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Genesis, sequestration and survival of *Plasmodium falciparum* gametocytes: parameter estimates from fitting a model to malariatherapy data

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Abstract

Plasmodium falciparum malaria is one of mankind's main killers. Part of the parasite's life-cycle is spent in human blood, mainly as asexual stages. A fraction of the asexual parasites develops into gametocytes (gamete precursors) while sequestered in deep tissues. After re-entering the circulation, gametocytes can be picked up by a mosquito to continue the parasite's life-cycle. We present estimates of the conversion probability from asexual parasites to circulating gametocytes and of the gametocytes' sequestration and circulation times, obtained for the first time by fitting a dynamic model to individual patients' histories (daily records of 113 neurosyphilitic patients undergoing malariatherapy). The model assumes that the conversion probability can vary among the successive waves of asexual parasitaemia of a patient, and that gametocytes die at an age-dependent rate which increases under high asexual parasite densities. On average, 1 gametocyte per 156 asexual parasites (range 7·4–3700) is produced. The most remarkable findings are the large individual variation of conversion probabilities and circulation times, the average gametocyte circulation time of 6·4 days (range 1·3–22·2 days) which is more than twice the currently accepted value, and the large variation of conversion probabilities among successive waves of asexual parasitaemia without any particular time pattern. The latter finding could be explained by an association between conversion probability and variation of PfEMP1.

Keywords: *Plasmodium falciparum*, life-cycle, gametocytes, dynamics, circulation time, mathematical modelling

Introduction

Infection with the malaria parasite *Plasmodium falciparum* (*P.f.*) is one of the most important causes of human morbidity and mortality in tropical countries, and gametocytes are the parasite's transmission stage (GARNHAM, 1988; DAY *et al.*, 1998). Development of *P.f.* parasites within human red blood cells (RBC), lasts 48 h, at the end of which the parasitized red blood cells (PRBC) burst, liberating on average 16 merozoites. A successful merozoite invades another RBC within minutes and either goes into another round of multiplication or develops into a single gametocyte. Newly invaded RBC circulate for a day as ring-infected PRBC, then sequester to the vascular endothelium of deep tissues for either a day until rupture and release of merozoites, or for the period it takes the gametocyte to mature (CARTER & MILLER, 1979). Mature gametocytes appear in the peripheral circulation, where they can be detected by microscopy, and can be picked up by an anopheline vector, in the midgut of which they become gametes and fertilization allows the life-cycle to continue (GARNHAM, 1988; DAY *et al.*, 1998). The dynamics of gametocytaemia (gametocyte density in the blood) is, therefore, important for the transmission of malaria and its control through either antimalarial drugs, vector control, or the malaria vaccines in process of development, in particular those which target the parasite's sexual stages (CARTER *et al.*, 2000).

In a previous paper (DIEBNER *et al.*, 2000), we formulated different models predicting gametocytaemia from asexual parasitaemia, supplied the models' biological background, fitted them to data, and identified the model that gave the best fits which is used in the present paper. In this model, (i) the conversion probability of asexual parasites to circulating gametocytes is allowed to vary among successive waves of asexual parasitaemia, (ii) the sequestration period is constant within each case-history, (iii) gametocytes die at a rate which grows exponentially with their age and are also destroyed by high asexual parasite densities. The model was fitted individually by maximum likelihood to 113 case-histories of adult neurosyphilitics not previously exposed to malaria, and yielded patient-specific estimates of the sequestration period D_s, the average conversion probability $\bar{g}$, the average gametocyte circulation time L as well as conversion probabilities $g(t)$ for each wave of asexual parasitaemia.

