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Population structure of *Plasmodium falciparum* gametocyte sex ratios in malarious children in an endemic area

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Abstract

The gametocyte sex ratio (proportion of gametocytes that are male) of *Plasmodium falciparum* may influence transmission. The distribution of *P. falciparum* sex ratios, the extent of inbreeding, the relationship between clone multiplicity and sex ratio, and the pre- and post-treatment factors influencing a sex ratio of 0.5 were determined in 1609 children, with acute malaria. Gametocytes were sexed by morphological appearance and asexual clone multiplicity was determined by polymerase chain reaction (PCR) using polymorphic loci of merozoite surface proteins-1 and -2 (MSP-1, MSP-2) and glutamine-rich protein (GLURP). The weighted mean population sex ratio on presentation in 162 gametocyte carriers was 0.22, that is, 3.5 female to 1 male (95% CI 0.15–0.28), with an estimated inbreeding rate (f) (the proportion of a mother's daughters that is fertilized by her sons) of 0.56 (95% CI 0.44–0.70). Sex ratio was significantly higher when clone multiplicity was >1 infecting clone than when it was 1 ($P=0.02$). The frequency of a pre-treatment sex ratio of 0.5 was low (3%), and was significantly increased by non-artemisinin but not by artemisinin – mono or combination – drugs by day 7 after therapy commenced ($P=0.03$ and $P=0.44$, respectively). No factor was associated with a pre-treatment sex ratio of 0.5 but two factors were independent predictors of a sex ratio of 0.5 by day 7 after therapy commenced: an age ≥ 5 years and anaemia. These population data provide some empirical support for the predictions of local mate competition (LMC) theory and, in conjunction with effects of antimalarials on a sex ratio of 0.5, may have implications for malaria control efforts in endemic settings.

Keywords

P. falciparum; Gametocytes; Sex ratio; Transmission; Antimalarials; Children; Nigeria

1. Introduction

One of the most crucial steps in malaria transmission is the uptake and the development into gametes in the mosquito vector, of the gametocytes of *Plasmodium* spp. obtained from the host blood meals [1]. In the blood meals both female and male gametocytes need be present in

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appropriate densities and proportions and be viable to ensure successful fertilization of the female by the male gametes. In this context, the gametocyte sex ratio (proportion of gametocytes that are male [2–4]), may influence transmission. In *Plasmodium falciparum*, a male gametocyte can differentiate into a maximum of eight gametes and the female to one gamete within the mosquito vector [5].

Following a blood meal, mating occurs among parasite clones present in the few microlitres of blood taken up by the mosquito vector. If there are low numbers of clones present in the blood meal, inbreeding will occur and lead to local mate competition. The fewer the clones the more female biased the sex ratio is predicted to be, in order to minimize competition among brothers (that is, males from the same clone) and maximize the number of zygotes that can be formed. ‘Local mate competition’ (LMC) theory [6] predicts that the optimal sex allocation strategy, defined by the equation $z^* = (1 - f)/2$, where z^* is the proportion of male relative to female and f is Wright’s coefficient of inbreeding is 0.5; that is, when inbreeding does not occur, $f=0$, and there is complete crossing. Complete crossing is more likely when the number of co-infecting genotype is large [7]. Thus, if one assumes that sex ratio is influenced by the inbreeding rate, then the optimal sex ratio favored by natural selection should vary from 0.5 for complete crossing to 0 for complete inbreeding (selfing) [4]. This is because the sex ratio could also be influenced by among other factors anaemia, immunity etc. and in this case one might even predict a sex ratio over 0.5 (if for example, on average, less than 1 gamete is produced per male gametocyte).

In natural populations, *Plasmodium* spp. gametocyte sex ratio is female biased, but this ratio can vary significantly depending upon other biological variables such as parasite density and transmission rates, and during the course of individual infections [8–10].

Most *P. falciparum* infections in children in Ibadan, southwestern Nigeria, an endemic area of malaria [11], are multiple, with an average of 3–4 genotypes per child [12–14] suggesting some degree of outcrossing. Although *P. falciparum* population sex ratio in the area is female biased, the extent of female bias is unknown and individual infections tend to show significant variations as infection progresses or as antimalarial drugs are administered [15–17]. In this endemic population and many others from Africa, the theories and predictions of LMC have not been tested or evaluated. In addition, the frequency of a sex ratio of 0.5 (the sex ratio that is independent of selfing rate) at presentation in malarious children and the factors influencing this ratio as infection progresses or drugs are administered are unknown. Such information, and the optimal sex ratios, that maximize transmission in endemic communities, need be elucidated if strategies for the control of transmission are to be optimized in endemic populations.

