On the evolution of reproductive restraint in malaria

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Malaria is one of the leading causes of death among infectious diseases in the world, claiming over one million lives every year. By these standards, this highly complex parasite is extremely successful at generating new infections. Somewhat surprisingly, however, many malaria species seem to invest relatively little in gametocytes, converting only a small percentage of circulating asexual parasite forms into this transmissible form. In this article, we use mathematical models to explore three of the hypotheses that have been proposed to explain this apparent 'reproductive restraint' and develop a novel, fourth hypothesis. We find that only one of the previous three hypotheses we explore can explain such low gametocyte conversion rates, and this hypothesis involves a very specific form of density-dependent transmission-blocking immunity. Our fourth hypothesis also provides a potential explanation and is based on the occurrence of multiple infections and the resultant within-host competition between malaria strains that this entails. Further experimental work is needed to determine which of these two hypotheses provides the most likely explanation.

Keywords: malaria; evolution; gametocytes; conversion rate; R_0; multiple infections

1. INTRODUCTION

Malaria is responsible for a vast amount of mortality and morbidity in the developing world. The damage done to a human host infected with malaria is caused by merozoites, the asexual blood stage forms of the parasite. These merozoites infect red blood cells (RBCs), replicate and then rupture the RBC, releasing several daughter merozoites each with the potential to invade another RBC. Occasionally, instead of following this pathway, an infected RBC will produce a single sexually differentiated form of the parasite (gametocyte). While apparently benign inside a host, gametocytes are responsible for transmitting infections to mosquitoes through blood meals. Inside the vector, the parasites undergo the sexual phase of their life cycle, producing infectious sporozoites that can be transmitted to another human host in a subsequent blood meal. Thus, gametocytes offer only the opportunities for transmission of malaria infections to new hosts, while asexual forms of the parasite allow for the maintenance of a given infection within a single host.

Were it not for the conversion of some infected RBCs to gametocytes, individual parasite 'lines' would be destined to die within a single host. Further, transmission of malaria to mosquitoes is generally positively related to gametocyte density within the host (Robert *et al.* 1996; Drakeley *et al.* 1999; Collins & Jeffery 2003). Despite their crucial role in transmission, gametocytes are vastly outnumbered by asexuals (reviewed by Taylor & Read 1997). It has been estimated, *in vitro*, that between 0 and 20% of infected RBCs go on to produce gametocytes (Carter & Miller 1979), and data collected from 113

human infections suggest a geometric mean of only 0.64% (Eichner *et al.* 2001).

Considering that malaria parasites would seem to benefit, evolutionarily, from high levels of gametocytes relative to the more harmful (to the host) asexuals, Taylor & Read (1997) posed the question: why is the gametocyte conversion rate so low? The first step towards an answer is recognizing the trade-off between replication and transmission (as described for parasites with two life-cycle stages by Koella & Antia (1995)). Converting to gametocytes at a high rate reduces the number of asexuals available for the next bout of RBC invasion, probably reducing the total number of RBCs infected over the course of the infection and limiting total gametocyte production. On the other hand, while investing a lot in asexual proliferation early on provides a large pool of potential gametocytes later in infection, the benefit of continuing this investment would be mitigated once a host's immune system is waging its attack on the infection. Animal models provide evidence of increasing conversion to gametocytes under stress (e.g. Buckling *et al.* 1999), but even the maximum estimates suggest only approximately 10% of infected cells produce gametocytes. The optimal strategy when clearance of the infection is imminent would be to convert everything to gametocytes. Even if there is some constraint on varying investment in gametocytes, a low conversion rate would lead to faster depletion of host resources and thus more host damage, reducing the duration of infection and limiting the total number of gametocytes transmitted. It would seem that malaria parasites could produce more transmission stages with modest increases in conversion rates.

Assuming that available methods of detecting gametocytes are reliable, we are left wondering why conversion rates and gametocyte densities are so low while the densities of harmful asexuals are relatively high. Taylor & Read (1997) offer two broad explanations: either

conversion rates are high and large numbers of gameto-cytes are produced but quickly removed by some host immune response, or evolution has favoured low conversion rates and the apparent reproductive restraint of malaria. We leave for the empiricists the possibility of an elusive arm of immunity disposing of large numbers of gametocytes and instead focus on the question of reproductive restraint.