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Materials and Methods

The data

The data were collected by the US Public Health Service in the National Institute of Health Laboratories in Columbia, South Carolina and Milledgeville, Georgia, between 1940 and 1963, when malariatherapy was a recommended treatment for neurosyphilis; consent was granted by the families or the courts (COLLINS & JEFFERY, 1999; WEIJER, 1999). The patients were neurosyphilitic adults with no history of prior exposure to malaria. Different strains of *P.f.* were inoculated, using sporozoites (generally through mosquito bite) or infected blood. Inoculations were preceded by variable sequences of blood and mosquito passages. It is not known whether the inoculations were mono- or polyclonal, but we obtained realistic simulations of asexual parasitaemia with a model assuming the inoculations to be monoclonal (MOLINEAUX *et al.*, 2001). Blood examination by microscopy was performed almost daily. In principle, 0·1 μL of blood was examined, less in the case of high parasite density, occasionally more; the detection threshold was thus about 10 PRBC/μL (EARLE & PEREZ, 1932). Asexual parasites and gametocytes were counted separately. Usually, the recorded gametocyte densities involve a 10-fold multiplication of the number actually counted. This is taken into account in the analysis. A curative treatment (generally chloroquine) was given early to a small number of clinically severe cases, excluded from this investigation. The others were given curative treatment before discharge. Low-dose suppressive treatments (most commonly quinine) were given to about half of the patients, for containment of high parasitaemias. We limited ourselves to primary *P.f.* inoculations ($n = 334$) and excluded all patients with any of the following features: (i) treatment with drugs reported to affect gametocytes (chlorguanide, pyrimethamine, primaquine) or unknown drugs; (ii) superinoculation with any malaria parasite in the course of the

