An integrated model of *Plasmodium falciparum* dynamics

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Abstract

The within-host and between-host dynamics of malaria are linked in myriad ways, but most obviously by gametocytes, the parasite blood forms transmissible from human to mosquito. Gametocyte dynamics depend on those of non-transmissible blood forms, which stimulate immune responses, impeding transmission as well as within-host parasite densities. These dynamics can, in turn, influence antigenic diversity and recombination between genetically distinct parasites. Here, we embed a differential-equation model of parasite-immune system interactions within each of the individual humans represented in a discrete-event model of *Plasmodium falciparum* transmission, and examine the effects of human population turnover, parasite antigenic diversity, recombination, and gametocyte production on the dynamics of malaria. Our results indicate that the local persistence of *P. falciparum* increases with turnover in the human population and antigenic diversity in the parasite, particularly in combination, and that antigenic diversity arising from meiotic recombination in the parasite has complex differential effects on the persistence of founder and progeny genotypes. We also find that reductions in the duration of individual human infectivity to mosquitoes, even if universal, produce population-level effects only if near-absolute, and that, in competition, the persistence and prevalence of parasite genotypes with gametocyte production concordant with data exceed those of genotypes with higher gametocyte production. This new, integrated approach provides a framework for investigating relationships between pathogen dynamics within an individual host and pathogen dynamics within interacting host and vector populations.

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1. Introduction

Models of malaria transmission and their applications to malaria control date back nearly a century, to Ross (1911), whose basic compartmental-modeling framework has been extended in many directions since (Dietz, 1988; Koella, 1991; Gupta et al., 1994). During the past decade our knowledge of the role of parasite, mosquito and human diversity in malaria has grown enormously, however, and it suggests that the classic modeling framework be expanded to integrate our understanding at multiple levels of biological organization (McKenzie, 2000). In particular, integrated models should help us better understand how the dynamics of parasites within an individual infected human relate to the dynamics of parasites within a population of humans, how the relationship is mediated by the dynamics of a vector mosquito population, and how these hierarchies of interactions shape the fate of anti-malaria interventions (Molineaux, 1985; Singer, 1990).

Here, we focus on *Plasmodium falciparum*, the species responsible for almost all of the 1–3 million annual deaths attributed directly to malaria. Malaria infection in a human begins with an inoculum of parasites from a blood-feeding *Anopheles* mosquito. The parasites penetrate liver cells, multiply, then burst the liver cells and...
enter the bloodstream, where they invade red blood cells; they again multiply, then burst the red blood cells, each releasing 8–32 “merozoites” that invade more red blood cells and continue the cycle. The pathology of malaria is associated with this blood-stage asexual replication cycle, which leads to geometric growth in parasite numbers, and to fevers, anemia, and sometimes death in the host. Some invading merozoites instead develop into “gametocytes,” the non-replicating sexual forms that, taken up in an *Anopheles* bloodmeal, recombine in meiosis and develop into the “sporozoite” forms infective to humans. If the male and female gametocytes are of different genotypes, recombination may unite or disrupt such phenotypic traits as high transmissibility, antigenic profile and drug resistance.

Thus *P. falciparum* diversity is determined in part by repeated samplings of parasite genotypes, in the form of sporozoites in inocula and gametocytes in bloodmeals, modulated by frequencies of host-vector contact. In turn, the relationship between sporozoites that enter and gametocytes that leave a host is modulated by a cascade of immune responses, the mechanisms of which remain largely mysterious but are known to vary in part with host genetics and in part with host infection history. Human immune responses are predominantly to blood-stage parasites, and are directed against the asexual forms; gametocytes provoke little if any immune response. In sum, *P. falciparum* diversity shapes and is shaped by individual host immune responses, and by the long- and short-term details of interactions between its host and vector populations.

Our previous work addressed *P. falciparum* dynamics in terms of the timing and densities of its life cycle stages in an individual human, and the prevalence and persistence of those stages in a human population, and considered the effects of immune responses and parasite diversity at each level. We developed differential-equation models of within-host malaria dynamics to examine a matrix of empirically based hypotheses about the regulation of gametocyte production and immune-response stimulation by asexual blood forms (McKenzie and Bossert, 1997), critical trade-offs in conversion from asexual blood forms to gametocytes, under superinfection (McKenzie and Bossert, 1998a), clinical implications of differing rates of gametocyte conversion (McKenzie and Bossert, 1998b), and effects of immune responses and drug intervention in mixed-species infections (Mason et al., 1999; Mason and McKenzie, 1999). We also developed discrete-event models of malaria transmission dynamics to examine the relative effects of vector mortality, host infectivity and individual host immunity to re-infection, in the presence of one or more parasite phenotypes (McKenzie et al., 1998, 1999), interactions between immunologic cross-reactivity and meiotic recombination among parasite genotypes (McKenzie et al., 2001a), seasonal fluctuations in vector populations (McKenzie et al., 2001b), and a range of approaches to community-level control (McKenzie et al., 2002a).

Here we merge the two approaches, embedding a differential-equation model of within-host dynamics in each of the humans represented in a discrete-event model of transmission dynamics, and use this new framework to examine the effects of human population turnover, *P. falciparum* antigenic diversity, meiotic recombination, and gametocyte production.

2. Methods

2.1. Within-host model

Our model of within-host dynamics is based on the system of ordinary differential equations developed in Mason et al. (1999). Here, we extend that system so as to encompass the possible involvement of more than two distinct parasite genotypes, and differential stimulation of acquired-immunity effectors by each genotype; we also include a parasite-removal-associated loss rate of innate-immunity effectors (as in McKenzie and Bossert, 1997), and the simplest of our previous representations of gametocyte dynamics (McKenzie and Bossert, 1997, 1998a, b), allowing the mortality rate of sequestered gametocytes to differ from that of the circulating ones.