In the present study we: evaluate the distribution of *P. falciparum* gametocyte sex ratios, use sex ratio data to estimate inbreeding levels as predicted by LMC theory, evaluate the relationship between asexual clone multiplicity and sex ratio since a key prediction of LMC theory is that parasites adjust their sex ratios in response to genetic diversity, and determine the factors influencing a sex ratio of 0.5 pre- and post-treatment in a group of children with acute *P. falciparum* malaria in an endemic area of southwestern Nigeria.

2. Materials and methods

2.1. Patients

Patients were recruited from 1999 to 2006 at the malaria clinic of the University College Hospital in Ibadan, southwest Nigeria, an endemic area of malaria [11] into various antimalarial efficacy studies and were enrolled if the following criteria were met: an age 0.5–14 years, fever or history of fever in the 24–48 h preceding presentation, pure *P. falciparum* parasitaemia

$\geq 2000/\mu\text{l}$ blood, absence of concomitant illness, negative urine tests for 4-aminoquinolines (i.e. chloroquine or amodiaquine) (Dill-Glazko) and sulfonamides (i.e. sulfadoxine or sulfalene) (lignin), and written informed consent of a parent or guardian. Patients with severe malaria [18] or serious underlying diseases (renal, cardiac or hepatic) or severe malnutrition were excluded from the study. The studies received approval from the local ethics committee.

2.2. Drug management

Drug treatment was according to standard schedules and has been described in detail elsewhere [19,20]. Briefly, at presentation, and for a few days after, depending on the treatment regimen, the children were given, orally, standard doses of chloroquine, amodiaquine, pyrimethamine–sulphadoxine, pyrimethamine–sulphadoxine plus probenecid, cotrimoxazole, artesunate, artemether–lumefantrine, amodiaquine plus artesunate, and amodiaquine plus sulfalene pyrimethamine. At enrolment (day 0) and at follow-up on days 1–7, 14, 21, and 28 (up to 2003) and on 1–3, 7, 14, 21, 28, 35 and 42 (after 2003), patients underwent full physical examination and thin and thick blood films examination for quantification of asexual and sexual parasitaemia.

2.3. Laboratory investigations

Quantification of asexual and sexual parasites in thick films was done against 500 and 1000 leukocytes, respectively assuming a leukocyte count of $6000/\mu\text{l}$ blood. All gametocytes were sexed if gametocytaemia $\geq 10/\mu\text{l}$ blood and according to the following criteria [21]: males (microgametocytes) are smaller than females (macro-gametocytes), the nucleus is larger in males than females, the ends of the cells are rounder in males and angular in females, with Giemsa the cytoplasm stains purple in males and deep blue in females, and the granules of malaria pigment are centrally located in females and more widely scattered in males. The sex ratio was defined as the proportion of gametocytes that were male [2–4].

Blood obtained from a finger prick into heparinized capillary tubes was used to estimate the haematocrit. In addition, two drops of blood were blotted onto 3MM filter paper at enrolment and follow-up for DNA extraction and asexual parasite population structure characterization by polymerase chain reaction (PCR).

2.3.1. PCR determination of parasite population in patient isolates of *P. falciparum*

—*P. falciparum* loci that exhibit repeated numbers of polymorphisms to distinguish between different parasite populations were used for characterization of *P. falciparum* population structure in each infection. Briefly, block2 of merozoite surface protein-1 (MSP-1), block3 of MSP-2, and region II of glutamine-rich protein (GLURP) were amplified by nested PCR using primers and amplification conditions described previously [12–14]. Ten microlitres of the PCR products was resolved by electrophoresis on a 2% agarose gel and sized against 100-basepair (bp) molecular weight marker (New England Biolabs, Beverly, MA). Primers sequences and PCR conditions for the nested PCR strategy are described in Table 1.

