Why So Few Transmission Stages?
Reproductive Restraint by Malaria Parasites

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Vast numbers of malaria parasites exist in a population: perhaps 10^{10} in just one vertebrate host. Yet gametocytes, the only stage capable of transmission, usually constitute just a few percent or even less of the circulating parasites. Why? Parasite fitness should be intimately linked with transmission probability and infectiousness rises with gametocyte density. Here, Louise Taylor and Andrew Read propose several testable hypotheses that might explain why natural selection has not favoured variants producing more transmission stages.

It may seem somewhat counter-intuitive to ask why malaria parasites do not produce more transmission stages. They obviously produce enough to ensure persistence, despite a massive onslaught from medical science. But evolution is about more than persistence: it is about maximizing genetic representation in subsequent generations. On the face of it, malaria parasites appear not to do that. Of the enormous numbers of parasites produced during infections, only a small fraction are capable of transmission, with the rest consigned to death within the host. Is this because hosts suppress gametocyte densities or because reproductive restraint by the parasite is favoured by natural selection? Either possibility implies the existence of factors limiting transmission. It would seem of more than just academic interest to identify these: in principle they could be enhanced (or, equally, inadvertently relaxed) by intervention strategies.

How many gametocytes?

Most people with clinical malaria exhibit few, if any, gametocytes. In *Plasmodium falciparum* infections, this may simply reflect the early onset of symptoms relative to gametocyte maturation, but it is also true for *P. vivax*, which has a much shorter gametocyte-maturation period. When gametocyte-positive people are found in epidemiological surveys, gametocyte densities in peripheral blood are at least an order of magnitude lower than those of asexual parasites (Table 1)[1–13]. The difference may actually be even greater than that because, at low densities, gametocytes are easily missed. Longitudinal studies of experimentally induced malaria infections in humans, chickens, primates and rodents all show that gametocyte densities are much lower than asexual densities and remain so throughout the infection (eg. Fig. 1; Ref. 14). Similarly, at any point in time, the proportion of hosts with detectable gametocytes is always lower than those with detectable asexuals, usually with substantially fewer than half of parasite positive people harbouring gametocytes (Table 1; Fig. 2). Taken together, these observations show that the vast majority of malaria parasites produced are asexuals incapable of transmission.

Is more better?

Defining fitness for any organism is not easy[15] and no less so for parasitic organisms[16]. For malaria parasites, one component of fitness is the number of mosquitoes infected. But there is also a second component. Mixed-clone infections are common[17], so that frequency relative to other genotypes in the gamete pool of a bloodmeal (and hence in the subsequent oocyst and sporozoite load) is also crucial. Combining these components into a measure of fitness is very difficult, not least because the total number of new infections generated by a clone (analogous to lifetime reproductive success) is likely to be the target of selection in an endemic region, whereas the number per unit time (analogous to the Malthusian growth parameter) may be the target in epidemic situations. Nevertheless, a formal and general definition is not crucial here. If greater gametocyte densities result in a greater contribution to the gamete pool in a bloodmeal and in more mosquitoes becoming infected, they should be favoured by natural selection.

Broadly speaking, higher gametocyte densities do lead to increased transmission. Ideally, the effect of gametocyte density *per se* would be examined by testing the infectiousness of a single progressively diluted or concentrated sample. To our knowledge, no such experiments using fresh blood samples from humans have been done, although this approach has been used to show that gametocyte density is an important determinant of *P. gallinaceum* infectiousness[18]. Work on cultured *P. falciparum* has found that up to tenfold dilutions of gametocyte preparations actually led to increased infectivity[19]. However, gametocyte densities used in those experiments resulted in oocyst burdens far in excess of those found in Nature (mean oocyst burdens >150 per gut compared with <5 in wild-caught mosquitoes) so that limiting factors not normally relevant may have been operating. Similar experiments that produce more realistic oocyst burdens would be of considerable interest. Due care to control for the effect of the concentration of any inhibitory factors in blood is also required.

