Problems with continuous-time malaria models in describing gametocytogenesis

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SUMMARY

Most mathematical models of malaria infection represent parasites as replicating continuously at a constant rate whereas in reality, malaria parasites replicate at a fixed age. The behaviour of continuous-time models when gametocytogenesis is included, in comparison to a more realistic discrete-time model that incorporates a fixed replication age was evaluated. Both the infection dynamics under gametocytogenesis and implications for predicting the amount parasites should invest into gametocytes (level of investment favoured by natural selection) are considered. It is shown that the many malaria models with constant replication rates can be represented by just 3 basic types. For these 3 types, it is then shown that under gametocytogenesis (i) in 2 cases, parasite multiplication and gametocyte production is mostly much too low, (ii) in the third, parasite multiplication and gametocyte production is mostly much too high, (iii) the effect of gametocyte investment on parasite multiplication is mostly too high, (iv) the effect of gametocyte investment on gametocyte production is nearly always too low and (v) with a simple approximation of fitness, the predicted level of gametocyte investment is mostly much too low. However, a continuous model with 48 age-compartments compares well to the discrete model. These findings are a further argument for modelling malaria infections in discrete time.

Key words: malaria, *Plasmodium falciparum*, mathematical models, within-host dynamics, gametocytes, optimal gametocyte investment.

INTRODUCTION

There has been a long history of mathematical modelling in malaria research, beginning with the pioneering work of Ross on malaria epidemiology (Ross, 1911). Within-host models have been developed to explore a number of aspects of malaria biology, including pathogenesis, red blood cell dynamics, immunity, drug action and antigenic variation. A few models have considered gametocytogenesis, the production of malaria transmission stages, which is important for understanding the incidence of disease and how malaria might be controlled. Following the influential model of Anderson *et al*. (1989), most within-host malaria models were formulated as differential equations in continuous time with parasites replicating at a constant rate. This formulation results in replication occurring at very variable ages (Gravenor and Lloyd, 1998; Saul, 1998) but malaria parasites actually replicate at a fixed age. For example, in *P. falciparum*, one replication cycle takes 48 h (Garnham, 1966). Saul (1998) showed that because of the difference between replication in Anderson *et al*.'s (1989) model and the biological process, the model could generate hugely inflated growth rates. To correct the growth rate, Anderson *et al*. (1989) used an artificially low value for the parameter governing parasite invasion of red blood cells, which meant that the infections appeared unrealistically easy to control (Molineaux and Dietz, 1999). In contrast, a fixed replication age is easily incorporated in a discrete-time model. By setting the time-step to one cycle period and using multiplication factors to describe the dynamics between each time-point, appropriate growth rates are ensured. Early discrete models were proposed by Marcus-Roberts and Roberts (1983) and Kwiatkowski and Nowak (1991). Because of the problems with the growth rate in Anderson *et al*.'s (1989) model, Saul (1998) and Molineaux and Dietz (1999) recommended modelling malaria infections in discrete time. Gravenor and Lloyd (1998) instead suggested defining several age compartments, which parasites move through in turn, and representing the dynamics by a series of differential equations.

Gametocytes are sexual forms that derive from replicating parasites, which are asexual. When ingested by a mosquito, gametocytes transform into male and female gametes. Following fertilization, parasites penetrate the midgut wall to form oocysts on the

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outside surface of the midgut. Oocysts produce sporozoites, which migrate to the salivary glands and result in the mosquito being infectious to new hosts. In *P. falciparum*, by capturing the progeny (merozoites) of single asexuals in clusters on a red blood cell monolayer, it was shown that commitment to gametocyte production is made by the parental asexual, with all the merozoites from one parent developing in the same way (Bruce *et al*. 1990). The timing of gametocyte commitment may, however, be different in other *Plasmodium* species. Mons (1986) compared the number of gametocytes developing in the rodent malaria species *P. berghei in vivo*, and when parasites were taken from infections and cultured. Similar proportions of gametocytes were found only when parasites were taken part-way through the replication cycle, suggesting that gametocyte commitment in *P. berghei* does not occur until parasites are growing inside cells. There are few data on the level of gametocytogenesis during *P. falciparum* infections. Measurement is difficult because *P. falciparum* gametocytes have a maturation period of around 8–10 days (Day *et al*. 1998) and immature gametocytes sequester from the peripheral circulation. In particular, this means that the ratio of gametocytes to asexuals at a given time is not a good indicator of gametocyte investment. Smalley *et al*. (1981) estimated gametocyte investment by collecting parasites from natural infections and culturing them until young gametocytes could be distinguished. On average, 8% of parasites became gametocytes in infections where gametocytes were already present and 1% in infections without gametocytes, with substantial variation. *In vitro*, between $<0{\cdot}3\%$ and 70% of *P. falciparum* parasites have been found to develop into gametocytes (Bruce *et al*. 1990) and almost 100% gametocyte conversion was measured when cyclic AMP was added to cultures (Kaushal *et al*. 1980).

Anderson *et al*.'s (1989) model ignores gametocytogenesis. Because gametocytogenesis represents a diversion of resources away from parasite growth, including gametocytogenesis in within-host malaria models will have an effect on asexual dynamics. In this paper, the dynamics of continuous-time models with gametocytogenesis or when gametocytogenesis is added are investigated, by comparing their behaviour to a discrete time model. Additionally, the possible implications of using these models to address questions relating to malaria transmission are considered. An unresolved problem in gametocyte biology is why the density and prevalence of gametocytes tends to be so much lower than that of asexuals (Taylor and Read, 1997). Two studies have performed optimality analyses based on continuous-time models to predict the level of investment into transmission stages that maximizes parasite transmission (Koella and Antia, 1995; McKenzie and Bossert, 1998). Here, the results of optimality analyses on gametocyte investment from the continuous models and the discrete model are compared. In the Methods section, the conditions under which the models are compared is described. Two parameters for comparing the model dynamics are introduced and a simple cost-benefit analysis to predict optimal gametocyte investment is outlined. A discrete-time model, to evaluate the continuous models against, is proposed. In the Results section, the behaviour of the discrete model is described. The continuous models with constant replication rates are classified into 3 types and the model dynamics and optimal gametocyte investment for each type are examined in turn. In the Discussion section, it is explained why gametocyte investment has a different effect in continuous rather than discrete models, and the 3 types of continuous model are compared. Also, it is indicated how the problems revealed by gametocytogenesis might affect other aspects of continuous within-host malaria models.

METHODS

Model dynamics

The aim here is to examine the fundamental behaviour of the continuous-time models. Therefore, basic forms of the models are taken, ignoring factors causing asexual death before replication, for example, host immune responses and red blood cell dynamics. Also, only infections of 1 parasite strain and species are considered. If the continuous models cannot reproduce the underlying parasite biology, then the impact of additional complexities may be misrepresented and parameter values estimated from fitting the model to data are likely to be misleading. Gametocyte production is added to the models that do not include it by designating a proportion of merozoites as gametocytes.

