An analysis of the temperature effects of fever on the intra-host population dynamics of *Plasmodium falciparum*

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SUMMARY

Observations that growth of *Plasmodium falciparum in vitro* is inhibited by high temperatures have led to hypotheses that malaria fever may influence the parasite population dynamics, regulating parasite density and synchronizing parasite growth. In order to investigate the fever hypotheses, we have developed an age-structured coupled Markov chain model that describes the parasite erythrocyte cycle and its interaction with the host fever response. We estimated the model parameters using data collected from laboratory parasite cultures that were exposed to febrile or normal temperature. Using the experimental parameter values, quantitative predictions were made of the effect of fever in determining the parasite population dynamics. It was concluded from the model behaviour that, during the primary infection of a non-immune host, a typical episode of fever can effect density-dependent regulation of the parasite population, maintaining cycles of parasitaemia and promoting synchronous parasite growth.

Key words: *Plasmodium falciparum*, fever, population dynamics, synchrony.

INTRODUCTION

The malaria parasite maintains its infection of the human host by successive cycles of invasion and replication within host erythrocytes. *Plasmodium falciparum*, the most virulent species, has a high capacity for population increase. Due to erythrocyte destruction alone, an unchecked infection would overwhelm the host a little over a week after parasites become detectable in the blood. However, untreated infections in non-immunes can last for several months. An initial exponential increase in parasitaemia terminates after a few days, and is followed by a period of relatively low and constant density. This regulation, or balance between growth and decline over many parasite generations, reflects both parasite evasion of immune clearance and host control of parasite density at a level that is not directly lethal. The mechanisms involved are unknown.

The most striking clinical manifestation of malaria is periodic fever. The febrile response is linked directly with the underlying parasite population dynamics, each episode being triggered when the parasitaemia reaches a pyrogenic threshold. This density-dependent nature of the fever response, and the observation that febrile temperatures inhibited the growth of laboratory cultures of *P. falciparum* (Kwiatkowski, 1989) led to the suggestion that fever may exert a large influence on the parasite population dynamics, acting as a non-specific immune response involved in stopping the initial exponential parasite population growth and its subsequent regulation at low density (Kwiatkowski & Greenwood, 1989; Kwiatkowski & Nowak, 1991).

The fever hypothesis presents an attractive opportunity for the investigation of parasite regulation, since of the many immune mechanisms that are known to interact with *P. falciparum*, few are as easy to observe and quantify. Detailed records of episodes of fever and parasite dynamics in primary infections of non-immunes are available from malaria therapy research carried out during the first half of the century. Here we attempt a quantitative appraisal of the role of fever in the early infection of non-immunes. We develop a mathematical framework for the analysis of the interaction between parasitaemia and the host fever response. We estimate model parameters from experiments performed on laboratory populations of *P. falciparum*, and predict the direct effects of fever on *P. falciparum* population dynamics. The results of the model are compared to *in vivo* data.

MATERIALS AND METHODS

Clinical data

Records from the use of induced malaria as a treatment for neurosyphilis provide some of the most detailed information on *P. falciparum* parasitaemia time-series in non-immune and largely

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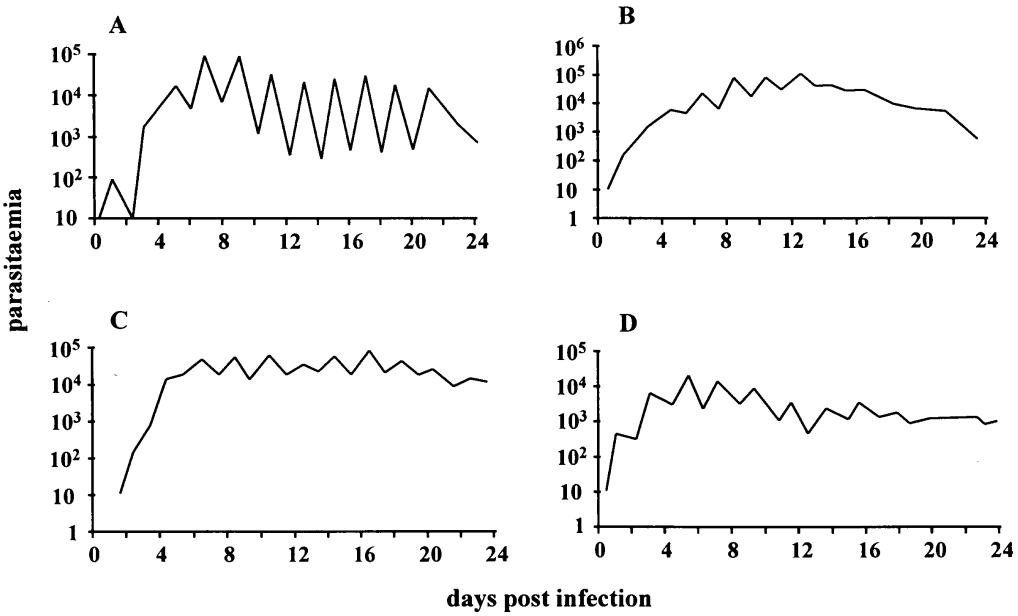

