Mosquito metabolism shapes life-history strategies of *Plasmodium* parasites

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ABSTRACT

The life-history of multicellular organisms is a collection of traits determining fitness described by growth, survival, and reproduction. Within-host survival and between-host transmission are key life-history traits of single-celled malaria parasites. Therefore, understanding the evolutionary forces that shape these components is crucial to predict malaria epidemiology, drug resistance, and virulence. The evolutionary strategies of *Plasmodium* parasites have been largely investigated in the vertebrate host. In contrast, very little is known about their adaptation strategies in the mosquito vector, possibly due to the experimental challenges encountered while studying vector-parasite interactions. Mathematical models offer a unique tool to study such complex biological systems, and have been extensively employed in malaria epidemiology. However, all models developed so far do not consider mosquito physiology. Here, we examine the life-history evolution of *Plasmodium* parasites with a novel individual-based model of malaria transmission that includes mosquito metabolism. Specifically, we model the metabolic cascade of resource allocation induced by blood-feeding, as well as the influence of multiple blood meals on parasite development. Our model shows that successful vector-to-human transmission events are rare, and are caused by long-lived mosquitoes. Interestingly, we observe that the life-history strategies of malaria parasites depend on the mosquito metabolic status. In our model, additional resources provided by multiple blood meals benefit selection for parasites with slow or intermediate developmental time. These results challenge the current concept that evolution selects for fast developing parasites to maximize their chances to complete their within-mosquito life cycle. We propose that the long sporogonic cycle observed for *Plasmodium* is not a constraint but rather an adaptation to increase transmission potential.

Introduction

Malaria, a parasitic disease caused by *Plasmodium spp*, poses one of the greatest medical and economical challenges in our society. *Plasmodium* parasites infected 229 million people in 2019 alone and claimed the lives of 409 000 individuals[1]. Malaria parasites exhibit a complex life cycle, invading and developing in a wide range of host environments, both within blood-feeding mosquitoes (their definitive host) and in vertebrates (their intermediate hosts). For malaria parasites, within-host survival and transmission between hosts are major components of fitness, consequently natural selection is expected to benefit adaptations

that maximize these components. Understanding the mechanisms within each host that affect parasite's life-history traits, and how parasites adapt to changes in their environment is crucial to determine potential bottlenecks in transmission, and predict the course of malaria epidemics.

Given the parasite's complex life cycle, the study of *Plasmodium* evolution is not trivial. Important insights have been obtained by applying ecological and evolutionary theories to study some reproductive strategies utilized by malaria parasites in the vertebrate host[2–7]. Here, malaria parasites face life-history trade-offs typical to all sexually reproducing organisms, when resources must be divided between growth (asexual replication in red blood cells) and reproduction (production of non-replicating sexual stages, gametocytes)[8]. Malaria parasites indeed deploy several strategies to alter their conversion rate (investment between asexuals and gametocytes)[3,5,6,9], and sex allocation (investment into female vs male gametes) to adapt to changes in their host environment[10,11], demonstrating a high level of adaptive phenotypic plasticity.

Although the mosquito is *Plasmodium* definitive host, the evolution of malaria life-history traits in the vector remains largely unexplored, and trade-offs are expected to occur here as well. For transmission to be successful, the within-vector development period must be shorter than the mosquito's average life span. Given the assumed short life-span of mosquitoes in the field, the fitness of malaria parasites is thought to increase with a short sporogonic period. Yet, a parasite developing too fast might not accumulate sufficient resources, which could limit the quantity and/or quality of produced sporozoites - its transmissible form. The trade-off between within-vector survival and between-host transmission might be further affected by numerous aspects of mosquito biology that have a direct effect on parasite development. For example, the acquisition of multiple blood meals increase oocyst size[12], growth rates[13–15], and sporozoite numbers[16], possibly contributing to a rapid yet successful parasite development.

Similarly, the metabolic status of the mosquito is crucial for *Plasmodium* development. After the acquisition of a blood meal, essential metabolic pathways are triggered in the mosquito to ensure a robust egg development within a three-day reproductive cycle. The major physiological event is vitellogenesis, a process finely orchestrated by several signaling pathways regulated by the steroid hormone 20-hydroxyecdyson (20E)[17–20], during which essential yolk proteins are massively secreted by the insect's fat body, and transferred to the ovaries where they are provide nutrients for growing eggs[21]. As malaria parasites rely on the same resources for their growth, the metabolically reproductive investment and nutrient allocation in the mosquito strongly influence oocyst and sporozoite development[13,14,22,23]. During this single blood meal scenario, parasites are thought to interact non-competitively with their vector, as they scavenge the surplus internal metabolic resources only after successful oviposition[13,22,24]. However, whether this symbiotic interaction would remain in more natural settings of multiple feedings, remains unexplored and is difficult to study experimentally. How would malaria parasites adapt to the metabolic status of their mosquito-host, and what would be the consequences for *Plasmodium* transmission and evolution?

Here, we present a theoretical framework that integrates within-vector metabolism (i.e. the metabolically induced resource allocation initiated by blood feeding) into an individual-based model of malaria transmission, with the aim to answer the following questions:

1. how does mosquito feeding behavior and metabolism affect *Plasmodium* development?

2. how does mosquito metabolism shape the evolution of *Plasmodium* life-history traits?

We show that malaria parasites exploit a small proportion of the mosquito population for transmission: rare mosquitoes that are long lived and take multiple blood meals during their life span. Moreover, our mosquito metabolism model demonstrates that malaria parasites compete for metabolic resources within their vector and benefit from the nutrients acquired after the second blood meal at the expense of mosquito reproduction. Importantly, we find that our model selects for parasites with longer sporogony time to maximize transmission potential. The evolution of long sporogony time critically depends on mosquito metabolism: when we let parasites evolve without considering mosquito metabolism, we observe that short developmental times are instead selected. We therefore conclude that mosquito metabolism profoundly affects the evolution of *Plasmodium* parasites, offering a new perspective for understanding malaria epidemiology and transmission.

Results

Rare, long-lived mosquitoes transmit malaria parasites

To study the mosquito traits crucial for malaria transmission, we developed a stochastic individual-based model that considers female mosquitoes, humans, and parasites. In this model, individual humans and mosquitoes are randomly selected during every time step of one day to be confronted with one of the randomly chosen events: birth, infection, and death. We considered the complete mosquito life cycle, including larval and adult stages. The time of pupation, growth, and death rates vary among individual larvae, thus generating heterogeneity in the population. We assumed that these traits depend on ecological variables, such as density, temperature, and carrying capacity. After a mosquito reaches the adult stage, it seeks a human blood meal. If successful, fully fed mosquitoes are considered to be digesting for three days before they lay eggs and seek a new blood meal. The cycle of seeking, digesting, and egg-laying is continued during their entire life span, and mosquito death is modeled as an age-dependent function. With these parameters we could model stable mosquito population dynamics (Figure 1A) that matches the age-distribution of mosquitoes observed in field settings[25] (Figure S1).