(a) Why might reproductive restraint be adaptive?
Taylor & Read (1997) put forth three reasons why it may be selectively advantageous to have low gametocyte conversion rates: (i) higher densities of gametocytes may ensure transmission to a mosquito through a blood meal, but may subsequently decrease the vector's survival, (ii) gametocyte densities remain low to avoid eliciting specific transmission-blocking immune responses, and (iii) gameto-cyte densities remain low *relative to asexual densities* so that transmissible forms of the parasite are masked from non-specific immune responses by asexual forms. Mckenzie & Bossert (1998) have also modelled another possibility, namely, that apparent competition between co-infecting parasites, via the immune responses that they elicit, can select for low conversion rates. Our purpose here is to formulate the first three of these hypotheses precisely, in terms of mathematical models, and to determine the conditions under which each of them predicts low gametocyte conversion rates. We also develop a fourth hypothesis, related to that of Mckenzie & Bossert (1998), but this focuses on the competition for access to RBCs that occurs upon co-infection. We formulate all four hypotheses within a common framework to clearly illustrate the connections among them and show that only two of the four hypotheses provide plausible evolutionary explanations for low conversion rates: (i) some forms of relative density-dependent transmission-blocking immunity and (ii) multiple infections, either through the process of apparent competition presented by Mckenzie & Bossert (1998) or through competition for RBCs as modelled here. These findings should help to sharpen the focus of empirical research on this important issue.

2. MODEL AND APPROACH
All of the results presented here are based on a modified version of the Ross–Macdonald model for malaria transmission (Macdonald 1957; Koella 1991), which assumes no acquired host immunity, but allows for host mortality. We suppose that the host population is homogeneous with respect to contact rates with mosqui-toes and to the probability of becoming infected when bitten by an infected mosquito. Biting rates of infected and uninfected mosquitoes are equal and the size of blood meals is constant. Finally, we make the simplifying assumption that the epidemiological parameters reflecting transmission and clearance rates in infected humans are constant during an infection. This is clearly not the case for malaria, but it greatly simplifies our analysis without sacrificing the important qualitative features under investigation. As such our analysis should be viewed as an examination of the validity of each of the four proposed hypotheses rather than an attempt to make quantitative predictions about gametocyte conversion rates.

We use an invasion analysis approach for modelling malaria evolution. Specifically, we suppose that a single strain of malaria is present in the population and examine the conditions under which a mutant strain with different properties can invade (Otto & Day 2007). Of particular interest are strains that, once present, can exclude all other possible mutants (i.e. evolutionarily stable strains). For the Ross–Macdonald model, it can be shown (appendix A in the electronic supplementary material) that the evolutionarily stable strain is the one that maximizes the following basic reproductive number:

$$R_0 = \frac{ma^2 bp e^{-\mu T}}{(r+d)\mu}, \tag{2.1}$$

where m is mosquito density per human; a is the biting rate; b is the transmission rate from an infected host to a susceptible mosquito; p is the transmission rate from an infected mosquito to a susceptible host; μ is the death rate of mosquitoes; T is the incubation period of malaria within a mosquito; r is the recovery rate of infected hosts; and d is the death rate of humans.

Each of the hypotheses proposed for the evolution of reproductive restraint assumes different relationships between the model parameters and the parasite traits of interest. To formalize these relationships, we denote the total number of RBCs infected during an infection by $A(\epsilon, \phi)$, where this is written as a function of both the proportion of infected cells that are converted to gameto-cytes, ϵ and the within-host asexual growth factor, ϕ. Formally, ϵ is a proportion but it is often incorrectly referred to as a 'rate' in the empirical literature, and we retain this convention here for consistency. The growth factor, ϕ represents the per parasite rate of invasion of RBCs multiplied by the burst size of an infected RBC and the expected lifespan of a merozoite in the bloodstream. For any value of ϵ, increasing ϕ results in an increased number of RBCs infected during the course of infection. The total number of gametocytes produced is $G = \epsilon A(\epsilon, \phi)$. Each of the hypotheses can then be formalized by specifying the assumed relationships between the par-ameters in equation (2.1) and A and G. Malaria strains will be assumed to differ in their values of ϵ and ϕ, which produces different values of A and G, and therefore different values of R_0 in equation (2.1). Since evolution maximizes the basic reproductive number, we can determine the direction that selection acts on ϵ and ϕ by differentiating equation (2.1) with respect to these variables (Otto & Day 2007). Our aim is to find the conditions under which evolution favours low rates of conversion to gametocytes.

