

*Breaking barriers: a leap ahead in Plasmodium biology*

# Do malaria parasites manipulate mosquitoes?

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**Malaria parasites have been suggested to alter the behavior of mosquito vectors to increase the likelihood of transmission. Some empirical evidence supports this hypothesis, yet the role of manipulation is ignored in most epidemiological models, and behavioral differences between infected and uninfected females are not considered in the development or implementation of control measures. We suggest that this disconnect exists because the link between behavioral alteration and actual transmission in the field has yet to be demonstrated or quantified fully. We review and discuss the current evidence for manipulation, explore its potential significance for malaria transmission, and suggest ways to move this hypothesis forward from theory to potential application in malaria control.**

## Manipulation by parasites

Transmission is a key determinant of parasite fitness. There are numerous examples of parasites altering the behavior of their host to increase the probability of transmission [1]. For example, ants infected with particular species of fungi ascend leaves at the appropriate moment before death to position themselves for prime dispersal of fungal spores [2]. Crab parasites castrate their crustacean hosts and manipulate them into caring for the parasite as they would their own offspring [3]. Normally terrestrial crickets leap into water, allowing their hairworm parasite to complete its life cycle [4]. A variety of studies have shown that malaria parasites can alter mosquito feeding behavior. These results have been interpreted as adaptive manipulation of vector behavior by the malaria parasite to enhance transmission (the ‘manipulation hypothesis’). The majority of evidence for manipulation, however, comes from avian or rodent model systems rather than from human malaria, and focuses on isolated components of mosquito behavior. Accordingly, the implications for human malaria transmission are uncertain. Here we explore the evidence, evaluate what impacts such manipulation might have upon transmission, and propose further research that might allow manipulation to be incorporated into our understanding of transmission dynamics and applied to disease control.

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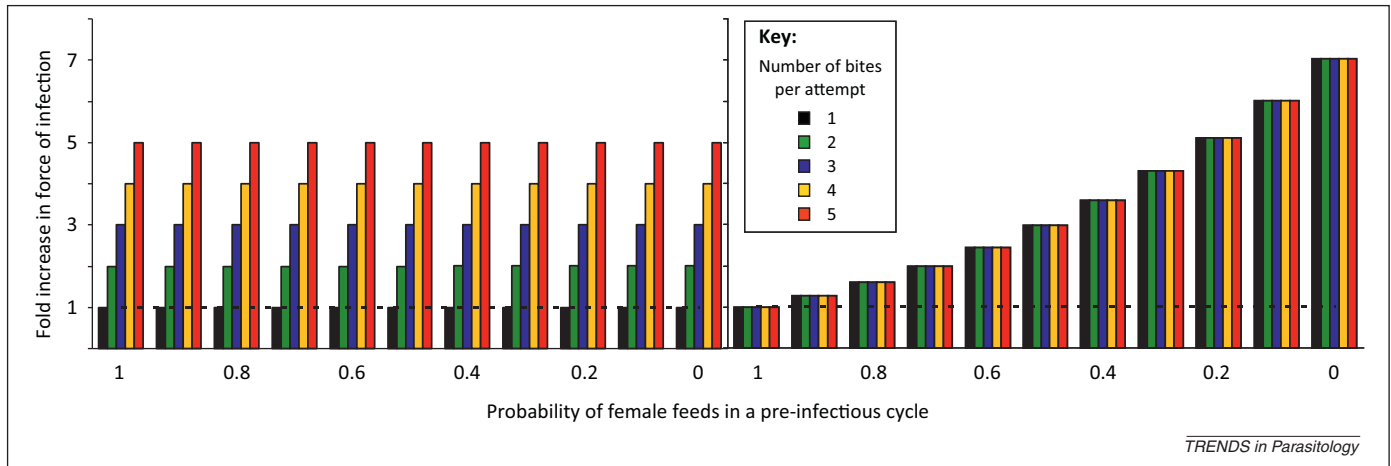
## Malaria and mosquitoes

It is not difficult to imagine that natural selection might favor a malaria parasite that could stimulate its own transmission. According to the manipulation hypothesis, malaria parasites decrease mosquito blood-feeding and other risky behaviors during the pre-infectious phase, thereby decreasing the risk of host death during parasite development (oocyst stage). Parasites then increase vector feeding rate once they have become infectious (sporozoite stage) (reviewed in [5]).

There is evidence that such behavioral changes take place. In laboratory studies, female mosquitoes infected with oocyst-stage malaria parasites were less persistent at blood-feeding and less likely to resume feeding if interrupted [6,7]. Other studies reported that sporozoite-infected females probed more frequently than uninfected controls [6,8–10] and were more persistent at attempts to obtain a blood meal [7]. In one study, sporozoite-infected females also took less blood per meal – which in the field may lead these females to feed more than once within a gonotrophic cycle [6]. However, other studies found that infection status did not affect blood-meal size [11] or probing duration [12].