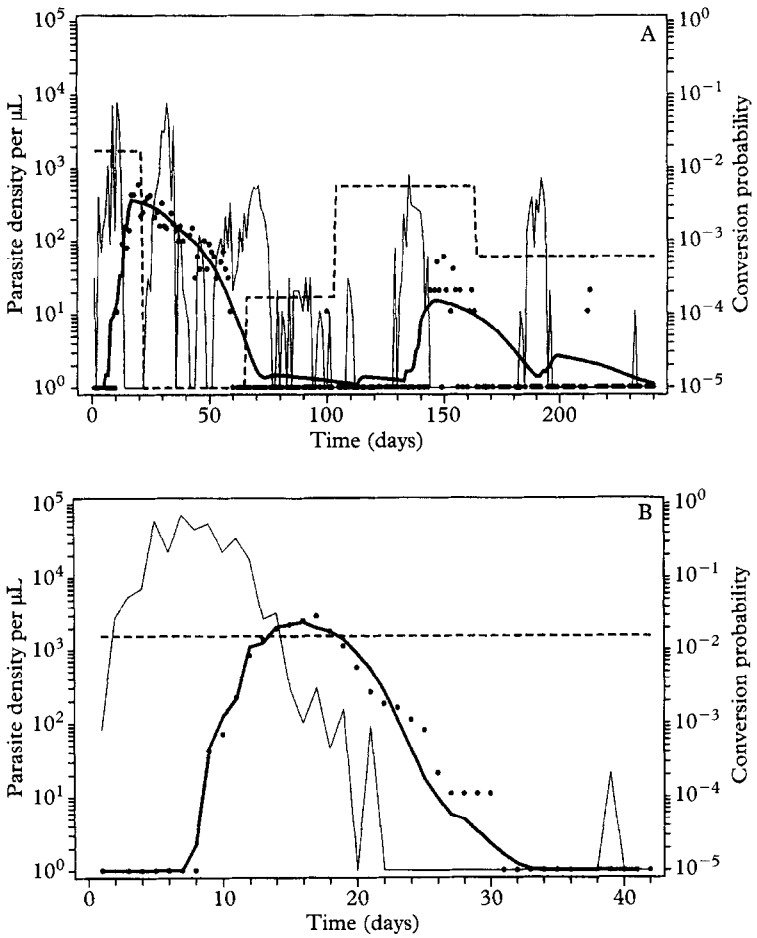

Figure A–D. Comparisons between model output and data of 4 patients. The asexual parasitaemia (thin line) serves as input from which the gametocytaemia is predicted. Parameter values are estimated by maximum likelihood to minimize the difference between observed (dots) and predicted gametocytaemia (fat line), assuming Poisson-distributed errors. The dashed step functions indicate the duration of asexual waves. The height of each step shows the estimate of the wave-dependent conversion probabilities $g(t)$ as indicated on the right-hand scale. The 4 patients were selected to illustrate the effect of variation in the gametocyte circulation time L (compare A and B) and of variation in the conversion probability $g(t)$ among a patient's waves of asexual parasitaemia (compare A, C and D). A, patient G221, mean conversion probability $\bar{g} = 0{\cdot}0069$, sequestration period $D_s = 5$ days, $L = 21{\cdot}1$ days; B, patient G54, $\bar{g} = 0{\cdot}015$, $D_s = 7$ days, $L = 3{\cdot}1$ days; C, patient S1050, $\bar{g} = 0{\cdot}038$, $D_s = 8$ days, $L = 6{\cdot}1$ days; D, patient S1204, $\bar{g} = 0{\cdot}014$, $D_s = 6$ days, $L = 10{\cdot}1$ days.

infection; (iii) parasitological status on the day before onset of curative treatment positive or uncertain; (iv) gametocyte counts never exceeding 100/μL (otherwise a reliable estimation of parameters would have been impossible); (v) inoculation of a rarely used strain. This left 113 patients (see Table 2): 49 received the SanteeCooper strain (South Carolina, 1946), 33 received the McLendon strain (South Carolina, 1940), and 31 received the ElLimon strain (Panama, 1948).

The model

Assumptions: (i) a fraction $g(t)$ of the circulating asexual parasites $A(t)$ at time t become circulating gametocytes after the sequestration period D_s; note that $g(t)$ is the product of the fraction entering into gametocytogenesis by the fraction surviving through sequestration; (ii) $g(t)$ is constant within each asexual wave, defined as follows: (a) an asexual parasite density of at least 100 parasites/μL is called a local maximum if it is preceded by 6 consecutive lower densities and followed by 6 consecutive lower (or equally high) densities; (b) to each local maximum corresponds an asexual wave, extending half-way to the preceding and the following maximum (or to the first or last positive observation, respectively); for illustration see Figure A–D; (iii) circulating gametocytes $G(t, a)$ at time t of age a die at a rate which grows exponentially with age; (iv) gametocytes are also destroyed in the presence of asexual parasites $A(t)$ at a daily probability $1 - (A(t) + 1)^{-\beta}$. Combining these 2 sources of mortality yields the time- and age-dependent mortality rate $\mu(t, a) = \mu_0 \exp(\alpha_G a) + \beta \ln(A(t) + 1)$. The predicted gametocyte density is then given by

$$G(t, a) = g(t - a - D_s)A(t - a - D_s) * \exp\left(-\left(\frac{\mu_0}{\alpha_G}(e^{\alpha_G a} - 1) + \beta \sum_{\tau=t-a}^{t} \ln(A(\tau) + 1)\right)\right).$$

The expected number of gametocytes at time t is $\bar{G}(t) = \sum_{a=0}^{t-D_s} G(t, a)$. For each patient, the average conversion probability is $\bar{g} = \sum_{t=1}^{t^*-D_s} g(t)A(t) / \sum_{t=1}^{t^*-D_s} A(t)$ where t^* is the last observation day, and the average circulation time is $L = \sum_{t=D_s}^{t^*} \bar{G}(t) / \sum_{t=1}^{t^*-D_s} g(t)A(t)$. A separate set of parameters was estimated for each patient using a maximum likelihood procedure where we assume that the reported gametocyte densities per 0·1 μL blood are Poisson distributed.