3. RESULTS
All four hypotheses assume that transmission to mosqui-toes, b, is an increasing function of G, as without a benefit to increasing gametocyte production it would make no sense to ask why there are so few. This assumption is therefore implicit in all analyses below.

(a) Mosquito survival
The first hypothesis is that low numbers of gametocytes may be selectively advantageous if ingesting gametocytes leads to an increased risk of death in the mosquito vector, i.e. if μ is an increasing function of G. Without defining explicit

forms of the functions $b(G)$ and $\mu(G)$, we can rewrite equation (2.1) to show these functional relationships as

$$R_0 = \frac{ma^2 b(G) p e^{-\mu(G)T}}{(r+d)\mu(G)}. \tag{3.1}$$

Evolution is expected to produce the values of ϵ and ϕ that maximize equation (3.1). The dependence of equation (3.1) on the traits ϵ and ϕ enters only through the dependence of G on these traits, and therefore we obtain the same condition for evolutionary stability for each trait. Taking the derivative of R_0 with respect to each trait and setting it equal to zero, we see that the optimal values ϵ and ϕ must produce a gametocyte level that satisfies the equation

$$\mu(G^*)\frac{db}{dG}\Big|_{G=G^*} - b(G^*)\frac{d\mu}{dG}\Big|_{G=G^*}(\mu(G^*)T+1) = 0. \tag{3.2}$$

The actual value of this optimal gametocyte production level, G^*, will depend on the forms of the functions $b(G)$ and $\mu(G)$. Thus, gametocyte levels can be high or low depending on these functions. Regardless of what this optimum is, however, from the standpoint of conversion rates, there is typically a continuum of values of ϵ and ϕ that give rise to the same level of gametocytes, G^*. In particular, for each growth factor there are typically two conversion rates that obtain the optimal G^*, one high and one low. The reason is that, for a fixed value of ϕ, the same total number of gametocytes can be produced by having a low conversion rate, which results in a high total amount of parasite replication, or vice versa (appendix B in the electronic supplementary material). Consequently, selection does not unequivocally favour reproductive restraint. Rather, an entire set of paired conversion rates and growth factors should be selectively neutral (figure 1). This suggests that, barring other constraints, we would expect to see different conversion rates in different populations of the same species of malaria. It should be noted, however, that we have neglected the fact that strategies resulting in high levels of asexuals (i.e. those with low conversion rates) will probably cause their hosts to suffer the highest mortality rate. This suggests that, if any directional evolution on conversion rate is expected under this hypothesis, it would be towards high conversion rates (and low growth factors).

(b) *Density-dependent transmission-blocking immunity*

Low gametocyte production may be selectively advantageous if specific immune responses are elicited by gametocytes in a density-dependent manner. While transmission to mosquitoes should directly increase with gametocytes, high densities may inhibit successful transmission by eliciting a strong, specific immune response. To formalize this idea, we suppose that 'immune cell activity' against gametocytes is an increasing function of total gametocyte production, G, and that the transmission rate, b, is a function of both G and immune cell activity, $I(G)$. For completeness, we keep mosquito mortality as an increasing function of G as well, but the same qualitative results are obtained if we leave out this dependency. With these functional relationships, equation (2.1) is written as

$$R_0 = \frac{ma^2 b(G, I(G)) p e^{-\mu(G)T}}{(r+d)\mu(G)}. \tag{3.3}$$

Again, the dependence of equation (3.3) on ϵ and ϕ enters only through the dependence of G on these traits, and
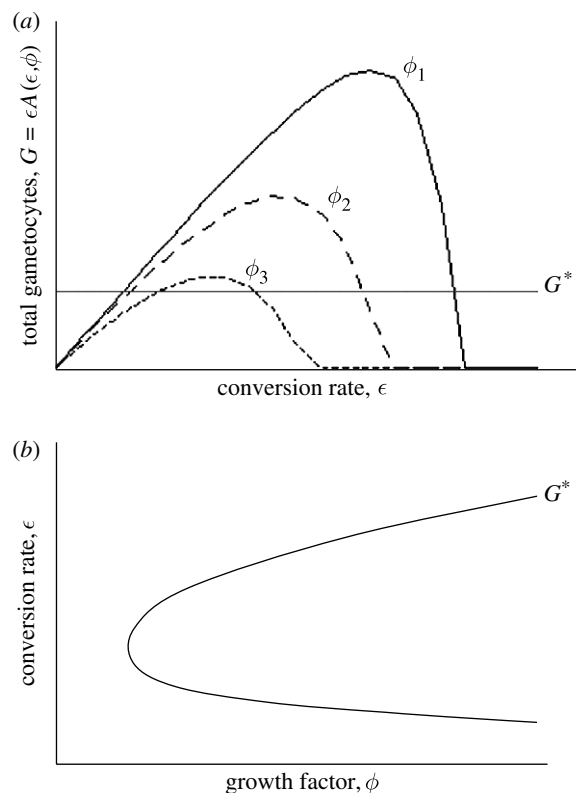