Some field data are also consistent with infection-induced changes in mosquito behavior. For example, in human-baited catches, infected and uninfected *An. punctulatus* were observed to take blood meals at different times of night [13]. Koella and others [14] found that sporozoite-infected *An. gambiae* females were more likely than uninfected females to take blood from multiple hosts in the same night, and also suffered higher feeding-associated mortality [15]. A clinical study in the Gambia reported instances in which children sharing a room appeared to have received infectious bites from the same female on the same night [16]. Infected *An. punctulatus* females have been reported to exhibit different engorgement rates over a feeding period compared to uninfected females [17].

These examples suggest that manipulation might occur in the human malaria system, but determining how behavioral changes relate to human malaria transmission is difficult. Notably, most relevant data derive from studies using various vector–parasite combinations. Several studies have measured the behavioral effect of the bird malaria parasite, *P. gallinaceum*, on *Aedes aegypti*, a mosquito that



**Figure 1.** The effect of manipulation on transmission. The effect of increased number of bites per attempt (different colored bars) and the probability that a female feeds in a pre-infectious cycle (x axis) on the force of malaria infection. For the left panel, daily mortality is assumed to be the most important source of mortality; in the right panel, mortality is assumed to be almost entirely feeding-associated. The dashed line indicates force of infection for unmanipulated females that always feed during the pre-infectious cycle and bite one person per feeding attempt. These two mortality extremes illustrate the interactions between mortality distribution and manipulation phenotypes. When mortality is evenly distributed through the feeding cycle, and is not associated with the feeding event, the biggest increase in force of infection comes from the number of bites per attempt (the differences between different colored bars). When mortality is attributed to feeding, the biggest increases in relative force of infection are mediated by the decreased mortality experienced by females in the oocyst-stage.

does not transmit human malaria parasites [6,8]. Studies utilizing human-relevant vector species often test the effects of rodent malaria infection [7,9,12,18]. Although such research is tantalizing, to our knowledge no studies have directly compared manipulation in model and human malaria systems, leaving the connection between them uncertain.

Furthermore, much of the behavioral data concern the effect of *Plasmodium* infection on the ‘at-host’ feeding behavior of mosquitoes, generally using anesthetized hosts placed very close to mosquitoes [8,10,12]. ‘Blood-feeding’ describes a suite of behaviors including detecting the host, alighting on it, probing, piercing, locating blood, ingesting it, and terminating the feed [19]. Each of these components represents an isolated stimulus response, with the output of one event becoming the input of the next [19]. The narrowly focused assays conducted to date make it difficult to characterize how infection affects this complex set of behaviors.

### Implications for transmission

The reproductive rate of the malaria parasite (which provides a measure of transmission intensity) is captured in the Ross–MacDonald framework,  $R_0 = ma^2bcp^n / r \ln(p)$  [20]. In this equation, ‘ $m$ ’ is the ratio of vectors to humans, ‘ $a$ ’ the mosquito biting frequency, ‘ $b$ ’ the efficiency at which the parasite passes from mosquito to human, ‘ $c$ ’ the efficiency with which the parasite passes from human to mosquito, ‘ $p$ ’ the daily survival of the vector, ‘ $n$ ’ the extrinsic incubation period of the parasite, and ‘ $r$ ’ the recovery rate of human hosts. Current evidence for manipulation indicates that the parasite might alter vector biting frequency ( $a$ ) and the likelihood that a feeding attempt results in transmission ( $b$ ), by increasing the feeding rate and altering the probing behavior of sporozoite-infected females. It might also alter daily survival ( $p$ ) by decreasing feeding and reproductive stressors during parasite development before transmission [21].

Quantifying these effects in a simple model (Box 1) reveals that relatively small behavioral changes can have

substantial effects on transmission (Figure 1). The predictions of the model concerning quantitative increases in transmission are crucially dependent upon mortality schedules, particularly the mortality risks associated with feeding. Unfortunately, remarkably little is known about adult mortality in the field. Even so, our analysis suggests that, for plausible parameter values, parasite manipulation of vector behavior could increase the force of infection by fivefold or more (Figure 1). To put this figure in perspective, a recent study found that high coverage with insecticide-treated nets reduced entomological inoculation rates (number of infectious bites per person per unit time) by a similar order of magnitude [22]. Another reported a sevenfold increase in the number of infectious bites per person between transmission study sites, and a several-fold increase at these sites between the dry and wet seasons [23]. Thus, behavioral alterations could impact upon malaria transmission ecology and overall disease dynamics as much as bed nets or major environmental variation.