Fig. 1. *Plasmodium falciparum* parasitaemia time-series in non-immune patients undergoing malariatherapy. Data from (A) Boyd & Kitchen (1937*a*), (B) and (D) Kitchen (1949), (C) Boyd & Kitchen (1937*b*). Parasitaemia refers to number of parasites/μl of blood.

untreated patients. Much of this illuminating work has been summarized by Boyd (1949). Following the short exponential growth phase, considerable variation exists between individual parasite growth curves. However, in many cases the parasite population is seen to oscillate with a frequency of approximately 2 days, and over an order of magnitude of parasitaemia. This period of cycling can last for only a few days or up to several weeks, and is followed by a decline in parasitaemia and possible recrudescence before eventual clearance. Examples of this population behaviour are given in Fig. 1. Note that these parasitaemia time-series, which provide an independent source of data with which to test model predictions, describe only the younger proportion of the total parasite population since mature parasites leave the peripheral circulation as they bind to microvascular endothelium. Accordingly, our model of the erythrocyte cycle describes an age-structured parasite population.

The parasitaemia cycles coincide with episodes of fever, and it has long been established that malaria fever does not occur until a certain degree of parasitaemia is reached (Ross & Thompson, 1910). Estimates of this pyrogenic threshold in children are in the order of 10^4 infected erythrocytes/μl (Miller, 1958), and there is a negative relationship between threshold and age (Rogier, Commenges & Trape, 1996). If the parasitaemia falls significantly below the threshold no fever is observed until the parasite population once again recovers. The common fever pattern is 'tertian' or every 48 h, although falciparum fever is often irregular and can occur daily or every 36 h (subtertian) early in infection. The records of Horton Hospital in Surrey contain detailed measurements of temperature taken during episodes of induced-falciparum fever, and were used to estimate a typical fever temperature profile (see *Experimental data for model parameter estimation*).

An age-structured model of the parasite erythrocyte cycle

Following a period of reproduction in the liver parasites enter the host bloodstream and begin the asexual erythrocyte cycle that maintains infection and is the direct cause of disease symptoms. Intra-erythrocytic parasites grow and reproduce over a period of 48 h, after which the erythrocyte ruptures releasing daughter parasites that quickly invade a fresh erythrocyte to renew the cycle. The cycle of growth within the erythrocyte is often categorized into age classes based on parasite morphology. We have modelled this cycle as a Markov process. The parasite can exist in 5 different states, with constant rates governing the transition between classes, and constant age-specific death rates. The 5 stages of the life-cycle represent the maturation of the parasite inside the erythrocyte, they are (1) young rings having a thin ring of cytoplasm; (2) old rings with a prominent central vacuole but fleshy cytoplasm; (3) young trophozoites with no vacuole but smaller than half of the erythrocyte; (4) old trophozoites occupying half or more of the erythrocyte; (5) schizonts with 2 or more daughter nuclei clearly visible (defined here as 'segmenters'). Stages 1 and 2 provide the majority of the 'parasitaemia' measured in a sample from the peripheral blood, the other age groups supply the 'sequestered mass' of parasites hidden in the microvasculature.

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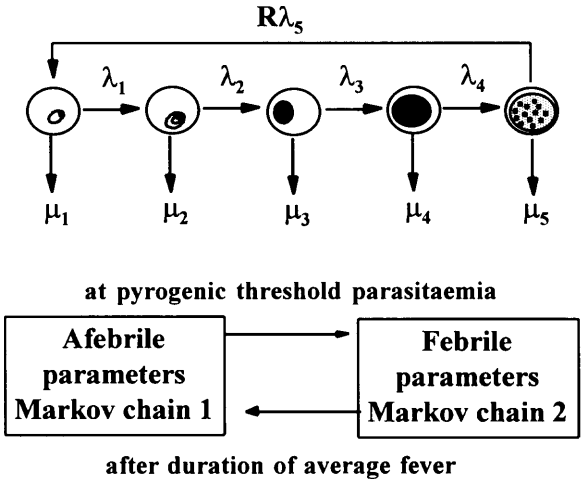

Fig. 2. Schematic representation of model. Two coupled Markov chains are used to simulate the age-structured parasite population changes during febrile and afebrile periods.

The mean of the total parasite population in each age class at time t is determined by the following equations:

$$\frac{dn_1(t)}{dt} = \rho\lambda_5 n_5(t) - (\mu_1 + \lambda_1) n_1(t),$$

$$\frac{dn_i(t)}{dt} = \lambda_{i-\lambda} n_{i-1}(t) - (\mu_i + \lambda_i) n_i(t),$$

$i = 2, 3, 4, 5.$

$n_i(t)$ is the number of parasites in state i at time t. λ_i is the transition rate from state i to state $i+1 (i = 1–4)$ and $\rho\lambda_5$ is the transition rate from state 5 to state 1 (ρ being the average number of secondary asexual intra-erythrocytic parasites produced at the end of 1 parasite cycle). Parasite death rates at each stage are denoted by μ_i. The dormant sexual parasite stages formed during the erythrocyte cycle are not investigated here.