Infection occurs when mosquitoes bite a malaria-infected human. The development of *Plasmodium* is complex: after ingestion, blood-borne sexual forms of the parasite fuse and convert into motile ookinetes, which in turn traverse the midgut epithelium and by day three after infection round-up to form oocysts. Mature oocysts generate then thousands of infective-to-human sporozoites that ultimately accumulate in the salivary glands. This parasite development process, hereafter called sporogonic cycle (T_{sp}), lasts approximately 10-14 days in natural systems[26]. We abstracted these processes and defined parasites only by their sporogonic cycle. Thus, upon an infectious bite, mosquitoes carry the parasite (*exposed*), but are *infectious* only after $T_{sp} = 13$ days. Infectious mosquitoes transmit the parasite to susceptible humans. We simplified parasite development in humans as well and consider a development period of $T_{EIP} = 28$ days, after which exposed humans become *infectious*[27]. During infection, humans have a higher death rate, simulating a high level of parasitemia. Infectious humans can recover at a rate p_r, becoming immune to the parasite. Humans that fail to clear the infection become chronically infected, but harbor lower

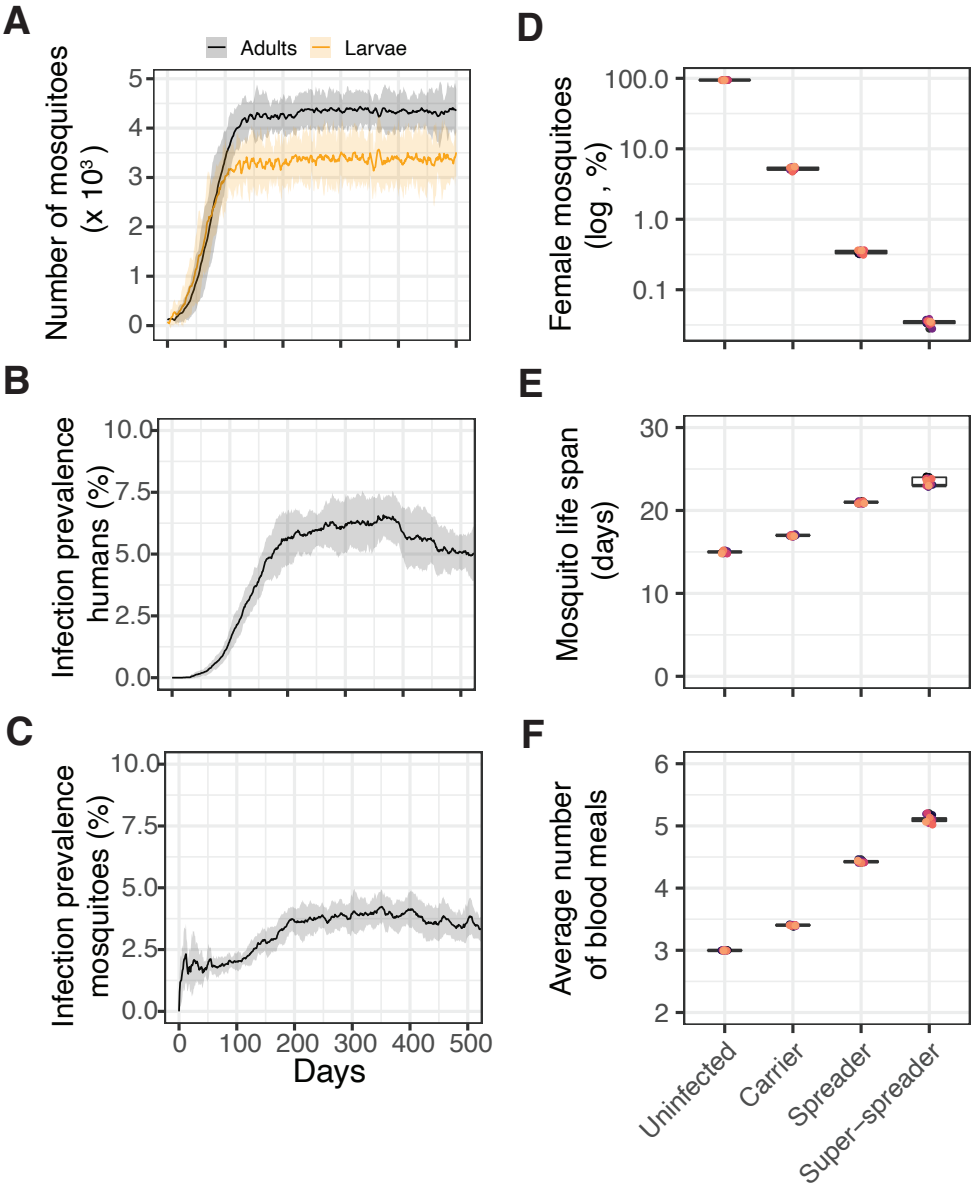

Figure 1. Events of malaria transmission are rare and caused by long-lived mosquitoes. Simulations of 15 mosquito populations that grow with the start of a rainy season in the vicinity of human hosts. (A) Initialized with 100 larvae and 100 adults, the mosquito populations readily grow over time, reaching a steady state defined by their carrying capacity. Infection prevalence given by the number of *new* infections in humans (B) and vectors (C). At the beginning of every simulation, 20% of humans are assumed to be asymptomatic carriers. With the growing mosquito population, parasite transmission increases, infecting on average more than 6% of humans, and 4% of vectors. Solid lines depict the average of 15 simulations, and the shaded areas show the standard deviation. (D) Percentage of mosquitoes in every category of infection status: uninfected, carriers (i.e. mosquitoes that do not transmit the parasite), spreaders (mosquitoes that infect one human host), and super-spreaders (mosquitoes that infect more than human host). The percentages were calculated by counting the number of mosquitoes throughout the entire simulation. (E) Life span of mosquitoes calculated in every infection category by measuring the median life span per simulation. (F) Mean number of blood meals taken per mosquito. Box plots show the median with first and third quartile, whiskers depict min and max values. Colors represent the replicates of each independent simulation.

parasitemia and a lower probability of transmitting the parasite. We considered these individuals to be asymptomatic carriers. We modeled a homogeneous human population in which individuals die at a fixed rate, and are immediately replaced by new susceptible ones. These infection dynamics, albeit simplified, allowed us to model a natural course of infection, during which more than 60 % of individuals remain susceptible (Figure 1B-C and Figure S2).

We first simulated a mosquito population that grows with the start of a rainy season in the vicinity of human hosts (Figure 1A). At the beginning of every simulation, 20% of humans are assumed to be asymptomatic carriers. With the growing mosquito population, parasite transmission increases, causing a prevalence in *new* infections of 6% in humans (Figure 1 B), and 4% in vectors (Figure 1C), epidemic parameters similar to those that have been reported in natural populations[28]. To investigate malaria transmission events, we next categorized all mosquitoes in every simulated population into four groups: uninfected, carriers (*exposed* mosquitoes), spreaders (*infectious* mosquitoes that caused one human infection), and super-spreaders (*infectious* mosquitoes responsible for more than one human infection). Surprisingly, the number of spreaders and super-spreaders is at least one order of magnitude lower than the observed prevalence (Figure 1 D), indicating that rare transmission events are sufficient to maintain a stable epidemic. Spreader mosquitoes are older (Figure 1 E), and consequently feed multiple times during their life span (Figure 1 F). Taken together, these results confirm the conventional expectation that transmission of *Plasmodium* parasites is driven by rare, long-lived vectors that acquire multiple blood meals.

Multiple blood meals result in competitive parasite-vector interactions

Plasmodium develops more rapidly when its mosquito vector acquires a second blood meal[16]. However, the effect of multiple (> 2) feeding cycles (which are observed in natural scenarios and our simulations) on malaria development remains unknown. To assess the effect of multiple blood meals on *Plasmodium* parasites, we next developed a simple mathematical model that focuses on how nutrients are allocated after the ingestion of a blood meal (Figure 2A). In this model, the within-host energy reserves of a female mosquito (R) grow after the ingestion of a blood meal ($\sigma(t)_{\mathrm{BM}}$), activating the necessary signal for the steroid hormone 20E synthesis in the ovaries, here represented with a blood meal-activated variable $\beta_E(t)$. Once activated, a proportion of the host resources are mobilized into the fat body for the production of the necessary yolk proteins, which are then utilized by developing oocytes (E) in the ovaries. The remaining host resources are invested into other physiological processes, including immunity, physical activity, and waste, at a rate δ_R. If a blood meal is infected with *Plasmodium*, oocysts (O) grow by accumulating resources taken from the vector's reserves at a constant rate β_P. Once mature, oocysts transfer their internal energy into sporozoites (S) at a rate $\gamma(t)$. The model consists of a system of ordinary differential equations (ODE). See Methods for details.