The models can be compared by their dynamics over 1 cycle period. For this purpose, 2 parameters are defined. The asexual multiplication factor (X) is the number of asexuals after 1 cycle period divided by the number of asexuals at the start of the period. Relative gametocyte production (P) is the number of gametocytes produced during 1 cycle period divided by the number of asexuals at the start of the period. If there are transient initial dynamics, the long-term values for X and P are given. Mathematically,

$$X = A[T+1]/A[T], \tag{1}$$

$$P = (G[T+1] - G[T])/A[T], \tag{2}$$

where A is the number of asexuals, T is the time in cycle periods and G is the total number of gametocytes that have been produced up to that point.

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Optimal gametocyte investment

Parasites should evolve in a way that maximizes transmission to new hosts (fitness) (Anderson and May, 1982). Optimal gametocyte investment is the level of investment that maximizes fitness and therefore that we would expect to see in malaria infections, provided the assumptions made are correct (see Parker and Maynard Smith (1990) for a review of optimality theory). I assume that fitness is proportional to the number of gametocytes produced over an infection. This is based on the assumption that the probability of infecting a new host is proportional to the intensity of infection in the mosquito and that, therefore, the mean number of oocysts per mosquito is a better indicator of transmission than the percentage of mosquitoes infected. It also assumes a linear relationship between gametocyte density and mean oocyst number, which generally has been found (Eyles, 1951; Tchuinkam *et al*. 1993; Robert *et al*. 1996) and fixed gametocyte mortality. The effect of representing transmission by a saturating function of gametocyte density, as would be the case if the percentage of mosquitoes infected were used as the measure of transmission, is considered in the Discussion section.

It is assumed that gametocyte investment is constant over an infection. The scarcity of *in vivo* estimates means that the pattern of gametocyte investment during *P. falciparum* infections is not known; although the report of Smalley *et al*. (1981) of higher average gametocyte conversion in infections with mature gametocytes suggests that investment is higher several days into an infection than at the start. *In vitro*, *P. falciparum* has been shown to alter gametocyte investment in response to a number of factors (for a review see Dyer and Day, 2000) but for many of these factors, their relevance to the time-course of investment *in vivo* is not clear. Findings that may be pertinent are increased gametocyte conversion when asexual multiplication slows down after a period of growth (Carter and Miller, 1979; Bruce *et al*. 1990; Williams, 1999), and higher conversion when serum and lymphocytes from infected children were added to cultures (Smalley and Brown, 1981). Koella and Antia (1995) have shown that if investment into transmission can vary, the pattern that optimizes the total number of gametocytes produced is zero investment until just before peak parasite density would have been reached, followed by complete conversion to gametocytes. The prevalences and relative densities of gametocytes in epidemiological surveys (see Taylor and Read, 1997), the chronicity of infections and gametocytaemia recorded in malariatherapy patients (Collins and Jeffery, 1999; Diebner *et al*. 2000) strongly argue against this being the pattern in *P. falciparum*. Given that *P. falciparum* does not behave as predicted if investment is allowed to vary freely, the uncertainty in the actual pattern of investment and that it is biologically plausible that malaria parasites should spread transmission opportunities throughout an infection, assuming constant investment is a reasonable starting point. Further studies could then explore the effects of modulating investment from this baseline level.

Some simplifications are made for the purpose of illustration. The intention is not to give a definitive prediction of optimal gametocyte investment but rather to show in an intuitive way how the effects of gametocyte investment on asexual multiplication and relative gametocyte production influence such predictions. It is first assumed that there is negligible effect of immunity up to the time of peak parasitaemia, which reasonably represents the situation for naïve individuals. There is a time lag of about 1 week before an acquired immune response becomes effective (Janeway *et al*. 2001) and the typical infection profile in naïve individuals is an initial exponential rise in parasitaemia (implying unrestricted parasite growth) (Boyd, 1949). It is also assumed that red blood cell dynamics have no effect up to the time of peak parasitaemia, which should be the case before any significant anaemia has developed (see Hoshen *et al*. 2000*a*) and that over this time-period no other factors cause asexual death before replication. Only single-strain, single-species infections are considered. For constant gametocyte investment, the number of gametocytes that are produced at any time is proportional to the number of asexuals. Therefore, provided, due to immunity or other mechanisms, there is a large drop in parasitaemia following the peak; when gametocyte investment is constant, considerably more gametocytes will be produced in the last cycle period before peak parasitaemia than at any other time. Hence, the total number of gametocytes produced over an infection is approximated by the number produced in the last cycle period before peak parasitaemia. This is a reasonable approximation for the situation where malaria is epidemic but is less appropriate to the case where malaria is endemic and transmission during the chronic phase of infection may be an important component of parasite fitness. With these simplifications, fitness (ω) is given, or approximately given (see below), by

$$\omega = PX^{l-1}A[0], \tag{3}$$

where l is the time (in cycle periods) to peak parasitaemia. Optimal gametocyte investment can be found by solving $d\omega/dg = 0$, where g is gametocyte investment, to obtain the value of g that maximizes fitness. By differentiating equation (3), after dividing through by $A[0]$, P and X^{l-1}, optimal investment is given by

$$\frac{1}{P}\frac{dP}{dg} + (l-1)\frac{1}{X}\frac{dX}{dg} = 0. \tag{4}$$

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Hence, from equation (4), the fitness benefit (B) and cost (C) of increasing gametocyte investment are defined as

$$B=\frac{1}{P}\frac{dP}{dg}, \tag{5}$$

$$C=-(l-1)\frac{1}{X}\frac{dX}{dg}, \tag{6}$$

respectively. Optimal investment occurs when the fitness benefit of increasing gametocyte investment equals the cost. The benefit and cost segregate the effects of gametocyte investment on gametocyte production and on asexual multiplication and are easily interpretable in biological terms. The benefit is the proportional increase in relative gametocyte production with increasing gametocyte investment. The cost is the proportional decrease in the asexual multiplication factor, multiplied by the number of cycles for which this is paid before gametocyte production is measured.

Equation (3) is only approximate if there are transient initial dynamics and X and P are not good approximations of the behaviour at the start of infection. However, equations (5) and (6) will be good approximations of the actual fitness benefit and cost provided the proportional change in the asexual multiplication factor with investment for the first $l-1$ cycle periods is close to the long-term value and the proportional change in relative gametocyte production with investment in the lth cycle period is close to the long-term value.

A proposed discrete-time model of malaria infection

A simple discrete-time model of malaria dynamics with a time-step of one cycle period is proposed. Asexuals die after one cycle period, each releasing m merozoites, which are all assumed to successfully invade uninfected red blood cells. Hence, in this model, asexuals have a fixed replication age, as described (Garnham, 1966). A proportion g of merozoites become gametocytes. The dynamics of the asexuals (A) are described by

$$A[T+1]=m(1-g)A[T] \tag{7}$$

and the dynamics of gametocyte production (G), by

$$G[T+1]-G[T]=mgA[T]. \tag{8}$$

Note that G does not represent the number of gametocytes at a given time but the total number that have been produced up to that point. For the purpose of this paper, only gametocyte production is needed and this avoids potential difficulties with representing gametocyte mortality and maturation under the different model formulations.