Figure 1. A particular optimal gametocyte production level can be obtained from multiple selectively neutral trait pairs. (*a*) Total gametocyte production as predicted by the within-host model described in the electronic supplementary material, appendix B at steady state (with $\theta = 1$, $\eta = 0.01$, $\zeta = 2$ and φ_1 $\varphi_2 > \varphi_3$). The total number of gametocytes, G, is the product of conversion rate, ϵ, and total RBCs infected, $A(\epsilon, \phi)$. As conversion rate increases, fewer merozoites are available to infect RBCs, so A decreases with ϵ, resulting in the 'hump' shape of G. The total number of RBCs infected increases with ϕ. In response to selection pressures, evolution favours a certain optimal level of gametocyte production, G^*, denoted by the grey line. For each ϕ we see two values of ϵ that obtain G^* gametocytes. (*b*) Extending these results we find that a continuum of trait pairs give rise to G^* gametocytes.

therefore we again obtain a single condition for the evolutionary stability of both traits,

$$\mu\frac{\partial b}{\partial G} - b\frac{d\mu}{dG}(\mu T + 1) + \mu\frac{\partial b}{\partial I}\frac{dI}{dG} = 0. \tag{3.4}$$

As before, there will be an optimal G^* that satisfies this equation, but since $\partial b/\partial I < 0$ and $dI/dG > 0$, density-dependent immune responses select for lower optimal gametocyte numbers than did mosquito survival alone. Nevertheless, these results again show that there will be both the high and low conversion rate strategies that give rise to the same value G^*, meaning reproductive restraint is not unequivocally favoured under this hypothesis either.

(c) *Relative density-dependent transmission-blocking immunity*

Alternatively, low gametocyte production may be selectively favourable if it is the relative density of gametocytes to asexuals that determines the success of transmission-blocking immune responses. In other words, non-specific immune responses with the ability to target both asexuals and gametocytes may be 'distracted' by asexuals if they are relatively more abundant, allowing gametocytes to be

transmitted. As the ratio of gametocytes to asexuals increases, transmission will decrease. In this case, immune cell activity against gametocytes, I, is an increasing function of ϵ because I is now an increasing function of $G/A = \epsilon A/A = \epsilon$. To show this relationship, we write the basic reproductive number as

$$R_0 = \frac{ma^2 b(G, I(\epsilon)) p e^{-\mu(G)T}}{(r + d)\mu(G)}. \tag{3.5}$$

Differentiating equation (3.5) with respect to ϵ and ϕ now produces two separate equations that must be satisfied by these traits at evolutionary equilibrium, because ϵ now appears independently of G in this equation. The derivative with respect to ϕ gives the condition

$$\mu \frac{\partial b}{\partial G} - b \frac{d\mu}{dG}(\mu T + 1) = 0, \tag{3.6}$$

which is what we found in the mosquito survival case. The derivative with respect to ϵ gives the condition

$$\frac{\partial G}{\partial \epsilon}\left(\mu \frac{\partial b}{\partial G} - b \frac{d\mu}{dG}(\mu T + 1)\right) + \mu \frac{\partial b}{\partial I}\frac{dI}{d\epsilon} = 0. \tag{3.7}$$

This reveals that, if we were at the evolutionary equilibrium of the mosquito survival case, then selection acts on ϵ in a direction given by the sign of μ $(\partial b/\partial I)$ $(dI/d\epsilon)$. Thus, selection acts to decrease the conversion rate, as postulated by Taylor & Read (1997). In addition, as a consequence of this selection, optimal growth factors will be higher, explaining the high asexual densities that are common in infections.

A criticism of the above formulation of this hypothesis might be that, while immune attack of gametocytes will increase with their relative density, the strength of the immune response elicited (in particular, the number of 'immune cells' recruited) will increase with the total number of infected cells as well. In other words, it may be more accurate to consider I as an increasing function of both ϵ and $A(\epsilon, \phi)$. In this case, selection no longer necessarily favours low conversion rates because such conversion rates require a high number of asexuals to reach the optimal level of gametocyte production, and such strategies are selected against by the increased immune response that they elicit. Therefore, to determine whether or not the relative density-dependent transmission-blocking immune responses can unequivocally select for reproductive restraint through low conversion rates, we would need to know the precise details of the action of the immune mechanism.

