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Predicting optimal transmission investment in malaria parasites

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In vertebrate hosts, malaria parasites face a tradeoff between replicating and the production of transmission stages that can be passed onto mosquitoes. This tradeoff is analogous to growth-reproduction tradeoffs in multicellular organisms. We use a mathematical model tailored to the life cycle and dynamics of malaria parasites to identify allocation strategies that maximize cumulative transmission potential to mosquitoes. We show that plastic strategies can substantially outperform fixed allocation because parasites can achieve greater fitness by investing in proliferation early and delaying the production of transmission stages. Parasites should further benefit from restraining transmission investment later in infection, because such a strategy can help maintain parasite numbers in the face of resource depletion. Early allocation decisions are predicted to have the greatest impact on parasite fitness. If the immune response saturates as parasite numbers increase, parasites should benefit from even longer delays prior to transmission investment. The presence of a competing strain selects for consistently lower levels of transmission investment and dramatically increased exploitation of the red blood cell resource. While we provide a detailed analysis of tradeoffs pertaining to malaria life history, our approach for identifying optimal plastic allocation strategies may be broadly applicable.

KEY WORDS: **Coinfection, malaria, reproductive restraint, transmission investment, virulence.**

Tradeoffs between reproduction and growth are ubiquitous, with tension arising between immediate fitness gains and potentially greater fitness gains in the uncertain future (e.g., Bell 1980). The optimal balance depends on available resources and the probability of survival (reviewed in Clutton-Brock 1984), environmental variability and the odds of offspring establishing (Metcalf et al. 2008). For organisms capable of reproducing more than once, the potential reproductive output depends on serial allocation to reproduction (Charlesworth and Leon 1976). Sexual organisms face the added difficulty of finding mates (reviewed in Courchamp et al. 2008), and all organisms require some time to respond to environmental change, which can considerably narrow the range of viable strategies (Padilla and Adolph 1996). While the dilemma is often discussed for free-living organisms (Bell 1980; Clutton-Brock 1984), parasites experience the same conflicting selection pressures as they must balance in-host replication and transmission/colonization of new hosts (Reece et al. 2009).

The tradeoff in parasites is most conspicuous for species that employ specialized forms to infect new hosts, a life-history trait seen in viruses, bacteria, fungi (Anderson and May 1981), and the enormously diverse group of parasites that make up the Apicomplexa. Despite their varied life cycles, Apicomplexan parasites all undergo asexual replication within the host, while sexual reproduction is required for onward transmission (either through vectors or environmental cysts, reviewed in Smith et al. 2002). For these organisms, in-host proliferation is analogous to somatic growth and reproductive investment is synonymous with transmission investment (Reece et al. 2009). Some Apicomplexans—like *Eimeria spp.*—persist only a short time within the host, proliferating for a predictable number of rounds of replication before committing entirely to transmission/reproduction (Smith et al. 2002), analogous to monocarpic free-living species. In contrast, and more akin to iteroparous free-living organisms, malaria parasites (*Plasmodium spp.*) persist for variable lengths of time within

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the host, with infections lasting for days, weeks (Daubersies et al. 1996), or months (Miller et al. 1994) in human hosts with the possibility of investing in transmission throughout. *Plasmodium* parasites exhibit strikingly variable patterns of transmission investment (denoted the "conversion rate" by convention in the malaria literature, Bruce et al. 1990). Even in the relatively simple environment of artificial culture, the proportion of parasites developing into transmission stages has been shown to vary from less than one percent to 70% as crowding increases (Bruce et al. 1990). In addition to plastic shifts in allocation through time, transmission investment strategies are capable of evolving, and passaging in culture often selects for parasites with reduced ability to produce transmission stages (reviewed in Bousema and Drakeley 2011).

For malaria parasites, success within the host—or within artificial culture—depends on asexual population growth (i.e., cycles of replication within red blood cells) but transmission to mosquitoes requires the production of gametocytes. Upon infecting a red blood cell, each parasite can give rise to either one gametocyte (either a male or female) or several asexual parasites capable of infecting new red blood cells. In the case of the murine species *P. chabaudi*, 2–13 asexual parasites can emerge from an infected red blood cell (Mideo et al. 2011), while in the human parasite *P. falciparum* the number is 8–32 (Garnham 1966). Gametocytes cannot invade red blood cells but instead differentiate into gametes upon ingestion by a mosquito, where successful fertilization of a macrogamete by a microgamete is necessary to produce a viable infection in the vector (reviewed in Bousema and Drakeley 2011).

When gametocytes are rare, modest increases in gametocyte numbers have been observed to yield disproportionate gains in transmission success in both human and rodent malarias (Huijben et al. 2010; Bell et al. 2012, respectively). This pattern is thought to result from low odds of a blood meal containing both a male and female gametocyte when few gametocytes are circulating within the host (Bell et al. 2012)—a mate-finding Allee effect in ecological parlance (Courchamp et al. 2008). In contrast, when large numbers of gametocytes are present, transmission gains saturate (Paul et al. 2007; Huijben et al. 2010; Bell et al. 2012), presumably because nearly all blood meals contain a sufficient number of male and female gametocytes. Producing more gametocytes would not be expected to provide transmission gains and would reduce the potential for proliferation within the host (Taylor and Read 1997). Thus high early transmission investment could reduce the number of gametocytes produced over the duration of the infection (Mideo and Day 2008), creating a tradeoff between current and future reproduction.

Rapid in-host replication may convey a fitness advantage when parasites encounter immune defenses of limited capacity or competition from coinfecting strains. Analysis of *P. chabaudi* infections of mice suggests that parasites inoculated at higher numbers are able to proliferate faster early in infection, suggestive of an innate immune response that—while efficient at removing small numbers of parasites—saturates as parasite numbers increase (Metcalf et al. 2011). Correspondingly, in vitro experiments show that some innate immune effectors have reduced efficacy as parasite numbers increase (including platelets, McMorran et al. 2009, and γδ T cells, Costa et al. 2011). Theory also predicts that coinfection with multiple strains should select for reduced transmission investment because it allows parasites to secure a larger share of host resources in the face of competition (McKenzie and Bossert 1998; Mideo and Day 2008), a pattern supported by experimental rodent malaria infections (Pollitt et al. 2011).

It is not obvious how these various and often conflicting selection pressures will change through the course of infection. The optimal level of allocation to transmission should vary with resource availability, pressure from the immune system, as well as the allocation patterns of any competing strains, which could themselves be changing dynamically through time. *Plasmodium* parasites can sustain lengthy reproductive lifespans, with the potential for more complex and diverse strategies than "monocarpic" parasites like *Eimeria*. This complexity can be simplified to some extent by focusing on the early or acute part of infection, which corresponds to the largest peak in parasite numbers (e.g., Miller et al. 1994) and the steepest drop in red blood cell numbers (Huijben et al. 2010). Modifying transmission investment during acute infection would be expected to have a disproportionate impact on the fitness of parasite and host (though persistent asymptomatic infections may be important for maintaining infections in human populations during seasonal troughs in vector abundance, reviewed in Bousema and Drakeley 2011). We use a mechanistic in-host model of the acute phase of infection to identify optimal fixed and time-varying transmission investment strategies for single and dual strain infections. Our simulation-based approach allows us to explore key biological details, including proliferation rates that vary with parasite and red blood cell numbers, realistic time lags for the development of transmission and replicative forms, and density-dependent transmission success. We find that parasites can enhance overall transmission success by initially delaying transmission investment—especially when faced with immune defenses or competition—and by scaling back gametocyte production when in-host parasite populations decline due to transient overexploitation of host resources. While we focus on malaria infections, our approach is applicable to host–parasite systems where selection acts at the within-host and between-host levels (e.g., Gilchrist and Coombs 2006) or when within-population growth undermines metapopulation persistence (King et al. 2009; Shrestha et al. 2014). More broadly, the approach could be used to identify optimal plastic responses

when life-history tradeoffs vary dynamically through time or space.

Model

We modify an existing model of infections by the rodent malaria *Plasmodium chabaudi*, allowing reproductive investment to remain constant or vary through time. The model framework was previously used to assess the fitness consequences of synchronous cycles of asexual growth in malaria parasites and so describes the sexual and asexual portions of the parasite life cycle using a set of delayed differential equations (Greischar et al. 2014). In the context of reproductive investment, the model allows for realistic time lags between the "decision" to invest in gametocyte production, and the point at which mature gametocytes can contribute to transmission success. For all investment strategies, we calculate cumulative transmission potential when each infection is terminated (on an arbitrary day, after the acute phase) and determine optimal strategies for infections of different lengths. Additional simulations identify how selection pressures vary through time, and how immune clearance and competition would be expected to shift the balance.