Each *P. falciparum* infection was characterized on the basis of the fragment size of the PCR products for each locus and determining size of the alleles of *MSP-1*, *MSP-2* and *GLURP*. Infections were defined as polyclonal if parasites from the same patient showed more than one allele on one or more genes. If an isolate had one allele at each of the three loci, the clone was taken to be one. The complexity of infection was calculated for each typing reaction (*MSP-1*, *MSP-2* and *GLURP*) independently as the average number of distinct fragments per PCR-positive sample.

2.4. Data analysis

Data were analyzed using version 6 of the *Epi-Info* software [22], and the statistical programme *SPSS for Windows* version 10.01 [23]. Variables considered in the analysis were related to the densities of *P. falciparum* gametocytes and trophozoites. Proportions were compared by calculating χ^2 with Yates' correction or by Fisher exact test. Normally distributed, continuous data were compared by Student's *t*-test, and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann–Whitney *U*-tests or the Kruskal–Wallis tests or by Wilcoxon ranked sum test. A multiple logistic regression model was used to test the association between a sex ratio=0.5 (yes or no at presentation or during follow-up) and factors that were significant at univariate analysis: an age ≥ 5 years and a haematocrit $< 30\%$. Because the study was conducted over a period of eight years, time in years since the commencement of the study was included as a covariate in the model for pre-treatment and post-treatment sex ratio of 0.5. All tests of significance were two-tailed. *P* values of ≤ 0.05 were taken to indicate significant differences. Data were (double)-entered serially using the patients' codes and were only analyzed at the end of the study.

3. Results

3.1. Patients

The characteristics of the 1609 children with acute falciparum malaria who were enrolled in the study have been reported elsewhere [24]. Of these children, 162 were gametocyte carriers. The characteristics of the gametocyte carriers are shown in Table 2.

3.1.1. Population structure of *P. falciparum* gametocyte sex ratios on day 0—

Fig. 1 (day 0) shows the pre-treatment distribution of *P. falciparum* sex ratios. The weighted mean population sex ratio was 0.18 (95% confidence interval, CI, 0.13–0.25). If a more reliable estimate of gametocyte density $\geq 15/\mu\text{l}$ were used, the mean population sex ratio in gametocyte carriers was 0.22, that is, 3.5 female to 1 male (95% CI 0.15–0.28). Therefore the inbreeding rate given by the equation, $f=1-2z^*$ [3,25,26], where z^* is the proportion of male relative to female, is 0.56 (95% CI 0.44–0.70). Extremes of sex ratios were as follows: 0 in thirty one children (19%) and 1 in three children (2%); a sex ratio of 0.5 was seen in 4 children (3%).

3.1.2. Clone multiplicity and sex ratio—In order to examine the effect of clone multiplicity on sex ratio, we excluded from the analyses all children with potential confounding factors: those with anaemia (haematocrit < 30) and low gametocytaemia ($< 15/\mu\text{l}$ blood) at enrolment. Clone data were available for analyses in 49 children who had gametocytaemia at enrolment and for additional 50 children who did not carry gametocyte during the three days of commencing therapy. In the latter children, evaluation of the relationship between clone multiplicity and sex ratio at presentation was not possible. Mean number of *P. falciparum* infecting clone in the 49 children with gametocytaemia at enrolment was 3.4 ± 1.5 (sd), range 1–8. The mean sex ratio when patients were infected with one parasite clone was 0 ($n=6$), 0.12 ± 0.04 (standard error of mean, sem) when the infecting parasite clone was 2 ($n=15$), 0.27 ± 0.1 when the infecting clone was 3 ($n=10$), 0.15 ± 0.07 when the infecting was 4 ($n=8$), and 0.14 ± 0.08 when the infecting clone was > 4 ($n=10$) (Fig. 2). Wilcoxon rank sum test showed that sex ratio was significantly higher ($F=-3.06$, $P<0.001$) when *P. falciparum* clone multiplicity was 2 compared to when the number of parasite clone infecting the patient was 1. Similarly, sex ratio was significantly higher ($F=-2.81$, $P<0.001$) when clone multiplicity was 3 than when it was 2. The sex ratio when 4 or > 4 clones are present is not significantly different from when 3 clones are present.

In children with 1 parasite clone, the mean sex ratio of 0 was significantly lower than the mean sex ratio of 0.29 ± 0.03 (sem) when parasite clone was > 1 ($F=-2.22$, $P=0.02$). However, in

children with ≤ 2 parasite clones, the mean sex ratio of 0.17 ± 0.04 (sem) was not significantly different from a mean sex ratio of 0.30 ± 0.04 (sem) when the parasite clone was > 2 ($F = -1.78$, $P = 0.07$).