Manipulation-induced behavioral changes would also affect understanding of how current control tools such as insecticide treated nets (ITNs) actually work. At present, infected and uninfected mosquitoes are presumed to behave in identical ways, feeding every 2–3 days across their lifetime [24–27]. This repeat feeding means mosquitoes have multiple chances of contacting a lethal ITN before they become infectious, which is why even moderate coverage of ITNs can theoretically lead to reduced transmission intensity [28]. However, if manipulation reduces this exposure risk, higher coverage of ITNs could be required to achieve a given level of control (and by extension, manipulation could make eradication that much harder).

### Research priorities to resolve the issues

Various lines of research would enable better characterization of the putative manipulation phenotype, a crucial step in assessing its effect on transmission (Box 1). Not least, the current evidence for manipulation needs to be greatly

**Box 1. What would the impact of manipulation be on transmission intensity?**

To model the relative effect of manipulation on force of infection we compared the number of predicted infectious bites per female ( $B$ ) for mosquitoes that either express or do not express manipulation phenotypes. We calculated  $B$  for all groups using the equation:

$$B = Wk((1-s)(1-f)(1+M(\lambda-1)))^n \left( \frac{1-k^A}{(1-k)(1-k^A W)} \right) \quad [1]$$

where  $W$  is the probability that the female survives one cycle of resting, search, and laying between feeding attempts,  $s$  is background mortality associated with time spent searching for blood hosts,  $f$  is background mortality associated the time spent searching for an oviposition site,  $M$  is the probability an oocyst-positive female attempts to feed in each feeding cycle,  $A$  is the number of times a sporozoite-positive female attempts to bite per feeding cycle,  $n$  is the number of feeding cycles that the female experiences between taking an infected feed and becoming infectious (sporogony),  $k$  is the probability that the female survives a bite, incorporating both pre- and post-bite mortality, and  $\lambda$  is the probability of surviving the combined incremental mortality associated with a single feed plus an attempt to lay.

Unmanipulated females feed once during each cycle ( $M = 1$ ) and attempt to bite once per feeding cycle ( $A = 1$ ). Therefore, the ratio of force of infection for manipulated ( $B$ ) to unmanipulated ( $B_0$ ) females would be:

$$R = \frac{B}{B_0} = \frac{(1+M(\lambda-1))^n(1-k^A)(1-kW)}{\lambda^n(1-k)(1-k^A W)} \quad [2]$$

Three days were assumed between blood meals [53], four feeding cycles (12 days) from infectious blood-meal to sporozoite-positivity

[54], and females were assumed to attempt to bite no more than five times during a single feeding cycle.

The degree that mortality is associated with feeding events is unknown. Thus, we used two contrasting scenarios. For both we started with a commonly used assumption of 15% mortality per day [24,55] to give 38.6% per feeding cycle, splitting feeding-associated mortality evenly between pre- and post-bite mortality. We assumed that this 38.6% mortality per feeding cycle was either almost completely associated with the feeding event (99.9%) or that this mortality was not at all associated with a feeding event (0.001%).

Under either scenario, manipulation can have a substantial impact on the force of infection. Under our assumptions, changes in the number of bites per feeding attempt in the sporozoite stage can increase the relative force of infection (number of infectious bites per female) by as much as 500%, while oocyst-stage manipulation could increase force of infection by as much as 700%. In a scenario assuming heavy feeding-associated mortality, even a 10% decrease in the probability of a female feeding during the pre-infectious stage results in a 27% increase in relative force of infection.

The actual pattern of mortality in the field is likely to be intermediate between our two extreme mortality scenarios, with changes in relative force of infection resulting from effects on both non-feeding- and feeding-related mortality. The impact of manipulation on transmission can, however, still be considerable. For example, if we split the mortality evenly between feeding events and daily mortality, then the increase in force of infection can still be over 400%. Such estimates, tentative as they are, show that manipulation could be of substantial epidemiological significance.

improved before we can understand the impact of parasite-induced behavior change on disease epidemiology.

**Expanded range of behavioral assays**

There is evidence from both the laboratory and the field for the manipulation hypothesis. However, laboratory experiments have been conducted almost exclusively on model species, and field experiments (justifiably) do not control for confounding variables, such as female mosquito age. Bringing a natural system into the laboratory, conducting controlled experiments, and then confirming results in the field would greatly strengthen the current evidence.

Most of the laboratory work on the feeding behaviors of malaria-infected mosquitoes has occurred over a distance of less than 36 cm [6–10,12]. Although these studies have revealed differences in behavior, it is not clear how relevant these differences are in the context of natural host-seeking over distances of many meters. Expanding behavioral assays to include greater distances, and the entire sequence of searching and feeding behaviors, would allow for better assessment of how manipulation might actually impact on transmission. Such assays have already been used to study host-seeking in uninfected mosquitoes [29–31].