The most common approach used to examine the role of gametocyte density on infectivity has been to feed mosquitoes, either directly or through membrane feeders, on the blood of naturally infected humans. These studies typically show that gametocyte density is indeed positively correlated with infectiousness (both the proportion of mosquitoes infected and mean oocyst burden)[3,6,13,18,20–23]. In all cases there was considerable scatter, and some studies found that gametocyte density was related to the proportion of mosquitoes infected but not to oocyst loads[24] while others have found no correlation at all[25]. The overdispersion

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Table 1. Prevalence and densities of gametocytaemia in people infected with various *Plasmodium* species

	Number of people (*n*)	Infections with gametocytes (%)	In infected people, density (per μl blood) of: Asexuals	Gametocytes	Ref.
Plasmodium falciparum					
Symptomatics[a]					
Brazil	15 179	17			1
Sri Lanka	1175	9			2
Cameroon	10 781	5	5 212	163	3
European neurosyphilitics	35	86	26 379	485	4
Asymptomatics					
Sri Lanka	1787	40			2
Netherlands New Guinea[b]	1566	8			5
Papua New Guinea	1541	10			6
Papua New Guinea	11 816	13			7
Papua New Guinea	1805	10			8
Nigeria	~5000	24			9
Nigeria	800	7			10
Mozambique	1782	5	2 098	146	11
Gambia	127	18			12
Plasmodium vivax					
Symptomatics					
Thailand	496	[d]	7191	455	13
Sri Lanka[c]	1175	62			2
Asymptomatics					
Sri Lanka	1787	62			2
Papua New Guinea	1541	36			6
Netherlands New Guinea[b]	1566	11			5
Plasmodium malariae					
Asymptomatics					
Papua New Guinea	1541	32			6
Netherlands New Guinea[b]	1566	17.5			5

[a] Studies of symptomatics are those involving patients seeking medical treatment (*n* = number of people seeking treatment); asymptomatics are those found in passive detection surveys (*n* = number of people surveyed).
[b] Now Irian Jaya.
[c] Mean of four point surveys.
[d] Study only included gametocyte-positive people.

of oocysts per vector[26] and the necessarily small samples involved must add considerable noise, but there are also biological reasons to expect variability. Many host-, parasite- and vector-derived factors are known to affect infectivity[18]. In *P. berghei* infections in mice, for example, infectiousness is reduced by a reversible humoral response against asexual parasites[27]. In field studies, infected human blood comes from people of different ages, with different exposure histories and at different stages of a malaria infection.

A clearer correlation between gametocyte density and the proportion of mosquitoes infected is found in *Plasmodium*-treated syphilitic patients with no previous malaria exposure[4] and for rodent malarias when mosquitoes are fed on naive mice at fixed points in the infections (L.H. Taylor *et al.*, unpublished). These data, and the fact that correlations between gametocyte density and infectiousness are frequently detected in the field despite considerable noise, argue that, all other things being equal, higher gametocyte density in a bloodmeal is associated with greater infectivity.

So why not more?

Since parasite infectiousness increases with gametocyte density, why does selection not favour variants producing more gametocytes? Higher densities are biologically feasible. *Plasmodium* infections in lizards can be dominated by gametocytes[28] and maximum recorded gametocyte densities in human infections are usually well above average values. For example, 68400 μl^{-1} and 17000 μl^{-1} have been found while treating neurosyphilitics[4,10] and, in the field, maxima of 1200 μl^{-1} and 1208 μl^{-1} were about an order of magnitude greater than the average densities found in the same studies[3,21]. How much of this variation is due to differences between parasites is unclear, but it is well known that there is genetic variation in rates of gametocytogenesis[18,29–31] on which selection for increased infectiousness could act.

So why does it not? Assuming no undiscovered variation in the timing or sites of gametocyte circulation (ie. that observed gametocyte densities are indeed those occurring in bloodmeals), two broad categories of explanation seem plausible: (1) observed densities are determined by host immune responses, with vast numbers of gametocytes being rapidly cleared; or (2) gametocyte densities are determined by the rate at which they are produced, but some other source of natural selection acting on the parasite is favouring reproductive restraint.