3.1.3. Temporal changes in sex ratio of 0.5—Four out of 71 children who had reliable estimates of gametocyte density had a sex ratio of 0.5 prior to treatment (Fig. 1, day 0). Overall, the number of patients with gametocyte sex ratio of 0.5 increased to 9 of 54, and 11 of 51 by days 3 and 7, respectively (Fig. 1). The increases on days 3 and 7 were significantly greater than on day 0 ($\chi^2 = 7.01$, $df = 2$, $P = 0.03$). However, the increase on day 7 was not significantly different from that on day 3 ($\chi^2 = 0.15$, $P = 0.69$). In children treated with amodiaquine ($n = 52$), 1 of 25, 6 of 22 and 7 of 19 children had a sex ratio of 0.5 at enrolment and on days 3 and 7, respectively. The increases on days 3 and 7 were significantly greater than on day 0 ($\chi^2 = 7.69$, $df = 2$, $P = 0.02$). However, the increase on day 7 was not significantly different from that on day 3 ($\chi^2 = 0.10$, $P = 0.7$). In general, in children treated with non-artemisinin drugs (chloroquine, pyrimethamine–sulphadoxine, pyrimethamine–sulphadoxine plus probenecid, cotrimoxazole) 3 of 51, 9 of 41, and 10 of 42 children had a sex ratio of 0.5 at enrolment and on days 3 and 7, respectively ($\chi^2 = 6.71$, $df = 2$, $P = 0.034$). However, the increase on day 7 was not significantly different from that on day 3 ($\chi^2 = 0.04$, $P = 0.8$). In children treated with artemisinin mono- or combination-therapy (artesunate, artemether–lumefantrine or artesunate plus amodiaquine) ($n = 35$), the corresponding values were 1 of 17, 0 of 11 and 1 of 7, respectively ($\chi^2 = 1.62$, $df = 2$, $P = 0.44$). Only one of the children treated with artemisinin derivatives had a sex ratio of 0.5 during the entire period of follow-up.

3.1.4. Antimalarial drugs and time to attainment of a sex ratio of 0.5 after start of treatment—Of the children who did not have a sex ratio of 0.5 at enrolment, 20 had a sex ratio of 0.5 within 7 days. Because of the small numbers in each treatment group 4, 12, 1, 1, 1, 1, treated with chloroquine, amodiaquine, pyrimethamine–sulphadoxine, pyrimethamine–sulphadoxine plus pro-benecid, cotrimoxazole and artesunate, respectively, these were divided into two groups for the purpose of analysis: non-artemisinin and artemisinin. In the non-artemisinin group ($n = 19$), the mean time was 1.14 ± 0.38 days (95% CI 0.79–1.49, $n = 19$). The only patient treated with an artemisinin had an unbeatable sex ratio of 0.5 on day 7.

3.1.5. Predictors of a sex ratio of 0.5 pre- and post-treatment—At presentation, the following factors were found not to be associated with a sex ratio of 0.5: age, gender, duration of illness before presentation, haematocrit, and fever. Table 3 shows that in a multiple regression model, an age ≥ 5 years and a haematocrit $< 30\%$ at enrolment are independent predictors of a sex ratio of 0.5 within seven days after initiation of treatment.

4. Discussion

Plasmodium species sex ratios are often female biased [2–4,7–9,27,28], and many workers have suggested that local mate competition may be responsible [29] and for the female-biased sex ratios in other apicomplexan parasites [27]. In the present study, the weighted mean population sex ratio was 0.22 suggesting that this is the maximum degree of female bias expected if the number of viable gametes released after exflagellation, by one male gametocyte was four [25]. It is of note that the mean population sex ratio in this endemic area is similar to that reported from Yaounde, Cameroon (0.217, [28]), higher than that reported from Papua New Guinea (PNG) (0.18, [30]) suggesting a higher potential for outcrossing in southwestern Nigeria where transmission intensity and hence the average number of clones per host of 3–4 [12–14], are greater than in PNG. However, the sex ratio is somewhat lower than 0.346 reported in Dielmo, Senegal [9]. It is of note that each infection is important.