Results

The observed and predicted gametocyte densities of 4 patients are shown in Figure A–D, together with their asexual parasite densities and estimated conversion probabilities $g(t)$ (for a summary of all estimates, see

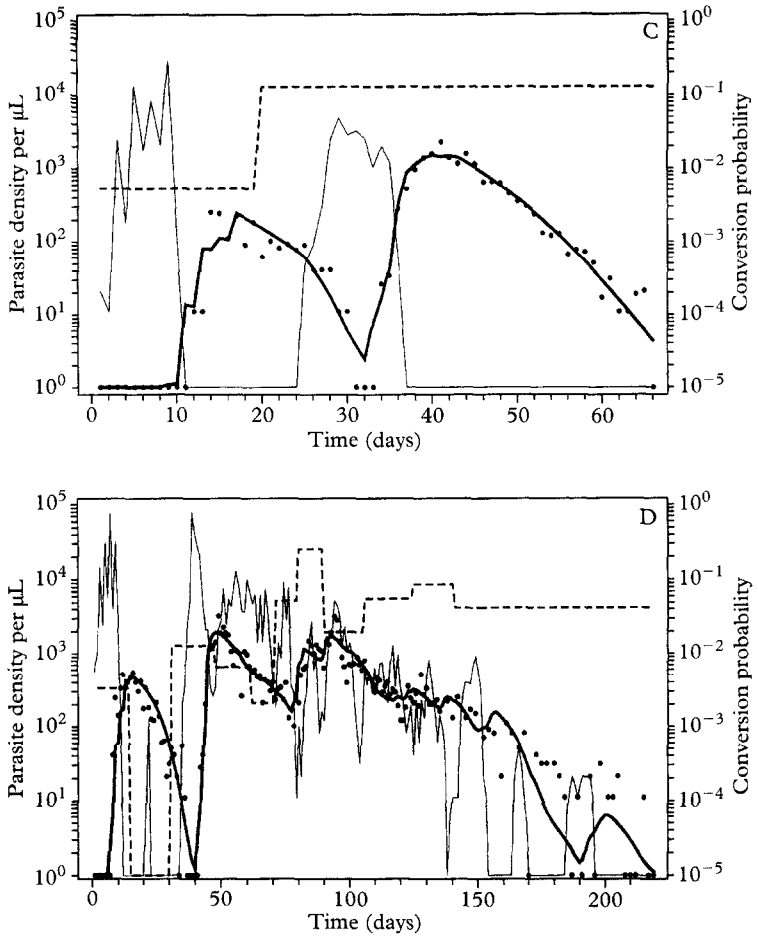

Figure *continued.*

Tables 1 and 2). The distribution of D_s is approximately normal, the distributions of $\bar{g}$ and L are approximately log-normal. Possible influences of the patient's mode of infection (blood transfusion vs sporozoites), the parasite strain, or their combination, were examined by ANOVA. The observed ranges of the results are shown in Table 1 and the least square means (stratified by strain and mode of infection) are given in Table 2. Sequestration time D_s and average conversion probability $\bar{g}$ were significantly affected only by mode of infection (mean D_s: 6·9 days by blood vs 7·8 days by sporozoites; $P = 0{\cdot}002$; geometric mean (GM) of $\bar{g}$: 0·0086 by blood vs 0·0048 by sporozoites; $P = 0{\cdot}047$. Circulation time L was significantly affected by mode of infection (GM 5·6 days by blood vs 7·4 days by sporozoites; $P = 0{\cdot}01$) and by strain (GM SanteeCooper 4·9 days, ElLimon 6·2 days, McLendon 8·7 days; $P < 0{\cdot}001$). Inter-individual variation of $\bar{g}$ and L was much larger than variation by mode of infection or strain. Among patients, it took on average 7·4 to 3700 (GM 156) asexual parasites to produce 1 circulating gametocyte which then survived on average for 1·3 to 22·2 days (GM 6·4 days). There was no significant correlation between $\log_{10}(L)$ and D_s or $\log_{10}(\bar{g})$, but $\log_{10}(\bar{g})$ and $\log_{10}(L)$ were both negatively correlated with the log-density of asexual parasites at the first local maximum of the patient ($\log_{10}(\bar{g})$: $r = -0{\cdot}23$, $P < 0{\cdot}015$; $\log_{10}(L)$: $r = -0{\cdot}38$, $P < 0{\cdot}0001$). The wave-specific conversion probability $g(t)$ varied among and within patients. By random effects ANOVA these 2 variances were found to be approximately equal. A possible association between the magnitude of a patient's estimates of $g(t)$ and their order of occurrence was examined by rank correlation (for patients with only 2 estimates, the sign test was used), but no particular time-pattern could be detected.