SINGLE INFECTIONS

In experimental infections, mice are typically inoculated with infected red blood cells (e.g., Reece et al. 2008), which burst to release red blood cell-invasive merozoites. The host replaces red blood cells lost to infection, but it may take a week or more for red blood cell abundance to return to pre-infection values (e.g., Huijben et al. 2010). Merozoites can only survive a short time outside of red blood cells (Boyle et al. 2010). Following successful invasion, parasites may either replicate inside red blood cells, which burst open 24 hours later to release new merozoites (Landau and Boulard 1978; O'Donnell et al. 2011), or mature into gametocytes following a 48-hour developmental period (Gautret et al. 1996). Infectious gametocytes usually persist less than a day for *P. chabaudi* (Gautret et al. 1996; Reece et al. 2003). The probability that a mosquito becomes infected by a host depends on the gametocyte abundance in a sigmoidal fashion (e.g., Huijben et al. 2010). We use an experimentally derived curve from *P. chabaudi* (drug-sensitive clone, Bell et al. 2012) to calculate the probability of infection τ:

$$\tau(t) = \frac{\exp[-12.69 + 3.6 \log_{10} G(t)]}{1 + \exp[-12.69 + 3.6 \log_{10} G(t)]}, \quad (1)$$

where $G(t)$ is the abundance of mature gametocytes per microliter. Equation (1) represents the probability of mosquitoes becoming infected assuming unrestricted access to a host with a given gametocyte abundance, rather than a per-bite probability. The mate-finding difficulties thought to be experienced by small numbers of gametocytes are implicit in the way τ accelerates when G increases from scarcity. The cumulative transmission potential at time ϵ would then be

$$f(\epsilon) = \int_0^{\epsilon} \tau(t) dt. \quad (2)$$

We assume that parasites are under selection to maximize their odds of infecting mosquitoes over the lifespan of infection, analogous to maximizing lifetime reproductive success of free-living organisms (e.g., Maynard Smith 1978). For clarity, we treat each time point as equally valuable in the calculation of cumulative transmission potential. Thus, a unit increase in infectivity (τ) is assumed to be equally beneficial whether it happens early or late in infection, allowing us to focus on how the cost of increasing transmission changes over the course of infection.

Infectivity varies with gametocyte abundance, which depends on the dynamics of red blood cells, asexual growth and reproductive investment, and survival through developmental periods. In the absence of infection but with background mortality (μ), red blood cells (R) maintain stable numbers at a homeostatic equilibrium (K). When depleted, red blood cells are assumed to be replenished in a logistic fashion, with realized replenishment rate approaching the maximum (λ) as red blood cell numbers move further from equilibrium:

$$\frac{dR}{dt} = \lambda \left(1 - \frac{R(t)}{K}\right) - \mu R(t) - pR(t)M(t), \quad (3)$$

where p is the rate at which merozoites (M) invade uninfected red blood cells given contact. At the point of invasion, infected red blood cells are either committed to developing into transmissible gametocytes or asexual merozoites. The proportion $c(t)$ recruited to sexual differentiation is the reproductive investment—by convention referred to as the "conversion rate" (Bruce et al. 1990)—which we either assume to be constant or define as a time-varying cubic spline to minimize functional constraints on possible strategies (details of simulation and optimization in supplement). A proportion $1 - c(t)$ invaded red blood cells and commit to asexual development (I) according to,

$$\frac{dI}{dt} = (1 - c(t))pR(t)M(t) - \mu I(t) - \frac{a}{b + I(t)} I(t) - (1 - c(t - \alpha))pR(t - \alpha)M(t - \alpha)S, \quad (4)$$

with infected red blood cells removed by saturating immunity at a maximum rate of a and a half-saturation constant of b. Time series data from rodent infections suggest that early immune defenses saturate with increasing parasite numbers (Metcalf et al. 2011), a pattern also seen in *P. falciparum* parasites cultured with early immune effectors (McMorran et al. 2009; Costa et al. 2011). The delay between invasion and bursting is given by α (24 hours for

P. chabaudi, Landau and Boulard 1978), and survival S through this period described by

$$S = \exp\left(-\int_{t-\alpha}^{t} \mu + \frac{a}{b + I(\omega)} d\omega\right). \quad (5)$$

The infected red blood cells that persist through the developmental period, α, will each burst to release β merozoites. Thus the overall change in the merozoite population is:

$$\frac{dM}{dt} = \beta(1 - c(t-\alpha))pR(t-\alpha)M(t-\alpha)S - \mu_z M(t) - pR(t)M(t), \quad (6)$$

where μ_z is the intrinsic mortality rate of merozoites. A proportion of invaded red blood cells, $c(t)$, instead commit to sexual development in the I_G class of infected red blood cells:

$$\frac{dI_G}{dt} = c(t)pR(t)M(t) - \mu I_G(t) - c(t-\alpha_G) \times pR(t-\alpha_G)M(t-\alpha_G)S_{G,} \quad (7)$$

where α_G represents the delay from invasion to maturation for developing gametocytes. Survival through this period is given by:

$$S_G = e^{-\mu\alpha_{G.}} \quad (8)$$

Infected red blood cells that persist through the developmental period become mature gametocytes:

$$\frac{dG}{dt} = c(t-\alpha_G)pR(t-\alpha_G)M(t-\alpha_G)S_G - \mu_G G(t), \quad (9)$$

where μ_G describes the background mortality rate of gametocytes. We assume that gametocytes are not cleared by immunity, because gametocytes do not elicit a strong innate immune response (reviewed in Riley and Stewart 2013). Thus, equation (9) gives the abundance of mature gametocytes that can contribute to transmission potential as defined in equations (1) and (2).

Note that equations (4–6) are defined for $t > \alpha$, and equations (7–9) for $t > \alpha_G$, so we must define the fate of initially inoculated parasites. We assume that no reproductive investment occurs until the simulation begins, or in other words, that all of the infected red blood cells inoculated at the beginning are asexual. Therefore, when $t \leq \alpha$:

$$\frac{dI}{dt} = (1 - c(t))pR(t)M(t) - \mu I(t) - \frac{a}{b + I(t)} I(t) - I_0 \text{Beta}(s_P, s_P)(t)S_0(t), \quad (10)$$

where I_0 is the number of parasites inoculated. The initial age structure of the population is given by a Beta distribution with shape parameter s_P (see Greischar et al. 2014); unless otherwise noted, we simulated asynchronous infections, which are initiated with parasites uniformly distributed throughout the asexual blood stages ($s_P = 1$). Survival until bursting is described by

$$S_0(t) = \exp\left(-\int_0^t \mu + \frac{a}{b + I(\omega)} d\omega\right), \quad (11)$$

so that the change in merozoite numbers follows

$$\frac{dM}{dt} = \beta I_0 \text{Beta}(s_P, s_P)(t)S_0(t) - \mu_z M(t) - pR(t)M(t). \quad (12)$$

Invaded red blood cells can be committed to sexual differentiation as soon as the simulation begins:

$$\frac{dI_G}{dt} = c(t)pR(t)M(t) - \mu I_G(t), \quad (13)$$

but since no developing gametocytes are inoculated, no mature gametocytes can be produced or transmission potential accrued while $t \leq \alpha_G$.

We simulate infection dynamics using the parameter values given in Table S1 and use the cumulative transmission potential (eq. 2) as the measure of parasite fitness. It is computationally expensive to calculate the cumulative transmission potential within the delayed differential equation solver in **R** so we approximate fitness by summing $\tau(t)$ at each simulated time point and multiplying it by the step size, which was kept very small (0.01 days). To examine the effects of varying the duration of infection, we estimate the cumulative transmission potential for infections lasting 20, 30, 40, 45, and 50 days for a range of fixed conversion rates (i.e., constant reproductive investment). For comparison, the acute portion of infection is thought to last approximately two weeks in *P. chabaudi* (Bell et al. 2006). We also examine the optimal plastic reproductive investment for 10-, 20-, 30-, and 50-day infections, as well as the optimal strategy if parasites experience infections with varying length (assuming that 10-, 20-, and 30-day infections are equally likely). By comparing fixed and time-varying investment, we see when selection favors restrained versus increased reproductive investment over the course of an infection. Focusing on 20 day infections, we compare optimal reproductive investment in the face of saturating immunity that removes red blood cells invaded by proliferative parasites ($a = 150$, $b = 100$ in eqs. 4, 5, 10, and 11).