Rapid immune clearance

Several observations are consistent with the idea that antigametocyte responses by the host might directly suppress gametocyte densities. After a peak in very young children, gametocyte and asexual prevalences in endemic regions decline with host age, but more rapidly for gametocytes (eg. Fig. 2; Refs 6, 9, 32), as might be expected if there are acquired, gametocyte-specific clearance mechanisms. Some studies have shown that intensive vector and drug control leads to a marked drop in parasite prevalence but an increase in gametocyte-positive infections in all age groups[5,9]. One explanation for this is that reduced exposure allows levels of natural anti-gametocyte immunity to wane[5]. In Irian Jaya, semi-immune people had lower gametocytaemias than recent (non-immune) immigrants, a difference which could not be attributed to differences in asexual densities, drug use or illness[32]. Gametocyte prevalence among the recent migrants waned over a 14 month period after arrival, even though asexual prevalence did not. In artificially induced *P. falciparum* infections in non-immune (neurosyphilitic) patients, gametocytes were produced more frequently and at greater densities than in areas from which the parasites were originally isolated[10].

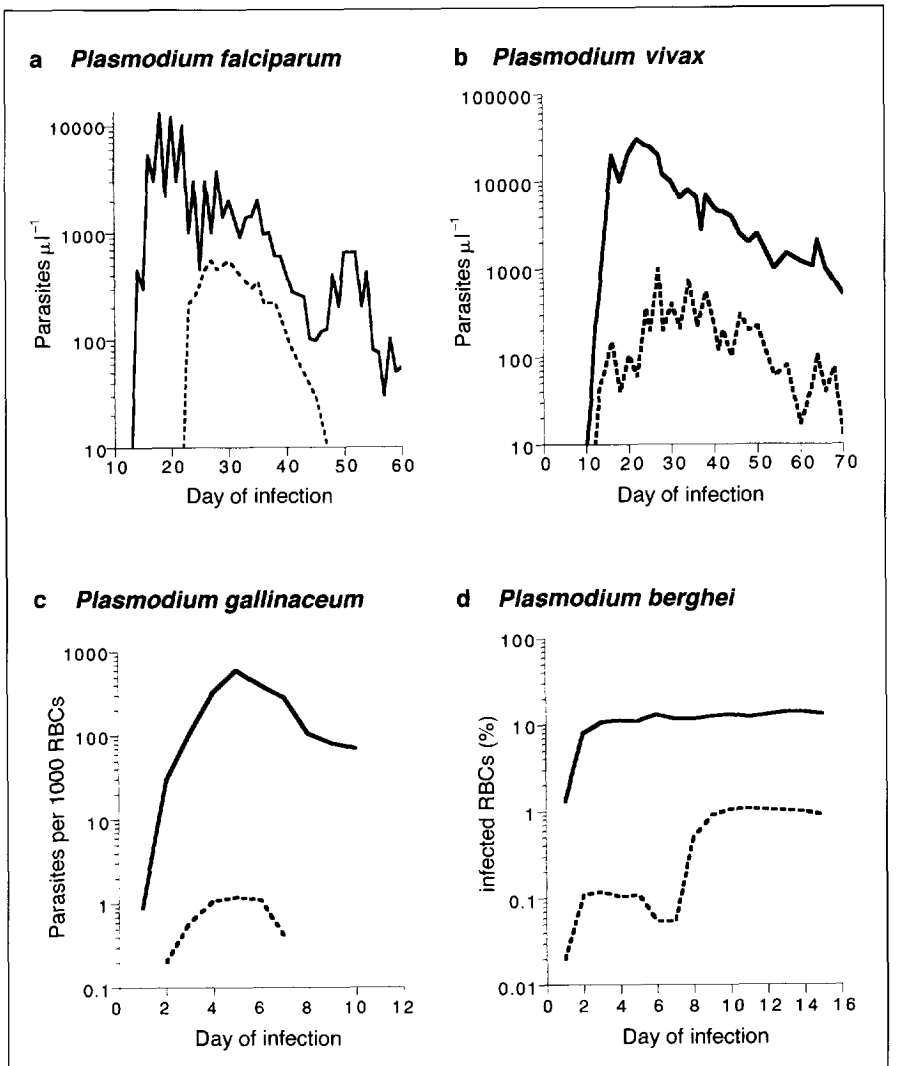

Fig. 1. Densities of asexual parasites (solid lines) and gametocytes (dotted lines) through time in experimentally induced infections of a single host. Sporozoite-induced *P. falciparum* in a non-immune human (a); sporozoite-induced *P. vivax* in a non-immune human (b); sporozoite-induced *P. gallinaceum* in chickens (c); blood-stage induced *P. berghei* in three mice (d). RBC, red blood cell. (Redrawn, with permission, from Refs 18, 29, 61.)