Table 1. Means and extreme values for *P. falciparum* gametocyte sequestration time, conversion probability and mature gametocyte circulation time ($N = 113$)

Parameter	Minimum	Mean[a]	Maximum
Gametocyte sequestration time, D_s (days)	4	7·4	12
Conversion probability $\bar{g}$ from circulating asexual parasites to circulating gametocytes	0·00027	0·0064	0·135
Mature gametocyte circulation time, L (days)	1·3	6·4	22·2

[a]Arithmetic mean for D_s and geometric mean for $\bar{g}$ and L.

Table 2. Least square means[a] of *P. falciparum* gametocyte sequestration time, conversion probability and mature gametocyte circulation time, stratified by parasite strain and mode of infection

	Strain		
Parameter/mode of infection	SanteeCooper	ElLimon	McLendon
Gametocyte sequestration time D_s in days			
Sporozoites	7·5	7·8	8·2
Blood	6·3	6·8	7·4
Conversion probability $\bar{g}$ to mature gametocytes			
Sporozoites	$6{\cdot}8 \times 10^{-3}$	$3{\cdot}9 \times 10^{-3}$	$4{\cdot}2 \times 10^{-3}$
Blood	$9{\cdot}4 \times 10^{-3}$	$15{\cdot}7 \times 10^{-3}$	$4{\cdot}3 \times 10^{-3}$
Mature gametocyte circulation time L in days			
Sporozoites	5·2	7·4	10·6
Blood	4·6	5·3	7·0
Number of patients			
Sporozoites	12	19	10
Blood	37	12	23

[a]Arithmetic means for D_s and geometric means for $\bar{g}$ and L.

Discussion

Our estimates of the sequestration time D_s (4 to 12 days; mean 7·4 days) are low compared to the values reported in the literature (7 to 15 days) (DAY *et al.*, 1998), where estimates were derived from the maturation period *in vitro* or from the delay *in vivo* between the detection of asexual parasites and the detection of gametocytes. In the latter approach, D_s is overestimated by the time needed to reach a gametocytaemia that can be detected by microscopy. This problem does not arise with the simultaneous estimation of D_s and $g(t)$, as done here. The average (GM) gametocyte circulation time after inoculation of sporozoites is 7·4 days, i.e. more than twice the expected circulation time of 3·4 days implied by the half-life of 2·4 days reported in the current standard reference. SMALLEY & SINDEN (1977) obtained this estimate from residual survival of gametocytes which had been circulating for varying periods, after eliminating asexual parasites by chloroquine, whereas we estimated L from emergence into the circulation. Their estimate is based on 7 Gambian children with variable prior exposure to malaria. They assumed that gametocyte survival is independent of gametocyte age, and that there is no individual variation. Our estimates are based on 113 adults without history of malaria, and we allow for age-dependent gametocyte mortality (which considerably improved the fits of the model) (DIEBNER *et al.*, 2000) and for individual variation (found to be large).