As a control, we first simulated the allocation of internal energy resources after a mosquito has acquired one non-infectious (BM) and one infectious blood meal (iBM) (Figure 2B, left columns). After ingestion of a blood meal, the mosquito's internal reserves are directed to the energy compartment used by reproduction, resulting in the rapid development of eggs. Importantly, the dynamics of reproduction are not affected in the presence of *Plasmodium*. Given the different time scales of egg and parasite development, the parasite is not in direct competition with its vector but scavenges the surplus internal metabolic resources

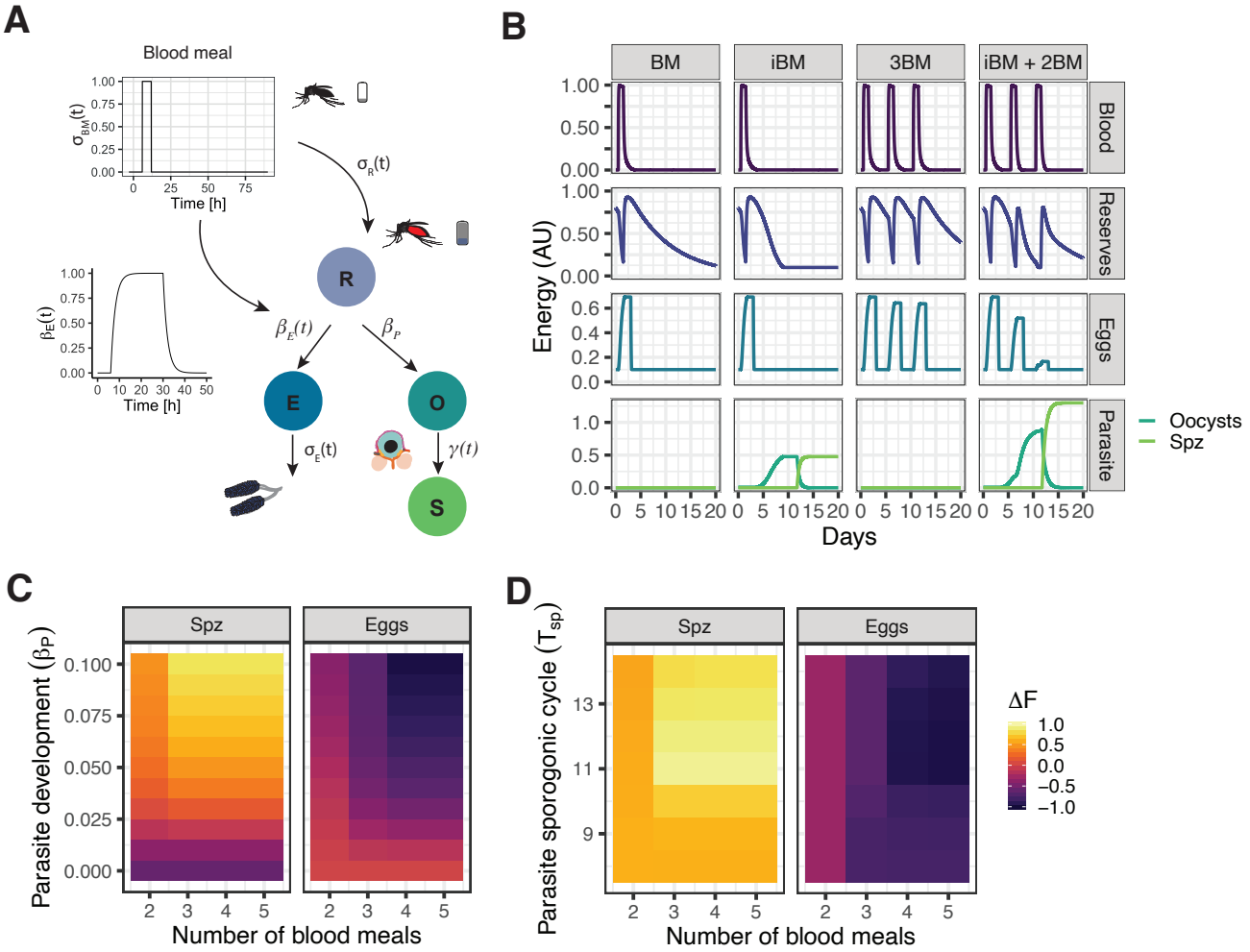

Figure 2. Parasite interacts competitively with its mosquito host after second blood meal (A) Schematic representation of the within-vector model of metabolic resource allocation A successful blood meal replenishes the initial energy resources, a small proportion of which will be used for the development of eggs and *Plasmodium* parasites. The nutrient mobilization is modeled considering a periodic blood meal behavior given by $\sigma(t)_{\mathrm{BM}}$ and four different compartments: within-host energy reserves (R), energy invested in reproduction (E), and developing oocysts (O) and sporozoites (S). The full model is described by equations 4-8 and the parameters are given in Table 2. (B) Simulation of internal energy resources after one (BM / iBM) and three (3BM/ iBM + 2BM) blood meals (depicted in purple). The ingestion of one blood meal (BM) activates the mobilization of mosquito resources (depicted in dark blue) to the ovaries, where energy accumulates in developing oocytes (depicted in light blue). The investment in reproduction remains unaffected during an infectious blood meal (iBM). After successful oviposition, the remaining internal reserves are used by the parasite, accumulating mosquito resources for the development of oocysts (depicted in dark green) and subsequently its transmissible form, sporozoites (depicted in light green). The ingestion of additional BM benefits the parasite. Parameter sweep of different parasite strength (C) and sporogonic cycle T_{Sp} (D) under different feeding regimes. The colors show the difference in fitness ΔF of Sporozoites (Spz) and Eggs in every simulated scenario.

after successful oviposition in agreement with experimental observations[13,22,24]. We next simulated the acquisition of three blood meals (Figure 2B, right columns). Interestingly, the energy accumulated by developing eggs during the second and third gonotrophic cycle decreases only in infected mosquitoes. Because the mosquito's second and third gonotrophic cycles and oocysts development now occur simultaneously, there is direct competition for resources.

We further assessed how parasites and vectors compete for resources by varying simultaneously the rate at which parasites scavenge mosquito resources and the number of additional blood meals (Figure 2C). We then quantified the differences in 'fitness' ΔF of both parasites and eggs, by calculating the difference in total accumulated energy to the control simulations (i.e. simulations without parasites, and with only one iBM for eggs and parasites, respectively). We observed a strong competitive vector-parasite interaction, with the largest fitness difference detected after 3 blood meals.

As in this model parasites strongly compete for resources only if the additional blood meal is given *during* oocyst development, we hypothesized that the duration of the sporogonic cycle T_{sp} will also play a role in the parasite's competition strength. We tested this hypothesis by running different simulations, varying the T_{sp} from 8 to 14 days together with the number of blood meals (Figure 2D). While all parasites (fast and slow) benefit from 2 additional blood meals, only parasites with long sporogonic cycles have the opportunity to scavenge resources acquired during subsequent feedings, becoming fitter than fast parasites. In conclusion, our model of nutrient allocation shows that parasites scavenge progressively more resources from their mosquito host after the second blood meal, suggesting that malaria parasites would benefit from long sporogonic cycles.

Mosquito metabolism restraints the advantage of shorter sporogonic cycles for malaria transmission

To explore how mosquito metabolism affects transmission, we integrated the model of nutrient allocation into our individual-based model. For simplicity, we assumed that the parasite's energy accumulated in the sporozoites is related to the mosquito-to-human transmission probability p_t. We modified the original model by describing p_t as a function of the number of blood meals acquired during oocyst development (i.e., starting from 3 days post-infection until the end of the sporogonic cycle T_{sp}). Because in our stochastic simulations mosquitoes display individual feeding patterns (i.e., every mosquito will obtain a different number of blood meals during their life span and consequently also during oocyst development), this description of transmission probability ($p_t(N_{BM})$) gives rise to a large heterogeneity in transmission potential. An increase in transmission results in a lower number of eggs per female, allowing us to model the competitive vector-parasite interactions in a simple manner (see Methods). Integrating the properties of mosquito metabolism (while keeping all other model parameters equal) slightly changes the infection prevalence (Figure 3A), a result of the heterogeneity in transmission probabilities and, with it, the number of spreader mosquitoes (Figure 3B). All other transmission patterns, including the age and blood meal distribution of spreaders, remained similar to the control simulations (Figure 3C, D).