There is, however, rather little direct experimental evidence of gametocyte clearance independent of asexual clearance. One mechanism which could reduce gametocytaemia involves specific T-cell mediated immunity. Non-exposed human T cells are known to proliferate and secrete interferon gamma (IFN-γ) in response to gametocyte-specific antigens[33], though there is no direct evidence that they remove gametocytes. In mice vaccinated with *P. yoelii* gametes, infections have reduced gametocytaemias, and this effect can be passively transferred with splenic T cells[34]. In *P. falciparum* infections, T cells stimulated by gametocytes could act to destroy immature gametocytes via cytokine action in their sequestering sites. This could remove large numbers of gametocytes from the host before they ever reach the bloodstream. However, these mechanisms are probably just as potent against sequestering asexuals, and would not explain low gametocyte densities in *P. vivax*, where gametocyte sequestration probably does not occur. Furthermore, if T-cell mediated gametocyte removal was important, there would be strong selection for total conversion to gametocytes early in the infection before the development of the T-cell response. That is not what is generally observed (eg. Fig. 1).

Could an as yet undiscovered mechanism be responsible for large-scale removal of gametocytes and hence their relative rarity? The most compelling evidence against this idea is that higher gametocyte densities can be induced *in vivo* without obviously altering immune responses. This should not be possible if host immunity was entirely responsible for suppressing densities of circulating gametocytes. In rodent models, for example, blood transfusion[35], treatment with phenylhydrazine[36] and subcurative doses of antimalarial drugs[37] increase gametocyte densities. More ambiguously, several selection experiments have inadvertently resulted in lines that produce more gametocytes[30,31].

Selection favours restraint

Thus, at least in animal models, gametocyte densities can be increased. That, coupled with the need to invoke an undiscovered yet highly effective immune response, argues against the idea that host-mediated gametocyte killing is wholly responsible for suppressing gametocyte densities to observed levels. If hosts

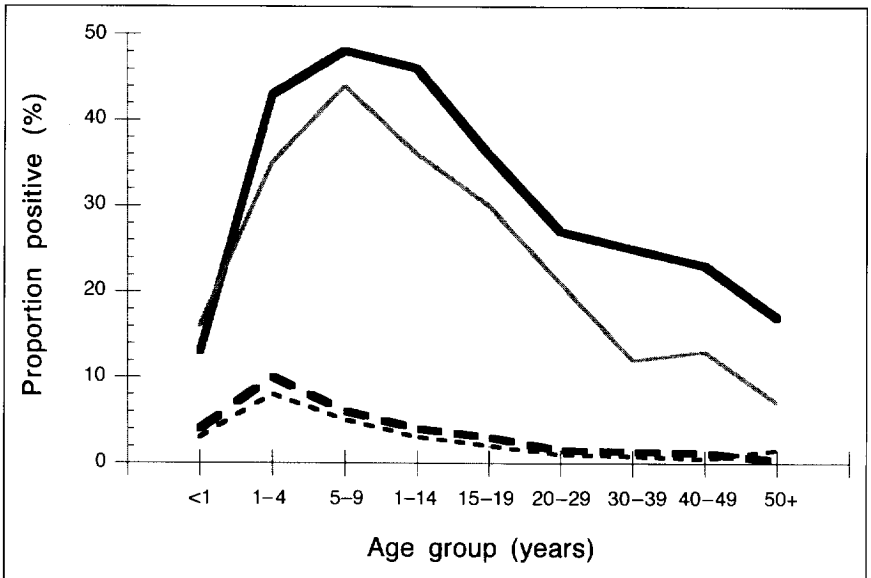

Fig. 2. Age prevalence curves for asexual parasites (solid lines) and gametocytes (dotted lines) of *P. falciparum* in the wet (bold lines) and dry (normal lines) seasons in Papua New Guinea. (Redrawn, with permission, from Ref. 7.)

are not directly suppressing gametocyte densities, the explanation for their relative rarity must lie in low rates of conversion of asexuals to gametocytes. What selection pressures might counter the fitness benefits of producing more transmission stages?