The within-host blood-stage dynamics of each of an arbitrary number of distinct parasite genotypes are represented by a set of three simultaneous ordinary differential equations; an additional equation is shared over all genotypes. For each genotype, $u$, the equations are:

$$dM_u/dt = (a_u - z_u - c_u I - \Sigma b_{u,v} J_v) M_u,$$

$$dG_u/dt = z_u y_u M_u(t - \tau) - p G_u,$$

$$dJ_u/dt = \Sigma r_{u,v} M_v - w_u J_u$$

with the shared equation

$$dI/dt = \Sigma s_v M_v - \Sigma k_v I_v - q I,$$

and the summations all taken with subscript, $v$, ranging over all genotypes. Except for the delay $\tau$ in the second equation, all variables are evaluated at time $t$. Fig. 1 is a schematic of this subsystem.

The four variables represent per-microliter densities: $M$ for asexual forms, $G$ for gametocytes, $J$ for acquired-immunity effectors, and $I$ for innate-immunity effectors. The parameters $a$, $z$ and $p$ are the daily replication rate of asexual blood forms, conversion rate of asexual forms to gametocytes and decay rate of mature gametocytes, respectively. Newly produced gametocytes sequester for a period before emerging into the circulation; the parameter $\gamma_v$ gives their survivorship over this period, as the fraction surviving, and $\tau$ its duration, in days. In
accord with the preponderance of current knowledge, immune responses are directed against asexual forms, and not gametocytes, viz. “the most striking aspect of the host’s reaction to gametocytes of the Haemosporina is its virtual absence” (Carter and Graves, 1988). However, it was reported in 1999 that late-stage sequestered asexual forms and early stage sequestered gametocytes of \textit{P. falciparum} may share a form of an antigenic protein involved in adhesion, and therefore might share the effects of some immune responses (Piper et al., 1999). We are well prepared to alter the more conventional assumption we make here, if and when confirmation and follow-up studies appear, given that we have already investigated a very similar possibility, with the added life history trade-offs it would imply (McKenzie and Bossert, 1998a).

The parameters $c$, $s$, $k$ and $q$ are the daily removal rate of asexual forms by innate-immunity effectors (asexual forms per effector), rate of stimulation of the innate immune response (effectors per asexual form), removal rate of innate-immunity effectors (effectors per effector per asexual form), and decay rate of innate-immunity effectors, respectively. The parameters, $b$, $r$ and $w$ are the analogs of $c$, $s$ and $q$ for the acquired immune response. Cross-reactivity is represented by the rate at which the density of asexual blood forms of one genotype stimulates immune responses to another and the rate at which the immune response to one genotype removes asexual blood forms of another. Although the equations for each genotype are distinct, the dynamics of several genotypes may be linked by their cross-reactivity with respect to acquired as well as innate host immune responses: $b$ and $r$ are matrices that define the degree of acquired-immunity effector cross-reactivity between parasite genotypes. Thus $c$, $s$, $b$ and $r$ form a table that defines the degree to which genotypes share host immune responses.

The separate consideration of “innate” and “acquired” responses in our model is well grounded in empirical data. A recent review (Stevenson and Riley, 2004) observes that, driven by the quest for a vaccine, “research on the immunology of malaria has tended to focus on adaptive immunity,” but “accumulating evidence...indicates a crucial role for innate immune responses in protective immunity to malaria.” In particular, “innate responses are essential to limit the initial phase of parasite replication, controlling the first wave of parasitemia and allowing the host time to develop specific adaptive responses that will enable the infection to be cleared.” This modern appreciation has been prompted in part by interactions between theory and laboratory, clinic and field studies (Kwiatkowski, 1995). Molineaux and Dietz (1999) provide a critical review of the immunological and parasitological features of within-host models, including our previous work.

The liver stages of the parasite are simply represented by a time lag, so that the initial merozoites appear in the bloodstream at a specified density a specified number of days after inoculation. Each genotype is accounted for separately during the liver (and all other) stages. The model is not intended to address the approximately 48-h replication cycle of \textit{P. falciparum} in red blood cells (Rouzine and McKenzie, 2003), during the last half of which asexual forms sequester and are not seen in the circulation; these very short-term features are averaged by the fitted model. Nor is it intended to represent clonal antigenic variation (Molineaux et al., 2001; Paget-McNicol et al., 2002), the process by which a single \textit{P. falciparum} genotype may switch the expression of an antigenic surface protein from one variant to any of ~50 others during the course of an infection, which may underlie pronounced long-term recrudescences in asexual-form density; in a within-host model, Antia et al. (1996) found that antigenic variation can increase the duration of an infection. Our model does not attempt to account for the dynamics of red blood cell populations (McQueen and McKenzie, 2004), or any stochastic elements that may affect sampling of parasite densities.

In summary, the following parameters for each genotype specify the within-host model: (1) the post-inoculation delay before blood stages emerge from the liver, (2) the initial concentration of asexual blood forms, (3) the intrinsic replication rate of asexual blood forms, (4) the conversion rate of asexual forms to gametocytes, (5) the time required for gametocyte maturation, (6) the decay rate of sequestered gametocytes, (7) the decay rate of circulating gametocytes, (8) the rate at which asexual forms stimulate the innate immune response, (9) the rate at which asexual forms are removed by the innate response, (10) the decay rate of the innate response, (11) the rate at which asexual-form densities of the genotype and its cross-reactors stimulate the acquired immune response, (12) the rate at which asexual forms of the genotype are removed by the
acquired immune response, and (13) the decay rate of the acquired immune response.