COINFECTIONS

Coinfecting malaria strains are thought to interact via resource competition (Mideo and Day 2008; Pollitt et al. 2011) but also have the potential to interact as mates, as evidenced by high rates of recombination (e.g., Su et al. 1999). Describing fitness in coinfections is therefore more complex. The probability of transmitting to a mosquito is given by equation (1), with $G(t) = G_1(t) + G_2(t)$ where G_1 and G_2 represent the gametocyte abundance of each strain. The strain-specific fitness (f) is then defined as the probability of transmission—corresponding to the

total gametocyte abundance—weighted by its representation in the gametocyte pool. We again estimate the integral of $f(t)$ over the duration of the infection:

$$f_1(t) = \tau(t)\frac{G_1(t)}{G_1(t) + G_2(t)} \quad (14)$$

$$f_2(t) = \tau(t)\frac{G_2(t)}{G_1(t) + G_2(t)}. \quad (15)$$

These fitness functions quantify two empirical observations that (1) transmission to mosquitoes is limited by low numbers of gametocytes, presumably because mate-finding is difficult when gametocytes are rare (Huijben et al. 2010; Bell et al. 2012); and that (2) outcrossing is possible (e.g., Wellems et al. 1990; Reece et al. 2008). We assume no inherent benefit or cost to outcrossing, since the fitness consequences are still being debated (Ramírez and Llewellyn 2014). Rather we expect that because onward transmission is enhanced by greater gametocyte numbers, a strain may benefit from the presence of a competitor's gametocytes when its own gametocytes are too rare to ensure efficient transmission in the absence of outcrossing. Nevertheless, the strain that has increased its gametocyte production improves its current representation in the mosquito vector, at the expense of the competing strain. Thus parasite fitness is assumed to reflect a balance between the costs and benefits of sharing a host with a coinfecting strain, both of which vary over the course of the infection.

We consider the simplest coinfection scenario: both strains infect the host simultaneously with the same starting inoculum and both have identical characteristics, save for their reproductive investment. Both strains are therefore equally capable of infecting red blood cells and equally vulnerable to immune clearance (at least at a given parasite abundance). While this is an oversimplification, it allows us to examine the impact of differing levels of reproductive investment in the absence of any other differences. We simulate coinfections for pairs of fixed conversion rates spanning the range of previously reported values (zero to 20%, Greischar et al. 2016) to identify the evolutionarily stable strategy (ESS) for this simple game. That is, we locate the level of reproductive investment at which neither strain can increase their relative fitness by changing their fixed strategy.

Taking the best single-infection strategy as a given, we find the optimal time-varying strategy in response. We then set the competitor's response to the best time-varying strategy and repeat the process. This approach is similar to best response dynamics in that we take the competitor's strategy as a given (whether fixed or time varying) for each iteration of the game (Matsui 1992), but with important differences: We find the optimal strategy assuming that the competing strategies begin at equal frequency, rather than finding the best response strategy for a small cluster of mutants within the population (Gilboa and Matsui 1991). Though the competitor's strategy is set, the course of infection represents a dynamic interplay between competing strategies, with both competitive and cooperative dynamics potentially favored by the payoff function (eq. 15). We model coinfection dynamics by splitting the infected red blood cell, merozoite, and gametocyte classes in two and setting the starting inoculum to $I_0/2$ for each strain. Since we assume no immunity, equation (4) becomes:

$$\frac{dI_1}{dt} = (1 - c_1(t))pR(t)M_1(t) - \mu I_1(t) - (1 - c_1(t - \alpha)) \times pR(t - \alpha)M_1(t - \alpha)S \quad (16)$$

$$\frac{dI_2}{dt} = (1 - c_2(t))pR(t)M_2(t) - \mu I_2(t) - (1 - c_2(t - \alpha)) \times pR(t - \alpha)M_2(t - \alpha)S. \quad (17)$$

As before, we assume a red blood cell may only be invaded once. Survival through the developmental period α is given by:

$$S = \exp\left(-\int_{t-\alpha}^{t} \mu d\omega\right) = \exp(-\mu\alpha). \quad (18)$$

Each strain likewise has its own merozoite class,

$$\frac{dM_1}{dt} = \beta(1 - c_1(t - \alpha))pR(t - \alpha)M_1(t - \alpha)S - \mu_z M_1(t) - pR(t)M_1(t) \quad (19)$$

$$\frac{dM_2}{dt} = \beta(1 - c_2(t - \alpha))pR(t - \alpha)M_2(t - \alpha)S - \mu_z M_2(t) - pR(t)M_2(t), \quad (20)$$

and each a separate class for infected red blood cells committed to developing into gametocytes,

$$\frac{dI_{G1}}{dt} = c_1(t)pR(t)M_1(t) - \mu I_{G1}(t) - c_1(t - \alpha_G) \times pR(t - \alpha_G)M_1(t - \alpha_G)S_G \quad (21)$$

$$\frac{dI_{G2}}{dt} = c_2(t)pR(t)M_2(t) - \mu I_{G2}(t) - c_2(t - \alpha_G) \times pR(t - \alpha_G)M_2(t - \alpha_G)S_G, \quad (22)$$

with S_G as in equation (8). The gametocyte abundance for each strain is defined by

$$\frac{dG_1}{dt} = c_1(t - \alpha_G)pR(t - \alpha_G)M_1(t - \alpha_G) \times S_G - \mu_G G_1(t) \quad (23)$$

$$\frac{dG_2}{dt} = c_2(t - \alpha_G)pR(t - \alpha_G)M_2(t - \alpha_G)S_G - \mu_G G_2(t). \quad (24)$$

As before, a separate set of equations describes the stage transitions for the initially inoculated parasites. When $t \leq \alpha$,

$$\frac{dI_1}{dt} = (1 - c_1(t))pR(t)M_1(t) - \mu I_1(t) - (I_0/2)\text{Beta}(s_P, s_P)(t)S \quad (25)$$

$$\frac{dI_2}{dt} = (1 - c_2(t))pR(t)M_2(t) - \mu I_2(t) - (I_0/2)\text{Beta}(s_P, s_P)(t)S \quad (26)$$

with

$$S = \exp\left(-\int_0^t \mu d\omega\right) = \exp(-\mu t). \quad (27)$$

Merozoite classes are therefore

$$\frac{dM_1}{dt} = \beta(I_0/2)\text{Beta}(s_P, s_P)(t)S - \mu_z M_1(t) - pR(t)M_1(t) \quad (28)$$

$$\frac{dM_2}{dt} = \beta(I_0/2)\text{Beta}(s_P, s_P)(t)S - \mu_z M_2(t) - pR(t)M_2(t) \quad (29)$$

and developing gametocytes follow

$$\frac{dI_{G1}}{dt} = c_1(t)pR(t)M_1(t) - \mu I_{G1}(t) \quad (30)$$

$$\frac{dI_{G2}}{dt} = c_2(t)pR(t)M_2(t) - \mu I_{G2}(t) \quad (31)$$

Again, we assume no mature gametocytes can be produced while $t \leq \alpha_G$.

Results

TRANSMISSION INVESTMENT DELAYS INFECTIVITY

We first examine the infection dynamics assuming that allocation to transmission is constant through time and omitting immunity. Increasing the level of transmission investment constrains the expansion of the parasite population, causing the peak in asexual parasite abundance to be smaller and later (Fig. 1A). Since transmission investment is fixed, gametocyte dynamics mirror those of the asexual stages with a time lag of 2 days corresponding to the time required for gametocyte development (Fig. 1B). Infectivity to mosquitoes is simulated as a sigmoidal function of gametocyte numbers, and all three levels of transmission investment saturate to the maximum transmission probability (Fig. 1C). Thus the fitness differences between these strains are due to differences in the timing of peak infectivity, rather than the maximum level of infectiousness. Low transmission investment yields a rapid but brief peak in infectivity, while a high level of transmission investment leads to a delayed but prolonged peak in infectivity. We plot simulated infectivity through time as a surface for different levels of transmission investment, finding that parasites face a tradeoff between attaining a highly infective state quickly and maintaining that state for an extended period (Fig. 1D). The optimal balance between these conflicting selection pressures falls at approximately 42% transmission investment when the infection is terminated at 20 days (details in Methods). Thus assuming allocation is fixed through time, 42% transmission investment maximizes the integral of transmission potential (τ) over 20 days.