As the length of the sporogonic cycle is one of the most influential parameters in classical mathematical models of malaria transmission, small reductions in T_{sp} are expected to have a large effect on parasite transmission[26,29]. We next tested how infection dynamics are affected by fast-developing parasites and simulated host populations infected with *Plasmodium* parasites, varying in their sporogonic development from $T_{sp} = 11$ to 13 days. We measured infection prevalence at steady-state in both hosts (at $t = 1,000$ days, Figure 4A), and the number of spreader mosquitoes (Figure 4B). As expected from classical predictions, malaria transmission increased with shorter T_{sp} following a linear trend in control simulations. However, when we explicitly modeled within-vector metabolism, this increase was significantly smaller, suggesting that mosquito metabolism limits the transmission advantage of short developmental times.

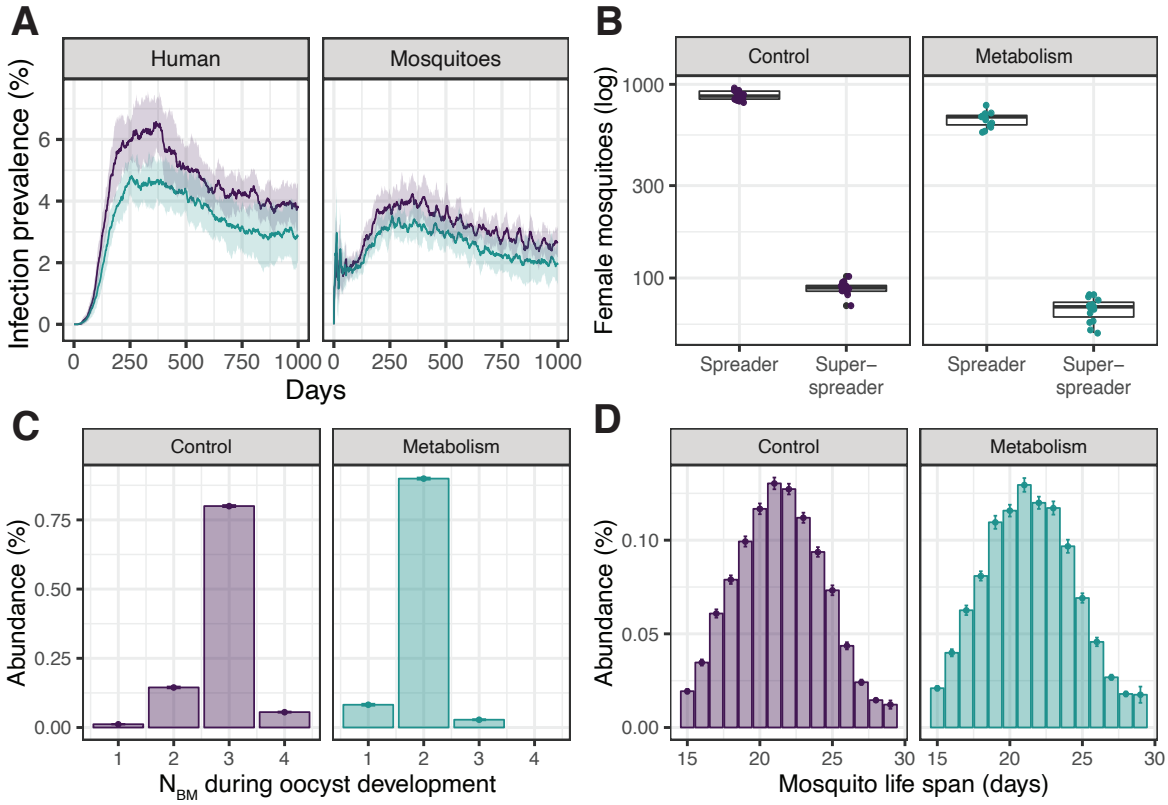

Figure 3. Model of *Plasmodium* transmission including mosquito metabolism. Summary of N=15 stochastic simulations with (cyan) or without (purple) mosquito metabolism. (A) Infection prevalence given by the percentage of novel infections in humans and mosquitoes. The solid line depict the mean and the shaded areas the standard deviation. (B) Number of spreaders and super-spreaders throughout the *entire* simulation as defined in Figure 1. (C) Distribution of the number of blood meals acquired by single mosquitoes (spreaders and super-spreaders) during oocyst development. (D) Age distribution of spreaders and super-spreaders. Bar plots show the mean, and error bars the standard deviation. Box plots depict the median with first and third quartile, whiskers depict min and max values.

Mosquito metabolism shapes the evolution of *Plasmodium* parasites

To study whether parasites would indeed benefit from long sporogonic cycles in the context of mosquito metabolism, we next performed evolutionary simulations. First, we allowed mosquito and human individuals to reach stable population dynamics. After this 'burn-in' period of 1,000 days, we introduced the malaria parasites. We also ensured that infection dynamics reach equilibrium and switched on mutation only after 5,000 days. Mutations occur during transmission events and can increase or decrease the length of the sporogonic cycle. We followed the population for further 5,000 days and used as control evolutionary simulations without metabolism (Figure 5A).

There was no difference between the models in the first days of infection dynamics before mutation is switched on. Once evolution started, there was a clear divergence between the simulations excluding (control) and including metabolism (Figure 5B). In the control simulations, infection prevalence suddenly rose, infecting twice as many humans and mosquitoes. As the fitness of the parasite is independent of mosquito metabolism, a short sporogonic development T_{sp} evolves because fast-developing parasites spread more rapidly in the population, reaching the minimum of $T_{sp} = 5$ days allowed in our simulations