The interaction between parasite growth and host temperature

The above equations describe population growth in a constant environment. In order to model the change in environment caused by fever, 2 such Markov chains, differing only in their parameter values, were coupled as follows. Initially the population behaviour is determined by the equations of chain 1, the parameters of which are relevant to normal body temperature. If, and when, a certain parasite density is reached (defined at the pyrogenic threshold), control of the population switches to chain 2, which has parameter values relevant to the temperatures of a typical fever. Chain 2 controls the population dynamics for a fixed time-period. This period represents the average duration of a falciparum malaria fever attack and is estimated in the following section at 24 h. After this period, chain 1 regains control until the pyrogenic threshold is again reached. Fig. 2 is a schematic of the model structure.

In vitro *cultures of* P. falciparum

Established isolates of *P. falciparum* were maintained in erythrocytic culture according to the method of Trager & Jensen (1976). The strain used for the study was IT04, derived from isolate IT4/25/5 (Berendt *et al.* 1989). The culture medium was RPMI-1640 with added glucose (preservative free, 2 g/l), L-glutamine (2 mM), gentamicin (15 mg/l), HEPES (25 mM) and fresh human serum (A+, 10%). A gas mixture of 1% oxygen, 3% carbon dioxide and 96% nitrogen was used, and O+ erythrocytes were used to maintain compatibility with the serum. Throughout the experiments medium was changed daily, the gas mixture was renewed each time a flask was opened, and cell volume was maintained at approximately 30 μl in 10 ml of medium (25 ml culture flasks used in each case). Population changes were monitored with thin blood smears stained with Giemsa. Slides were read blind, and at least 1000 erythrocytes were counted per slide. Parasites were considered viable unless they were substantially pyknotic, small and of uniform staining with no definition in the cytoplasm.

Experimental data for model parameter estimation

In order to estimate parameters for parasite growth in the absence of fever (Markov chain 1) parasites were maintained in culture at 37 °C. The transition in the numbers of each parasite stage was recorded over 12 h intervals in 40 cultures, a total of 156 transitions. To estimate the parameters during a fever (Markov chain 2), 65 parasite cultures initially grown at 37 °C were monitored during exposure to temperatures of an average fever episode. This temperature profile was estimated from the malaria-therapy records of Horton Hospital. From a patient experiencing several episodes of tertian fever before anti-malarial treatment, a typical fever was calculated by superimposing the fever episodes and averaging the temperature at each time-point. The derived temperature profile, showing a fever of approximately 24 h duration, is shown in Fig. 3 along with the clinical data. In the experimental exposure of parasite populations to fever *in vitro*, a discrete version of this curve was used, calculated from the area under the curve over 4 equal time-periods, resulting in 6 h periods of 37·7, 38·9, 39·4 and 38·8 °C. Blood smears from the 65 cultures were taken before and after this 24 h period to monitor changes in the parasite density and age structure. All parasite cultures were initialized at different age structures, and at less than 1 parasite per 100 erythrocytes, to minimize density dependent effects.

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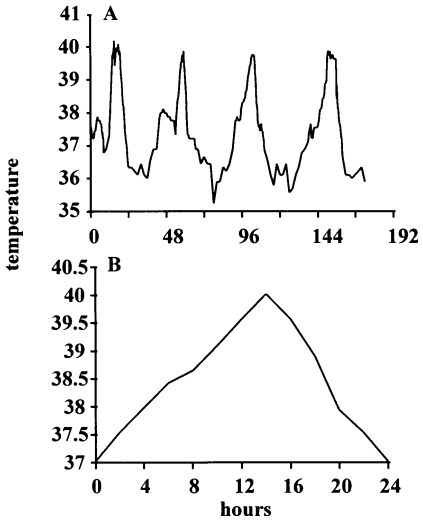

Fig. 3. (A) Tertian pattern of fever observed in a malariatherapy patient. (B) Temperature profile (°C) for 'average' fever episode.

Statistical methods for model parameter estimation

Parameters were estimated by fitting each Markov model chain to the appropriate experimental data using the non-linear least squares algorithm E04UPF from the NAG FORTRAN Library (NAG Central Office, 7 Banbury Road, Oxford, UK). The constraint

$$\sum_{i}^{5} \frac{1}{\lambda_i} = 48$$

was used since the average duration of the life-cycle is approximately 48 h. It was also assumed that the duration of each parasite morphological class was relatively independent of temperature, therefore λ_i was estimated from the cultures at 37 °C thus reducing the number of independent parameters needed to be estimated from the cultures undergoing 'fever'. Estimates of ρ at 37 °C were available from clinical data (Gravenor, McLean & Kwiatkowski, 1995). Computer simulations of the model were performed, and parameter estimates made, using time-intervals corresponding to 4·8 min.