Extending the duration of infection allows the possibility of more than one peak in infectivity (Fig. S1), with more peaks possible when transmission investment is restrained in favor of proliferation. For infections lasting 30 and 45 days, there are two competing optima: a "slow growth" strategy with high transmission investment and a single prolonged peak in infectivity, and a "fast growth" strategy with restrained transmission investment and multiple peaks in infectivity (Fig. S2). Whether the fast or the slow strategy maximizes fitness depends on the length of infection, with restrained transmission investment favored in a 45 day-infection (Fig. S2B) and a higher level of investment favored when infection is truncated at 30 days (Fig. S2C). As the duration of infection increases, the fitness landscape becomes flatter, so that by 50 days intermediate values of transmission investment yield similar fitness gains (Fig. S2A).

To examine the changing selection pressures over the course of infection, we relax the assumption of constant transmission investment. Allowing investment to vary smoothly through time as a cubic spline, we find that the optimal strategy is to invest heavily in asexual growth early in infection before switching to high levels of transmission investment (Fig. 2A). Similar strategies are favored for infections of different durations, such that the optimal level of transmission investment is lowest initially and also restrained later in the infection. When we allow the infection duration to fluctuate, so that parasites experience a 10-, a 20-, and a 30-day infection, we find that the optimal strategy is nearly identical to the best transmission investment assuming infections always last 30 days (Fig. S3). The strategy is weighted toward the optimal for a 30 day infection because we assume each infection length is equally likely but much greater cumulative transmission success is possible in longer infections (Fig. 2B).

To assess the fitness consequences of these strategies, we plot the rate at which they accrue transmission potential, comparing it to the maximum possible rate of fitness gain (a one-to-one line representing perfect transmission to mosquitoes for the entire duration of infection). After an initial time delay to grow in numbers, the spline strategies accrue transmission potential at nearly maximal rates (Fig. 2B). This pattern suggests that the shape of the spline is adaptive rather than an artifact of using a

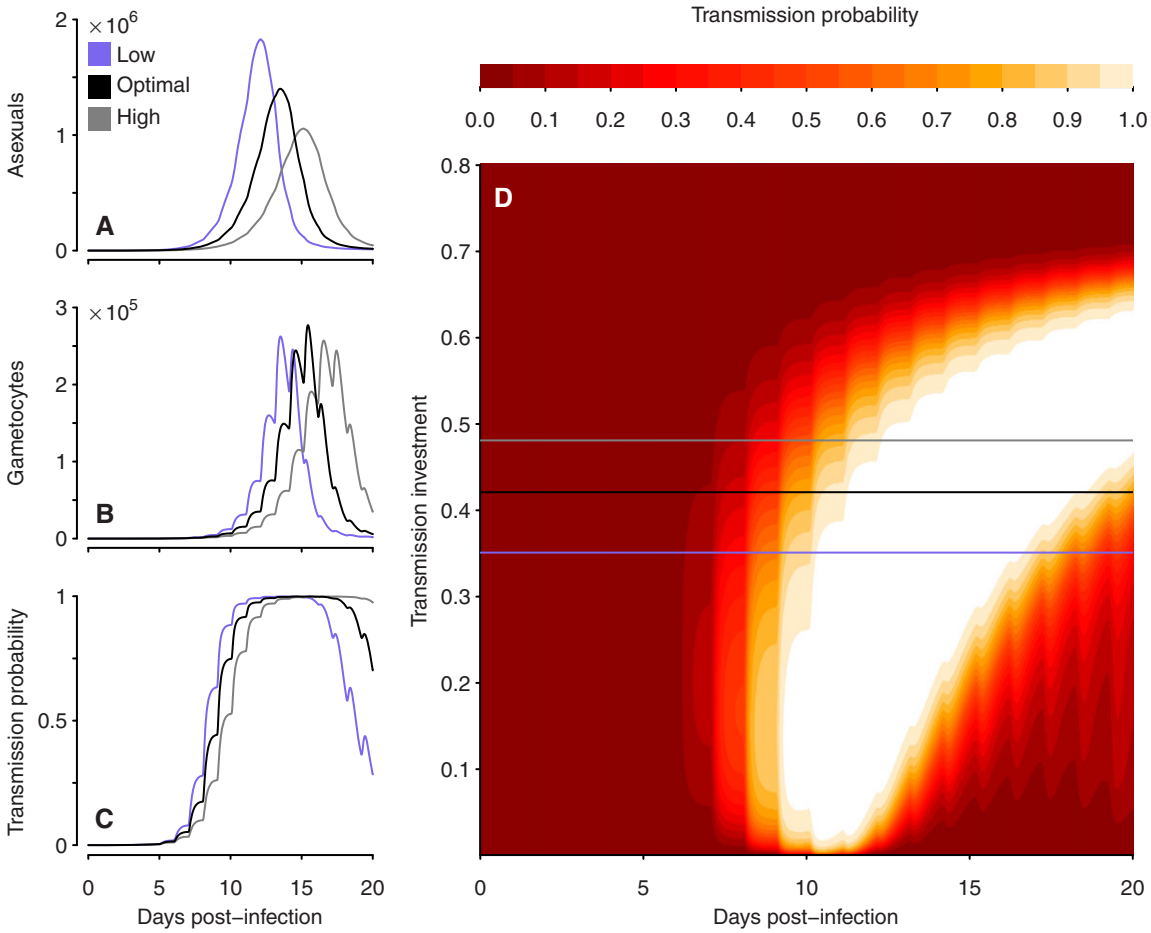

Figure 1. **Greater transmission investment slows within-host proliferation, thereby delaying gametocyte production and infectivity to mosquitoes. Assuming that the level of transmission investment is constant, we show simulated dynamics of red blood cells infected by asexual (proliferative) forms (A), of transmissible gametocytes (B), and the probability of transmission over 20 days post-infection for three levels of transmission investment: low (purple, 35.1%), optimal (black, 42.1%), and high (gray, 48.1%). The probability of transmission to mosquitoes over time is shown as a surface for varying levels of transmission investment (D), with horizontal gray, black and purple lines to indicate the levels of investment (48.1, 42.1, and 35.1%, respectively) that are plotted in panel C.**

cubic polynomial; in contrast, if the optimization were constrained by flexibility of the spline, we would expect fitness to accrue at a slower than maximal pace. Further, more complex functions yield comparable transmission potential and similar shapes (Fig. S4). Specifically, reproductive restraint is favored later in infection whenever the function is complex enough to allow for nonmonotonic strategies.

We explore the fitness consequences of reproductive restraint by returning to the best-fixed level of transmission investment (42%) and simulating the fitness gains of modifying that level of investment in a piecewise fashion for each day independently (Fig. 3). We find that the greatest potential gains or losses occur early in infection—with reproductive restraint favored early—and that allocation decisions made later on have a smaller impact on cumulative transmission potential (Fig. 3A). The point at which restraint switches from beneficial to costly (day six) corresponds to the inflection point of the sigmoidal relationship between gametocyte numbers and infectivity (eq. 1, Fig. 3B). Below this inflection point, there are disproportionate fitness gains associated with increasing gametocyte numbers, thought to be due to the difficulty of obtaining a male and a female gametocyte in the same blood meal when gametocytes are rare (Bell et al. 2012). Therefore, reproductive restraint is favored because it allows the parasite population to expand to the point that gametocytes can be produced in large numbers, escaping the part of the parameter space where mate finding difficulties would severely limit transmission to mosquitoes.