2.2. Within-host data and model fitting

Knowledge of *Plasmodium* dynamics in infected humans derives almost entirely from 40 years of work with malaria induced to treat neurosyphilis. The parameter ranges we examined in previous work on *P. falciparum* blood-stage dynamics conformed to the 14 complete and 10 partial malarialovery charts that had been published by that date, which reflect a variety of parasite “strains,” facilities, and procedures. Since then, several 100 charts from the United States Public Health Service (USPHS) malariotherapy clinics in South Carolina and Georgia that were active from 1940 to 1963 have become available; the treatment and data collection procedures at those clinics are described in detail in Collins and Jeffery (1999) and references therein. Accordingly, here we fit model parameters to the same subset of those charts we have used elsewhere for comparative purposes (McKenzie et al., 2002b,c), i.e. those of the 63 patients with no known history of previous malaria infection, inoculated with the McLendon strain of *P. falciparum* in the South Carolina facility, whose charts included >21 days of patent parasitemia preceding any drug or other intervention, and daily records of per-microliter densities of asexual blood forms and (male) gametocytes for >99% of those days. The genetic composition of the various “strains” used in malarialotherapy remains unknown, and no samples were preserved, but it is almost certain that, like most modern isolates, each included multiple genotypes; nonetheless, characteristic differences between the entities seemed to persist. Here we consider the McLendon strain as a single parasite entity. We use only those sections of charts that preceded any drug or other invention, because the interventions were explicitly intended to modify or clear infections; those sections ranged from 22 to 159 days of patency, with a mean of 69.6 days. Infections were initiated by inoculation of whole blood, in 49 cases, or sporozoites, in 14 cases; the two inoculation modes led to differences in prepatency, as expected, but no other differences relevant to the analyses here. The first reported day of patent parasitemia was, in effect, the first day on which asexual-form density reached 10 per µl of blood, the threshold of detection with conventional microscopy; we designate this as day “0,” in relation to which all time series were evaluated.

Many model parameters carry over from our previous work, so we used the ranges of values that were consistent with previously published data as starting points for parameter estimation for the USPHS charts. Specifically, we began with an assumption that the sum of the exponential growth rates of the asexual and sexual forms combined, \( a + z \), was 1.39 per day, a value consistent with each merozoite producing 16 descendants after 48 h of development in a red blood cell (Note that in the equations the gametocyte conversion rate, \( z \), appears only in difference with \( a \), and in product with \( \gamma \), so the daily rate parameters to be estimated are \( a - z \) and \( \gamma z \)). As a starting point, we set the gametocyte maturatio delay at 7 days, and assumed that the half-life of a mature, circulating gametocyte was 2.5 days (an exponential decay rate of about 0.28 per day). We assumed that the half-life of acquired-immunity effectors was very long in relation to the length of the individual chart data series, and so fixed the decay rate \( w = 0 \) for the parameterization. While this makes intuitive sense, for the relatively short course of an initial infection in a malaria-naïve host, it could be varied in future work to generate population-level results, to test hypotheses about its value; here, having fit the other parameters to the data (see below), we explored a range of other values for \( w \), and found none that produced a significant reduction in the residual sum of squares. We assumed that the rise and fall of the innate immune response is on the order of 2 or 3 days, and so began our search for optimal values of \( s \) and \( q \) with a value of 0.2 for each (per asexual form per day, and per day, respectively). We know of no empirically based an priori values for the two remaining rate parameters of the acquired immune response, \( r \) and \( b \), or the two remaining rate parameters of the innate immune response \( c \) and \( k \).

We estimated 10 of the 11 parameters, keeping \( w = 0 \), by fitting the model to each of the 63 individual charts. We used a steepest-descent algorithm (Press et al., 1992) to determine the parameter set that minimized the sum of squared deviations of the model from the asexual-form and gametocyte density data over the time course of each chart; though not the most efficient minimization algorithm, it is stable, reliable and converges quickly enough, given the starting values based on the literature and our previous work. Fig. 2 shows examples of the fits.

There is wide variation in the infection dynamics among the individual cases, and the degree to which the model fit the data varied as well. For 45 of the 63 cases the residual sum of squares about the fitted curve was significantly less than the sum of squares about the mean at the \( p < 0.01 \) level using the \( F \) statistic. For 7 additional cases the fitted curve reduced the variance at the level \( 0.01 < p < 0.05 \). There was no significant reduction in the variance for the remaining 11 cases. The cases that the model did not fit well typically involved initial peaks that dominated the parameter-fitting such that fairly large subsequent peaks were missed or misrepresented. Future papers will examine the case-to-case variation in this data set and model fitting in detail, and the effects of incorporating similar inter-individual heterogeneity in
the integrated model’s representation of a human population. The fitted parameters varied widely over the cases, with coefficients of variation typically around 0.5 but ranging from 0.37 for the $a - z$ estimate to over 2 for $zy$. To represent a typical host, we calculated the average of each parameter value over the 63 charts, and used those values as our canonical parameter set: $a - z = 1.43, zy = 0.034, p = 0.50, s = 0.168, c = 4.7 \times 10^{-4}, k = 2.8 \times 10^{-5}, q = 0.285, b = 2.3 \times 10^{-4}, r = 0.067$ (all as rates per day), and $\tau = 9$ days.

2.3. Transmission model

The within-host model is integrated into an individual-based, population-level model of malaria transmission that extends our previous discrete-event models. Those models adapted the basic compartmental scheme of classic transmission models to the level of individuals by incorporating representations of $P. falciparum$ life cycle timing within interacting representations of individual humans and mosquitoes, using a single timeline variable to track each parasite life cycle within and between these individuals. The discrete-event approach allowed us to investigate many critical features of malaria transmission dynamics, including the effects of host–vector contact and host immunity on parasite sampling, but not those that may depend on the within-host densities of parasite stages or immune effectors.

Here we consider an isolated, local population of 500 humans interacting with 5000 mosquitoes, through the female mosquito bites taken for bloodmeals. Each day a given number of humans and mosquitoes are drawn at random, with replacement, for this interaction and potential parasite transfer; we generally assume that on average an Anopheles feeds on alternate days and only on humans, and so fix the number of such bites per day at one-half the number of vectors. These population sizes are readily adjusted, and a derived epidemiological power-law relationship allows extrapolation to

Fig. 2. Examples of model fits to the 63 individual patient charts. The solid lines show the fit, dashed lines the observed log 10/$\mu$l densities of asexual forms ($M$ observed, $M$ fit) and gametocytes ($G$ observed, $G$ fit) from the first day of patency to termination (see text). Patient identification numbers are (a) S-606, (b) S-548, (c) S-1246.
mosquito populations of different sizes or with other bloodmeal sources (McKenzie et al., 1998). No spatial heterogeneity or seasonal fluctuation is considered in the model presented here.