Sensitivity analysis

Sensitivity of the model predictions to variation in estimated parameters was explored with an analysis based on Latin hypercube sampling (LHS) methods (McKay, Conover & Beckman, 1979). If ranges are defined for parameters, LHS can be used to explore how the model predictions vary according to the ranges of parameters. We allowed each estimated parameter to vary over a range defined by a triangular distribution, with the mean of the distribution set at the least squares estimate from the experimental data, and the probability density function set to zero at $\pm 50\,\%$ of the mean. A total of 100 simulations was performed with parameter sets chosen at random from these ranges. Histograms were constructed for the model outputs of fever periodicity and the ratio of maximum : minimum parasitaemia. Note that the ranges sampled by LHS are arbitrary (to represent 'large' errors in parameter estimation) and equal weight is given to each prediction regardless of the fit of that parameter set to the data. Thus emphasis should be put on the range of behaviour in the sensitivity analysis rather than specific values.

RESULTS

Model behaviour

The matrix

$$\mathbf{A}\begin{pmatrix} -(\mu_1+\lambda_1) & 0 & 0 & 0 & \rho\lambda_5 \\ \lambda_1 & -(\mu_2+\lambda_2) & 0 & 0 & 0 \\ 0 & \lambda_2 & -(\mu_3+\lambda_3) & 0 & 0 \\ 0 & 0 & \lambda_3 & -(\mu_4+\lambda_4) & 0 \\ 0 & 0 & 0 & \lambda_4 & -(\lambda_5+\lambda_5) \end{pmatrix}$$

contains the parameters of a Markov chain. The behaviour of the chain can be understood in terms of γ, the dominant eigenvalue of the matrix. In the context of the full coupled Markov chain model, γ_1 and γ_2 are the dominant eigenvalues of 2 matrices $\mathbf{A}_1$ and $\mathbf{A}_2$ which have parameter values relevant to Markov chain 1 and 2 respectively. In general, at normal temperatures γ_1 is positive and the population grows exponentially (at the rate $e^{\gamma 1}$). The specific effects of temperature on the parasite population determine the value of γ_2.

The behaviour of the coupled model can have 3 general outcomes depending upon the model parameter values. (1) If the febrile temperature has little or no effect on reducing parasite growth the parameters of chain 2 will be similar to chain 1. The parasitaemia will then grow unchecked at a rate that tends to $e^{\gamma 2}$. (2) If the parasite death rates during fever are high, γ_2 will be negative, and the population will decline exponentially during the febrile period. This may drive the parasite population to extinction during the febrile period if γ_2 is large enough (in the deterministic formulation here extinction can be defined at less than 1 parasite per host). (3) Regulation occurs if the fever causes the parasitaemia to decline below the fever threshold, but not to extinction. After the febrile period the parasitaemia will recover under the control of Markov chain 1 and grow at a rate $e^{\gamma 1}$ until the threshold is once more reached. The parasitaemia is thus regulated in a density-dependent manner and a limit cycle begins due to the negative population growth during the fever, and the presence of a threshold parasitaemia before the fever is triggered.

Note that the pattern of regulation may vary widely, hence if the model exhibits limit cycles, 2 quantities are of particular interest: the wavelength

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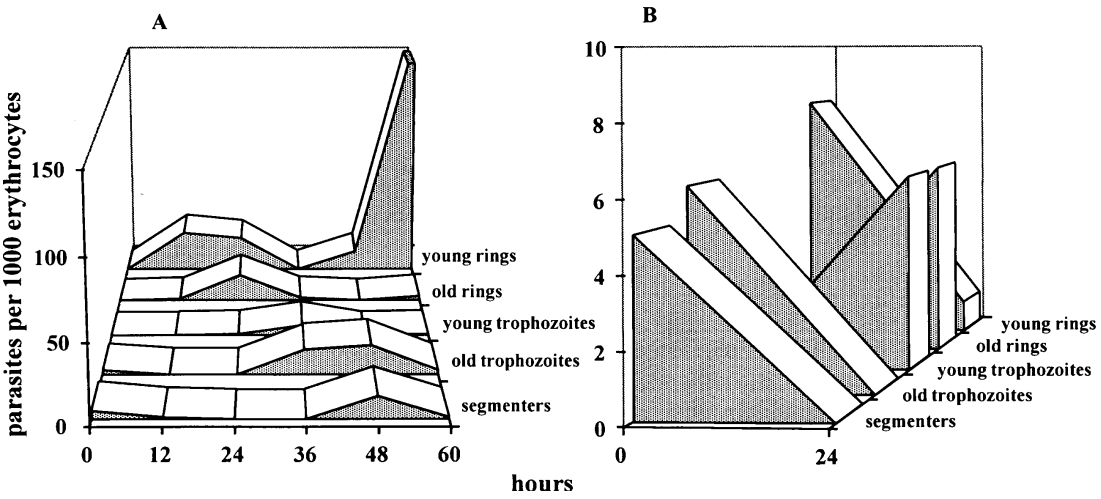

Fig. 4. Examples of *in vitro* data collected for parameter estimation. (A) Changes in parasite numbers and age structure over five 12 h periods in 1 culture growing at 37 °C. (B) Changes in parasite numbers and age structure in 1 culture over a 24 h period of febrile temperatures. Note different scales on time and parasite density axes.