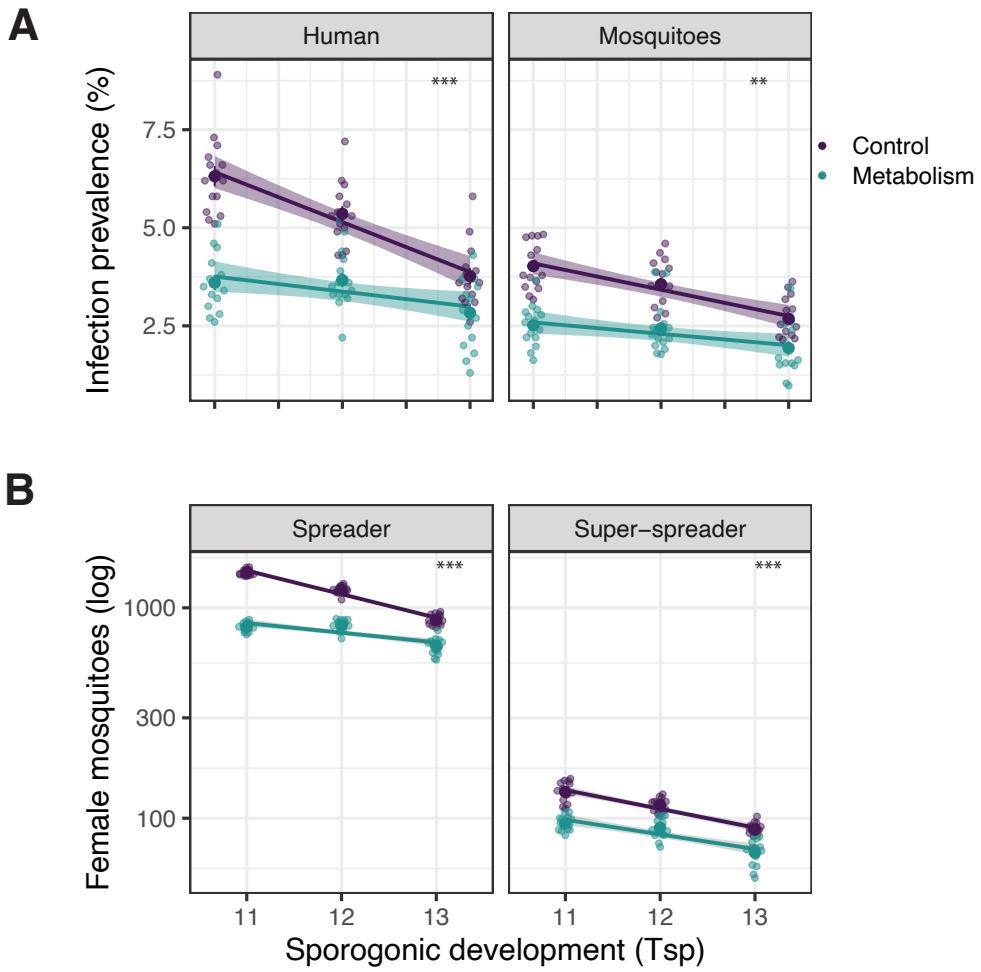

Figure 4. Mosquito metabolism restraints the transmission benefits of short sporogonic cycles. Simulations of malaria transmission assuming different sporogonic cycles (T_{sp}). We measure the infection prevalence in both hosts at setady-state, i.e., at $t = 1000$ days (A), and the number of spreader and super-spreader mosquitoes in the population throughout the entire simulation time (B). Note how *Plasmodium* transmission grows with shorter T_{sp} significantly more in the control simulations (purple) than in those including mosquito metabolism (cyan). The difference between groups was calculated with a t-test comparing the slopes of linear regressions (lines and shaded areas). *** depicts a p-value ≤ 0.001. Every dot represents one of N=15 stochastic simulations.

(Figure 5C, purple line). Accordingly, the number of blood meals acquired by every infectious mosquito during the parasite development time shifted during evolution (Figure 5D, purple bars): most parasites entered their mosquito vectors, and after 5 days, were mature to infect a new human host without acquiring any additional blood meal. In contrast, mosquito metabolism limited the evolution of very short sporogonic development times (Figure 5C, cyan line). As parasites require blood meals to increase their otherwise low transmission potential, there is selection pressure to acquire at least two blood meals during oocyst development (Figure 5D, cyan bars), resulting in parasites with an 'optimal' $T_{sp} = 12$ days. Importantly, the evolution of this long sporogonic development time was robust, and independent of the initial conditions: starting the simulations with a low $T_{sp} = 10$ days resulted in the same 'optimal' $T_{sp} = 12$ days (Figure 5E). Taken together, our evolutionary simulations show that parasites change their life-history strategies depending on mosquito metabolism, providing a plausible explanation for the long

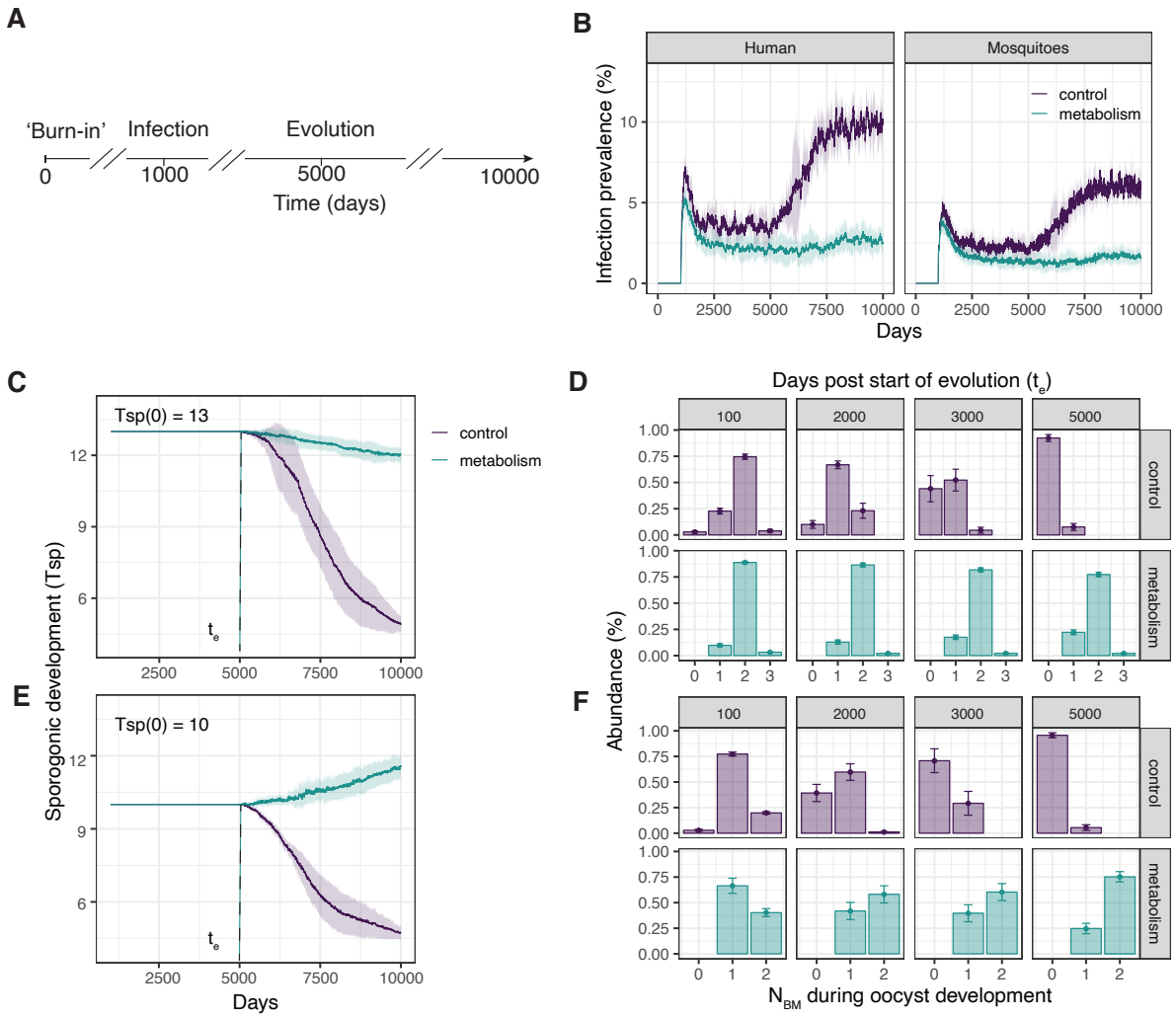

Figure 5. Mosquito metabolism shapes *Plasmodium* evolution. (A) Simulation protocol for evolutionary simulations. After 1000 days of 'burn-in' period, we infect 20% of the human population with malaria parasites. We allow infection dynamics to reach a steady state until 5000 days, where we switch on mutation. Simulations end after 10,000 days. We compare the evolutionary patterns observed in our original model (control, depicted in purple) to those emerging in the model including metabolism (depicted in cyan). (B) Time course of infection prevalence. Note how infection increases after mutation is turned on only in the control simulations. (C, E) Time course of the sporogonic development T_{sp}. (D, F) Distribution of blood meals acquired by individual mosquitoes during oocyst development. Every column summarizes the blood meals of individual mosquitoes 100, 2000, 3000, and 5000 days after turning on evolution, in control (upper row) and simulations including vector metabolism (lower row). To test the effect of initial conditions we run two sets of simulations: one with $T_{sp}(0) = 13$ (C,D) and another with $T_{sp}(0) = 10$ (E,F). Independent of the initial conditions, a minimal $T_{sp} = 5$ naturally evolves in the control simulations, while mosquito metabolism maintains a long $T_{sp} \approx 12$. Solid lines in the time courses depict the mean, with shaded areas displaying standard deviation. Bar plots show the mean, and error bars the standard deviation. Summary of N=10 stochastic simulations.

sporogonic cycles observed in natural systems.