A simple replacement scheme maintains a constant human population size, with turnover. Each day a number of randomly selected humans are removed from the population and immediately replaced by malaria-naïve humans. The number of individuals replaced each day is described by a Poisson random variable with a mean given as a parameter (the “turnover”), here with values between 0 and 1. A simple conversion gives the percentage annual population turnover: the parameter value is multiplied by 360 days (our standard year), divided by the population size (to get an annual fractional turnover), then multiplied by 100 to get a percent. Thus a parameter value of 0.01 individuals per day for a population of 500 yields a replacement rate of about 0.7%, and a value of 0.1 yields about 7%. Other than the elapsed time since an individual is introduced into the population, there is no age structure in the host population in the current model.

The mosquito population dynamics are represented by a modified stochastic, logistic growth model. Mosquitoes are killed in daily Bernoulli trials, with a constant daily probability of death given as a parameter (here 0.069, giving a half-life of 10 days); the relation of this and other parameter values in the model to available data is described in detail elsewhere (McKenzie et al., 1998). Each dead mosquito is replaced by a number of newly emerged adults that is a Poisson random variable with parameter \( K/N \), where \( N \) is the current vector population size, and the value of \( K \) is a parameter.

In summary, the population-level parameters that specify the transmission model are: (1) the number of humans, (2) the mean of a daily Poisson replacement of humans, (4) the equilibrium mosquito population, (5) the daily mosquito mortality and (6) the average number of days between mosquito blood meals.

2.4. Model integration

If the individual mosquito or individual host selected for a bite is infectious, then sporozoites or gametocytes, respectively, of all parasite genotypes present pass from one to the other. The integration of the within-host and population-level models involves modeling these vector-to-host and host-to-vector transmission processes, the liver (non-circulating) stages of the parasite in the host, and the development of the parasite in the mosquito. When a mosquito and host are randomly selected for a “bite,” the following calculations take place:

(1) For each parasite genotype for which the mosquito is carrying sporozoites, if no latent, liver-stage parasites of that genotype are already present in the human, a timeline variable is set to track liver-stage development of that genotype in that host beginning on the current day. After a delay, specified as a parameter (here 10 days), merozoites of that genotype emerge from the liver into the blood at a specified initial concentration (here 0.01 per \( \mu l \)); the state of the host blood environment those merozoites enter is specified by the differential equations given above. In accord with the current understanding of \( P. falciparum \), parasites do not persist in the liver or re-invade it after the initial release of merozoites.

(2) For each parasite genotype for which the human is carrying mature gametocytes (those calculated, in the within-host model, as having been produced at least the specified \( \tau \) days ago) the number of gametocytes in a 1 \( \mu l \) sample, a Poisson variable, is passed to the mosquito. Here we assume that male and female gametocytes appear in equal abundance, though the sex ratio is easily altered in the model, and that zygotes form in proportion to the frequency of the genotypes in the bloodmeal. We consider a mosquito infected when her bloodmeal contains at least one male and one female gametocyte.

Parasite development in the mosquito involves several intermediate stages (gamete, zygote, ookinete and oocyst) between gametocyte pair and sporozoite, and a delay that we account for by fixing the interval required for a mosquito to progress from an infected to an infectious state at 10 days, a parameter value that is easily altered. There have been very few relevant studies on oocyst development, but they show dramatic reductions in numbers from gametocyte pairs ingested to oocysts produced; for instance, the most comprehensive laboratory study to date indicates that the production of a single \( P. falciparum \) oocyst in an \( A. gambiae \) requires, on average, 31,600 female gametocytes (Vaughan et al., 1992, 1994), albeit with enormous variation. It is not at all clear how such data might be reconciled with information from field studies (see below), and we do not explicitly model this reduction. Instead, as before (McKenzie et al., 2001a), in accord with the literature (Pringle, 1966; Haji et al., 1996), we assume that the number of oocysts from a single blood meal is a Poisson random variable with parameter (mean and variance) 0.8; this parameter is readily adjusted. In the current model, a Poisson number of oocysts is generated and that number of samples from the calculated distribution of oocyst genotypes is taken. With this representation, approximately 55% of mosquito feeds on gametocyte carriers produce oocysts, again in accord with field data from Africa (e.g. Carter and Graves, 1988; Toure et al., 1998; Gouagna et al., 2004); in these as in most field studies, relationships
between gametocyte density and transmission success are loose at best, and tend to disappear when analyses include patent gametocyte carriers who fail to infect any mosquito, and individuals without detectable gametocytes who infect many mosquitoes. In our model, after the specified delay (here 10 days), sporozoites from the oocysts are available for inoculation into a host. Fig. 3 is a schematic of the integrated system.

We also considered an alternative model in which the number of oocysts varies with gametocyte density. In the alternative model, we assume that the number of oocysts is proportional to the logarithm of the number of gametocytes in the blood meal (a Poisson sample of $G$, as before), and relate this model to the data noted above through the arbitrary assumption that it is 10 male and 10 female gametocytes that produce 0.8 oocysts, the same average number of oocysts as before. Thus, with 100 gametocytes of each sex, the expected number of oocysts is 1.6, with 1000 it is 2.4, and so forth; with 1 or fewer of each sex, the expected number of oocysts is 0.

This alternative model produces some quantitative changes in several results, but no qualitative change in any conclusion. The quantitative differences arise because the duration of the infectious period is shortened in the alternative model, since the long tail of $G$ values in the 1–2 range has a lower probability of producing an oocyst. We point out the quantitative differences below, in the results on host turnover, antigenic diversity and gametocyte production; we found no notable differences in the results on meiotic recombination or gametocyte survivorship. If we shift the alternative model so that the expected oocyst production with only 1 gametocyte of each sex in the bloodmeal is 0.8 (and with 10 of each is 1.6, etc.), the differences are removed. We infer that, in general, with respect to the results reported here, once past some threshold density, the presence of gametocytes is more important than their number. Because the alternative model could not be calibrated to both parts of the field data just noted, we chose to emphasize the simpler model here, and to argue for more research focused on gametocytes and parasite population dynamics within the vector.