The most obvious is that conversion to gametocytes necessarily results in the loss of potential asexual replication. For example, in the ten days the ~20 progeny of a *P. falciparum* schizont take to mature into gametocytes, the same progeny could undergo five further rounds of asexual replication to generate 20^5 asexual parasites. Such enormous potential may generate more antigenic variation, confer a competitive advantage in mixed-genotype infections, or maximize parasite numbers before the development of host immunity. Natural selection should therefore optimize the timing and rate of conversion and this probably explains why conversion rates are generally low early in infections[18]. However, the relative cost of gametocytes in terms of lost asexual reproduction is dramatically reduced if there is a low chance that a merozoite will successfully invade an erythrocyte, or if protective responses are directed preferentially against the more numerous asexuals, as might be expected. Once asexual population growth has been checked by the host, the transmission benefits of increased gametocyte densities should apply.

Why might natural selection favour variants which restrict gametocyte output? We suggest two testable hypotheses: density-dependent effects of gametocyte density on either mosquito survival or on the development of transmission-blocking immunity.

Mosquito survival. Malaria parasites migrating through the mosquito body can inflict considerable damage[38]. Whether this translates into reduced vectorial capacity is controversial. Work on animal models has reported density-dependent mortality[39,40], but critics have argued that the oocyst burdens that generate this effect are far larger than those found naturally[41]. Field evidence is harder to interpret. Wild-caught mosquitoes with more than ten oocysts are rare[42,43], whereas batches of laboratory mosquitoes fed on cultured or rodent gametocytes can develop an average of more than 100 oocysts[19,26]. This difference may arise because heavily infected mosquitoes in the wild suffer greater mortality or because laboratory lines are chosen for their susceptibility to infection. In Sri Lanka and Burkina Faso, laboratory-reared mosquitoes fed on blood from humans with gametocytes had mortality rates similar to controls fed uninfected blood[20,44]. And survivorship of wild-caught *Anopheles funestus* and *An. gambiae* subsequently maintained in the lab was almost identical for sporozoite-positive and -negative mosquitoes[41]. Thus, the most direct evidence from the field provides no support for the idea that density-dependent gametocyte-induced mortality might select for lower conversion rates.

Transmission-blocking immunity. If circulating gametocytes elicit host responses in a density-dependent manner, natural selection may favour parasite variants that maintain low gametocyte densities to avoid or slow the development of transmission-blocking immunity. This idea presupposes that effective transmission-blocking immunity occurs in the field, that its efficacy increases as gametocyte density rises, that it acts during the course of a single infection, and that it is not wholly crossreactive with immunity against asexuals.

There is good evidence for each of these from animal models[18,45,46]. Several mechanisms of transmission-blocking immunity have been identified: antibody-mediated responses that interfere with fertilization or zygote/ookinete development in the mosquito midgut[18,47] and cytokine-mediated inactivation of circulating gametocytes during periods of peak parasitaemia[48,49], and the T-cell-mediated removal of gametocytes we discussed above[34]. Experiments involving vaccinations with attenuated (dead) gametocytes or gametes show that serum-mediated transmission blockage is dose-dependent[45,46,50] and can be boosted by reinfection[51].

In the field, the picture is more complex. Most of the work has focused on antibody-mediated responses, and there is compelling evidence that these occur naturally and can block transmission[21,52–54]. For example, in Papua New Guinea, 76% of serum samples reduced *P. falciparum* infectivity[52], and in an endemic region of Sri Lanka, 70% of serum samples reduced transmission of *P. vivax*, and 22% blocked it completely[53]. However, there is a strong impression that immune memory either does not develop or wanes rather rapidly after infection[51] and, to be effective, needs frequent reboosting[55]. This may explain the variable responsiveness to gametocytes of serum samples from people in endemic areas, and why non-responders are common[11,47,51,56].