We also find that reproductive restraint is favored later in infection, at the point when the number of merozoites bursting out of red blood cells begins to decline (Fig. 3B, day 14). At that point, restraint can slow the decline in merozoite recruitment, ultimately leading to greater numbers of parasites that are

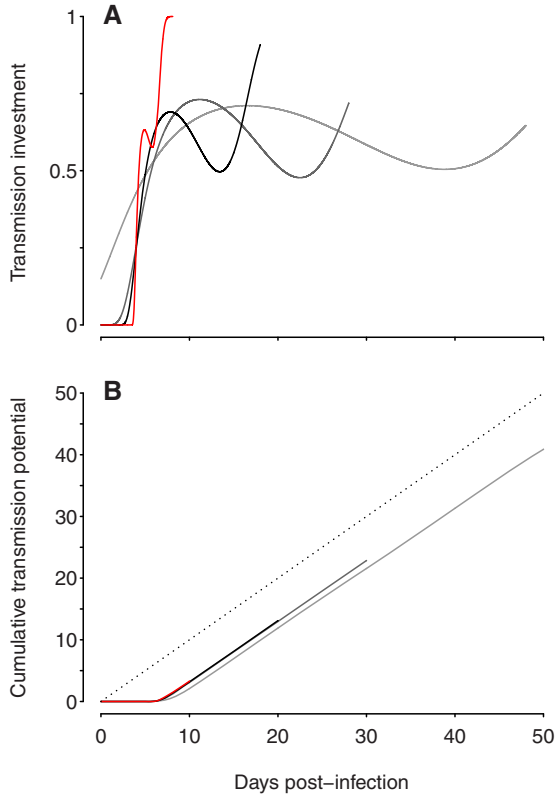

Figure 2. Plastic transmission strategies accrue transmission success at the maximum possible rate, after an initial delay. Optimal smoothly varying transmission investment strategies (A) for infections lasting 10 (red), 20 (black), 30 (dark gray), and 50 days (light gray). The corresponding gains in cumulative transmission potential are shown below (B, same colors), with the dotted one-to-one line representing the maximum rate of fitness gain (equivalent to infecting mosquitoes with a probability of one in each time step). Infections were simulated in the absence of host immunity, and the parameters defining each spline can be found in Table S2.

capable of developing into gametocytes. Deviating from the fixed strategy after day 18 has no fitness consequence in the 20 days simulated because gametocytes require 2 days to develop to maturity. These simulations provide additional support for the best cubic spline strategy: early investment in proliferation may allow parasites to avoid mate-finding difficulties, while later investment in proliferation slows the decline in merozoite recruitment, enabling parasites to continue producing gametocytes at relatively high levels.

SATURATING IMMUNITY SELECTS FOR REPRODUCTIVE RESTRAINT

If immunity saturates with increasing parasite numbers, parasites benefit from delaying reproductive investment even further (Fig. 4). Parasites must invest in asexual growth—conversely dedicating less to transmission—to ensure that the infection grows fast enough to optimize the timing of gametocyte production. Investing in asexual proliferation reduces gametocyte production, so the cumulative transmission potential is necessarily lower in the presence of immune defenses. Later in infection, parasites have reached higher numbers and the per capita rate of immune clearance approaches zero. Thus the optimal strategy for transmission investment is very similar later in infection whether immunity is present or absent.

COMPETITION FAVORS IN-HOST REPLICATION

Competition from a coinfecting malaria strain dramatically reduces the optimal level of transmission investment from the 42% optimum observed for single infections. When the focal strain has higher relative fitness, its relative fitness is largely insensitive to changes in its own level of transmission investment (Fig. 5). However, the focal strain's success does vary with the transmission investment of the competitor, and vice versa. Selection would be expected to push the system towards the point where neither strain can increase its relative fitness by modifying its investment in transmission (the evolutionarily stable level of investment, marked with an open circle in Fig. 5).

We again use splines to identify an optimal strategy for transmission investment in coinfections, taking as a starting point the optimal strategy for single infections lasting 20 days (Fig. S2). Taking as a given that the competitor utilizes the best strategy for single infections, we determine the pattern of transmission investment that maximizes fitness according to equation (15). Assuming a competitor takes that new optimal strategy, we then find the optimal response strategy. By iterating this process, each time retaining the strategy predicted to be optimal and competing it against a strain with plastic conversion rates subject to optimization, we can identify a candidate ESS. The best single infection strategy is at a substantial disadvantage—nearly sixfold—when the other strain is employing an optimal strategy for coinfections, demonstrating the dramatic fitness consequences of allocation to transmission. For comparison, drug-resistant *P. chabaudi* parasites are expected to experience a two to threefold fitness cost compared to drug-sensitive parasites—that is, they are predicted to infect two to threefold fewer mosquitoes—in the absence of drugs (Huijben et al. 2010). Subsequent optimizations tend to increase the delay before transmission investment and further reduce the level of transmission investment, and the relative fitness advantage of the best time varying strategy tends to decrease through successive optimizations, as would be expected if the competing strains were converging on an ESS (Fig. 6). These iterated optimizations suggest that frequent coinfections should select for parasites that leave transmission investment until later and invest substantially less than they would in single infections. From the perspective of host resources, coinfection selects for greater red blood cell loss than would result if both strains were employing

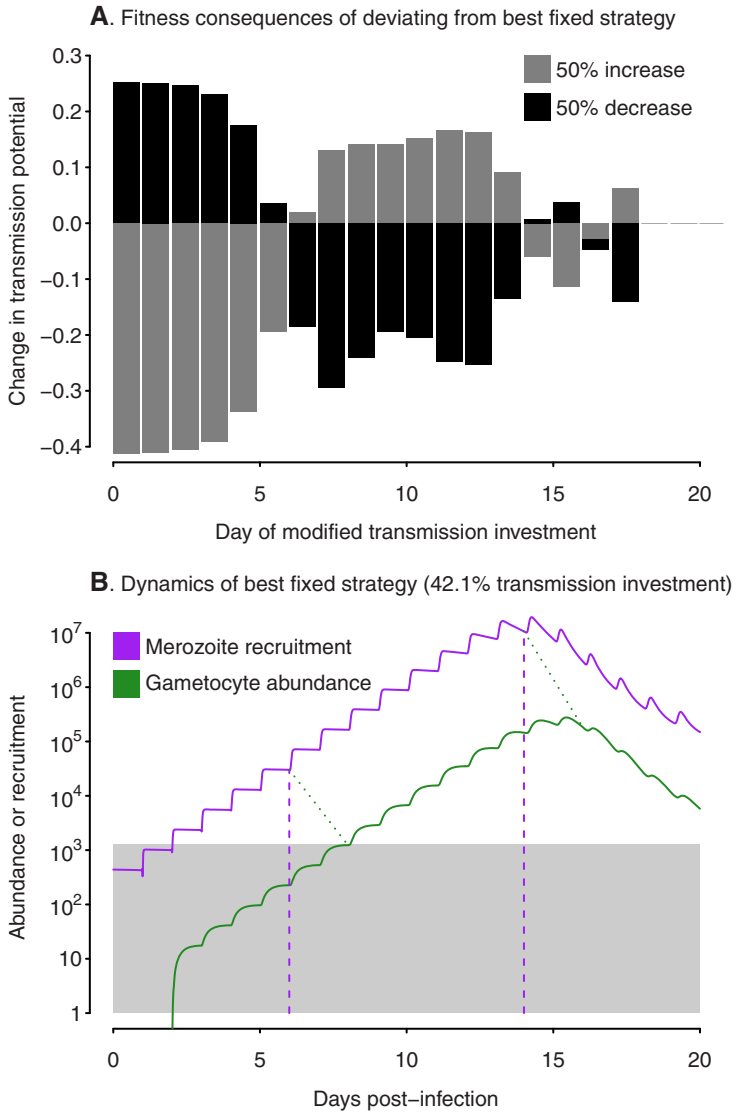

Figure 3. **Reproductive restraint is favored early in infection, and again when parasite numbers begin to decline. The fitness gains or losses compared with the best-fixed transmission investment strategy (42.1%) are shown for simulations in which parasites are assumed to follow that fixed strategy except on a single day post-infection (A). Thus each bar represents an independent simulation with parasites deviating from 42.1% on a particular day and subsequently resuming the best fixed level of investment. The dynamics of the best-fixed transmission investment strategy are shown below (B), for both the number of merozoites bursting out of red blood cells at each time point (merozoite recruitment, purple) and gametocyte abundance (green). The gray shaded region indicates the region of dynamics where increases in gametocyte abundance incur accelerating fitness gains; above this level, the probability of transmission saturates with increasing numbers of gametocytes.**

the optimal single infection strategy for transmission investment (Fig. S5).

SENSITIVITY TO BURST SIZE, RED BLOOD CELL REPLENISHMENT, AND SYNCHRONY

The optimal level of reproductive investment is likely to be influenced by parasites' maximum replication rate, by how quickly hosts can replenish resources, by gametocyte lifespan, and by the level of synchronization of the infection. We therefore recalculate the optimal conversion rate for different values of the burst size, the maximum rate of erythropoiesis, and the gametocyte mortality rate. Finally, we reexamine the role of immunity in depressing the conversion rates in synchronous infections. For computational ease, we examine the effects of these parameters in single infections assuming fixed conversion rates.