The individual variation of $\bar{g}$ and L and their negative correlation with the first maximum of asexual parasitaemia might be explained by several host-factors. (i) Phagocytosis by the spleen is likely to affect gametocyte survival. (ii) Tumour necrosis factor (TNF), and associated pro-inflammatory cytokines, released in response to the rupture of asexual PRBC, (KWIATKOWSKI, 1995) might affect survival of sequestered and circulating gametocytes (NAOTUNNE *et al.*, 1991, 1993; KARUNAWEERA *et al.*, 1992) and of asexual parasites (KWIATKOWSKI, 1995). (iii) As cytoadherence receptors of asexual parasites and gametocytes overlap (DAY *et al.*, 1998), competition with large numbers of asexual parasites could reduce the success rate of maturing gametocytes. Increased overlap may also increase the exposure of sequestered gametocytes to high local TNF concentrations.

Our estimates of the successful conversion probability ($\bar{g}$, $g(t)$; see Table 1) cannot be compared directly to literature values. The only related estimates are point-estimates of the conversion probability *per se* (excluding survival through sequestration), obtained *in vitro*, where they vary rapidly and reach very high levels (CARTER & MILLER, 1979; KAUSHAL *et al.*, 1980; SMALLEY & BROWN, 1981; ONO *et al.*, 1986; BRUCE *et al.*, 1990; TRAGER & GILL, 1992; WILLIAMS, 1999), and *ex vivo* in Gambian children where estimates showed a large individual variation (SMALLEY *et al.*, 1981). Some of the wave-specific estimates of $g(t)$ have wide confidence intervals, but allowing for wave-specificity considerably improved the fits (DIEBNER *et al.*, 2000), which strongly suggests that the observed trendless wave-specific variation of the conversion probability is real. This finding is new and remarkable. It cannot be explained by any of the factors advocated, on the basis of experiments *in vitro*: immunity (SMALLEY & BROWN, 1981; ONO *et al.*, 1986), asexual parasite density (CARTER & MILLER, 1979; KAUSHAL *et al.*, 1980) or age of RBC (BRUCE *et al.*, 1990; TRAGER & GILL, 1992; WILLIAMS, 1999). Fever is probably also not a significant factor: asexual parasitaemia, gametocytaemia and fever all decrease in the course of an infection (COLLINS & JEFFERY, 1999), but the conversion probability shows no time trend and does not seem to be associated with the presence or absence of fever. A trendless wave-specific variation of the conversion probability could, however, be explained by: (i) an association between asexual waves and different PfEMP1 variants (BORST *et al.*, 1995), now widely accepted; and (ii) a variant-specific conversion probability, which is plausible on the basis of the reported linkage between gametocytogenesis and PfEMP1 expression, and the latter's involvement in the sequestration of asexual parasites and gametocytes (DAY *et al.*, 1998; HAYWARD *et al.*, 1999; PIPER *et al.*, 1999). A recent review of factors and mechanisms involved in gametocytogenesis (DYER & DAY, 2000) does not mention a possible link with expression of PfEMP1 variants. We have proposed a model of asexual parasitaemia, which includes variant-specific and variant-transcending immunity (MOLINEAUX *et al.*, 2001), and we will merge the 2 models to examine whether variant-specific gametocyte production generates realistic patterns of conversion probabilities. Acquired immunity during primary infections seems to have little or no effect on sequestered gametocytes, as this would have gradually lowered the estimates of $g(t)$, and we have seen no such trend. The question whether acquired immunity reduces survival of circulating gametocytes was addressed in our model

comparison paper (DIEBNER *et al.*, 2000): the fits improved when changing a constant mortality rate to one which grows over time, but even better fits were obtained when allowing mortality to grow with the gametocyte's age.

Our findings should be relevant for the planning and evaluation of malaria control, as well as for further modelling of malaria transmission and of the effects of natural selection on the trade-off between asexual multiplication and gametocytogenesis (KOELLA & ANTIA, 1995; TAYLOR & READ, 1997; MCKENZIE & BOSSERT, 1998). The finding of a significant variation of gametocyte survival among strains of *P.f.* adds a factor to the problem of coexistence of sympatric strains.

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