In the following section, expressions for the asexual multiplication factor (equation (1)), relative gametocyte production (equation (2)) and the fitness cost and benefit of increasing gametocyte investment (equations (5) and (6)) are given for this discrete model. Because the model incorporates a fixed replication age, it should realistically represent the biological process. The results from this model are used as a standard against which to compare the behaviour of the continuous models. Throughout this paper, quantitative results and model comparisons are for *P. falciparum* infections.

RESULTS

The discrete model

From equations (7) and (8), the asexual multiplication factor and relative gametocyte production are given by

$$X=m(1-g), \tag{9}$$

$$P=mg, \tag{10}$$

respectively. The fitness benefit and cost of increasing gametocyte investment are given by

$$B=1/g, \tag{11}$$

$$C=(l-1)/(1-g), \tag{12}$$

respectively. Under this model, as gametocyte investment rises, relative gametocyte production increases linearly and the asexual multiplication factor decreases linearly. From equating the fitness benefit and cost, with $l=4$ (average time to peak parasite density for blood-induced infections in Collins and Jeffery, 1999) optimal gametocyte investment is 0·25.

Model classification

A classification of continuous within-host malaria models with constant replication rates into 3 types is presented in Table 1. Type 1 models have a single equation for asexual dynamics. Several of these models include gametocytogenesis. Koella and Antia's (1995) model is a general one for parasites with separate stages for replication and transmission. Their aim was to predict the pattern of investment into transmission stages that would maximize transmission and they considered both constant and variable investment. McKenzie and Bossert (1997) devised a series of equations to investigate the interplay of host immunity and gametocytogenesis in *P. falciparum* and compared the results to empirical data. They explored 3 different functions for gametocyte conversion: a constant proportion of asexuals, proportional to the product of asexual and immune effector densities and proportional to the square of the asexual density. They then used the simplest of these models, with constant gametocyte investment, to predict optimal gametocyte production, including

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Table 1. Classification of continuous within-host malaria models with constant replication rates

Type 1: single asexual equation	Type 2: additional merozoite equation	Type 3: compartmental
Koella and Antia (1995)*	Anderson *et al*. (1989)	Gravenor and Kwiatkowski (1998)
McKenzie and Bossert (1997, 1998)*	Hellriegel (1992)*	Gravenor *et al*. (1998)
Hoshen *et al*. (1998, 2001, 2002)	Gravenor *et al*. (1995)	Gravenor *et al*. (2002)
Mason and McKenzie (1999)*	Hetzel and Anderson (1996)	
Mason *et al*. (1999)*	Swinton (1996)	
Simpson *et al*. (2002)	Anderson (1998)	
Gurarie *et al*. (2006)	Austin *et al*. (1998)	
	Recker *et al*. (2005)	

* Includes gametocyte production.

when 2 parasite strains infect the same host (McKenzie and Bossert, 1998). The purpose of the models of Hoshen *et al*. (1998, 2001, 2002) was to suggest improved drug-treatment protocols by combining data on drug kinetics and efficacy with *P. falciparum* dynamics. Mason *et al*.'s (1999) model describes mixed infections of *P. falciparum* and *P. malariae* and examines the consequences of cross-immunity between the two species. They include gametocyte production as the loss of a fixed proportion of asexuals, but do not model gametocyte dynamics. Mason and McKenzie (1999) used essentially the same model to look at mixed infections of *P. falciparum* and *P. vivax*. Simpson *et al*.'s (2002) motivation was to characterize the initial population dynamics of *P. falciparum* before there was a significant effect of host immunity. Gurarie *et al*.'s (2006) model was developed to assess the importance of non-specific and species-specific immune responses in controlling malaria parasitaemia and is similar to Mason *et al*.'s (1999) model, with the addition of a fever response and time lags in the onset of immunity.

Type 2 models have an additional equation for merozoite dynamics. The model of Hellriegel (1992) is the only one that incorporates gametocytogenesis; she assumes a constant rate of asexual conversion to gametocytes. Anderson *et al*.'s (1989) model was designed to look at the relative impact of immune responses against merozoites and against asexuals during *P. falciparum* infections. Gravenor *et al*. (1995) used the same model to evaluate the extent to which parasite destruction of red blood cells regulates parasitaemia and causes anaemia. The models of Hellriegel (1992), Hetzel and Anderson (1996), Swinton (1996), Anderson (1998) and Austin *et al*. (1998) were all based on Anderson *et al*.'s (1989) model. Hellriegel's (1992) model describes competition between 2 *P. falciparum* clones for red blood cells in the presence of an immune response, and addressed the effect of timing of superinfection. The aim of Hetzel and Anderson (1996) was to determine the criteria governing parasite invasion and persistence in the host and to reproduce the infection pattern of the rodent malaria parasite *P. berghei*. They also examined the effect of immunity against merozoites compared to that against asexuals. Swinton (1996) formulated a model of infection with multiple strains, intended for exploring strain-specific and strain-transcending immunity, but did not present any results. Anderson (1998) looked at the relationship between equilibrium densities of malaria parasites and responding immune cells. The purpose of Austin *et al*.'s (1998) study was to determine drug effectiveness and dose criteria for successful prophylaxis or treatment of *P. falciparum* based on the within-host basic reproduction number, R_0. Recker *et al*.'s (2005) model, unlike the other Type 2 models, does not explicitly incorporate red blood cell dynamics and was aimed at investigating antigenic variation of merozoite surface proteins in the rodent malaria parasite, *P. yoelli*.

Type 3 models are compartmental models consisting of a series of asexual equations, which Gravenor and Lloyd (1998) suggested would resolve growth rate problems by reducing the variation in parasite replication age. None of these models include gametocytogenesis. Gravenor and Kwiatkowski's (1998) model was developed to evaluate whether fever could regulate *P. falciparum* parasitaemia and promote parasite synchronization. The goal of Gravenor *et al*.'s (1998) study was to determine the effect of drug treatment on sequestered parasites in cerebral *P. falciparum* malaria. Similarly, Gravenor *et al*.'s (2002) model was designed to estimate sequestered parasite dynamics in *P. falciparum* patients undergoing drug therapy.

Type 1: single equation for asexual dynamics

In most Type 1 models (Table 1), asexuals replicate at rate a in the absence of gametocyte production and, in the models that include gametocytogenesis, produce gametocytes at rate g'. To represent gametocyte investment as a proportion rather than a rate, a can be replaced by $r(m-1)$ and g' by rmg,

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where r is the rate at which asexuals rupture. Hence asexuals die at rate r, each releasing m merozoites. All merozoites are assumed to instantaneously invade uninfected red blood cells and a proportion g becomes gametocytes. The basic form of the models is

$$dA/dt = r(m-1)A - rmgA, \tag{13}$$

$$dG/dt = rmgA. \tag{14}$$

Note that gametocyte mortality is not included in equation (14) because it describes the total number of gametocytes produced, not gametocyte numbers. Koella and Antia's (1995) model differs from the standard Type 1 model described above. They omit the rate term in equation (14), which makes their equation dimensionally inconsistent. They assume that gametocyte commitment occurs not at the merozoite stage but once parasites are already growing in cells. Hence, the m in equation (14) is absent. Koella and Antia (1995) also assumed that parasites replicate by repeated division into two and therefore set $m=2$ and increase r.