Malaria parasites can replicate into multiple asexual merozoites within a red blood cell or generate a single gametocyte. The cost of gametocyte investment should therefore depend on how

many merozoites can be produced by each infected red blood cell (the burst size). We find that the optimal-fixed conversion rate increases with the burst size (β) in single infections (Fig. S6). Thus, the relative cost of investing in gametocytes is much higher when burst size is lower, while strains with high burst sizes should be able to maintain robust asexual growth even while investing heavily in transmission.

The optimal gametocyte investment should also depend on how quickly the host can replenish red blood cells, or in other words, the cost of in-host replication should depend on the rate of erythropoiesis. Though we assume continued host survival, if red blood cell replenishment is sluggish, investing too heavily in asexual proliferation may put parasites at risk of depleting host resources, thereby limiting future opportunities for parasite population growth. A low rate of erythropoiesis may benefit strains that invest more in transmission. In contrast, if the host rapidly replaces red blood cells, red blood cell depletion may be unlikely, and parasites may benefit from investing more into growth and less into transmission. Accordingly, we find that increasing the maximum rate of erythropoiesis (λ) reduces gametocyte investment (Fig. S7). We note that even dramatic changes in λ—in this case, 75 and 125% of the baseline λ—modify the optimal conversion rate by only a few percent, a change dwarfed by the effects of adding saturating immunity (Fig. 4) or a competing parasite strain (Figs. 5, 6).

There is uncertainty in gametocyte longevity, even in the well-studied case of *P. chabaudi* (Greischar et al. 2016). Longer gametocyte lifespans would be expected to reduce the optimal level of transmission investment, making it easier to sustain large numbers of gametocytes. We have assumed that the transmissible lifespan of a gametocyte is 6 hours, the length of time gametocytes have been reported to be maximally infectious (Gautret et al. 1996). However, *P. chabaudi* gametocytes have been estimated to persist for 20 hours on average (equivalent to a 14 hour half-life, Reece et al. 2003). In *P. falciparum*, gametocytes may be infectious for longer than the duration of the proliferative cycle (Lensen et al. 1999). We find that, as expected, longer gametocytes lifespans reduce the optimal level of transmission investment and also flatten the fitness landscape so that a greater range of transmission investment values yield similar fitness returns (Fig. S8).

Synchronous development of gametocytes is predicted to help parasites overcome mating finding difficulties early in infection (Greischar et al. 2014), so we might expect synchrony to alter the optimal conversion rate. However, we find a similar qualitative pattern for synchronous infections (Fig. S9), where the optimal level of transmission investment is nearly identical to that of asynchronous infections. Adding saturating host defenses reduces the optimal transmission investment in a similar manner.

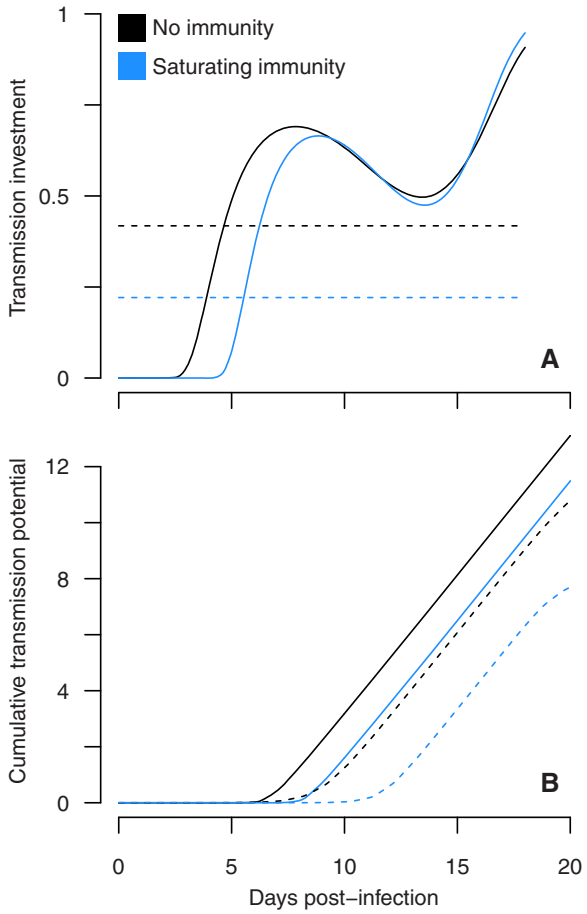

Figure 4. Saturating immunity favors reduced reproductive investment early in infection (A) despite the cost to cumulative transmission potential (B). The parameters for the best spline strategies are in Table S2.

Discussion

Reproduction often requires investment in specialized tissues or cell types, diverting resources away from increasing or maintaining biomass. If increased biomass translates into sufficiently greater potential fecundity, delays prior to reproductive investment are predicted to be adaptive (Bell 1980). The same selection pressures would be expected to influence allocation strategies in parasites where reproduction is synonymous with transmission, and the production of specialized transmission stages trades off with proliferation within the host (Reece et al. 2009). Previous theory has shown that greater numbers of gametocytes can be achieved by more rapid proliferation or by increased transmission investment or both, so that selection for more gametocytes need not alter transmission investment (Mideo and Day 2008). We expand on that theory by incorporating reasonable constraints on parasite proliferation—a maximum burst size (β) and invasion success that varies with red blood cell availability (eq. 3)—to show that selection can favor restrained transmission investment

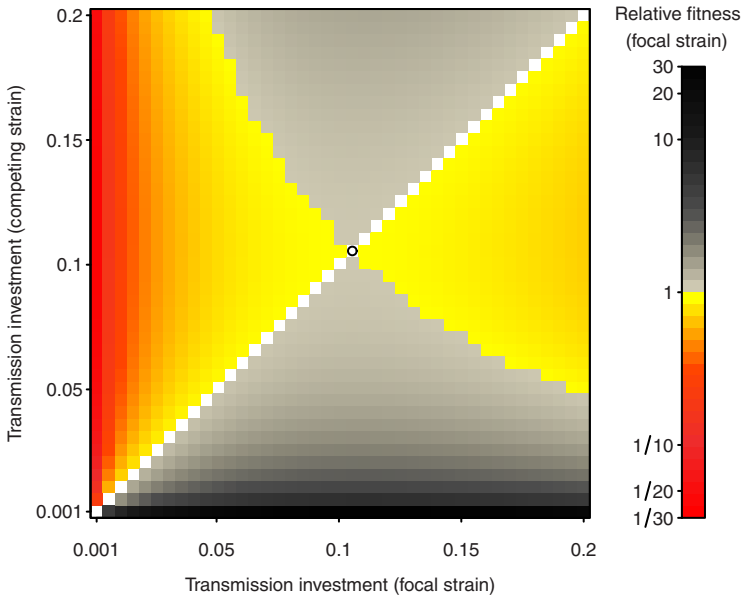

Figure 5. Coinfection reduces optimal transmission investment. Relative fitness (transmission potential of focal/competing strain) is shown as both strains modify their level of transmission investment. Parasites are assumed to have constant transmission investment and immunity is absent. Red regions indicate where the competing strain has higher relative fitness, while the gray area denotes where the focal strain has higher relative fitness. White boxes indicate that both strains have the same transmission potential (relative fitness of one). An open circle indicates the evolutionarily stable level of transmission investment.

precisely because it enhances parasite proliferation and subsequent gametocyte production.