(d) Multiple infection

To this point, our analysis has focused on infections generated by a single 'strain' of a particular malaria species. If, however, infections are composed of multiple strains (or multiple species) of malaria, these strains may be in direct competition for both host resources (i.e. RBCs) and access to mosquitoes. Many studies have documented the high prevalence of people infected with multiple *Plasmodium* species (e.g. Snounou *et al.* 1993; Bruce *et al.* 2000; Ebrahimzadeh *et al.* 2007) and with multiple strains of the same species (e.g. Paul *et al.* 1995; Babiker *et al.* 1999; Konaté *et al.* 1999; Bruce *et al.* 2000; Engelbrecht *et al.* 2000; Magesa *et al.* 2002; Cole-Tobian *et al.* 2005). In stable endemic regions, where malaria transmission is consistently high, such as Nigeria (Engelbrecht *et al.* 2000), Tanzania (Babiker *et al.* 1999) and some parts of Senegal

(Konaté *et al.* 1999), it has been estimated that between 70 and 90% of all infections harbour multiple strains.

Mckenzie & Bossert (1998) examined the potential for multiple infections to select for low gametocyte conversion rates, under the assumption that different strains interact indirectly through a shared immune response. They found that low conversion rates were optimal, provided that the immune response resulted in a form of apparent competition between co-infecting strains. Here we explore an even simpler hypothesis that multiple infection results in direct competition between strains for access to RBCs, and this drives the evolution of lower conversion rates.

There are two main theoretical approaches for exploring multiple infections. A co-infection framework allows multiple strains to coexist within a host (May & Nowak 1995) whereas a superinfection framework (Nowak & May 1994) assumes that, although multiple infections can occur, one strain always immediately excludes the other in any given host, owing to the competition between the two. While co-infection is certainly closer to reality for malaria, an assumption of superinfection still accounts for the main qualitatively important evolutionary consequences of multiple infections with respect to conversion rates. Superinfection also results in much more transparent mathematical analyses and, since our main purpose is to use the models to draw out important conceptual aspects of multiple infection, it makes the most sense to employ this assumption here (qualitatively similar findings result from a model of co-infection; N. Mideo & T. Day 2007, unpublished results).

The hypothesis is that competition for RBCs alone can explain the evolution of low gametocyte conversion rates. To this end, we ignore any immune response of the host and suppose that within-host dynamics of malaria are solely determined by the exploitation of RBCs. In this case the strain with the largest, within-host reproduction number will ultimately exclude all others from the infection (see appendix B in the electronic supplementary material). Using ω to denote the number of merozoites produced by a single infected RBC, β to denote the invasion rate of RBCs and δ to denote the death rate of free-living merozoites, the strain that wins the within-host competition is that with the largest value of

$$\kappa_i = \phi_i(1 - \epsilon_i), \tag{3.8}$$

where a strain's within-host growth factor is given by (see appendix B in the electronic supplementary material)

$$\phi = \frac{\omega\beta}{\delta}. \tag{3.9}$$

From equation (3.8) it can be seen that within-host competition always favours strains with high growth factors and low conversion rates.