For constant g, the analytical solutions to equations (13) and (14) are given by

$$A[t] = A[0]e^{r(m(1-g)-1)t}, \tag{15}$$

$$G[t] = \frac{A[0]mg}{m(1-g)-1}\left(e^{r(m(1-g)-1)t}-1\right), \tag{16}$$

respectively. Hence, the asexual multiplication factor (equation (1)) and relative gametocyte production (equation (2)) are given by

$$X = e^{r(m(1-g)-1)\tau}, \tag{17}$$

$$P = \frac{mg}{m(1-g)-1}\left(e^{r(m(1-g)-1)\tau}-1\right), \tag{18}$$

respectively, where τ is the cycle period. The fitness benefit (equation (5)) and the cost (equation (6)) of increasing gametocyte investment are given by

$$B = \frac{1}{g} + \frac{m}{m(1-g)-1} + \frac{rm\tau}{e^{-r(m(1-g)-1)\tau}-1}, \tag{19}$$

$$C = (l-1)rm\tau, \tag{20}$$

respectively. The models can be set up with either r or m reduced and both cases are considered. Note that for the average age at replication to be τ, r must be set to $1/\tau$.

Fig. 1A shows asexual multiplication as a function of gametocyte investment for the discrete model (equation (9)) and for Type 1 models with r or m reduced (equation (17)). The asexual multiplication factor decreases exponentially with rising gametocyte investment for Type 1 models instead of declining linearly as in the discrete model. It is nearly always much lower than for the discrete model and is lower when m is reduced than when r is (except for $g=0$). The asexual multiplication factor is lower still for Koella and Antia's (1995) model.

Fig. 1B shows relative gametocyte production as a function of investment for the discrete model (equation (10)) and for Type 1 models with r or m reduced (equation (18)). Relative gametocyte production in Type 1 models initially increases with gametocyte investment but reaches a maximum and then drops, rather than increasing linearly as in the discrete model. Except at low values of g, it is much lower than for the discrete model. The difference between reducing r and reducing m is only slight. Relative gametocyte production in Koella and Antia's (1995) model is affected by their different timing of gametocyte commitment, which alters it by a factor of m. Taking this into account, relative gametocyte production for Koella and Antia's (1995) model is mostly much higher than in a discrete model. Table 2 describes the biggest differences in Type 1 dynamics from the discrete model.

Fig. 2A shows the fitness benefit and cost of increasing gametocyte investment for the discrete model (equations (11) and (12)) and for Type 1 models (equations (19) and (20)). The benefit in Type 1 models decreases with rising investment similarly to the discrete model, but is nearly always much lower and is negative for high investments. The difference from the discrete model is bigger when m is reduced than when r is reduced. The cost of increasing investment is independent of investment in Type 1 models rather than increasing with investment as it does in the discrete model. It is mostly much higher than for the discrete model and is higher when m is reduced than when r is reduced. In Koella and Antia's (1995) model, the benefit of increasing gametocyte investment is lower (for $g>0$) and the cost is higher. Table 3 gives the optimal gametocyte investments for Type 1 models. The values are much lower than for the discrete model.

Type 2: additional equation for merozoite dynamics

In these models (Table 1), asexuals rupture at rate r, each releasing m merozoites. In most of the models, there is an equation for red blood cell dynamics and the rate at which merozoites invade uninfected blood cells is given by $\beta' x$, where x is the density of uninfected blood cells. When red blood cell dynamics are ignored, $\beta' x$ can be replaced by β. Merozoites die at rate δ. With gametocyte production added, the basic form of the models is

$$dA/dt = \beta(1-g)M - rA, \tag{21}$$

$$dM/dt = rmA - \delta M - \beta M, \tag{22}$$

$$dG/dt = \beta gM, \tag{23}$$

where M is the number of merozoites. Hellriegel's (1992) model has several differences from the

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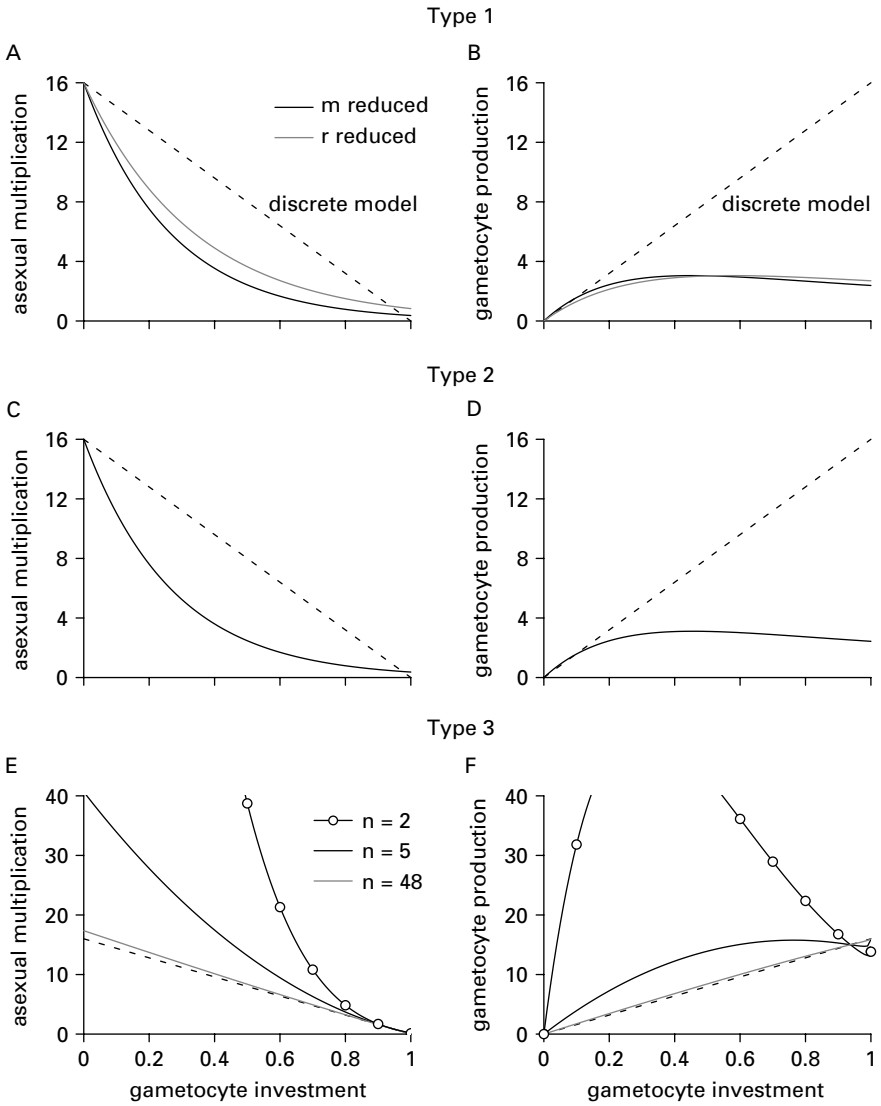