Intriguingly in a fifth of the infections no male gametocytes were found. Similarly, in 2% of the infections no female gametocytes were found; we wonder why these are so, and how these parasites would transmit. However, both extremes raise a question: in nature (and in children with acute falciparum malaria), what factors predict extremes of sex ratios and how can sex ratio be manipulated genetically, drug- or chemical-wise to produce these extremes?

Exploration of these factors would contribute to designing modalities for reducing transmission. The low frequency (3%) at enrolment of a sex ratio of 0.5 suggests that gamete contribution from many genotypes (clones) which will maximize the genetic representation of each clone in the zygote population is uncommon.

Nee et al. [26] argued that since sex ratio can be predicted from the inbreeding rates, sex ratio data offer a relatively cheap and easy method for indirectly estimating inbreeding rate with respect to population structure in malaria parasites. In addition, these authors suggested that it may be preferable to measure inbreeding rate (f) in malaria and other parasites by measuring sex ratios. We used the sex ratios of parasite population in naturally infected children to determine inbreeding rate in malaria parasites from this endemic area. This rate, 0.44–0.70, provides a baseline for which future changes in inbreeding rate in malaria parasite population from this area may be measured or compared, and may be relevant in the evolution of drug resistance to the newly introduced artemisinin-based combination therapies (ACTs) in the region in the future. The latter drugs are known to reduce gametocyte carriage and intensity of transmission [31] and to favor the emergence of a female-biased sex ratio following therapy [15,24].

The extent of local mate competition clearly depends on how many different *P. falciparum* clones are circulating in the blood of an infected host. Using polymerase chain reaction (PCR) technique, we were able to detect on the average 3–4 clones per patient, and in about 5% of the children more than six parasite clones were detected. In our data set, it would appear that sex ratio was more female biased when there was one infecting parasite clone compared with more than one infecting parasite clone. These findings provide some support for the local mate competition theory that predicts that, the greater the clone multiplicity within the host, the less female biased the sex ratio should be [7,26].

It is generally known that in animal infections, female-biased sex ratio decreases as the infection progresses [7,8,32] usually as a response to anaemia, host immune response and parasite density or competition from other parasite genotypes. In our data set female-biased sex allocation in response to local mate competition also decreased not only as response to competition from other genotypes at enrolment, but also as a response to drug treatment particularly to non-artemisinin antimalarials during and after the course of treatment. There are several reasons for the latter effect. (i). Drug-related fall in haematocrit: non-artemisinin drugs produce significantly greater fall in haematocrit than artemisinin drugs in children from this endemic area [33]. (ii). Antimalarial drugs may increase the circulating half life of male gametocytes [24]. (iii). A form of fertility insurance as parasites adjust their reproductive strategies to maximize transmission [4]. It is unclear why parasites fail to increase their allocation to males during and after treatment with artemisinin derivatives: with these drugs, a sex ratio of 0.5 was only observed in one child throughout the entire follow-up period. Understanding the mechanism(s) of how artemisinins produce this effect may be crucial to designing new drugs to reduce transmission. It has been suggested [15,16,24] that artemisinin drugs may selectively kill male gametocytes or prolong the survival of female gametocytes, but larger, longer and more sophisticated studies are needed to confirm these suggestions.

A sex ratio of 0.5 may be indicative of low or no inbreeding. Theoretically, this ratio might indicate a high level of recombination among different parasite genotypes. This would allow the generation of parasite genotypes with new diversity and chromosomal rearrangements that

may have varying degrees of virulence, drug susceptibility profiles and immune responses. The low frequency of this sex ratio in parasite population pre-treatment suggests that inbreeding is relatively common. However, its increased frequency post-treatment indicates that a sex ratio of 0.5 occurs more frequently due to non-artemisinin drugs, increasing immunity (≥ 5 years), and anaemia (Table 2). These three factors are more likely to have negative impact on male than female gametocytes [8,9,34], and lead to the production of male gametocytes as a form of fertility insurance [4]. The frequent occurrence of malaria-associated anaemia in children [35], its association with increased gametocyte carriage [36], and the proclivity of drug-related increased frequency of a sex ratio of 0.5 may have implications for transmission in endemic areas.