Table 1. Model parameter values estimated from experimental and clinical data

	Transition rates between parasite age groups/12 h
Young ring to old ring	0·98
Old ring to young trophozoite	1·89
Young trophozoite to old trophozoite	1·43
Old trophozoite to segmenter	0·88
Segmenter to young ring	1·63

	Parasite death rate at 37 °C/12 h	Parasite death rate during fever/12 h
Young ring	0·00	0·35
Old ring	0·93	0·94
Young trophozoite	0·00	0·01
Old trophozoite	0·05	0·71
Segmenter	0·00	8·00

	ρ at 37 °C	ρ during fever
	10·0	6·4

and amplitude of the oscillations in parasitaemia. Depending on the model parameters, the periodicity of the triggering of a fever can vary along a full range from continuous fever to fever at more than a weekly interval. Similarly, the ratio of maximum to minimum parasitaemia can vary over orders of magnitude.

Parameters estimated from experimental data

Examples of experimental data used for parameter estimation are given in Fig. 4. Fig. 4A shows 5 consecutive population transitions in a culture maintained throughout at 37 °C. At this temperature all parasite death rates are low, and since this culture is fairly synchronous at $t = 0$, the changes in age structure can be seen as a single 'wave' of parasitaemia. Fig. 4B shows population changes in a culture over a 24 h period at febrile temperatures. The older stages do not survive the fever (shown by the decline in stages 4 and 5, and the failure of the ring population to increase), whilst the young ring stage matures successfully into stages 2 and 3. The parameter values derived from fitting the model to 156 populations growing at normal temperatures (e.g. Fig. 4A), and 65 cultures grown at febrile temperature (e.g. Fig. 4B) are presented in Table 1.

The transition rates are the inverse of the time a parasite spends in each class. These time-intervals are roughly known from observations on parasite cultures (see Silamut & White (1993) although different morphological definitions are used), and therefore provide an informal test of the estimation procedure. Our parameters predict that a parasite spends 12·2, 6·4, 8·4, 13·6 and 7·4 h in stages 1–5 respectively. These values are consistent with *in vitro* observations.

At 37 °C parasite death rates are very low, allowing the population to increase exponentially. The average parameter estimates during the febrile period indicate that the effect of the fever is considerable increase in parasite death rate, which in model simulations lead to an exponential decline in parasitaemia over the febrile period. The most marked effect of the temperature is on the older stages of parasites, with segmenters being particularly susceptible to temperature.

Predicted model behaviour with estimated parameters

The model simulation with the estimated parameters is given in Fig. 5. By setting the pyrogenic threshold

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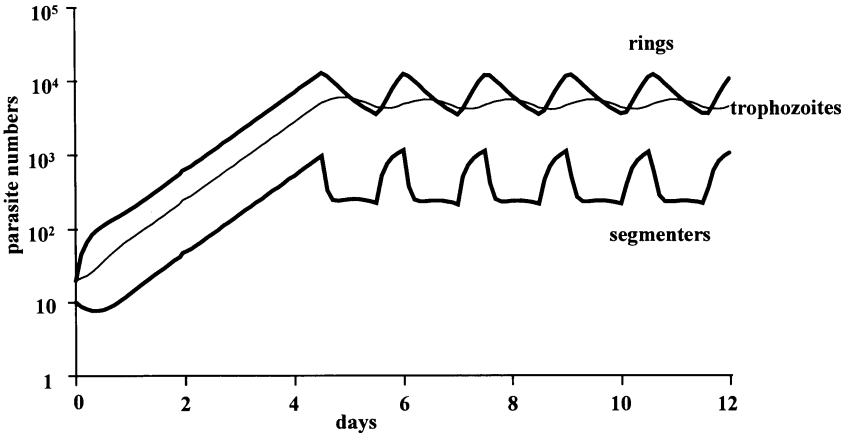

Fig. 5. Model prediction of parasite population dynamics using experimental parameter estimates (Table 1). Parasitaemia refers to the number of parasites/μl of peripheral blood. Pyrogenic threshold set at 10^4 rings. Here, rings refer to model stages 1 and 2, trophozoites to stages 3 and 4, and segmenters to stage 5.

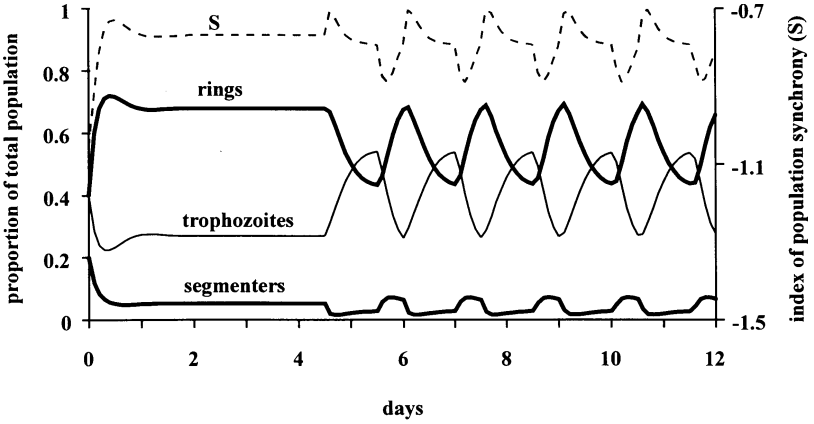

Fig. 6. Model prediction of changes in relative age structure of the parasite population (parameters from Table 1). Rings refer to model stages 1 and 2, trophozoites to stages 3 and 4, and segmenters to stage 5. Each population expressed as a percentage of the total parasite number. Degree of population synchrony (S) plotted on second y-axis (dashed line).