Our intra-mosquito model is structured to track the sporozoite genotypes that might be produced from a single infecting bloodmeal. *Plasmodium* parasites are otherwise haploid throughout their life cycles, but briefly diploid as zygotes. Thus, given male and female gametocytes of different genotypes, recombination of genetic loci can occur immediately following formation of the zygote, either by cross-over events or chromosome re-association in meiosis.

For our purposes here, we consider two representations of parasite genetics:

(1) The first takes the form of a single locus with 16 alleles, for which we assume that there is no intra-locus recombination, and that oocysts formed from a single zygote contain both parental haploid genotypes; hence at most two parasite genotypes are present in a single oocyst.

(2) The second takes the form of two loci with four alleles at each, with recombination between the loci, and thus is a direct extension of our previous work with two biallelic loci (McKenzie et al., 2001b, 2002).

We labeled the alleles A, B, C, D at each locus, and began each simulation run with one host, randomly selected, with replacement, infected with each of the “parental” genotypes AA, BB, CC, or DD. Thus with the 12 recombinant or “successor” genotypes AB, AC, AD, BA, BC, BD, CA, CB, CD, DA, DB, DC, there were again 16 parasite genotypes in the system, each with the canonical parameter set given above.

In summary, the parameters that specify the intra-mosquito model here are: (1) the time delay between bloodmeal and availability of sporozoites, and (2) the mean number of oocysts produced per blood meal, and (3) the probability of meiotic recombination among the parasite genotypes.

### 2.5. Initial conditions

The parasite genotypes considered in our analyses of host population turnover and parasite antigenic diversity are all identical, in the sense that each is defined by the canonical parameter set for the within-host dynamic model, but they are immunologically distinct and independent with respect to the host’s acquired immune response: the off-diagonal elements of the cross-reactivity matrices $r$ and $b$ are 0. In this sense the genotypes can be considered different phenotypes, each presenting different antigens to which the host’s acquired immune responses differ.
The last two analyses examine the effects of altering the value of a single gametocyte-related parameter in the within-host model, with no turnover in the host population, no recombination among parasites, and all other parameters at their canonical values.

(1) The first increases the decay rate of mature gametocytes, \( p \), in the context of a single parasite genotype, in a human population with no turnover. Here, in line with previous work, we report (a) the frequency of “establishment,” defined as the fraction of replicate runs in which at least 1 host other than the initial carrier became infected, and (b) given establishment, the total number of hosts who became infected, prior to the extinction time (or the 20-year limit).

(2) The second introduces a parasite genotype with a higher or lower gametocyte conversion rate, \( z \), in the context of another, co-circulating genotype with the canonical rate. In the equations, \( z \) appears only in combination with \( a \) and \( y \), so we must know one of these values independently to estimate the other two. We can set a lower bound on \( a \): since our estimate of \( a - z \) is 1.43 per day and \( y \cdot z \) is 0.034 per day, \( a \) must be \( > 1.464 \); \( a = 1.464 \) would imply \( y = 1 \), that is, no mortality whatsoever for the sequestered gametocytes. We can set an upper bound on \( a \) by assuming a maximum growth rate of asexual forms. A rate of 32 asexuals per asexual, per 48-h cycle (Garnham, 1966), would imply \( a = (\ln 32)/2 = 1.73 \) and \( y = 0.113 \).

Subsequent papers will examine the effects of other phenotypic distinctions among parasite genotypes, cross-reactivity with respect to acquired immunity, and other factors.

We considered 10 rates of human population turnover, from a mean of 0.01 per day to a mean of 0.1 per day; birth and death rates in most of the world fall between these extremes, though migration could lead to larger values. For the single-locus model, at the start of each replicate run a single human was drawn at random, infected with one of the parasite genotypes, and replaced; this process was repeated for another genotype, and then another, up to the given number (1–16) of genotypes to be introduced, at which point the run began. For the two-locus model, we varied the recombination probability from 0.00001 to 0.5, to vary recombination rates from a level that produced each of the recombinants in only about 10% of replicates to a level of random assortment. We began each run with one randomly selected host infected with each of the 4 “parental” genotypes; we define the time of recombinant appearance as the day on which a recombinant form first appears in a host other than the initial carrier. With each parameter set and scenario, each replicate ran for 7200 days (20 of our standard years), much longer than the persistence of a single parasite genotype in the absence of turnover in the human population (see below). We completed 100 replicate runs for each set of values, i.e. for each particular human population turnover, number of parasite genotypes introduced, or recombination rate.

We define the extinction of a genotype as the point at which, simultaneously, no mosquitoes are infected with that genotype, and for more than 60 days no human has had an asexual-blood-form density > 1 per μl of that genotype; the persistence of a genotype is the absence of its extinction. Within each set of runs, the mean time to extinction of an individual genotype is calculated by summing the extinction dates for each of the genotypes in each replicate, then dividing this sum by the total number of genotypes and replicates. The mean time to extinction for all genotypes is calculated by summing the date at which this definition is satisfied for all genotypes introduced in a replicate, then dividing this sum by the total number of replicates. In each set we also calculated the fraction of the genotypes that persisted throughout. If a genotype persisted to the end of a replicate run, its extinction time was included in the average as 7200 days; thus the average persistence given (if > 0) for a given turnover in the human population is a lower bound.

The differential equations were integrated numerically by a fourth-order Runge–Kutta method with a time step of 1/2 day; this time step reduces integration errors in the models to well under 1% of the densities being calculated. The model was implemented in C using the GCC compiler and libraries, and run on a Windows or LINUX Athlon PC.

3. Results

Because our canonical parameter set fixes the decay of the acquired immune response at 0, when every host in an isolated local population has been infected sufficiently to sustain a high acquired immunity to a genotype, that genotype, and eventually all genotypes, must go extinct. A number of factors might promote parasite persistence, however. Most obviously, introductions of new genotypes with distinct antigenic profiles could delay extinction, perhaps indefinitely (McKenzie et al., 2001a). We address this possibility first with the model structured such that antigenic diversity in the parasite population is synonymous with an initial number of genotypes, and then structured so that antigenic diversity arises through meiotic recombination. Future work will examine the effects of parasite mutation per se.