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References

1. Sinden RE. Sexual development of malaria parasites. *Adv Parasitol* 1983;22:153–216. [PubMed: 6141715]
2. Pickering J, Read AF, Guerrero S, West SA. Sex ratio and virulence in two species of lizard malaria parasites. *Evol Ecol Res* 2000;2:171–84.
3. West SA, Reece SE, Read AF. Evolution of gametocyte sex ratios in malaria and related apicomplexan (protozoan) parasites. *Trends Parasitol* 2001;17:525–31. [PubMed: 11872397]
4. West SA, Smith TG, Nee S, Read AF. Fertility insurance and the sex ratios of malaria and related hemosporin blood parasites. *J Parasitol* 2002;88:258–63. [PubMed: 12053995]
5. Sinden RE, Canning EU, Bray RS, Smalley ME. Gametocytes and gamete development in *Plasmodium falciparum*. *Proc R Soc Lond Ser B* 1978;201:375–99. [PubMed: 27809]
6. Hamilton WD. Extraordinary sex ratios. *Science* 1967;156:477–81. [PubMed: 6021675]
7. Reece SE, Drew DR, Gardner A. Sex ratio adjustment and kin discrimination in malaria parasites. *Nature* 2008;453:609–14. [PubMed: 18509435]
8. Paul REL, Coulson TN, Raibaud A, Brey PT. Sex determination in malaria parasites. *Science* 2000;287:128–31. [PubMed: 10615046]
9. Robert V, Sokhna CS, Rogier C, Arieu F, Trape JF. Sex ratio of *Plasmodium falciparum* gametocytes in inhabitants of Dielmo, Senegal. *Parasitol* 2003;127:1–8.
10. Reece SE, Duncan AB, West SA, Read AF. Host cell preference and variable transmission strategies in malaria parasites. *Proc R Soc Lond Ser B* 2005;272:511–7.
11. Salako LA, Ajayi FO, Sowunmi A, Walker O. Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol* 1990;84:435–45. [PubMed: 2256767]
12. Happi CT, Gbotosho GO, Falade CO, Akinboye DO, Gerena L, Hudson T, et al. Point mutation in the *pfprt* and *pfmdr-1* genes of *Plasmodium falciparum* and clinical response to chloroquine among malaria patients from Nigeria. *Ann Trop Med Parasitol* 2003;97:439–51. [PubMed: 12930607]
13. Happi CT, Gbotosho GO, Sowunmi A, Falade CO, Akinboye DO, Hudson T, et al. Molecular analysis of *Plasmodium falciparum* recrudescence malaria infections in children treated with chloroquine in Nigeria. *Am J Trop Med Hyg* 2004;70:20–6. [PubMed: 14971693]
14. Happi CT, Gbotosho GO, Folarin OA, Bolaji OM, Sowunmi A, Kyle DE, et al. Association between mutations in *pfprt* and *pfmdr-1* genes and *in vivo* amodiaquine resistance in falciparum malaria-infected children in Nigeria. *Am J Trop Med Hyg* 2006;75:155–61. [PubMed: 16837724]