The present model predicts a delay prior to any transmission investment, in direct analogy to adaptive delays in reproductive investment predicted for macroorganisms (e.g., Bell 1980; Koons et al. 2008). In support of this pattern, experimental infections of mice (Koella and Antia 1995) and humans (*Plasmodium falciparum*, Taylor and Read 1997; Collins and Jeffery 2003) show increases in gametocyte abundance following periods of rapid asexual growth. Gametocyte numbers are expected to lag behind asexual parasitemia because sexual differentiation is a lengthy process, but gametocytes appear even later than expected given the lengthy period required for sexual differentiation (7 to 8 days for the human malaria parasite *Plasmodium falciparum*, 2 days for the murine parasite *P. chabaudi*, Gautret et al. 1996; Lensen et al. 1999, respectively). Similarly, in experimentally infected human volunteers, asexual parasites rise to detectable levels before markers for early gametocyte differentiation can be detected (Schneider et al. 2004). Despite theoretical and empirical support for a delay, it has recently been hypothesized that a constitutive low level of transmission investment—some gametocyte production in each cycle of proliferation—will extend the period over which the host is infectious (Eksi et al. 2012) and aid transmission by allowing earlier production of gametocytes (Morahan and Garcia-Bustos 2014). The present model demonstrates two flaws in that line of reasoning. First, constitutive investment in gametocyte production restricts parasite proliferation and is therefore predicted to delay peak infectiousness (Fig. 1), whereas parasites that delay transmission investment should be able to accumulate transmission success much faster (Fig. 4). Second, early infectivity need not be associated with a lengthy period of infectiousness nor greater cumulative transmission potential (Fig. S1), since early gametocyte production may compromise persistence within the host and future transmission opportunities. Notably, artificial selection for attenuated *Eimeria* strains has also selected for earlier transmission investment, which coincides with a 75–90% reduction in the reproductive capacity compared with wild-type strains (McDonald and Shirley 2009). In malaria infections, early transmission investment could actually abbreviate the infectious window, since it puts parasites at greater risk of succumbing to immune clearance or competition.

If transmission investment jeopardizes survival within the host and future transmission success, parasites might be expected to delay gametocyte production for as long as possible. Previous theory suggests that such a strategy makes sense when parasites proliferate exponentially (Koella and Antia 1995). The optimal strategy should then be "bang-bang," as when a monocarpic plant invests exclusively in growth before investing completely and terminally in seed production (Maynard Smith 1978). That life history is likely to be a good description of Apicomplexan parasites like *Eimeria spp.*, which typically undergo a fixed number of rounds of proliferation before a single, terminal round of gametocytes is produced (reviewed in Walker et al. 2013). Intermediate values of transmission investment are more challenging to explain,

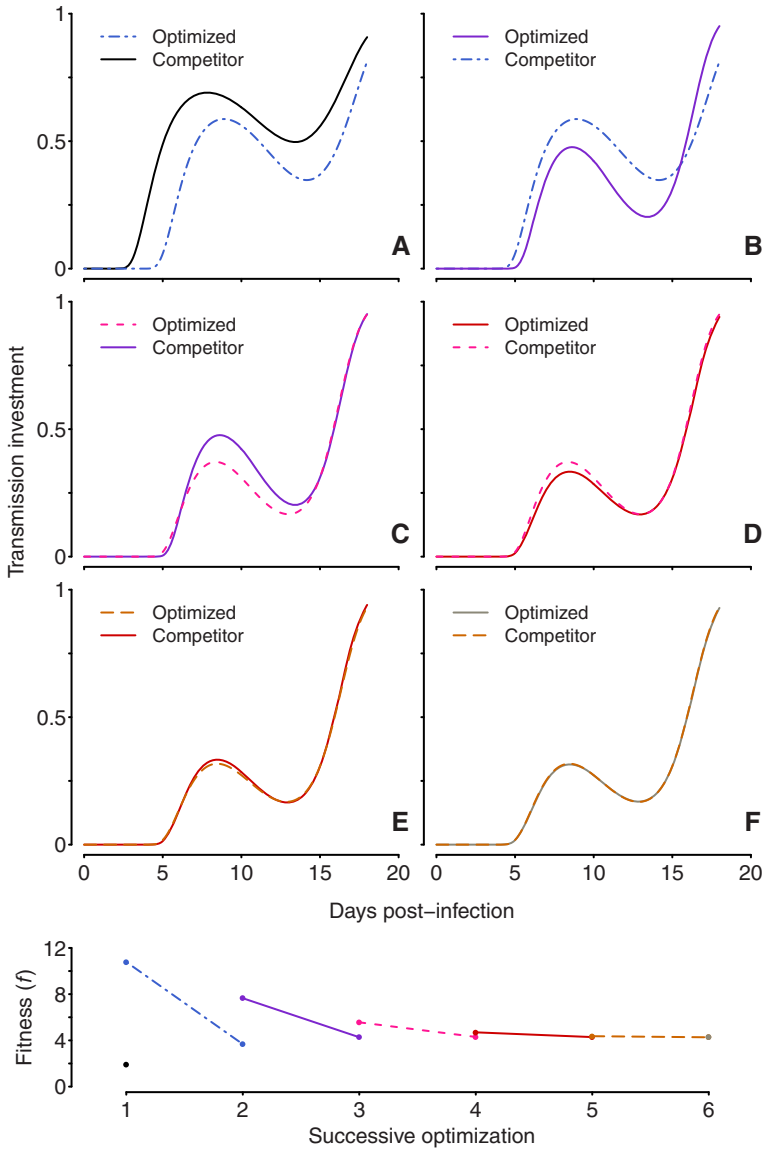

Figure 6. **Dueling splines in successive optimizations: When two parasite strains infect the same host and differ only in their allocation to transmission, they should converge on an optimal strategy. In the first case (A), we assume the competitor uses the best strategy for single infections lasting 20 days (black curve in Fig. S2) and find the optimal cubic spline strategy. In the lettered panels (A-F), we retain the optimal cubic spline strategy from the previous simulation and find the best cubic spline response. In the bottom panel, we show how fitness of the two strains changes over the successive optimizations. For simplicity, we assume that host immunity is absent. Spline parameters are given in Table S2.**

but simulations suggest that rapidly proliferating parasites might invest in transmission to avoid reaching a density lethal to the host and thereby extend the duration of gametocyte production (Koella and Antia 1995). The present model shows instead that intermediate levels of transmission investment would be expected given two characteristics of malaria biology: (1) the probability of infecting mosquitoes saturates with increasing numbers of gametocytes (Paul et al. 2007; Huijben et al. 2010; Bell et al. 2012), meaning that at some point greater numbers of gametocytes cannot improve transmission. Thus, the difficulty of finding conditions that select for intermediate levels of transmission investment may arise in part from the assumption that selection will maximize cumulative gametocyte production (Koella and Antia 1995)—as might be expected in environmentally transmitted Apicomplexans like *Eimeria*—instead of cumulative transmission potential, as would be predicted for vector-transmitted parasites; (2) parasites cannot sustain exponential proliferation as red blood cells become depleted (e.g., *P. chabaudi* infections, Metcalf et al. 2011; Pollitt et al. 2015). At the extreme, if parasites cannot modulate transmission investment, declining red blood cell numbers would

be predicted to cause a severe drop in infectivity (Fig. 1), since *P. chabaudi* gametocytes have been reported to be maximally infectious for only six hours (Gautret et al. 1996). Our simulations suggest that sustaining consistently high levels of infectivity in the face of resource depletion requires modulating transmission investment (Fig. 3B).

Adding to the challenge of coping with dramatic changes in resource availability, malaria parasites may also experience considerable variation in the duration of infection, which in *P. falciparum* can vary from days to weeks (Daubersies et al. 1996) up to hundreds of days (Miller et al. 1994). The extent to which parasite proliferation shapes this variability is an open question. Malaria parasites can reach lethal densities as has been assumed previously (Koella and Antia 1995), but parasite biomass only appears to increase the risk of severe disease beyond a certain threshold density (*P. falciparum*, reviewed in Cunnington et al. 2013). Infections are frequently asymptomatic, and these infections may be critical to parasite fitness in the long term, maintaining infections in the dry season when mosquitoes are absent (reviewed in Bousema and Drakeley 2011). Transmission investment might then be expected to be shaped by immunity, but again, it has yet to be determined how parasite proliferation alters the time required for immune clearance. Analysis of experimental rodent infections suggests that rapid parasite proliferation lessens the impact of early immunity and hastens escalation of the adaptive immune response, but neither of these host responses terminate infection, at least over the 50 days examined (Metcalf et al. 2011). Model simulations of human infections suggest that the time required for immune clearance is an extremely complex function of the parasites' proliferation rates coupled with their antigenic repertoires, although parasites that replicate rapidly initially should be better able to modulate immune defenses and prolong infection (Klein et al. 2014). Despite these unknowns, we find that infection length is unlikely to alter selection against early transmission investment or to eliminate the benefit of restraining reproduction when parasite populations are in decline (Fig. 2).