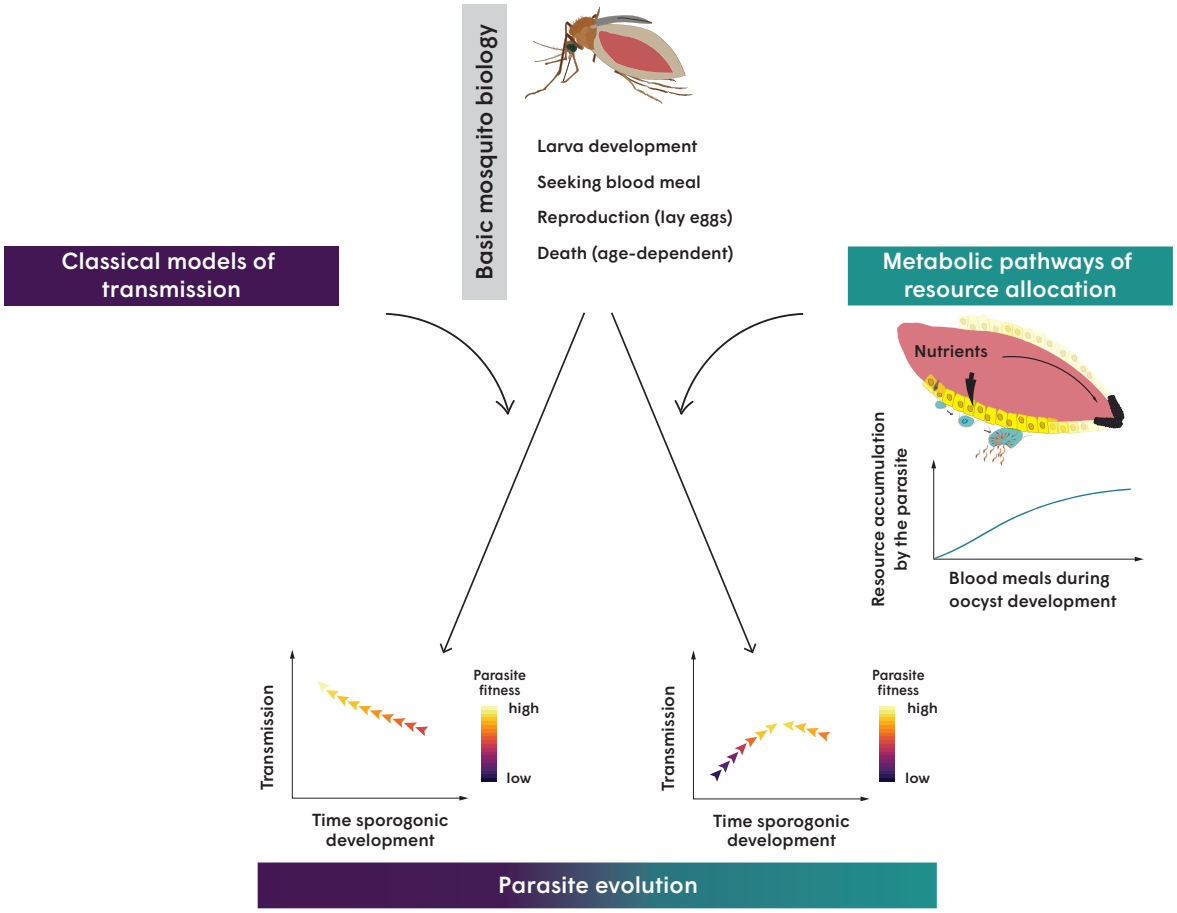

Figure 6. Summary of how mosquito metabolism shapes life-history traits of *Plasmodium* parasites. By only considering basic life-history traits of mosquitoes, i.e., larval stages, biting behavior, reproduction, and death, classical models of malaria transmission predict that a reduction of the sporogony time (T_{sp}) increases parasite's fitness and aids transmission success (left). In contrast, when mosquito metabolism (and with it the associated parasite competition for mosquito resources during multiple blood meals) is also included, our model reveals a novel evolutionary scenario in which intermediate long sporogony is optimal for transmission (right).

Discussion

We provide a framework that integrates complex mosquito metabolic traits and its interactions with *Plasmodium* parasites into a model of transmission. Our model demonstrates that mosquito metabolism shapes the parasite's evolutionary life-history strategies. Specifically, we show that (1) *Plasmodium* is transmitted by rare long-lived "superspreader" mosquitoes that take multiple blood meals; (2) successive blood feeding introduces a competitive parasite behavior within the female mosquito that restricts the allocation of nutrients into reproduction, and aids the parasite's own development; and consequently, (3) parasites with long sporogony are selected during evolution.

Our results challenge the current concept that malaria parasites strive to shorten their development to maximize transmission. Given the assumed short life span of the mosquito observed in the field[30,31], it has remained puzzling why malaria parasites develop so slowly. Until now, two major explanations have been provided that are based on the premise that long sporogony

guarantees a large number of sporozoites in the salivary glands. First, this high number of sporozoites is necessary to transmit even few to the vertebrate host[32,33]; and second, a high number of sporozoites induces a change in the mosquito biting behavior to increase transmission[32,34]. Here, we introduce a novel mechanism in which long sporogony is beneficial because parasites scavenge increasingly more resources with successive blood meals. Our model identifies two different mosquito traits that shape the fitness landscape of *Plasmodium* parasites: 1) mosquito longevity, exerting selection pressure for the parasite to develop fast, and 2) mosquito metabolism which determines the nutrient availability for malaria parasites, resulting in pressure *Plasmodium* to develop slowly (Figure 6). We, therefore, propose that the long sporogonic cycles observed in nature are not a constraint but rather an adaptation, potentially resulting in a higher transmission success.

A critical parameter in our model is the age-distribution of adult mosquitoes, as it directly determines the number of long-lived vectors and, consequently, of spreaders. The survival rates we use to parameterize our model were obtained under laboratory conditions and result in an older age structure than previously estimated[30,31]. However, the age distribution of our simulated mosquito populations is remarkably similar to wild *An.coluzzi* mosquitoes, as recently measured with novel age-grading methods[25]. Like in our simulations, these natural mosquito populations have a very small proportion of old individuals (>17 days) that feed frequently (> 4 blood meals). Our work suggests that these rare individuals are the major malaria spreaders and should therefore be studied in more detail.

Our study demonstrates the importance of integrating complex parasite-vector interactions into models of *Plasmodium* transmission to realistically predict malaria epidemics and evolution. Since the first mathematical model developed by Ross[35], there has been an expansion of theoretical approaches that consider a variety of geographical, ecological and epidemiological complexities (reviewed in[36–38]), as well as multiple mosquito life-history traits (e.g., larval stages[39], biting frequency[40,41], feeding, and movement patterns[42]). However, all these models assume the same exponential relationship between sporogonic cycle and mosquito life-span[26,29], thus closely resembling the original description[36,43]. We show that if mosquito metabolism is explicitly modeled, this relationship no longer holds (Figure 6), indicating that T_{sp} is not as sensitive in determining transmission intensity as was originally suggested[15] . While recent experimental evidence has shown that reductions in sporogony time does not diminish the infectivity to primary hepatocytes[13], these early sporozoites are very low in numbers. Whether such low sporozoite numbers will be sufficient for a successful infectious bite, remains to be demonstrated. Further studies are needed to demonstrate the effect of shorter extrinsic incubation periods on parasite transmission efficiency.