at 10^4 young rings, the unit of volume represented in the model simulation corresponds approximately to 1 μl of blood. After a transient period of exponential growth, the population settles down to a limit cycle, hence the predicted effect of the fever is to regulate the parasite population in a density-dependent manner.

The parasitaemia stops increasing soon after it reaches the fever threshold, and the troughs occur at approximately 1/5 of the threshold (2000 parasites/μl). The wavelength is proportional to the effect of the fever and also gives a prediction of the interfebrile period. In Fig. 5, the young ring population reaches the threshold (causing a fever episode) every 1·5 days.

Predicted parasite age structure and population synchrony

The relative changes in age structure of the parasite population are illustrated in Fig. 6. A complicated picture emerges, with the proportion of the total parasite population in each age class oscillating out of phase. It appears that the asymmetric effect of the fever on parasites of different ages ($\mu_5 > \mu_4 > \mu_1$) generates a degree of synchrony in parasite development, but synchrony is not complete and ring stages tend to predominate through most of the infection.

The level of synchrony was explored further with the formula

$$s_{(t)} = \sum_{i=1}^{5} p_i \ln p_i,$$

where p_i is the proportion of the total population in stage i at time t. Here, $s_{(t)}$ (based on the Shannon entropy index) is at a maximum of 0 when the population is completely synchronous (all parasites in one stage) and at a minimum of $-1{\cdot}6$ during complete asynchrony. We have plotted $s_{(t)}$ in Fig. 6 alongside the relative changes in the population age structure. Soon after the triggering of a fever, the level of population synchrony rises to a peak. Over the 24 h febrile period, the level of synchrony

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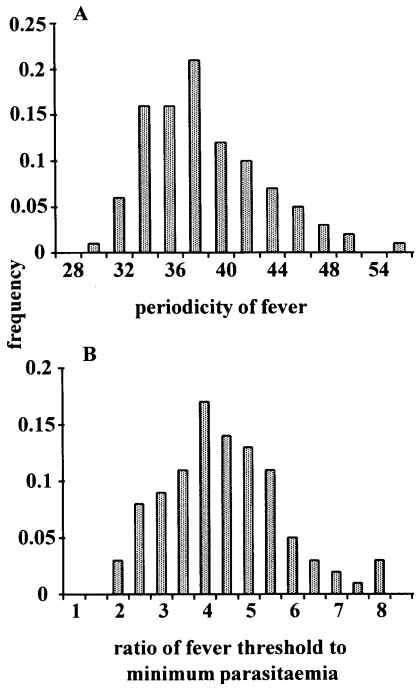

Fig. 7. Sensitivity of model output to variation in estimated parameters. A total of 100 model simulations run with parameters selected at random by Latin hypercube sampling. (A) Histogram of fever periodicity, (B) histogram of magnitude of parasitaemia oscillations measured as the ratio of fever threshold parasitaemia to minimum parasitaemia.

declines. When the fever stops, there is a further drop in synchrony to its lowest point before increasing again under the afebrile parameters.

The age structure under any one Markov chain will tend over time towards a steady state where a constant proportion of parasites occupy each age class (see period 0–4 days in Fig. 6). Reminiscent behaviour can be observed in parasite cultures which tend to an asynchronous state over time. Transient changes in age structure can occur, and are dependent on both the parameters and the starting conditions or age structure at the moment the parameters begin to act. The equilibrium age structure can be determined from the eigenvectors of the matrix of parameters for that chain. It can be seen in Fig. 6 that the equilibrium age structure is marginally more synchronized at normal than febrile temperatures, yet the highest synchrony occurs during the fever and the lowest at normal temperature. It is therefore the interaction between both temperature regimes that introduces the high degree of synchrony, and maintains it over the period of population regulation.

Sensitivity analysis

The results of the sensitivity analyses are shown in Fig. 7. Qualitatively the model output is not sensitive to substantial deviation from the best set of parameter estimates: in all cases parasitaemia was regulated by the fever. From the Latin hypercube sampling, 95% confidence intervals for the model output were (28–48 h) for fever periodicity and (1·5–6·9) for the ratio of the fever threshold parasitaemia to minimum parasitaemia.