Our previous studies showed that an increased vector population size or deceased interval between human blood meals would increase the effective transmission rate, and so increase the average persistence of a parasite
We also showed that a pool of susceptibles sufficient to avoid extinction of the existing genotypes could be maintained by an introduction of hosts immunologically naïve with respect to the parasite genotypes in circulation (McKenzie et al., 2002a). This is the factor we consider in the first set of results below, as a rough approximation of birth, death and migration processes.

Finally, we revisit the crucial but underappreciated role of gametocytes (McKenzie and Bossert, 1997). Gametocytes become the gametes that recombine in meiosis. Gametocytes seem to be clinically benign, and, as noted above, few if any immune responses are directed against them, at least in their mature, circulating form. Gametocyte production may actually benefit the individual host (McKenzie and Bossert, 1998b) but, if infectivity tracks gametocyte density, harm the host population. With the integrated model, we again investigate whether gametocyte dynamics might evolve in response to immune-mediated competition among parasite genotypes (McKenzie and Bossert, 1998a).

3.1. The effect of host population turnover on parasite persistence

Given that a single genotype is introduced at day 0, we want to determine the rate of turnover in the host population that allows a genotype to persist for 20 years or more. Fig. 4a confirms the general result noted above: increased turnover in the host population prolongs parasite genotype persistence. The transition from rare to near-certain persistence is fairly abrupt, with the critical value (to achieve a 50% chance of persisting 7200 days) being a host turnover of about 0.035 individuals per day. This value translates to an annual population turnover of 2.5%, which is within the range of natural birth–death rates, and below the rate of natural increase, in several developing tropical countries (e.g. see http://www.census.gov/ipc/www/idbprint.html), without taking migration into account.

With the alternative model of oocyst production (see Methods: Model Integration), we also found an abrupt transition from rare to near-certain persistence, but at a much higher turnover rate: 0.13 individuals per day, an annual population turnover of 9.3%. This value is much higher than natural birth and death rates, which suggests that, if the alternative model is correct, other factors such as migration or loss of acquired immunity would be required to support the long-term persistence of a single parasite genotype in a population.

3.2. The effect of antigenic diversity on parasite persistence

Given that 1–16 genotypes (a single locus with 16 alleles) are introduced at day 0, we want to determine whether the number of genotypes affects parasite persistence. Fig. 4b shows that the average persistence of each individual genotype, and of malaria infection overall, increase as the number of genotypes introduced increases. For a single genotype the average persistence was 394 days, identical to the 0-turnover case above; with only one genotype the individual-genotype and overall extinction times are identical. With two genotypes, the overall persistence was 1258 days, and the average persistence of an individual genotype was 1237 days. That is, the persistence of each individual genotype was greatly increased by the presence of the others. With 16 genotypes, overall persistence reaches 3159 days, and individual-genotype persistence 3077 days. Thus, an increase in initial diversity from 1 to 16 genotypes increases average parasite persistence from just over 1 year to nearly 9 years (With the alternative model of oocyst production, as diversity increases from 1 to 16 genotypes, the average individual-genotype persistence increases from 240 to 2980 days.)

Though the result may not be intuitive, it has a straightforward explanation. The genotypes are interacting through the innate immune response, to which
each is sensitive. When a host is infected with one genotype, the innate immune response is elevated, so, if the host is superinfected with another genotype, the superinfecting genotype is inhibited, or proceeds at a very low density. Therefore the host does not build up an acquired immune response to the superinfecting genotype, or at least not one sufficient to inhibit later infection. As the host’s acquired immune response clears the initial infection, the host is still (relatively) immunologically naive to the second genotype. This inhibition of immediate superinfection maintains a population of susceptible hosts for each of the genotypes beyond the time at which they would all have been infected with, and acquired permanent immunity to, a single genotype.

3.3. The interaction of host population turnover and antigenic diversity

As Fig. 5 shows, increases in parasite antigenic diversity greatly reduce the rate of host population turnover required to support long-term parasite persistence. That is, as antigenic diversity goes up, the population turnover required for individual parasite genotypes to persist in the host population for at least 20 years goes down. The critical turnover rate of about 0.035 for 1 genotype, noted above, falls to about 0.015 (1.08%) for four genotypes and to 0.01 for 12–16 genotypes. These values are well within the range for human populations (see above), so that neither net immigration of naive hosts or high birth rates would be required for malaria infection to persist. This interaction may bear on differences reported from recent studies of *P. falciparum* antigenic diversity at different field sites (Snounou et al., 1999; Anderson et al., 2000; Sallenave-Sales et al., 2000; Branch et al., 2001; Hoffmann et al., 2001; Leclerc et al., 2002).

The alternative model produces a similar result, though again at higher turnover values: the critical turnover rate for 20-year persistence declines from 0.13 individuals per day for one genotype to 0.025 for 16 genotypes. Only this last value is consonant with natural birth and death rates, which suggests that, if the alternative model is correct, high (but not low) antigenic diversity might combine with host turnover to support long-term parasite persistence.

3.4. The effect of antigenic diversity generated by meiotic recombination

Because antigenic diversity can be generated through recombination events, it seems likely that these events would increase the local persistence of the parasite. We used the representation of parasite genetics with two loci, four alleles at each, to investigate this hypothesis. As Fig. 6 shows, the introduction of new genotypes by recombination does increase parasite persistence, but it affects parental and recombinant genotypes differently. This is in general agreement with our earlier results (with recombination rates \( \geq 0.0005 \) in the discrete-event transmission model) on varying recombination rates and the time of introduction of new parental genotypes.