Fig. 1. Dynamics in continuous-time models. (Left column) asexual multiplication per cycle period. (Right column) relative gametocyte production per cycle period. Dashed lines show values for discrete model. (A and B) Type 1. (C and D) Type 2 (long-term behaviour). (E and F) Type 3 (long-term behaviour), n is the number of compartments. Parameter values are: $m=16$, $r=0.5$, $\tau=2$; (A and B) reduced $m=3.8$, reduced $r=0.09$; (C and D) $\delta=72$, $\beta=22.6$; (E and F) $\alpha=n/2$.

standard Type 2 model described above. Firstly, it is assumed, as in the model of Koella and Antia (1995), that gametocyte commitment occurs at the asexual rather than the merozoite stage. Hence $(1-g)$ is absent from equation (21) and equation (23) is altered. In Hellriegel's (1992) model, asexuals become gametocytes at rate c, but to represent gametocyte investment as a proportion rather than a rate, c can be replaced by rg. The equivalent of equation (23) is then $dG/dt=rgA$. Secondly, Hellriegel (1992) adds gametocyte production by an additional loss term, $-rgA$, in equation (21). This is unrealistic because it means that asexuals that become gametocytes are removed twice from the population and, as the rate of merozoite production is not reduced, they also replicate. Anderson's (1998) model differs from the standard Type 2 model by omitting the $-\beta M$ term in equation (22). This is unrealistic because it allows merozoites to invade more than 1 cell. Hetzel and Anderson (1996) include a loss term in equation (22) for merozoites invading already-infected cells. Numerical simulations showed that this had a negligible effect on the dynamics up to the end of the fourth cycle period and therefore Hetzel and Anderson's (1996) model is not considered separately.

The analytical results to equations (21)–(23) and the asexual multiplication factor, relative gametocyte production and the fitness benefit and cost of increasing gametocyte investment are given in the Appendix. There are transient initial dynamics and the results are based on the long-term dynamics but the initial behaviour for the full model solution is also described.

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Table 2. Summary of discrepancies in dynamics between continuous models and discrete model*

	Type 1		Type 2		Type 3	
Biggest difference in asexual multiplication factor from discrete model	r reduced:	$-4{\cdot}7$ $g=0{\cdot}37$		$-6{\cdot}1$ $g=0{\cdot}35$	$n=2$:	390 $g=0$
	m reduced:	$-6{\cdot}1$ $g=0{\cdot}35$	H:	6·0 $g=1$	$n=5$:	25 $g=0$
	KA:	$-8{\cdot}2$ $g=0{\cdot}31$	A:	33 $g=0$	$n=48$:	1·4 $g=0$
Biggest difference in relative gametocyte production from discrete model	r reduced:	-13 $g=1$		-14 $g=1$	$n=2$:	46 $g=0{\cdot}28$
	m reduced:	-14 $g=1$	H:	1·8† $g=1$	$n=5$:	6·0 $g=0{\cdot}46$
	KA:	0·84† $g=0{\cdot}23$	A:	-13 $g=1$	$n=48$:	0·43 $g=0{\cdot}53$

* Values given are the difference between the value for the continuous model and the value for the discrete model. The level of gametocyte investment for which this difference occurs is also reported. Values are given to 2 significant figures and where appropriate, are for long-term solutions.
KA = Koella and Antia's (1995) model; H = Hellriegel's (1992) model; A = Anderson's (1998) model.
† Compared to a discrete model where asexuals develop directly into gametocytes and relative gametocyte production ranges from 0–1.

The asexual multiplication factor in Type 2 models is shown in Fig. 1C. It is nearly always much lower than in the discrete model. In Hellriegel's (1992) model, the asexual multiplication factor is higher, and also higher than in the discrete model (except when $g=0$). For Anderson's (1998) model the multiplication factor is also higher (except when $g=1$) and is higher than in the discrete model for low gametocyte investments. For all the models, the multiplication factor for the full solution is very similar to the long-term value in the first cycle period.

Relative gametocyte production in Type 2 models is shown in Fig. 1D. It is much lower than in the discrete model except when gametocyte investment is low and declines at high investments. For Anderson's (1998) model, relative gametocyte production is higher (except for $g=0$) and is higher than in the discrete model for low gametocyte investments. Taking into account the different timing in gametocyte investment, relative gametocyte production is much higher in Hellriegel's (1992) model than in a discrete model, except for low investments. For all the models, relative gametocyte production for the full solution is very similar to the long-term value in the first cycle period. Table 2 describes when the dynamics in Type 2 models differ most from the discrete model.

Fig. 2B shows the fitness benefit and cost of increasing gametocyte investment in Type 2 models. The benefit is nearly always much lower than in the discrete model and is negative when gametocyte investment is high. The cost is mostly much higher than in the discrete model. In Hellriegel's (1992) model, the benefit of increasing gametocyte investment is higher (for $g>0$) and always positive but still less than in the discrete model and the cost is lower than in the discrete model. In contrast, in Anderson's (1998) model, the benefit of increasing gametocyte investment is lower (for $g>0$) and the cost is higher. Optimal gametocyte investments for Type 2 models are given in Table 3. They are much lower than for the discrete model except for Hellriegel's (1992) model.

Type 3: compartmental

These models (Table 1) have n asexual age compartments and asexuals move from one compartment to the next as they mature. Movement between compartments occurs at fixed rates and asexuals in the last compartment rupture to produce m merozoites each. All merozoites are assumed to instantaneously invade uninfected red blood cells and new asexuals enter the first compartment. In some of the models, compartment-specific transition rates are considered, but in order to give analytical results a single rate α is assumed for all the compartments. Numerical results with individual rates were similar. With gametocyte production added, the basic form of the models is

$$dA_1/dt=\alpha m(1-g)A_n-\alpha A_1, \tag{24}$$

$$dA_x/dt=\alpha A_{x-1}-\alpha A_x \quad \text{for} \quad 2\leqslant x\leqslant n, \tag{25}$$

$$dG/dt=\alpha mgA_n, \tag{26}$$

where A_x is the number of asexuals in compartment x. Similarly to Type 2 models (see Appendix), the full solution is a sum of exponential terms, which is dominated by the term with the most positive eigenvalue after some transient initial dynamics. The results are based on the long-term dynamics but the