By conceptualizing complex metabolic processes within the mosquito, we have obtained a new perspective for understanding malaria transmission and evolution. This work raises new questions that need to be addressed both experimentally and theoretically. Experimentally, the relationship between parasite competition strength and the number of blood meals must be quantified. How strong will mosquito reproduction be impaired by malaria parasites during natural feeding regimes (more than two blood meals)? Moreover, we assumed that the metabolic energy accumulated by the parasite can be translated into transmission probability. How do multiple feedings affect the number, and/or, quality of sporozoites, and their transmission potential into a vertebrate host? Recent studies have shown that additional blood meals influence parasite growth, resulting in

larger oocysts[44,45], and an accelerated invasion of sporozoites of the mosquito salivary glands reducing the time potentially required for transmission[15]. Our work suggests that the fitness of those early sporozoites should be low given the reduced metabolic resources they acquired during their fast development. Do parasites with different T_{sp} display differences in transmission potential? Measuring sporozoite numbers, and quality in infected mosquitoes fed under different regimes is necessary to further understand the effect of mosquito metabolic resources on *Plasmodium* development. Additionally, given that malaria parasites exhibit adaptation to their vertebrate hosts[2,6,10], it seems plausible that they also show phenotypic plasticity depending on the metabolic status of their mosquito-host: would they sense and modulate their sporogonic cycle in response to the nutrients available in different mosquitoes?

Theoretically, extensions of the model will help test hypotheses that are difficult to study in an experimental or field setting. For example, shifts in vector feeding schedules and life-spans can be studied computationally to assess the effect of different mosquito species on parasite transmission. Additionally, several genetic and environmental factors have also been identified as determinants in *Plasmodium* sporogony[26,46], including the mean environmental temperature[47–49], genetic diversity of both the vector and the parasite[50], as well as nutritional status during larval stages[46,51,52]. How these processes would interact together with the metabolic parasite-vector competition presented here can be addressed in future extensions of our model. Thus, our theoretical approach sets the basis for future investigations on the vector-parasite pairs that favor transmission, possibly revealing unexpected strategies parasites naturally evolve.

Methods

Individual-based model of malaria transmission

We develop an individual-based model consisting of three actors (humans, female mosquitoes and parasites) and four types of events (birth, growth, transmission and death). The basic time step of the model is one day, during which every human host and vector is chosen in a random order and confronted to one of the randomly chosen events. Parasites are embedded in every host, and will be copied into a new host upon every transmission event. Humans and vectors age over time, i.e., their age is updated after each time step. The cycle is repeated over several vector generations. All parameters are fully described in Table 1. The following is a detailed description of individuals and events.

Mosquitoes

We consider both phases of the mosquito life cycle and explicitly model larvae and adults. Individual larvae die at a temperature dependent rate, described as a quadratic function: $\delta_L(T) = a_\delta T^2 - h_\delta^2 + k_\delta$. Larvae develop into adults only after a pupation time that follows an exponential function: $T_P(T) = \frac{m_t}{1+\exp(Th_t)} + k_t$. After this pupation time, we allow larvae to grow with a density-dependent rate $\gamma(L) = 2^{-L/K_L} m_\gamma$, where K_L is the carrying capacity of the assumed aquatic habitat, and m_γ the maximal daily growth rate. We consider individual effects by assuming that every parameter j consists of population and individual effects, as follows: $\theta_{ij} = \mu_j e^{\eta_{ij}}$. Here μ_j is the population parameter, and $\eta_{ij} \sim \mathcal{N}(0, \omega_j^2)$ is the random effect for every individual larvae i. The larval parameters given in Table 1 were obtained by fitting a mechanistic mathematical model to data of

Table 1. Model parameters of the individual-based model.

Parameter	Value [Unit]
Time step	1 [day]
Environment temperature T	28 [°C]
Host parameters	
Carrying capacity K_{H}	1000 individuals
Death rate δ_H	1 [year$^-$1]
Vector parameters	
Carrying capacity of aquatic habitat K_{L}	5540 individuals
Coefficients of larval death rate $\delta_L(T) = aT^2 - bT + c$	
a_{pop}; η_a	0.00288; 1.12
b_{pop}; η_b	0.00663; 0.645
c_{pop}; η_c	0.0381; 0.701
Coefficients of larval growth rate $\gamma(L) = 2^{-L/K_{\mathrm{L}}} m_\gamma$	
$m_{\gamma,\mathrm{pop}}$; η_{m_γ}	1.19; 0.513
Coefficients of pupation time $T_P(T) = \frac{m_t}{1+\exp(Th_t)} + k_t$	
$m_{t,\mathrm{pop}}$;η_{m_t}	9.63; 0.104
$h_{t,\mathrm{pop}}$; η_{h_t}	0.0996; 0.0258
$k_{t,\mathrm{pop}}$:η_{k_t}	2.95; 0.188
Mosquito birth probability	0.03[53]
Number of eggs per oviposition	$\mathcal{N}(\mu = 55, \sigma = 24.5)$
Mosquito biting probability	0.97[53]
Mosquito hazard / death rate $h(\mathrm{age}) = b\exp(x\,\mathrm{age})$	$x = 0.09, b = 0.01$
Parasite parameters	
Parasite incubation period in humans T_{EIP}	$\mathcal{N}(\mu = 28, \sigma = 0.25)$[27] [day]$^{-1}$
Parasite incubation period in mosquitoes T_{sp}	$\mathcal{N}(\mu = 13, \sigma = 0.25)$ [day]$^{-1}$
Parasitemia in humans $\delta_{p,H}$	0.001
Infection parameters	
Transmission probability mosquito-to-human $p_{\mathrm{M}\to\mathrm{H}}$	0.7
Transmission probability human-to-mosquito $p_{\mathrm{H}\to\mathrm{M}}$	0.7
Recovery probability in humans p_r	0.75
Initial conditions	
Larva initial population L_0	100
Mosquito initial population M_0	100
Human initial population H_0	1000

larva development at 28°C (Estupiñán et al. unpublished). Of note, all rates (r) were converted into their respective probability as described by $p = 1 - \exp(-rt)$.

We allow female mosquitoes to seek a blood meal immediately after emerging as adults, as previously modeled[53]. During their seeking state, they bite a randomly chosen human depending on the number of human hosts in the population, as

follows: $p'_b = p_b \left(\frac{N_H^3}{N_H^3+h^3}\right)$, where p_b is the maximal biting probability, N_H is the number of human hosts, and h is the human population at which the probability is half maximal. After a successful blood meal, every mosquito digests for a period of approximately three days, after which it develops eggs. The number of eggs developed per female mosquito N_E follows a normal distribution$\mathcal{N}(\mu_E, \sigma_E)$, as typically observed in our laboratory. We assume that not all eggs survive, and model the effective probability of birth as a density-dependent function $b = \hat{b}(1 - L/K_L)$, where $\hat{b}$ is the maximal birth probability, L is the number of existing larvae, and K_L is the carrying capacity of the aquatic habitat. All surviving eggs contribute immediately to the larvae population.

Mosquitoes die with an age-dependent death rate described with a Gomepertz distribution with shape $a = 0.09$ and rate $b = 0.01$. This death rate function was obtained by fitting several distributions to survival curves of mosquitoes reared at 28°C in our laboratory. Note that in our laboratory mosquitoes did not show different survival profiles when infected, and therefore we do not simulate any infection-related survival cost.

Humans

We consider a homogeneous human population, in which every human has a death rate $\delta = \delta_H + \delta_{p,H}$, where $\delta_H = 1[\text{year}^{-1}]$ is the intrinsic death rate, and $\delta_{p,H} = 1x10^{-3}$ is the increase caused by the parasitic infection (see below). Humans live on average one year. While this is short, we do this to assure that sufficient susceptible individuals are available and new infections can constantly occur. For simplicity, we keep the human population constant, i.e., when an individual dies due to background mortality, or infection, it will be immediately replaced by a new susceptible individual.

Parasite

In our model, *Plasmodium* parasites are only described by the duration of their sporogonic development $T_{Sp} \sim \mathcal{N}(\mu = 13, \sigma = 0.25)$, thus generating a heterogeneous parasite population. Parasites mutate upon every transmission event with a probability $p_m = 0.5$ by randomly increasing or decreasing their T_{Sp} by 0.5. We set an arbitrary minimal value of $T_{Sp} = 5$ days.