Here, with high recombination rates the average persistence of each parental and recombinant genotype is about 2900 days, comparable to that for the 16 single-locus genotypes introduced without recombination (above). The average persistence declines with lower recombination rates, more dramatically (and non-monotonically) for the parental than the recombinant genotypes. With lower recombination rates, recombination events occur later, so the first appearance of the recombinant genotypes is delayed, and parental genotypes gain less from the diversity effect provided by the recombinants. However, with a recombination rate of 0, we would expect the average ("parental") persistence to be 1750–1800 days, in line with an initial introduction of four single-locus genotypes (above). In Fig. 4, note that the average parental persistence with a recombination rate of 0.00001 is about 1835 days.
3.5. The effect of decreased gametocyte survivorship

Because parasite transmission depends upon a period of potential infectivity of individual hosts to vectors, changes in gametocyte survivorship within hosts should alter malaria epidemiology. Here, given the introduction of a single parasite genotype on day 0, we want to determine the extent to which increases in the daily decay rate of gametocytes (the parameter \( p \) in the within-host model) decrease parasite persistence, the frequency of parasite "establishment" (model runs in which the parasite infects more than the initial host), and, when establishment occurs, the number of hosts infected.

Fig. 7 shows that these features are relatively insensitive to increases in \( p \): they decline appreciably only with 15–17-fold increases in the daily decay rate, i.e. roughly a 99.95% drop in gametocyte survivorship relative to the canonical case. This suggests that, even with 100% coverage, an anti-gametocyte drug would need to be astonishingly effective to have significant population-level consequences.

3.6. The effect of increased gametocyte production

In our earlier work with within-host models, we found that the potential transmission of a single parasite genotype in a single host was optimized by values of \( z \) much greater than those that conformed to field or laboratory data, but also that asymmetries in the interaction of two parasite genotypes in a single host negated this result. Given parasite genotypes with low and high values of \( z \), and a shared immune response, co-infections and super-infections inhibited the high-\( z \) genotype but had only minimal effects on the low-\( z \) genotype: “competition” generally favored genotypes with the lower \( z \) values, in accord with field or laboratory data. We conjectured that similar results would hold for persistence and prevalence in an integrated model.

Because our current within-host model is more detailed, and our estimates of parameter values are much improved, we first recalculate the “transmission potential” of a single genotype in a single host as a function of \( z \), for assumed values of \( a \) in the range 1.464–1.73 (see above). As before (McKenzie and Bossert, 1998a,b), we assume that mature gametocytes are distributed uniformly in the bloodstream, that for all levels of gametocytemia male and female gametocytes are present in equal proportion (though this assumption is easily altered), and that at any given moment, the probability that no male is present in a 1-\( m \)l sample of peripheral blood and the probability that no female is present in that sample are each equal to the zeroth term in a Poisson distribution function with parameter \((G/2)\). Therefore the probability that at least one male (or one female) is present in a given sample is \(1 - e^{-G/2}\), and the probability that at least one of each is present in that sample is \([1 - e^{-G/2}]^2\). Thus our index of “transmission potential” is the integral \(\int (1 - e^{-G/2})^2 \, dt\), taken over a 180-day period (so that the units are infectious days); beyond this the probability of a gametocyte in a bloodmeal is negligible.

As Fig. 8 shows, its value increases monotonically to a maximum at a value of \( z \) between 1.0 and 1.6, then monotonically decreases. These “optimal” \( z \) values are again much higher than those consistent with field or laboratory reports, but they produce much higher transmission potentials: 122–158 days, versus 95 for the canonical parameter set, for instance.

A very different picture emerges in the integrated model with the simultaneous introduction of two genotypes, completely co-immune (i.e. \( b_{12} = b_{21} \) and \( r_{12} = r_{21} \) for both) and defined by the canonical parameters, with the exception that for one the value of \( z \) is
higher or lower. Fig. 9 summarizes the results of 100 replicate runs with each pair of genotypes, for fixed values of a ranging from 1.464 to 1.73 per day (and, accordingly, of y from 1 to 0.113; see above), with z values ranging above and below the corresponding canonical value. For each combination, part a shows whether the persistence of the variant is \(>\), =, or < the canonical genotype; part b does the same for peak prevalence.

In competition, the persistence and peak prevalence of a genotype with a \(z\) value “optimal” in a single-host single-genotype context fall below those of the canonical: for a given value of \(a\), variants exceed the canonical if and only if their \(z\) values are lower. Similarly, for a given value of \(z\), variants exceed the canonical if and only if their \(a\) values are higher. Thus, in line with our earlier results with within-host models, it appears that in a host population parasite genotypes with lower gametocyte conversion rates may inhibit those with higher rates. Fig. 10 shows an example.

The alternative model of oocyst production produces qualitatively identical results: that is, it also shows that the transmission potential for a single genotype is maximized at values of \(z\) much higher than those consistent with field or laboratory observations, while in competition the lower \(z\) values prove superior. Notable quantitative differences occur at the combination of the highest \(a\) and lowest \(z\) values (the upper right corner of Fig. 9), where, in the alternative model, the persistence and peak prevalence of the canonical genotype exceed those of the variant genotype, again because the duration of the infectious period is shortened. Also, at \(a = 1.464\) per day and \(z = 0.1\) per day, in the alternative model the peak prevalence of the variant exceeds that of the canonical genotype by 19%, on average, though its persistence is 20% below the canonical.

4. Discussion

This model merges our earlier models of the within-host and transmission dynamics of \(P. falciparum\), and remains a relatively transparent representation of parasite genotypes circulating and interacting within populations of individual mosquitoes and individual humans. We used it here to investigate how human population turnover, parasite antigenic diversity, and gametocyte production may affect \(P. falciparum\) dynamics at the host population level.

The idea that host population turnover might have dramatic effects on malaria epidemiology is not new. For instance Macdonald (1957) noted that “persistence is most likely in those places where communal immunity is most rapidly diluted by the birth of new infants or arrival of other immigrants,” but he did not include any such factors in his models. We introduced turnover in a previous discrete-event transmission model (McKenzie et al., 2002a), and, in the most nearly comparable circumstances of perennial transmission, find its effect here as great or greater. Again, the effect interacts with, but does not depend on, the presence of multiple parasite genotypes. Our results in this more complex immunological landscape argue that high net birth rates...
could play a role in sustaining malaria, as they do with directly transmitted childhood diseases (Anderson and May, 1991).