15. Sowunmi A, Balogun T, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA. Activities of amodiaquine, artesunate, and artesunate–amodiaquine against asexual- and sexual-stage parasites in falciparum malaria in children. *Antimicrob Agents Chemother* 2007;51:1694–9. [PubMed: 17325222]
16. Sowunmi A, Balogun T, Gbotosho GO, Happi CT, Adedeji AA, Bolaji OM, et al. Activities of artemether–lumefantrine and amodiaquine–sulfalene–pyrimethamine against sexual-stage parasites in falciparum malaria in children. *Chemother* 2008;54:201–8.
17. Sowunmi A, Balogun ST, Gbotosho GO, Happi CT. *Plasmodium falciparum* gametocyte sex ratios in children with acute, symptomatic, uncomplicated infections treated with amodiaquine. *Malaria J* 2008;7:169.
18. World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2000;94(Suppl 1):1–90. [PubMed: 10748883]
19. Sowunmi A, Fateye BA. *Plasmodium falciparum* gametocytaemia in Nigerian children: before, during and after treatment with antimalarial drugs. *Trop Med Inter Health* 2003;8:783–92.
20. Sowunmi A, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA, Folarin OA, et al. Therapeutic efficacy and effects of artemether–lumefantrine and amodiaquine–sulfalene–pyrimethamine on gametocyte carriage in children with uncomplicated *Plasmodium falciparum* malaria in southwestern Nigeria. *Am J Trop Med Hyg* 2007;77:235–41. [PubMed: 17690392]
21. Carter, R.; Graves, PM. Gametocytes. In: Wernsdorfer, WH.; McGregor, I., editors. *Malaria: principles and practice of malariology*. Vol. I. Edinburgh: Churchill Livingstone; 1988. p. 253–303.
22. Epi Info version 6. A word processing data base and statistics program for public health on IBM-compatible microcomputers. Atlanta, GA: Centers for Disease Control and Prevention; 1994.
23. SPSS for Windows release 10.01 (standard version). Chicago, IL, USA: SPSS Inc; 1999.
24. Sowunmi A, Balogun ST, Gbotosho GO, Happi CT. *Plasmodium falciparum* sex ratios in acutely symptomatic children treated with antimalarial drugs. *Acta Tropica* 2009;109:108–17. [PubMed: 19027703]
25. Read AF, Narara A, Nee S, Keymer AE, Day KP. Gametocyte sex ratios as indirect measures of outcrossing rates in malaria. *Parasitol* 1992;104:387–95.
26. Nee S, West SA, Read AF. Inbreeding and parasite sex ratios. *Proc R Soc Lond Series B* 2002;269:755–60.
27. West SA, Smith TG, Read AF. Sex allocation and population structure in apicomplexan (protozoa) parasites. *Proc R Soc Lond Series B* 2000;267:257–63.
28. Robert V, Read AF, Essong J, Tchuinkam T, Mulder B, Verhave JP, et al. Effects of gametocyte sex ratio on infectivity of *Plasmodium falciparum* to *Anopheles gambiae*. *Trans R Soc Trop Med Hyg* 1996;90:621–4. [PubMed: 9015496]
29. Werren LH, Nur U, Wu C-I. Selfish genetic elements. *Trends Ecol Evol* 1988;3:297–302.
30. Paul REL, Packer MJ, Walmsley M, Lagog M, Randford-Cartwright LC, Paru R, et al. Mating patterns in malaria parasite populations of Papua New Guinea. *Science* 1995;269:1709–11. [PubMed: 7569897]
31. Price RN, Nosten F, Luxemburger C, ter Kuile KO, Paiphun L, Chongsuphajasiddhi T, et al. Effects of artemisinin derivatives on malaria transmissibility. *Lancet* 1996;347:1654–8. [PubMed: 8642959]
32. Drew DR, Reece SE. Development of reverse-transcription PCR techniques to analyse the density and sex ratio of gametocytes in genetically diverse *Plasmodium chabaudi* infections. *Mol Biochem Parasitol* 2007;156:199–209. [PubMed: 17889948]
33. Sowunmi A, Balogun ST, Gbotosho GO, Happi CT. Effects of amodiaquine, artesunate, artesunate–amodiaquine on *Plasmodium falciparum* associated anaemia in children. *Acta Tropica* 2009;109:55–60. [PubMed: 18992209]
34. Sowunmi A, Balogun ST, Gbotosho GO, Happi CT. Influence of anaemia on *Plasmodium falciparum* gametocyte sex ratios in acutely symptomatic children. *The Open Trop Med J* 2008;1:13–20.
35. Crawley J. Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: from evidence to action. *Am J Trop Med Hyg* 2004;71:25–34. [PubMed: 15331816]

36. Price RN, Nosten F, Simpson JA, Luxemburger C, Paiphun L, ter Kuile TO, et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am J Trop Med Hyg* 1999;60:1019–23. [PubMed: 10403336]

Fig. 1.

Frequency distribution of *P. falciparum* gametocyte sex ratios at presentation (day 0), and at follow-up (days 3 and 7). Hatched portions of bars refer to gametocyte carriers in whom the gametocyte densities $\geq 15/\mu\text{l}$ blood.

Fig. 2.

Sex ratio variations according to the number of infecting clones (genotypes). Horizontal bars indicate mean sex ratios.

Table 1

Primers sequences and thermocycling conditions for the amplification reactions.