DISCUSSION

Clinical observations on primary infections of *P. falciparum* in non-immune subjects suggest that the parasite population, with its enormous capacity for increase, is quickly brought under control by density-dependent influences. The development of natural immunity to *P. falciparum* is extremely complex, and despite progress in describing detailed interactions between the parasite and the host immune response, little is known about the relative role of these mechanisms in controlling parasite numbers *in vivo*. To investigate a candidate mechanism a number of questions can be posed. When is the mechanism turned on and off? Can its action on parasite numbers be measured directly? How do the consequent changes in parasite numbers change the action of the mechanism? Unlike many specific immune effectors, the nature of the fever response allows at least approximate answers to these questions through a combination of clinical observations and *in vitro* data.

Our simple coupled Markov chain model describes the basic interaction between the parasite population and the host fever response. The model parameters can be estimated from experimental data. Other immune mechanisms are omitted in order to investigate the role of fever in isolation. An additional omission is an interaction between the parasite and erythrocyte populations. Any limiting effects of erythrocyte availability on parasite growth are considered small. This assumption will only hold at low parasite densities and during the early phases of infection before the development of chronic anaemia (Gravenor *et al*. 1995). Because of these assumptions, we emphasize that the model is a first step in the analysis of fever in cases of acute primary infection in non-immunes.

Our results suggest that fever can regulate the parasite population, and that periodic fever is a result of a balance between population growth in the host when afebrile, and population decay when febrile. The classic periodicity of malaria is 48 h. What, then, is the meaning of the approximately 37 h periodicity predicted by our model in Fig. 5? If parasitaemia thresholds determine the onset of fever, a wide range of periodicities should be possible (e.g. the interruption of parasite growth by drugs will interrupt the fever pattern), and this behaviour is reflected in the model which can generate fever periodicities from weekly to continuous. Since the parameters estimated from the experimental data

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determine the model periodicity, our underestimates of the true periodicity suggest that, although the temperatures are able to regulate parasitaemia, the estimated febrile parasite death rates are not high enough to fully account for the regulation observed *in vivo*. Similarly, the troughs in parasitaemia are not as low as the data.

Since we do not expect fever to be the sole cause of parasite regulation, we may be satisfied with an unusual predicted fever periodicity. However, a further question needs addressing: why is tertian periodicity so common in nature? Although falciparum fever can be more frequent than 48 h early in infection and is often irregular, we would not have much confidence in a model that could not produce 48 h periodicity over a wide range of (realistic) parameters that represent individual host variation. Here, when the death rates of rings and young trophozoites are small, the fever periodicity can tend asymptotically to around 48 h as the death rates of both old trophozoites and schizonts increase. The specific model predictions here do not generate this periodicity because of a relatively low estimate of μ_4, the death rate of old trophozoites during fever. *In vivo*, this parameter could be increased by other immune responses absent from the model. Furthermore, experimental conditions cannot be expected to mimic exactly the *in vivo* effects of falciparum fever. Although wild isolates show a similar response to temperature as laboratory cultures (Kwiatkowski, 1989), parameters must vary according to the strain of parasite used. Our discrete and constant fever curve also greatly simplifies the system and parameter estimates. In addition it has been suggested that a circadian rhythm may be involved in training the population into 48 hourly cycles (Stauber, 1939; Hawking, Worms & Gammage, 1968).

In a simple sensitivity analysis of the model predictions, we allowed all the parameters to vary randomly around their estimated values. The result of this analysis suggested that large errors in parameter estimation must have occurred for us to reject our basic conclusion, that the fever is capable of density-dependent regulation of the parasitaemia. Although the sensitivity analysis introduced some heterogeneity into the erythrocyte cycle duration, our analysis centred on the effects of temperature on parasite death rate rather than on the transition rates between age classes. We have observed that young rings can complete their cycle in approximately 48 h even following febrile temperatures. However, the consequences of specific effects of febrile temperatures on the various transition rates (such as an induced stasis between stages) remain unexplored in terms of the population dynamics. A potential source of error in the parameter estimates is, of course, that we are restricted to the use of *in vitro* parasite populations and must extrapolate to *in vivo* infections. However, it is difficult to speculate on the errors this will introduce in the parameter values, and detailed *in vivo* parasitaemia time-series are rare, complicated by drug interference and immune status, and never fully age-structured. If sufficient data were available, a useful further test of the fever hypothesis might be a critical evaluation of the relationship between host temperature and parasite density.

Modelling the parasite population with age structure allows an investigation of the chronicity of *Plasmodium* infection, which tends to synchronous development of the intra-erythrocytic parasites. Since a number of anti-malarial drugs are not equally effective against all parasite stages (Geary, Divo & Jensen, 1989), the level of synchrony is of potential clinical importance (Landau *et al*. 1991).

Increased temperatures have been linked with the generation of synchronous cell growth in a number of organisms, including avian Plasmodia (Sherbaum & Zeuthen, 1954; Kovic & Zeuthen, 1967) and in *in vitro* cultures of *P. falciparum* (Kwiatkowski, 1989). Here, a density-dependent febrile response promotes synchrony by periodically killing proportionally more of the older parasites, but the model suggests that the parameters of the parasite life-cycle at normal temperatures play an integral role.