This within-host competition model can be readily included within the theoretical framework used in the previous hypotheses using the assumption of superinfection. Specifically, we suppose that the likelihood of a strain being replaced upon multiple infection depends on the difference between the new strain's and the existing strain's 'competitiveness'. Thus, the likelihood of strain i replacing strain j is given by $\sigma(\kappa_i - \kappa_j)$, where σ is an increasing function of its argument.
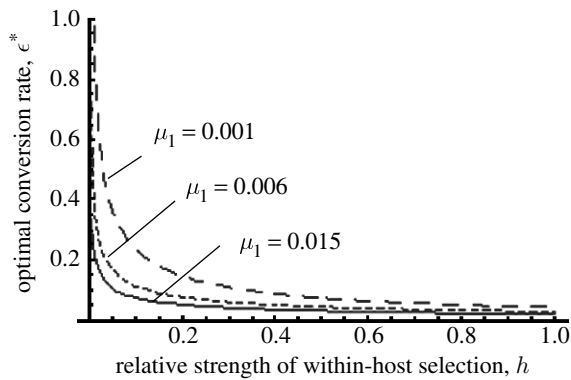
Figure 2. The effect of multiple infections on the optimal conversion rate. Evolutionarily stable conversion rate strategies as predicted by the model of superinfection in the electronic supplementary material, appendix C assuming a steady-state gametocyte production as described in the electronic supplementary material, appendix B. (Here, $\mu(G) = \mu_0 + \mu_1 G$, $b(G) = \lambda(G/\alpha + G)$, $\sigma(\kappa_i - \kappa_j) = \sigma_1(1 + \tanh(h(\kappa_i - \kappa_j)))/2$, $m = 1000$, $a = 1000$, $p = 1$, $\mu_0 = 10^{-4}$, $\lambda = 1$, $\alpha = 1$, $T = 10$, $r = 0.01$, $d = 0.001$, $\theta = 1$, $\eta = 0.01$, $\zeta = 2$, $\sigma_1 = 1$.) Under these conditions, selection always acts to increase ϕ. Therefore, the equilibrium value of ϕ will always be ϕ_{max} and here we have assumed $\phi_{max} = 10$. The parameter h describes the relationship between the likelihood of superinfection and the outcome of within-host competition. When $h = 0$, superinfection occurs at a constant rate irrespective of the within-host growth factors of the competing strains. Superinfection in this case does not result in selection at the within-host level. As h increases, the likelihood of superinfection becomes increasingly dependent on the competing strains' within-host growth factors, generating selection at this level. The equilibrium conversion rate therefore decreases as h increases. Also, as the cost of gametocytes goes up, i.e. increased mosquito mortality μ_1, lower conversion rates are favoured by selection.

As shown in the electronic supplementary material, appendix C, when multiple infections occur the fitness of a mutant (strain 2) in a resident population (strain 1) is

$$R_0(G_2, G_1) = \frac{ma^2 b_2(G_2) p e^{-\mu_2(G_2)T}}{\mu_2(G_2)}$$
$$\times \frac{x^* + y_1^* \sigma(\kappa_2 - \kappa_1)}{r_2 + d + h_1^* \sigma(\kappa_1 - \kappa_2)}, \qquad (3.10)$$

where, in the absence of superinfection, x^* and y_1^* are the equilibrium densities of susceptible hosts and individuals infected with strain 1 and h_1^* is the equilibrium inoculation rate of strain 1. The first factor in the above fitness expression is equivalent to expression (3.1) from the mosquito survival hypothesis, up to a multiplicative constant. Therefore, the effect of multiple infections is given by the second factor. Since σ is an increasing function, we can see that multiple infection always favours mutant strains with large values of κ, meaning low conversion rates and high growth factors (figure 2).

This makes intuitive sense since it is only the asexual forms that play any role in the competition for access to RBCs. Thus, although a continuum of pairs of conversion rates and growth factors, (ϵ, ϕ), can yield an optimal level of gametocyte production in singly infected hosts, if such a host should ever become multiply infected, it is those strains from this continuum that have a lower conversion rate and a higher growth factor that will do best. In the context of our assumption of superinfection, this is because

the strain with a lower conversion rate will most often immediately exclude the other, before it has any chance of further transmission. Under an assumption of co-infection a similar process occurs, but in this case the strain with the lower conversion rate does best, not by immediately excluding further transmission by the other strain but by simply reducing its output via competition for RBCs during the remainder of the infection. Therefore, quite generally, the occurrence of multiple infections can drive the evolution of conversion rate to low values, solely through its effects on competition for access to RBCs.

4. DISCUSSION

Our results show that evolution can favour reproductive restraint in malaria. While all of the mechanisms offered by Taylor & Read (1997) to explain reproductive restraint can generate selection for low numbers of gametocytes, in only one case does this come about through the selection for low conversion rates. Of the hypotheses we explore, multiple infection is the most likely explanation for the evolution of reproductive restraint in malaria (see also Mckenzie & Bossert 1998).