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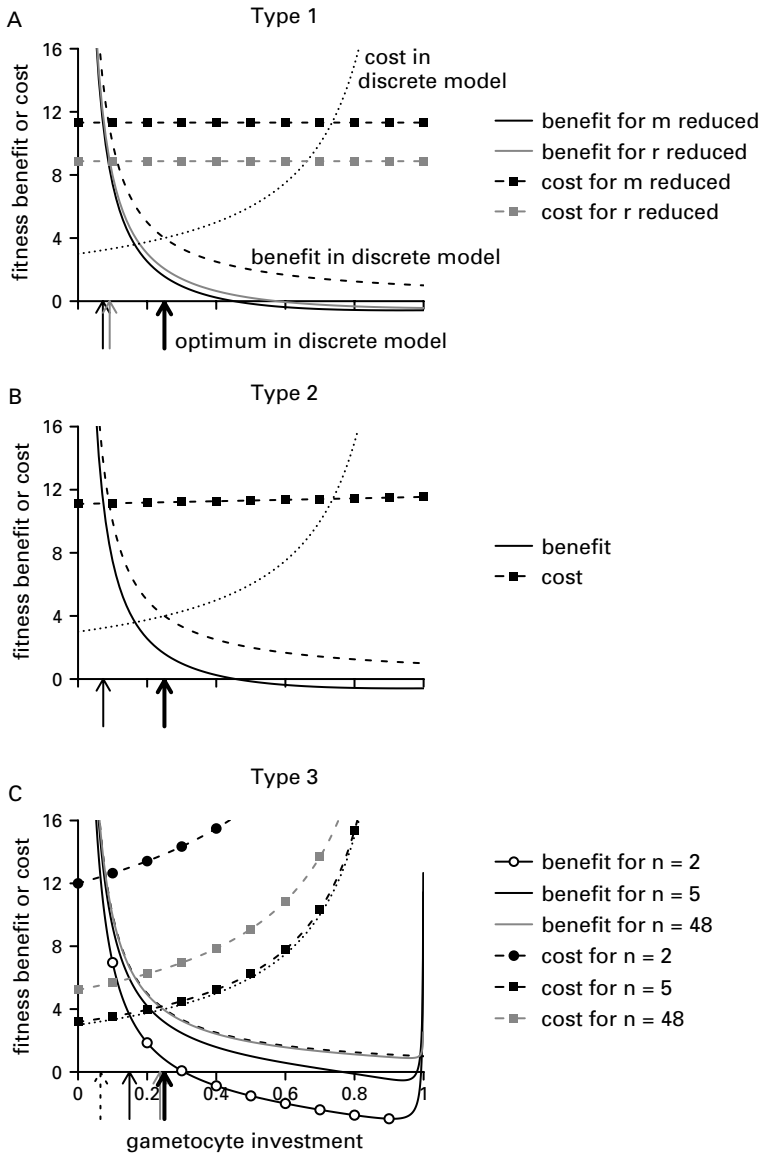

Fig. 2. Optimal gametocyte investment in continuous-time models. Fitness benefit and cost of increasing gametocyte investment is shown and arrows indicate optimum investment. Dashed line with no symbols shows fitness benefit for discrete model, dotted line shows fitness cost for discrete model and thick black arrow shows optimum investment for discrete model. (A) Type 1, thin black arrow shows optimal investment for reduced m, grey arrow optimal investment for reduced r. (B) Type 2 (based on long-term dynamics). (C) Type 3 (based on long-term dynamics), n is the number of compartments. Dashed arrow shows optimal investment for $n=2$, thin black arrow optimal investment for $n=5$, grey arrow optimal investment for $n=48$. Parameter values are $l=4$ and other values as in Fig. 1.

Table 3. Optimal gametocyte investment in the continuous models and the discrete model*

Discrete model	Type 1		Type 2		Type 3	
0·25	r reduced:	0·09		0·07	$n=2$:	0·06 (0·07)
	m reduced:	0·07	H:	0·28	$n=5$:	0·15
	KA:	0·05	A:	0·06	$n=48$:	0·24 (0·26)

* KA = Koella and Antia's (1995) model; H = Hellriegel's (1992) model; A = Anderson's (1998) model. Values in parentheses are for full model solutions, where these differ from the long-term approximations.

initial behaviour is also described for the full model solution. For constant g and the boundary condition that all asexuals start in the first compartment, the long-term solutions are

$$A[t]=\frac{A[0]}{n(m(1-g))^{(n-1)/n}}\,\frac{m(1-g)-1}{\sqrt[n]{m(1-g)}-1}\,e^{\alpha\left(\sqrt[n]{m(1-g)}-1\right)t}, \tag{27}$$

$$G[t]=\frac{mgA[0]}{n(m(1-g))^{(n-1)/n}}\,\frac{e^{\alpha\left(\sqrt[n]{m(1-g)}-1\right)t}}{\sqrt[n]{m(1-g)}-1}-\frac{mgA[0]}{m(1-g)-1}, \tag{28}$$

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respectively, where the number of asexuals is summed over all compartments. Hence,

$$X = e^{\alpha\left(\sqrt[n]{m(1-g)}-1\right)\tau}, \tag{29}$$

$$P = \frac{mg}{m(1-g)-1}\left(e^{\alpha\left(\sqrt[n]{m(1-g)}-1\right)\tau}-1\right). \tag{30}$$

The fitness benefit and the cost of increasing gametocyte investment are given by

$$B = \frac{1}{g} + \frac{m}{m(1-g)-1} - \frac{\alpha m\tau}{n(m(1-g))^{(n-1)/n}} \times \left(\frac{e^{\alpha(\sqrt[n]{m(1-g)}-1)\tau}}{e^{\alpha(\sqrt[n]{m(1-g)}-1)\tau}-1}\right), \tag{31}$$

$$C = \frac{(l-1)\alpha m\tau}{n(m(1-g))^{(n-1)/n}}, \tag{32}$$

respectively. Gravenor *et al.* (1998) used 2 asexual compartments, Gravenor and Kwiatkowski (1998) used 5 compartments and Gravenor *et al.* (2002) 48 compartments.

Fig. 1E shows the asexual multiplication factor against gametocyte investment for Type 3 models. For 2 and 5 compartments, the asexual multiplication factor decreases concavely with rising gametocyte investment and is mostly much higher than for the discrete model, with the difference being larger for 2 compartments than for 5. For 48 compartments, the asexual multiplication factor decreases close to linearly with rising investment and is similar to the value for the discrete model.

With the full solution, asexual multiplication in the first cycle period is mostly much lower than the long-term value. The values from the full solution are similar to the long-term values by the second cycle period.

Relative gametocyte production as a function of gametocyte investment for Type 3 models is shown in Fig. 1F. For 2 and 5 compartments, relative gametocyte production has a maximum at intermediate gametocyte investment and is nearly always much higher than in the discrete model. The difference from the discrete model is bigger for 2 compartments than for 5. For 48 compartments, relative gametocyte production increases close to linearly with investment and is similar to the value for the discrete model. With the full solution, gametocyte production in the first cycle period is nearly always much lower than the long-term approximation, but by the second cycle period the values from the full solution are similar to the long-term values. Table 3 details when the dynamics in Type 3 models are most different from the discrete model.

Fig. 2C shows the fitness benefit and cost of increasing gametocyte investment in Type 3 models. Both the benefit and cost have a similar relationship with investment to the discrete model. For 2 compartments, the fitness benefit is nearly always much lower than in the discrete model and is negative for a range of investments. For 5 compartments, the fitness benefit is also nearly always lower than in the discrete model, but not as low as for 2 compartments. With 48 compartments, the benefit is always positive and is close to the value for the discrete model. Using the full solution for $n=48$, the proportional change in relative gametocyte production with investment in the fourth cycle period is very similar to the long-term value. Hence, the benefit is a good approximation of the actual effect.

The fitness cost of increasing gametocyte investment is mostly much higher than for the discrete model for 2 and 5 compartments, with the difference being larger for 2 compartments than for 5. For 48 compartments, the cost is close to the value for the discrete model. Using the full model, the proportional change in the asexual multiplication factor with increasing investment in the first cycle period is lower than the long-term value for each case, but close to the approximation for at least $g<0{\cdot}5$. For the second and third cycle periods the values for the full solution are very similar to the long-term values. Hence, the cost is a good, but slightly high, representation of the actual effect, up to high gametocyte investments. Optimal gametocyte investments for Type 3 models are shown in Table 3. For 2 and 5 compartments the values are lower than for the discrete model, but for 48 compartments the result is very close to the value from the discrete model.