Locus and reactions	Primers names and sequences	Cycling conditions
<i>Msp-1</i> (block2)		
Primary amplification	CHM1-OF: 5'CTAGAAGCTTTAGAAGATGCAGTATTG-3' CHM1-OR: 5'CTTAAATAGTATTCTAATTCAAGTGGATCA-3'	95 °C/5 min; 45 cycles 94 °C/1 min; 58 °C/1 min; 72 °C/ 1 min 72 °C/10 min.
Secondary amplification	CHM1-KF: 5'AAATGAAGAAGAAATTACTACAAAAGGTGC-3' CHM1-KR: 5'GCTTGCATCAGCTGGAGGGCTGCACCAGA-3' CHM1-MF: 5'AAATGAAGGAACAAGTGGAACAGCTGTTAC-3' CHM1-MR: 5'ATCTGAAGGATTTGTACGTCTGAATTACC-3' CHM1-RF: 5'TAAAGGATGGAGCAAATACTCAAGTTGTTG-3' CHM1-RR: 5'CATTGAAGGATTGACGACCTGGAGATC-3'	95 °C/5 min; 35 cycles 94 °C/1 min; 61 °C/2 min; 72 °C/ 2 min 72 °C/10 min.
<i>Msp-2</i> (block3)		
Primary amplification	M2-OF: 5'ATGAAGGTAATTAACATTGTCTATTATA-3' M2-OR: 5'CTTTGTTACCATCGGTACATCTT-3'	95 °C/5 min; 45 cycles 94 °C/1 min; 55 °C/2 min; 72 °C/ 2 min 72 °C/10 min.
Secondary amplification	M2-FCF: 5'AATATTAAGAGTGTAGGTGCARATGCTCCA-3' M2-FCR: 5'TTTTATTTGGTGCATTGCCAGAACTGAAC-3' M2-ICF: 5'AGAAGTATGGCAGAAAGTAACCTYCTACT-3' M2-ICR: 5'GATTGTAATTCGGGGGATTCAGTTGTTTCG-3'	95 °C/5 min; 35 cycles 94 °C/1 min; 62 °C/2 min; 72 °C/ 2 min 72 °C/10 min.
<i>Glurp</i> (region II)		
Primary amplification	CHG-OF: 5'TGAATTGAAGATGTTCACTGAAC-3' CHG-OR: 5'GTGGAATTGCTTTTCTTCAACACTAA-3'	95 °C/5 min; 45 cycles 94 °C/30 s; 45 °C/1 min; 68 °C/2 min 72 °C/15 min.
Secondary amplification	CHG-OR: 5'GTGGAATTGCTTTTCTTCAACACTAA-3' CHG-NF: 5'TGTTCACTGAACAATTAGATTTAGATCA-3'	95 °C/5 min; 35 cycles 94 °C/30 s; 45 °C/1 min; 68 °C/2 min 72 °C/15 min.

Table 2

Demographic characteristics of 162 gametocyte carriers.

Parameter	Value
Age (year)	5.3±3.0 (0.5–14) ^a
Gender F:M	75:87
Weight (kg)	15.5±5.5 (6–32) ^a
Duration of illness (days)	3.2±1.4 (1–8) ^a
Haematocrit (%)	29.1±4.9 (18–40) ^a
Body temperature (°C)	37.9±1.2 (36–40.7) ^a
Asexual parasitaemia (/μl)	25,222 ^b (2000–1,376,551) ^a
Gametocyte density (/μl)	18 ^b (6–498) ^a

Median gametocyte density is 21/μl, interquartile range is 20–30/μl.

Values are mean±standard deviation.

^aRange.

^bGeometric mean density.

Table 3

Predictors of a sex ratio of 0.5 seven days post-treatment in 276 children with gametocytaemia on day 7.

Variables	No with gametocytaemia	No with unbeatable sex ratio	Crude odds ratios	P value	Adjusted odds ratios	P value
Gender						
Female	132	201				
Male	144	180.89 (0.63–1.28)		0.52	–	–
Age (years)						
<5	107	111			1	
≥5	101	242.31 (1.81–2.95)		0.027	4.55 (1.36–14.71)	0.014
Duration of illness (d)						
<3	86	141				
≥3	184	220.70 (0.58–0.83)		0.34	–	–
Packed cell volume (%)						
<30	71	121			1	
≥30	101	60.31 (0.23–0.41)		0.025	0.17 (0.05–0.58)	0.005
Fever						
Absent	88	81				
Present	179	261.70 (1.10–2.60)		0.20	–	–
Year						
Before 2004	192	291			–	–
2004 onwards	46	91.29 (0.66–2.29)		0.54	–	–