The consequences of later allocation decisions are also likely to be dwarfed by the potential gains of early reproductive restraint (Fig. 3A). Accordingly, evidence suggests that parasites may resist investing so much into gametocyte production that they preclude future proliferation. In vitro assays did not detect terminal investment even when parasites were confronted with conditions far more crowded than they would typically experience in vivo (Bruce et al. 1990). Much greater transmission potential is possible from longer infections (Fig. 2B), and when infection length is allowed to vary, the optimal strategy is biased toward the allocation pattern favored in the longest infection (Fig. S3). The allocation strategy should be weighted toward allowing continued transmission from longer infections when that possibility exists, especially given that parasites are unlikely to have perfect knowledge of when the infection will be cleared. Terminal investment is therefore likely to be selected against whenever there is a chance of a longer infection, potentially explaining why anti-malarial drugs—while increasing rates of gametocyte production in some cases—have not been shown to trigger conversion rates even close to terminal investment (Buckling et al. 1999). While inducing terminal investment has been proposed as a possible means of treating malaria infections (Carter et al. 2013), variability in the length of infection may impose strong counter-selection to keep parasites from investing everything into transmission.

Reproductive restraint has harsh implications for human health, since a malaria strain that invests more into transmission will tend to grow more slowly and to smaller population sizes (as shown in Fig. 1 A). There has been considerable interest in identifying the factors that select for reduced transmission investment and, all else being equal, increased virulence to the host. The present model predicts that saturating immunity against asexual stages will select for parasites that proliferate rapidly to greater numbers at the expense of gametocyte production, analogous to animals that delay reproductive maturity so as to outgrow predators (Reznick and Endler 1982). Any host defenses that target asexual stages and lose efficacy with increasing numbers of targets—including early immune effectors like platelets (McMorran et al. 2009) and γδ T cells (Costa et al. 2011)—should select for reproductive restraint and hence greater potential for host exploitation.

Theory suggests that coinfecting parasite strains should likewise select for reduced reproductive investment, as both strains jockey for a greater share of host resources (McKenzie and Bossert 1998; Mideo and Day 2008). The selection pressure of a competing strain should be common in human malaria infections (e.g., Färnert et al. 1999, 2008; Juliano et al. 2010). We show further that the presence of a competing strain should select for a longer delay prior to gametocyte production (Fig. 6). In contrast to saturating immunity, which selects for reproductive restraint only when parasite numbers are low, a competing strain provides continued selection for reduced transmission investment throughout an infection (Fig. 6). Coinfection selects for greater host exploitation, leading to earlier and more severe anemia than would be expected if both strains employed the optimal strategy for single infections (Fig. S5). Given the substantial fitness disadvantage predicted for a strain employing the optimal single infection strategy in a coinfection (Fig. 6), parasites would do well to facultatively alter allocation depending on whether the host is coinfected by another strain. Accordingly, rodent malaria parasites appear able to plastically alter their transmission investment in response to competition from a coinfecting strain (Pollitt et al. 2011). We focus on the case when two strains infect the host simultaneously and with equal inoculum size (i.e., the scenario examined in experimental rodent malaria infections, Pollitt et al. 2011). The situation

may also be relevant to some human malaria parasites: multistrain infections of humans show greater relatedness than expected by chance, consistent with repeated cotransmission of related parasites by mosquitoes (Nkhoma et al. 2012). Thus, different strains may be inoculated simultaneously in natural infections, and strains may frequently reencounter and interbreed with one another. A key question is whether initially distinct parasite lineages could become so highly related that evolution would proceed towards the optimal transmission investment strategy for single infections rather than for coinfections, a situation that could dramatically improve host health (Fig. S5).

Despite the advantages of plastic allocation strategies, it remains unclear whether parasites sense competitors directly or rely on other cues within the host (reviewed in Carter et al. 2013). The present model shows the dramatic fitness gains associated with allocation strategies that can change plastically through time and in response to immunity and competition. We assume that parasites have perfect knowledge of when the infection will end, so the model outputs represent optimal strategies under the best circumstances for parasite fitness. We know of no mechanism by which parasites could sense the age of infection, although *Eimeria spp.* appear to alter transmission investment based on the number of proliferative cycles completed (reviewed in Smith et al. 2002). It is possible that one or more cues may serve as good proxies. Parasites might be expected to respond to changes in red blood cell availability, parasite numbers, or even changes in parasite numbers (reviewed in Carter et al. 2013), but efforts to determine what cues parasites respond to have been hindered by the lack—until recently—of robust methods for inferring transmission investment from time series data (Greischar et al. 2016). When more is known about which within-host cues trigger changes in allocation, it will be possible to evaluate whether parasites can actually utilize optimal strategies, or whether imperfect information on the within-host environment forces parasites to make suboptimal allocation decisions.

We find that diverse selection pressures—immunity, variability in infection duration, coinfection—yields broadly similar optimal strategies: a delay prior to gametocyte production, followed by increasing transmission investment until red blood cells become limiting and reproductive restraint is needed to sustain sufficient parasite (and ultimately, gametocyte) numbers. The shape of the optimal strategy shows how selection pressures change over the course of infection, but real levels of transmission investment are likely to be much lower, less than 10 or 20% (Pollitt et al. 2011; Greischar et al. 2016). For clarity, here we consider one selection pressure at a time, but real parasites must cope with immune responses and uncertainty in infection duration simultaneously, along with the possibility of a coinfecting strain; we show that all of these complications are likely to reduce or delay gametocyte production to allow for greater proliferation within the host. Yet studies suggest diverse patterns of transmission investment across human patients (Eichner et al. 2001) and even replicate *P. chabaudi*-mouse combinations (Greischar et al. 2016). Nonadaptive explanations are possible, since the ability to accurately infer transmission investment rests in part on good assumptions about how long gametocytes persist, but the pattern could also be related to stochastic differences in the adaptive immune response (Greischar et al. 2016). The present model suggests that variability in burst size and gametocyte lifespan should influence the optimal transmission investment strategy (Fig. S6, S8). While burst sizes may vary plastically over the course of infection as mature red blood cells become depleted (Mideo et al. 2011), it is unknown whether that plasticity would vary across hosts. However, gametocyte circulation times do appear to vary across human hosts (Eichner et al. 2001) and genetically homogenous mice (Greischar et al. 2016). Though gametocytes trigger minimal innate immune response (Riley and Stewart 2013), their circulation times may be limited by antibody responses (reviewed in Bousema and Drakeley 2011). The adaptive immune response is thought to be inherently stochastic, dependent on which parasite antigens are predominantly expressed to the immune system at the beginning of infection (Klein et al. 2014). Further work is needed to determine if hosts rapidly diverge in their antibody responses in a way that impacts gametocyte longevity, and whether parasites can sense and respond to those changes; if so, we would expect parasites to benefit from employing divergent transmission investment strategies across otherwise similar hosts.

Identifying the optimal life-history allocation is a substantial challenge when there are dynamic feedbacks with resource availability and when fitness is density-dependent and strongly influenced by the allocation strategies of conspecifics. Here, we present a means of incorporating ecological detail into a model and locating optimal strategies. The system need not be at any kind of equilibrium, and the delay framework allows realistic time lags between modulating allocation and the fitness consequences of doing so. Such time lags are expected to determine when plastic strategies may be favored (Padilla and Adolph 1996). Using splines allows great flexibility in the shape of the candidate strategies, so that allocation can vary on whatever timescale or in response to whatever environmental gradient is deemed most relevant for the organism in question. The approach allows us to describe a dynamic tension in the selective forces acting on malaria parasites, and identify the factors that may select for replication—and host exploitation—over transmission investment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Increasing levels of constant transmission investment (*y*-axis) delay but prolong transmission success over the duration of infection (*x*-axis).
Figure S2. The adaptive landscape shifts as infections last longer, with a single optimal conversion rate splitting into two optima (A).
Figure S3. When infection length varies, the optimal strategy is weighted toward the ideal for longer infections.
Figure S4. Increasingly complicated strategies yield diminishing fitness returns.
Figure S5. Coinfection selects for greater host exploitation.
Figure S6. The optimal level of transmission investment increases with the burst size (β).
Figure S7. Parasites can achieve higher cumulative transmission potential with a greater maximum rate of erythropoiesis (λ), but the optimal level of transmission investment is similar.
Figure S8. Long-lived gametocytes reduce the optimal level of transmission investment.
Figure S9. Optimal level of transmission investment is similar for asynchronous and synchronous infections (solid and broken lines, respectively).
Table S1. Parameter values, units, and sources.
Table S2. Coefficients for fitted splines.