DISCUSSION

Gametocytogenesis in continuous-time malaria models with constant replication rates has been compared to a simple discrete-time model of infections where parasites replicate at a fixed age. Replication at a fixed age is an important aspect of malaria, which is not captured in the continuous models. Ignoring some models with unreasonable elements, the many models with constant replication rates reduce to 3 basic forms. Type 1 has a single equation for asexual dynamics. Type 2 has an additional equation for merozoite dynamics and Type 3 is composed of a series of asexual equations, representing the movement of asexuals through several age compartments. The behaviour of these basic forms was investigated. Asexual multiplication per cycle period and gametocyte production per asexual per cycle period is mostly much lower for Type 1 and Type 2 than in the discrete model and mostly much higher than in the discrete model for Type 3. However, with 48 compartments, Type 3 gives very similar asexual multiplication per cycle period and gametocyte production per asexual per cycle period to the discrete model, after one cycle period.

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How the continuous models performed in predicting the amount parasites should invest into gametocytes was also investigated, under the assumption that investment is constant. Some simplifying assumptions were made such that the fitness benefit of increasing investment equated to the relative increase in gametocyte production per asexual per cycle period and the fitness cost to a multiple of the relative decrease in asexual multiplication per cycle period. For all 3 model types, increasing gametocyte investment mostly has a lower relative effect on gametocyte production per asexual per cycle period than in the discrete model and a higher relative effect on asexual multiplication per cycle period. Therefore, the benefit of increasing investment was mostly much lower and the cost mostly much higher than in the discrete model and consequently optimal investments were mostly much lower. However, for Type 3 with 48 compartments, the relative effect of increasing gametocyte investment on gametocyte production per asexual per cycle period is very similar to the effect in the discrete model, and the relative effect on asexual multiplication per cycle period is very similar to the effect in the discrete model after one cycle period. Therefore, with 48 compartments, Type 3 gave a similar fitness benefit and cost to the discrete model and a similar optimal investment.

The problem with the continuous models is that some parasites replicate far earlier than they should. Some of the progeny of these asexuals also replicate early and consequently there can be several cycles of parasite replication within one cycle period. This can generate the hugely inflated growth rates described by Saul (1998). When gametocyte production is added, it alters the number of asexuals produced early in a cycle period, which influences the number replicating later in the same period and therefore has a cumulative effect on replication within one cycle period. As a result, the relationship between asexual multiplication per cycle period and gametocyte investment in the basic model forms is mostly concave instead of linear and the relative effect on asexual multiplication per cycle period of increasing gametocyte investment is mostly too high. By reducing the number of asexuals replicating later in a cycle period, gametocyte investment also impacts on the number of gametocytes that can be produced later in the period. Hence, the relationship between gametocyte production per asexual per cycle period and gametocyte investment in the basic model forms is mostly convex rather than linear and the relative effect on gametocyte production per asexual per cycle period of increasing investment is nearly always too low. In most cases, gametocyte production per cycle period even declines with increasing investment when investment is high.

In Type 1 and Type 2, the growth rate in the absence of gametocyte production is corrected by reducing one of the parameter values. Consequently, when gametocyte production is included, asexual multiplication per cycle period and gametocyte production per asexual per cycle period are nearly always too low. For Type 1, results were given both for a reduced number of merozoites produced per asexual and for a reduced asexual rupture rate. On the whole, the models perform slightly better when the rupture rate is reduced. This is because the effect of gametocyte production on replication is determined by the product rm, which (as $r(m-1)$ is fixed by the growth rate in the absence of gametocyte production) is smaller when r is reduced than when m is. In Type 2 the growth rate is corrected by reducing the rate at which merozoites invade blood cells. The results are very similar to those for Type 1 with a reduced number of merozoites per asexual. This is logical because reducing the merozoite invasion rate effectively reduces the number of merozoites per asexual by decreasing the number that successfully invade cells. The similarity between the models is revealed by a quasi-steady state approximation of Type 2 in which the merozoite population is assumed to be at equilibrium because of their rapid clearance (see Gravenor *et al*. 1995). Type 2 then reduces to almost the same equations as Type 1, but with m replaced by $m\beta/(\delta+\beta)=3{\cdot}83$, compared to the reduced $m=3{\cdot}77$ in Type 1.

In Type 3, the parameter values are set to give both a realistic average age at replication and number of merozoites per asexual. Hence, asexual multiplication per cycle period and gametocyte production per asexual per cycle period are mostly too high. The inflated growth rates are not apparent in the original models because they are compensated by increased parasite death rates. Gravenor *et al*.'s (1998) and (2002) models were designed to look at the effect of drug treatment and therefore actually show decreases in parasite numbers over 1 cycle period. Type 3 models address the underlying problem of variable replication age by the addition of more asexual compartments. The distribution of replication ages becomes the sum of a series of exponential distributions, rather than a more variable exponential distribution (Gravenor and Lloyd, 1998). With the same transition rate for each compartment, the variance is inversely proportional to the number of compartments (Gravenor *et al*. 2002). Hence, as the number of compartments is increased, fewer asexuals replicate early, there is less impact of gametocyte investment on replication and gametocyte production later in the cycle period and Type 3 approaches the behaviour of the discrete model.

Three models have unreasonable elements. Koella and Antia's (1995) model is missing a rate factor in the gametocyte equation. This makes their equation dimensionally unbalanced, although the outcome is relatively minor; it reduces the number of gametocytes produced by a factor of 0·7. Koella and Antia

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(1995) also used a reduced number of merozoites per asexual and increased rupture rate. Therefore the effect of gametocyte production on replication is greater in the basic form of Koella and Antia's (1995) model than in the standard Type 1 model. Consequently, asexual multiplication per cycle period is lower than in the standard Type 1 model and increasing gametocyte investment has a higher relative effect on asexual multiplication per cycle period and a lower relative effect on gametocyte production per asexual per cycle period. Hellriegel's (1992) model incorporates gametocyte production by an additional asexual loss term instead of a reduction in replication. As a result, the effect of gametocyte production on replication is smaller in the basic form of Hellriegel's (1992) model than in the standard Type 2 model, asexual multiplication per cycle period is higher than in the discrete model and the relative effect on asexual multiplication per cycle period of increasing gametocyte investment is lower. The relative effect of increasing gametocyte investment on gametocyte production per asexual per cycle period is higher than in the standard Type 2 model but remains less than in the discrete model. Hellriegel's (1992) model actually gave a similar optimal investment to the discrete model, but only because too low a cost of increasing gametocyte investment offset too low a benefit. In Anderson's (1998) model, merozoites are not removed when they infect cells, which effectively increases the number of merozoites per asexual. Hence, asexual multiplication per cycle period and gametocyte production per asexual per cycle period are higher in the basic form of Anderson's (1998) model than in the standard Type 2 model, the relative effect of increasing gametocyte investment on asexual multiplication per cycle period is higher and the relative effect on gametocyte production per asexual per cycle period is lower.

