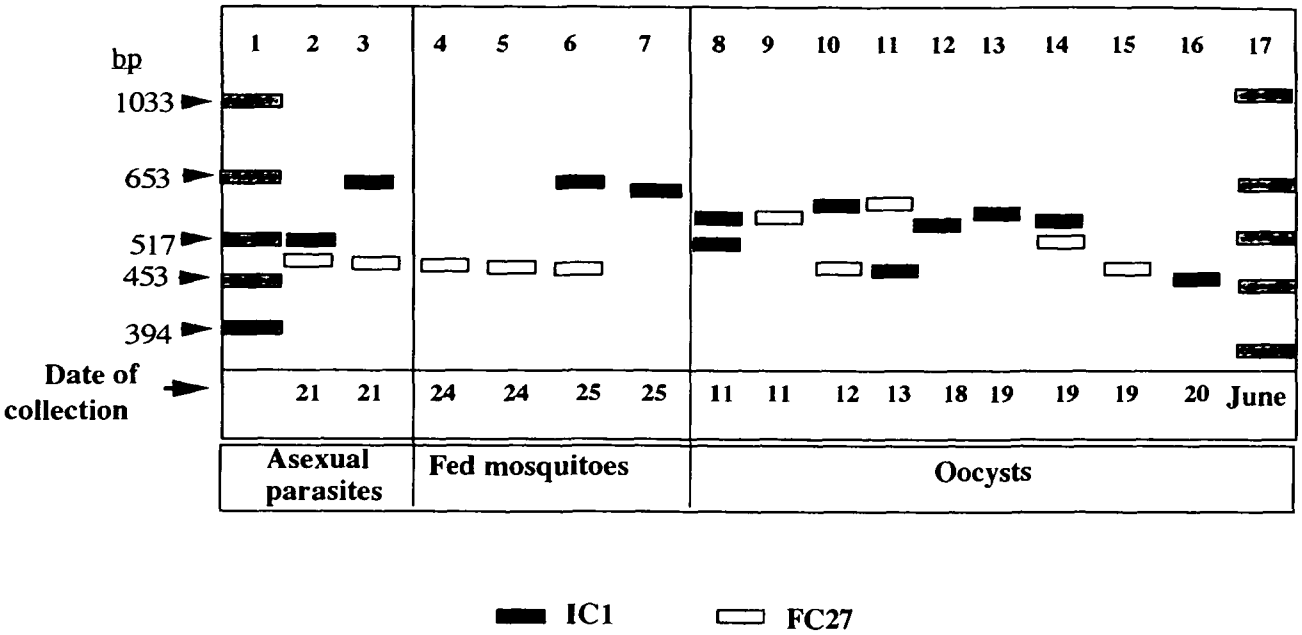

Fig. 2. Schematic illustration of the MSP-2 alleles of parasites in fingerprick blood samples (lanes 2 and 3), bloodmeals from fed mosquitoes (lanes 4–7), and oocysts from mosquitoes (lanes 8–16) collected from hut number 8001/1. Alleles are classified by size and sequence of PCR-amplified fragments. Lanes 1 and 17: size markers.

intensity of transmission, to determine whether such spatial clustering of parasites does occur in such places.

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APPENDIX

Estimate of the proportion of infected mosquitoes caught in nets with holes which carry oocysts from infections in different households

To estimate the proportion of infected mosquitoes caught in the nets with holes in each house in Kining'ina which carry oocysts from gametocytes acquired elsewhere, the following assumptions are made. (i) The chances of mosquitoes acquiring gametocytes giving rise to any oocysts (I) and to single oocysts (I') per feed are constant for all days and households. (ii) Oocysts detected may be derived from a previous feed, but rarely from an even earlier one. (iii) Parous mosquitoes move between households with probability E between feeds, E being the emigration rate which is constant over houses and time. (iv) The system is closed. (v) Intensity and risk of infection are independent of mosquito parity, infection and migration status.

Then, before feeding (a) Proportion of immigrant mosquitoes with any oocysts = I. (b) Proportion of immigrant mosquitoes with single oocysts = I'. (c) Proportion of immigrant mosquitoes with more than 1 oocyst = $I-I'$. (d) Proportion of fed mosquitoes which are parous = P. (e) Proportion of fed mosquitoes which are immigrants = EP. (f) Proportion of fed mosquitoes already having a single oocyst infection = PI'. (g) Proportion of fed mosquitoes already having any oocyst infection = PI. (h) Proportion of fed mosquitoes already having an alien single oocyst infection = EPI'. (i) Proportion of fed mosquitoes already having any alien oocyst infection = EPI.

After feeding, among fed mosquitoes in the net:- (j) Overall proportion of mosquitoes with an alien single oocyst infection and no superinfection = $EPI'(1-I)$. (k) Overall proportion of mosquitoes with a new single oocyst infection and no previous infection = $(1-PI)I'$. (l) Overall proportion of mosquitoes with a single oocyst (R') = $EPI'(1-I)+(1-PI)I'$. (m) Overall proportion of uninfected mosquitoes = $(1-PI)(1-I)$. (n) Overall proportion of infected mosquitoes (R) = $I(1+P-PI)$.

Solving for I, we obtain:

$$I = (-1-P \pm \sqrt{(1+2P+P^2-4PR)}/-2P.$$

To obtain I' we rearrange (l), to give $I' = R'/(EP(1-I)+(1-PI)$. In the current study, the relevant figures are as follows.

	A. gambiae	*A. funestus*
E	(11/19) 0·58	(7/28) 0·25
P	0·77	0·82
R	0·21	0·11
R'	(36/951) 0·0379*	(36/951) 0·0379*
I	0·125	0·062
I'	0·029	0·033
EPI	0·056	0·013

* Data for *A. gambiae* and *A. funestus* aggregated.

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