The synchrony predicted by the model is not exact, rings tend to be the most common stage for most of the infection. Although all stages cannot be observed *in vivo*, this behaviour is reminiscent of the clinical records at Horton, where rings could often be seen on consecutive days despite a tertian fever (personal observation). The changes in age structure shown in Fig. 6 illustrate the problems of trying to infer the density of the hidden parasite stages from data on the ring population. If, however, all parameters can be estimated from data on the ring stages only, it may be possible to use a model framework such as that developed here to shed light on the behaviour of sequestered parasites which are considered the more pathogenic and clinically important.

Within-host parasite models can be useful in determining the importance of the different arms of the immune response in controlling parasite populations (Anderson, 1994). However, in most cases, and in particular the case of malaria, the available data with which to compare the models or estimate parameters is limited. The fever response to *P. falciparum* is easy to observe, and its effects can be measured at least in the laboratory. Here, the combination of experimental observations, a population model and independent clinical data suggests that the temperatures of malaria fever may help control the primary infection of a non-immune. Much more detailed data on the dynamics of parasitaemia, along with a framework describing the complex interaction between host and parasite, are

needed before the effects of specific and non-specific immune candidates for control and eventual clearance of infection can be investigated in this manner.

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REFERENCES

ANDERSON, R. M. (1994). Mathematical studies on immunity and infection. *Science* **264**, 1884–1888.

BERENDT, A. R., SIMMONS, D. L., TANSLEY, J., NEWBOLD, C. I. & MARSH, K. (1989). Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature*, *London* **341**, 57–59.

BOYD, M. F. (1949). *Malariology*. Saunders, Philadelphia.

BOYD, M. F. & KITCHEN, S. F. (1937*a*). Simultaneous inoculation with *Plasmodium vivax* and *Plasmodium falciparum*. *American Journal of Tropical Medicine* **17**, 855–861.

BOYD, M. F. & KITCHEN, S. F. (1937*b*). On the infectiousness of patients infected with *Plasmodium vivax* and *Plasmodium falciparum*. *American Journal of Tropical Medicine* **17**, 253–262.

GEARY, T. G., DIVO, A. A. & JENSEN, J. B. (1989). Stage specific actions of antimalarial drugs on *Plasmodium falciparum* in culture. *American Journal of Tropical Medicine and Hygiene* **40**, 240–244.

GRAVENOR, M. B., MCLEAN, A. R. & KWIATKOWSKI, D. (1995). The regulation of malaria parasitaemia: parameter estimates for a population model. *Parasitology* **110**, 115–122.

HAWKING, F., WORMS, M. J. & GAMMAGE, K. (1968). 24- and 48-Hour cycles of malaria parasites in the blood; their purpose, production and control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **62**, 731–760.

KITCHEN, S. F. (1949). Falciparum malaria. In *Malariology* (ed. Boyd, M. F.), pp. 995–1016. Saunders, Philadelphia.

KOVIC, M. & ZEUTHEN, E. (1967). Malarial periodicity and body temperature. An experimental study of *Plasmodium lophurae* in chicken embryos. *Comptes Rendus Des Travaux du Laboratoire Carlsberg* **36**, 209–223.

KWIATKOWSKI, D. (1989). Febrile temperatures can synchronize the growth of *Plasmodium falciparum in vitro*. *Journal of Experimental Medicine* **169**, 357–361.

KWIATKOWSKI, D. & GREENWOOD, B. M. (1989). Why malaria fever is periodic: a hypothesis. *Parasitology Today* **5**, 264–266.

KWIATKOWSKI, D. & NOWAK, M. (1991). Periodic and chaotic host–parasite interactions in human malaria. *Proceedings of the National Academy of Sciences*, *USA* **88**, 5111–5113.

LANDAU, I., CHABAUD, A., CAMBIE, G. & GINSBURG, H. (1991). Chronotherapy of malaria: An approach to malaria chronotherapy. *Parasitology Today* **7**, 350–352.

MCKAY, M. D., CONOVER, W. J. & BECKMAN, R. J. (1979). A comparison of three methods for selecting values of input variables in the analysis of output from a computer code. *Technometrics* **21**, 239–245.

MILLER, M. J. (1958). Observations on the natural history of malaria in the semi-resistant West African. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **52**, 152–168.

ROGIER, C., COMMENGES, D. & TRAPE, F. (1996). Evidence for an age-dependent pyrogenic threshold parasitemia in highly endemic populations. *American Journal of Tropical Medicine and Hygiene* **54**, 613–619.

ROSS, R. & THOMPSON, D. (1910). Some enumerative studies on malarial fever. *Proceedings of the Royal Society of London*, *B* **83**, 159.

SHERBAUM, O. & ZEUTHEN, E. (1958). Induction of synchronous cell division in mass cultures of *Teterahymena pyriformis*. *Experimental Cell Research* **6**, 221–227.

SILAMUT, K. & WHITE, N. J. (1993). Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 436–443.

STAUBER, L. A. (1939). Factors influencing the asexual periodicity of avian malarias. *Journal of Parasitology* **25**, 95–116.

TRAGER, W. & JENSEN, J. B. (1976). Human malaria parasites in continuous culture. *Science* **193**, 673.