Similarly, malariologists long ago recognized that *P. falciparum* contains “races having different biologic properties such as virulence, pathogenic role, ability to develop in *Anopheles* of a given species, antigenic characters and reaction toward drugs” (Brumpt, 1949), and that many such “races” (or “strains”) may be in circulation at a single moment, at a single site, and even in a single individual. As modern molecular tools have begun to relate some of these phenotypic traits to genotypes, we have focused on circumstances and interactions that promote or restrain genetic diversity among parasites, for instance how immune responses may mediate parasite population genetics (McKenzie et al., 2001a,b). We expect that the degree to which interventions directed against particular genotypes hasten or retard the decline of their own efficacy will depend upon the degree to which they account for the contexts in which they are implemented.

Our results here show a strong effect of parasite antigenic diversity, through which the persistence of each genotype increases with increasing numbers of genotypes, and a striking synergy with host population turnover. Our results with antigenic diversity introduced through meiotic recombination highlight the complexity of immune-mediated interactions between founder genotypes and their progeny, and suggest further subtleties relating individual- and population-level parasite dynamics. Most obviously, even with this simplest immunological structure, the first generation of blood-stage parasites of a superinfecting genotype will be larger if the superinfection occurs months rather than days after the previous infection. Thus, given some cross-reactivity in acquired immunity, chronic low-level infection might prove to have unexpected value, not only to an individual host (“premunition;” Sergent, 1963), but, depending upon its influence on gametocyte production and transmission probability, to a host population. This in turn should depend upon frequencies of superinfection, however, which in malaria are mediated by the dynamics of vector mosquito populations. These issues, along with the relative roles of innate and acquired immunity, are among those to be addressed in future analyses.

Fig. 10. Prevalence (fraction of hosts infected, with $M > 0.1$) plotted against time. Examples for (a) a genotype with the canonical parameter set ($a-z = 1.43, yz = 0.034$; transmission potential 95), (b) a genotype with a $z$ value “optimal” in a single-host single-genotype context ($a-z = 0.28, yz = 0.459$; transmission potential 116–156), and (c) the canonical and “optimal” genotypes introduced simultaneously.
Gametocytes are a tangible link between the within-host and transmission dynamics of P. falciparum. P. falciparum infectivity lasts longer and the relation between infectivity and gametocyte density is weaker than is commonly recognized (McKenzie and Bossert, 1997, McKenzie et al., 2002b, c), but, unfortunately, our results here suggest that the impact of any intervention aimed solely at abbreviating P. falciparum infectivity would be minimal. The question clearly merits further attention, and more detailed examination. The paucity of data on gametocytes and their development within Anopheles remains enormously frustrating, given that the transition so clearly plays a central role in Plasmodium population dynamics (McKenzie and Bossert, 1998a). As our alternative model of oocyst production suggests, the research required is fundamental, and still conspicuously lacking.

The seemingly meager gametocyte production of P. falciparum has been a puzzle for many years. Bishop (1955) stated a conclusion obvious to all, before and since, that “the accidental introduction of a strain of parasite producing large numbers of gametocytes would lead to an increase in incidence,” and that selection would lead such “strains” to dominance. In previous work (McKenzie and Bossert, 1998a,b), we noted that higher gametocyte production by malaria parasites might be advantageous to individual humans, because the pathogenesis of malaria is associated almost exclusively with the density of asexual forms: each asexual form that becomes a gametocyte does not become multiple asexual forms; a gametocyte represents a diversion from the geometric growth of asexual replication. However, we also found that rates of gametocyte production that appear optimal for transmission in the context of a single parasite entity are much higher than those optimal in the context of several co-circulating parasite entities and that conform to rates typically observed in the field and laboratory. Our results here, with the more complex dynamics of the integrated hierarchical model, support that conclusion, in the more familiar form of persistence and prevalence.

Human malaria is an enormously complex system. Models can help us determine which of its features are decisive and which are incidental, and so can help us identify points of leverage for effective, sustainable interventions. Interventions aim to change the system, by definition. We need an integrated understanding of the mechanisms that contribute to success or failure at each level of biological organization in the system, because effects at one level typically proceed from causes at another. In epidemiology, however, “models commonly assume more uniformity than there is in reality, and in terms of control this usually leads to exaggerated optimism” (Molineaux, 1985). More modern approaches have begun to address this problem (Koopman and Longini, 1994; Levin and Durrett, 1996; Keeling and Grenfell, 2000), but, to the best of our knowledge, none have led to integrated models of the sort presented here, at least not of malaria.

This model provides a framework, to be elaborated in different ways for different purposes. There are many important features of vector populations (Hasibeder and Dye, 1988; Killeen et al., 2001) that could be incorporated in its transmission component, for example, though we know of only one model of the population dynamics of an Anopheles species (Haile and Weidhaas, 1977). As noted above, we have not attempted to represent the dynamics of parasite development within vectors. We have not explicitly considered microepidemiology (Greenwood, 1999), migration (Prothero, 1965) or other spatial factors, though these are clear concerns. In the within-host component, we have not accounted for possible responses to the liver stages of the parasite, and, though our caricatures capture the basic features of innate and acquired immune responses to the blood stages, and do so in more detail, we look forward to the point at which the information needed to depict the specific immunological mechanisms or agents involved is available.

Immune responses influence every aspect of human malaria. Though meiotic recombination in Plasmodium occurs in the mosquito, genetic variation depends on mutation, and mutation is more likely to occur within a human. In a mosquito, each mated pair of gametocytes may develop into 1–10 thousand sporozoites. In a human, an initial inoculum of about 10 parasites multiplies to 40–50 million before microscopy can detect them in the bloodstream, and then to 10 s of billions at peak density. Thus a blood stage of P. falciparum is likely the product of dozens more mitoses than a sporozoite, and, all else being equal, more likely a mutant. The probability that a given mutant is then successfully transmitted through a mosquito is necessarily biased by host immune responses, which must arise from interplay between the individual host characteristics and infection history and the parasite populations circulating in the host and vector populations—i.e. interplay of the sort that we have begun to model here. Nonetheless, like all other modelers of malaria, we have modeled the dynamics of infection, not of disease. Plasmodium infection is necessary but not sufficient for the development of clinical malaria; the connection between the two remains the outstanding mystery of malariology, and we hope that the framework presented here will help the search for its solution.

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