**Natural combinations of relevant hosts and parasites**

The evidence for behavioral manipulation comes from several vector–parasite combinations, but none conducted simultaneously, making it difficult to know whether systems differ or if results lack repeatability. For example, one study found stage-specific changes in the feeding behavior of *An. stephensi* infected with *P. yoelii* [7], while another found no effect when the same vector species was infected with *P. berghei* [12]. It is not clear if these differences are due to species combination, intensity of infection [32],

experimental conditions, or if the studies had truly disparate outcomes. Extending assays to human parasites and relevant malaria vectors is a key next step in investigating manipulation. Including natural and artificial vector–parasite pairings in the same experiments would enable us to determine which systems, if any, provide robust models for human malaria.

**Exploring effects beyond the flying syringe**

Although the number of infectious blood-meals a mosquito takes has the most direct link to parasite transmission, other aspects of mosquito biology highly relevant to malaria epidemiology [33] might also be altered by the parasite. Malaria parasites impact on mosquito fecundity [18,34–38], but we know of no studies investigating whether there are additional effects on, for example, the length of the gonotrophic cycle. If *Plasmodium* infection changes the duration and number of gonotrophic cycles, transmission dynamics could be altered through the frequency of blood-feeding and any associated mortality.

Malaria infection could also alter mosquito dispersal, an important component of transmission dynamics [39]. There is some evidence that *P. cynomolgi* [40] and *P. yoelii* [41] infection reduces the flight performance of *An. stephensi*. We know of no data on the flight capacity of mosquitoes infected with human malaria parasites. More broadly, do malaria parasites affect the decisions vectors make about which hosts to feed on? Several studies have reported that infected vertebrate hosts are most attractive during transmissible stages of infection [42,43]. In addition to affecting the choices mosquitoes make within vertebrate host species, malaria parasites could also affect decisions on feeding between species. The degree of anthrophily is a key determinant of malaria epidemiology. Again we know

of no relevant data, but it is an intriguing possibility that malaria infection might alter host preference; an effect that might be particularly important for the vectorial capacity of mosquito species which tend to be naturally more zoophilic.

#### Finding a mechanism

Across the many host–parasite systems where there is indisputable evidence of manipulation, the mechanisms by which parasites alter host behavior are not well understood [44]. The mosquito–malaria system is no exception. One possibility is that infection interferes with the ability of the mosquito to imbibe blood. The activity of apyrase, a key enzyme involved in feeding efficiency, has been shown to be reduced in sporozoite-infected females [8]. This reduced activity was associated with fine lesions in the salivary glands. Infection also affects the expression of salivary proteins [45].

Malaria parasites might also manipulate the satiation threshold, so that infected females take smaller and more frequent blood meals [6]. The head proteome of sporozoite-infected females differs from uninfected females [46]. Hairworms that manipulate the behavior of crickets have been found to produce molecules that may act directly on the cricket central nervous system, and proteomic changes can be detected in cricket hosts during manipulation [47,48]. Similar studies looking at the mechanisms by which human malaria parasites manipulate their vectors could identify interesting new targets for control.

#### Concluding remarks

Several studies suggest that malaria parasites manipulate mosquito behavior to facilitate transmission, but the nature and extent of the phenomenon remains unclear. Without transmission data, it is formally possible that these behavioral alterations could be side-effects of infection that do not increase transmission. Changes in mosquito behavior following parasite invasion could be a pathological consequence of infection, or a manifestation of the mosquito immune response to infection, or an interaction between the two [49]. Even if behavioral differences are not the direct result of manipulation, they are still relevant to our understanding of transmission ecology. ITNs are central to contemporary malaria control [50], and their efficacy depends on the patterns of mosquito feeding behavior and the resultant mortality [51]. If malaria alters feeding behavior, then infected mosquitoes might interact with ITNs differently. Understanding whether these differences exist and, if they do, the mechanisms that underlie them, could allow us to use this powerful control tool even more effectively. Moreover, if infected mosquitoes do behave differently, it might be possible to develop novel control tools to exploit this altered behavior. If oocyst-infected females are less likely to blood-feed, for example, then strategies which target sugar-feeding [52] or resting sites might lead to larger than expected benefits in terms of malaria control. More speculatively, determining the molecular and physiological mechanisms responsible for changes in mosquito behavior could lead to novel genetic, chemical, or semiochemical control strategies to target infected mosquitoes, possibly even ‘manipulating the

manipulation’. After more than 100 years since it was confirmed that mosquitoes transmit malaria, it simply should not be an open question whether human parasites manipulate the behavior of their mosquito hosts.

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