In the models of Hellriegel (1992) and Koella and Antia (1995), asexuals are assumed to develop directly into gametocytes rather than producing merozoites that develop into gametocytes. Although this is not appropriate for *P. falciparum*, it may be relevant to other *Plasmodium* species. The consequence for gametocyte production is interesting. Because the growth rate is corrected by reducing the number of successful merozoites per asexual whilst having a realistic (or increased) rupture rate, gametocyte production per asexual per cycle period is mostly much higher in the basic form of Hellriegel's (1992) and Koella and Antia's (1995) models than in the equivalent discrete model. The different timing of gametocyte commitment does not alter the relative effect on gametocyte production of increasing gametocyte investment because the factors that are independent of g cancel out.

Fitness was measured by the number of gametocytes produced in the last cycle period before peak parasite density. Two previous studies of optimal transmission investment (Koella and Antia, 1995; McKenzie and Bossert, 1998) used other fitness measures. Koella and Antia (1995) equated fitness with the integral of gametocyte density over an infection. As they assumed a fixed gametocyte death rate, their measure is proportional to the total number of gametocytes produced and therefore should give similar results to the measure used here. Using gametocytes produced over l cycle periods as fitness gave slightly higher but very similar optimal investments. McKenzie and Bossert's (1998) fitness measure is based on the probability of a blood meal containing at least 1 gametocyte of each sex, assuming gametocytes are Poisson distributed in the blood, integrated over the first 50 days of infection. It is less directly related to gametocyte production because the transmission probability at any time saturates at high gametocyte densities. As a result, the effect of gametocyte investment on asexual numbers later in the infection should be less important for this measure than the measure used here, especially when asexual multiplication per cycle period is high, and therefore optimal investments should be higher. Applying McKenzie and Bossert's (1998) measure over l cycle periods (using their function for gametocyte death and the same initial asexual density) gave optimal investments than were higher by 0·02–0·14. The biggest increases were for Type 3, Hellriegel's (1992) model and the discrete model. Therefore, with McKenzie and Bossert's (1998) fitness measure, the discrepancy in optimal investment between the discrete model and both Type 1 and Type 2 (although not Hellriegel's (1992) model) was increased.

Most of the continuous-time models did not originally include gametocyte production. Therefore, although these models generally do not perform well when gametocyte production is added, the original models are not affected. However, the difference in replication dynamics within a cycle period between continuous and discrete models could influence the model outcomes in other ways. Many of the models were aimed at understanding the impact of the immune response on the course of infection. Typically, the rate of increase in immune effectors is assumed to be a function of parasite density and the rate at which parasites are killed is assumed to be proportional to the product of the parasite and effector densities. Because parasite numbers rapidly increase within a cycle period in the continuous models but only at the end of the cycle period in the discrete model, immune stimulation might be greater in the continuous models and have a larger impact on parasite numbers. Exploratory simulations of Type 1 and Type 2 with some of the functions that have been used to model an immune response against asexuals (and no gametocyte production) showed higher effector densities up to peak parasite density than for the discrete

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model with the same function and that peak parasite densities could be substantially lower in the continuous models.

The models of Hoshen *et al*. (2000*a*) and Rouzine and McKenzie (2003) do not have the problems of the other continuous-time models. Both models restrict parasite replication by age and therefore prevent multiple cycles of replication from occurring within 1 cycle period. Hoshen *et al*. (2000*a*) incorporated a fixed time lag between parasites invading red cells and producing merozoites so that the number of asexuals replicating at any time depends on the number that invaded cells 1 cycle period earlier. Rouzine and McKenzie (2003) presented an age-structured model in which the rate of replication is a function of parasite age. They assumed a normal distribution of replication ages about a mean of 1 cycle period. For small enough variances, Rouzine and McKenzie's (2003) model should behave similarly to Type 3 with 48 compartments.

Since Saul (1998) and Gravenor and Lloyd (1998) pointed out the potential growth-rate problems with continuous-time malaria models, and Molineaux and Dietz (1999) advocated discrete modelling, more discrete within-host models of malaria have been formulated (Jakeman *et al*. 1999; Hoshen *et al*. 2000*b*; Molineaux *et al*. 2001; Paget-Nicol *et al*. 2002; Gatton and Cheng, 2004; Smith *et al*. 2004). However, continuous models with constant replication rates are still being developed (Hoshen *et al*. 2001, 2002; Simpson *et al*. 2002; Recker *et al*. 2005; Gurarie *et al*. 2006). One reason for using continuous-time models has been to accommodate a continually changing immune response. However, immunity can also be represented by survival probabilities between time steps, which could be estimated from data, or by combining continuous functions with discrete replication (see Haydon *et al*. 2003). Here it has been shown that continuous-time models generally perform poorly when gametocyte dynamics are included and argued that similar problems may arise with other aspects of the models. Although realistic dynamics can be generated in continuous models by defining a large number of asexual compartments or restricting parasite replication by age, solving these models is considerably more computationally demanding than a simple discrete model. Furthermore, parasite replication occurs synchronously in *P. falciparum* infections (White *et al*. 1992), as evidenced by the characteristic periodic fever and strong oscillations in parasitaemia, making a discrete model a more obvious choice. The problems of continuous-time models in describing gametocyte production demonstrate another benefit of discrete-time modelling.

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APPENDIX

Type 2 models

For constant g, the solution for $A[t]$ has the form $A[t]=K_1e^{\lambda_1 t}+K_2e^{\lambda_2 t}$, where K_i are constants and λ_i the eigenvalues of the system. After some transient dynamics, the solution is dominated by the term with the most positive eigenvalue. Boundary conditions need to be defined to determine the K_i; I set $M[0]=0$. Note that the choice of boundary conditions does not affect (the long-term) X and P. Hence, for constant g, the long-term solutions for the asexual dynamics and gametocyte production are given by

$$A[t]=\frac{\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}+\delta+\beta-r}{2\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}}A[0]e^{\frac{1}{2}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta+r)\right)t}, \tag{A1}$$

$$G[t]=\frac{2rmg\beta A[0]e^{\frac{1}{2}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta+r)\right)t}}{\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta+r)\right)}-\frac{mg\beta A[0]}{\beta(m(1-g)-1)-\delta}. \tag{A2}$$

Hence,

$$X=e^{\frac{1}{2}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta+r)\right)\tau}, \tag{A3}$$

$$P=\frac{2mg\beta\left(e^{\frac{1}{2}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta-r)\right)\tau}-1\right)}{2m\beta(1-g)-\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta-r)}. \tag{A4}$$

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The fitness benefit and cost of increasing gametocyte investment are given by

$$B = \frac{1}{g} - \frac{rm\beta\tau e^{\frac{1}{2}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta+r)\right)\tau}}{\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}\left(e^{\frac{1}{2}\left(\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta+r)\right)\tau}-1\right)} + \frac{2m\beta\left(1-r\Big/\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}\right)}{\left(2m\beta(1-g)-\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}-(\delta+\beta-r)\right)}, \tag{A5}$$

$$C = \frac{(l-1)rm\beta\tau}{\sqrt{(\delta+\beta-r)^2+4rm\beta(1-g)}}, \tag{A6}$$

respectively.

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