Thus, while evidence from animal models is compelling, evidence from the field for the sort of transmission-blocking immunity necessary to generate selection in favour of reduced gametocyte output is more equivocal. This is perhaps unsurprising, given

the (understandable) emphasis on immune mechanisms with a long-term memory component for vaccine development. As far as our hypothesis is concerned, memory is irrelevant. Even transitory blocking would be sufficient to impose selection. What matters is whether blocking is elicited by gametocytes in a density-dependent manner during a single infection. The timescale is right: effective transmission-blocking immunity can arise during a primary infection of both *P. vivax*[57,58] and *P. falciparum*[55]. Field tests for density-dependence would require experimental manipulation of levels of exposure to gametocytes analogous to the work on animal models and, unsurprisingly, have not been performed. It is an attractive possibility, amenable to theoretical analysis, that one source of noise in the positive but generally weak relationship between gametocyte density and infectivity across people is variation in their current or recent exposure to gametocytes.

An obvious experimental test of the hypothesis that transmission-blocking immunity is the crucial selective factor limiting gametocyte densities would be to induce artificially high gametocytaemias, and then determine whether responses to these reduced subsequent transmission efficiency. In the field, we should observe that gametocyte densities higher than normal are more immunogenic. As well as explaining why gametocyte densities remain low despite the selective benefits of higher densities in naive hosts, the hypothesis may also explain several related phenomena. For example, intuition and simple evolutionary models[59] suggest that, all other things being equal, conversion rates should continue to rise, culminating in whole-scale conversion to gametocytes as the parasite population begins to be cleared from the host. So far as we are aware, there has never been a report of malaria parasites in birds or mammals producing a gametocyte wave with a density approaching anything like that of the asexual populations from which they were derived. In infections in lizards, however, that situation may be common[28]; it should be possible experimentally to determine whether lizards develop transmission-blocking immunity. Various related Apicomplexa have asexual reproductive cycles which culminate in total conversion to gametocytes; none of these has unlimited potential for asexual replication and so, for any clone, the development of transmission-blocking immunity has no fitness consequences.

Finally, one possibility is that it is not the absolute level of gametocytes which is the target of any selection imposed by transmission-blocking immunity, but rather their density relative to asexuals. Asexual parasites are highly immunogenic, and being specialized to evade immunity could distract the immune system away from gametocytes. This could explain why gametocytes remain relatively rare in all age categories. Sinden[27] has actually argued the opposite because there is some evidence, principally from *P. berghei*, that host responses to asexual parasites may reduce infectiousness. If such a phenomenon is widespread and effective throughout an infection (ie. not just a consequence of crisis factors and paroxysm), there ought to be selection for high gametocyte production early in the infection, which is not generally observed (eg. Fig. 1). Indeed, large-scale conversion to gametocytes throughout an infection is one way a parasite could regulate population growth[59] were asexuals really inhibiting transmission.

Conclusions

We have proposed several hypotheses to explain why transmission stages are so rare in malaria populations, and no doubt there are others. Those we have discussed are testable, both empirically and theoretically, and need not be mutually contradictory. Two are plausible contenders. Large numbers of gametocytes could be removed by hosts before they enter peripheral blood. This requires an immune mechanism that is much more effective against gametocytes than any directed against asexual parasites, which has a substantial impact on transmission, and about which we know almost nothing. Given current levels of ignorance, that must remain an important possibility. An attractive alternative is that transmission-blocking responses elicited in proportion to gametocyte densities impose selection for reproductive restraint by the parasites.

Earlier this century, Garnham[60] was in no doubt about the 'scanty appearance' of gametocytes in tropical Africa but did not comment on why they should be so rare. These days, the absence of detectable transmission stages in the majority of human *Plasmodium* infections is frequently described but still draws little comment (except, as a referee pointed out, perhaps over coffee). In evolutionary ecology, the resolution of apparently paradoxical examples of reproductive restraint has almost always provided insight[15]; in the case of malaria parasites, it might also generate more informed decisions about the design and evaluation of control strategies.

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