Our results suggest that an increased risk of mosquito mortality associated with gametocytes does not clearly favour low conversion rates within a host. The empirical evidence for the mosquito survival hypothesis has itself been equivocal. While malaria has been shown to cause damage to its vector (reviewed by Ferguson & Read 2002*b*), studies have found mosquito mortality to be unrelated to gametocyte density (Robert *et al.* 1990; Ferguson & Read 2002*a*). How gametocyte density in a blood meal relates to mosquito mortality at later stages of the parasite's sexual cycle remains unclear. Mosquitoes harbouring sporozoites suffer greater mortality associated with feeding than uninfected mosquitoes (Anderson *et al.* 2000), but only a limited number of gametocytes can be converted to sporozoites and early on in development parasite numbers are checked by apoptosis (Al-Olayan *et al.* 2002). Regardless of the shape of the relationship between gametocyte density in a blood meal and sporozoite burden later in an infection, our results suggest that mosquito mortality could result in low optimal numbers of gametocytes but is not sufficient for generating selection to achieve these low levels via a low conversion rate.

Similarly, a recent study estimated the relationship between gametocyte density and infection rates of mosquitoes and found an upper threshold above which infection rates levelled off (Paul *et al.* 2007). The authors conclude that the lack of further benefits to increased gametocyte densities would favour reproductive restraint. While this might again explain low optimal numbers of gametocytes, this could be generated by a continuum of trait pairs, ϵ and ϕ, including relatively higher conversion rates and lower growth factors.

Transmission-blocking immune responses to specific gametocyte antigens have been described (e.g. Healer *et al.* 1999) and could impose a strong selective force against high gametocyte production. Our results show that indeed selection in response to this sort of immunity leads to lower optimal production of gametocytes but, again, not necessarily to low conversion rates. Transmission-blocking immune responses are generally considered to be mechanisms operating within the vector, for example by

blocking fertilization. In our model, we assume that it is specifically the transmission of gametocytes to mosquitoes that is blocked by these immune responses though the precise timing of their action should not qualitatively change our results.

We have shown that the relative density-dependent transmission-blocking immunity may generate selection for low conversion rates, but that this conclusion is dependent on the precise details of the immune response. In particular, it depends on the relative importance of immune stimulation versus immune evasion. Taylor & Read (1997) offer relative density dependence as a slight variation on the immune pressure hypothesis and in an argument against its plausibility, cite Sinden (1991) who showed that infectiousness to mosquitoes actually decreases with increasing asexuals. Other studies, however, have failed to find any relationship between asexual density and transmission success (Drakeley et al. 1999; Paul et al. 2007). In any case, the relationship between relative gametocyte density and transmission may be slightly more subtle. As transmission success depends on gametocyte maturity (Hallett et al. 2006), one of the biggest constraints on transmission might be the ability of gametocytes to successfully develop inside a host. Maturation of gametocytes takes approximately 10 days to complete during which time the dynamics of infection and immune activity are ongoing. Piper et al. (1999) have suggested that cross-stage immune responses against PfEMP-1 may help explain the low gametocyte numbers seen in malaria infections. PfEMP-1 is an antigen that is expressed on the surface of infected RBCs, both in which asexuals are developing and which harbour early stages of gametocytes. In later stages of development, gametocytes lose this antigen and so are protected from immune responses directed towards it (Hayward et al. 1999). While low gametocyte numbers relative to asexuals may do little to prevent activating this anti-PfEMP-1 response (considering the vast numbers of asexuals stimulating immunity), their relative scarcity would provide early stage gametocytes some shelter from these immune responses. With transmission success depending on maturity and gametocytes being more likely to survive the early stages of their development with many asexuals masking them from immune responses, the conditions would seem to be right for selection to favour low conversion rates.

Multiple infections have been suggested previously as an explanation for the apparent reproductive restraint of malaria and Mckenzie & Bossert (1998) use numerical simulations to show that low conversion rates are favoured in multi-strain infections. In their model, all immune activity is targeted against asexual parasite forms and is fully cross-reactive between strains. A strain that invests more in asexuals will be primarily responsible for eliciting the immune response, but this response will affect all strains. Thus, a strain with a low conversion rate that produces many asexuals can reduce the success of a competitor with fewer. Our results are similar in that multiple infections can select for lower optimal conversion rates, but suggest that in addition to 'apparent competition' mediated through a common immune response, competition for resources (i.e. RBCs) alone can provide the selective force. In particular, evolution favours strains with high growth factors and low conversion rates because these are best able to exploit the supply of RBCs within the host. This finding adds considerable further weight to the idea that multiple infections are responsible for low conversion rates since this mechanism does not even require specific assumptions about the nature of the immune response (e.g. target of response, level of cross-reactivity).

Both of these competition theories suggest that in areas with a considerable amount of multiple infections (i.e. high-transmission areas) we should be able to measure lower conversion rates in infected hosts. We are unaware of any studies that have compared this trait in particular, in high- and low-transmission settings. However, a recent review (Drakeley et al. 2006) cites evidence of high-transmission areas harbouring fewer gametocyte carriers among infectious individuals and a lower gametocyte to asexual parasite ratio within hosts. Further, there is more seasonal variation in both of these measures in low-transmission areas (see Drakeley et al. 2006 and references therein), suggesting that something is constraining the flexibility in conversion rates in high-transmission settings.

The two hypotheses that provide a plausible explanation for low conversion rates posit that there is some additional benefit to high densities of asexuals beyond future production of gametocytes. In the relative density-dependent transmission-blocking immunity case, asexuals mask gametocytes from immune responses and in the multiple infection case, higher asexual densities are equated with greater ability to exploit host resources and, consequently, a within-host competitive advantage. It should be noted that there are other plausible benefits for high asexual densities, which could be incorporated into future theoretical frameworks. For example, greater asexual production will lead to greater host anaemia and other measures of parasite-induced harm. If these 'symptoms' of high asexual densities translate to reduced anti-vector behaviour, overall transmission success could be enhanced (Ewald 1983). Alternatively, in the presence of specific immune responses, there could be substantial selection pressure towards generating immune-escaping antigenic variants (Brown & Brown 1965; Phillips et al. 1997) and increased asexual production could facilitate this.

We have claimed that selection for low optimal numbers of gametocytes does not necessarily lead to selection for low conversion rates, as the total number of gametocytes produced is the product of two traits that could equally be modified (i.e. growth factor and conversion rate). Our conclusions are contingent on this assumption and it is possible that we have overlooked some physiological constraint at play. It may be the case that to produce an optimal number of gametocytes a minimum number of asexuals must be produced, for example, if parasites are unavoidably programmed to switch to gametocytogenesis after a certain number of rounds of asexual multiplication. If this threshold number of asexuals were high enough and selection on asexual investment thus constrained, selection for low gametocyte production would result in low conversion rates. What determines the timing of gametocytogenesis is not completely clear though putative roles have been established for factors such as host immune responses, host hormones and anti-malarial drugs (reviewed by Dyer & Day (2000) and Talman et al. (2004)). In addition, gametocytogenesis is enhanced *in vitro* in cultures enriched with young RBCs (Trager & Gill 1992; Trager et al. 1999). These reticulocytes make up 1% of

normal blood, but this proportion can rise significantly with anaemia as the body flushes the system with new cells to compensate. Anaemia was able to predict the presence of gametocytes in patients in Thailand (Price *et al.* 1999) although the study's authors suggest that the length of infection may have been a confounding factor. Still, there is some evidence of a critical asexual threshold required to produce conditions favourable to gametocytogenesis. Exactly how high that threshold might be is unclear so we do not know how much (or if) the evolution of conversion rates is constrained, but indeed such constraints may exist.

Part of the motivation for resolving the case of reproductive restraint in malaria is the potential for new insights into control strategies (Taylor & Read 1997). One intriguing potential aim of interventions is reducing the occurrence of multiple infections. Experimental multi-strain malaria infections in mice have shown that both immune- and resource-mediated competition may be generating selection for increased virulence in multiple infections (Råberg *et al.* 2006). Thus by limiting these types of infections, a beneficial evolutionary consequence would be selection for less virulent parasites (Bell *et al.* 2006). Consistent with this, our results suggest that limiting multiple infection would reduce selection for high growth factors and would have the added benefit of eliminating selection against high conversion rates. While this might seem to suggest an increased risk of transmission, selection on the optimal gametocyte production level, G^*, in theory should not change, so the total transmission potential of an infection should remain the same. The benefit comes in the form of those infections with higher conversion rates destroying fewer RBCs of the infected host, while still achieving the same total gametocyte production.

An interesting line of future investigation would be to see if differing conversion rates in other taxa could be explained by differing levels of multiple infections. For example, gametocytes in lizard malaria, *Plasmodium mexicanum*, can make up the majority of parasites within a host (Bromwich & Schall 1986). Other apicomplexans closely related to *Plasmodium*, including *Hematocystis* and *Haemoproteus* spp., have life cycles that do not involve a period of asexual expansion within RBCs at all (Smith *et al.* 2002). Our results suggest that within-host competition is the main selective force favouring low conversion rates, so the occurrence of multiple infections with these related parasites should be lower than that with human malaria. To our knowledge, empirical estimates of clonal diversity in these related infections do not yet exist.

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