Infection dynamics

For simplicity, we do not model the individual stages of parasite development within each host. Instead, we capture infection dynamics with a classical "Susceptible-Exposed-Infectious-Recovered-Susceptible" (SEIRS) model for humans, and an "Susceptible-Exposed-Infectious' (SEI) model for mosquitoes. Susceptible mosquitoes are exposed to the parasite with a probabilty $p_{\text{H}\to\text{M}}$ when feeding on an infectious human. Mosquitoes become infectious after the parasite has completed its development, i.e., after a sporogonic development time T_{sp}. Infectious vectors can transmit the parasite to susceptible humans upon their next blood meal with a transmission probability $p_{\text{M}\to\text{H}}$. Like in mosquitoes, exposed humans become infectious after a development period T_{EIP}. During this period, their death rate increases by $\delta_{p,H}$, thereby modeling parasitemia. Infected individuals recover from the infection with a probability p_r. Humans that fail to recover become chronically infected with the parasite, and show lower transmission probability ($0.1p_{\text{H}\to\text{M}}$) and parasitemia ($0.001\delta_{p,H}$), effectively modeling asymptomatic carriers.

Table 2. Model parameters of the resource allocation model.

Parameter	Value [Unit]
Blood meal parameters	
Feeding interval T_σ	7 [days]
Duration of feeding λ_{BM}	6.00 [h]
Time delay of feeding τ_{BM}	6.00 [h]
Duration of 20E activation λ_{β_E}	24.00 [h]
Rate of 20E activation a	0.5 $[\mathrm{h}^{-1}]$
Rate of 20E inhibition b	0.5 $[\mathrm{h}^{-1}]$
Host parameters	
Duration of nutrient transport into the reserve compartment λ_{R}	60.00 [h]
Rate at which energy flows into physiological processes δ_R	$[5 \times 10^{-4}\mathrm{h}^{-1}]$
Energy flow rate for egg development c	0.3 $[\mathrm{h}^{-1}]$
Degradation rate of reproductive energy δ_E	0.5 $[\mathrm{h}^{-1}]$
Duration of oviposition λ_{E}	6.00 [h]
Time delay of oviposition τ_{d_E}	66 [h]
Parasite parameters	
Energy flow rate for parasite development β_P	0.045 $[\mathrm{h}^{-1}]$
Rate of sporozoite development γ	0.1 $[\mathrm{h}^{-1}]$
Sporogony time T_{sp}	11 [days]
Initial conditions	
Blood meal $B(0)$	0 [AU]
Internal reserves $R(0)$	0.8 [AU]
20E activation $\beta_E(0)$	0[AU]
Reproductive energy $E(0)$	0.1 [AU]
Energy of oocysts $O(0)$	0 [AU]
Energy of sporozoites $S(0)$	0 [AU]

Model initialization

The model was initialized with 100 larvae and 100 adult females, each with a random age between 1 - 10 days. Similarly, 1,000 human hosts were set at the start of the simulations. 20% of the human population are initialized as asymptomatic carriers.

Life span calculations

We categorized mosquitoes in every simulated population into four groups: uninfected, carriers (*exposed* mosquitoes that died before becoming infectious), spreaders (*infectious* mosquitoes that caused one human infection), and super-spreaders (*infectious* mosquitoes responsible for more than one human infection). We compute the life span by recording the age (in days) at which mosquitoes died, and calculated the median life span per category, per simulation.

ODE model of resource allocation

We model the mobilization of nutrients after a blood meal with a system of ordinary differential equations that consider three vector-specific compartments (blood digested during a blood meal (B), within-host energy reserves (R), and reproductive energy

for developing eggs (E)), and two parasite-specific compartments (internal energy resources for oocysts (O) and developing sporozoites (S)) (Figure 2). The energy flow from one compartment to the other is activated with rectangular pulse wave functions, described by:

$$\sigma(t) = \begin{cases} 0, & \text{if } t < \tau \;\&\; t - \tau - n_i T_\sigma > \lambda \\ 1, & \text{otherwise.} \end{cases} \tag{1}$$

$$\tag{2}$$

Here, a pulse starts after a delay of τ and lasts for a duration of λ. The pulse is repeated with a period T_σ and will be repeated for N_{BM} blood meals, thus $\sum_1^{N_{\mathrm{BM}}} n_i T_\sigma$. For every process different values of τ, and λ were chosen as described below.

After the ingestion of a blood meal $\sigma_{\mathrm{BM}}(t)$, the blood can be either digested at a rate δ_B, or its metabolic energy can be mobilized to the mosquito reserves R after a period $\tau_{\mathrm{BM}} = 24\mathrm{h}$. This blood-meal activates the signal for the steroid hormone 20E synthesis in the ovaries $\beta_E(t)$. A proportion of the host resources is then mobilized to the ovaries and is accumulated by developing eggs (E). The remaining resources are invested into other physiological processes, including immunity, physical activity, and waste, at a rate δ_R. If a blood meal is infected with *Plasmodium*, oocysts (O) grow by accumulating resources taken from the vector's reserves at a constant rate β_P. Once mature, oocysts transfer their internal energy into sporozoites (Sp) at a rate $\gamma(t)$. The dynamics of $\beta_E(t)$ are explicitly modeled by equation 8, where a and b represent the activation and inhibition rate of reproductive investment, respectively. Periodic blood meals, the thereby induced resource allocation, and the blood meal dependent signal for 20E activation occur with a period $T_\sigma = 7$days, durations $\lambda_{\mathrm{BM}} = 6\,\mathrm{h}$, $\lambda_{\mathrm{R}} = 60\,\mathrm{h}$, and $\lambda_{\beta_E} = 24\,\mathrm{h}$, and delays of $\tau_{\mathrm{BM}} = 6\,\mathrm{h}$, $\tau_{\mathrm{R}} = 30\,\mathrm{h}$, and $\tau_{\beta_E} = 6\,\mathrm{h}$, respectively. Similarly, the time for oviposition is activated with a time delay $\tau_{d_E} = 60\,\mathrm{h}$ and lasts $\lambda_E = 6\,\mathrm{h}$. The model is given as follows:

$$\dot{B} = \sigma_{\mathrm{BM}}(t)(1-B) - \sigma_{\mathrm{R}}(t)BR(1-R) - \delta_B B \tag{3}$$

$$\dot{R} = \sigma_{\mathrm{R}}(t)BR(1-R) - c\beta_E(t)RE(1-E) - \beta_P RO(1-O) - \delta_R R \tag{4}$$

$$\dot{E} = c\beta_E(t)RE(1-E) - \sigma_{\mathrm{E}}(t)\delta_E E \tag{5}$$

$$\dot{O} = \beta_P RO(1-O) - \gamma(t)O \tag{6}$$

$$\dot{S} = \gamma(t)O \tag{7}$$

$$\dot{\beta}_E = \sigma_{\beta_E}(t)a(1-\beta_E) - b\beta_E \tag{8}$$

$$\gamma(t) = \begin{cases} 0, & \text{if } t \leq T_{\mathrm{sp}} \\ \gamma, & \text{otherwise.} \end{cases} \tag{9}$$

Note that this is a conceptual framework that models the metabolic processes in the mosquito. Importantly, not all of the processes described here can be measured experimentally. Therefore, most parameter values have been chosen to qualitatively match empirical data describing the temporal dynamics of 20E activation and oviposition[23], as well as infection dynamics

typically observed during *Plasmodium* infections. All parameters are fully described in Table 2.

Parameter sweeps

To test for the effect of different parameter values, we performed several parameter sweeps, varying one parameter at a time in small intervals. We studied the effects of different parasite strengths in allocating resources by changing β_P from [0.0-0.1] in 0.05 steps. The impact of the number of blood meals was tested by running several simulations altering N_{BM} from [1-5] days. Finally, we simulated five different sporogony times T_{sp} ranging from [11 - 15] days in 2 days intervals.

Fitness calculations

We define arbitrary fitness functions for eggs and parasites. Here, reproductive fitness is defined as the sum of the maximal energy accumulated during every blood meal (equation 10), where as parasite fitness is defined the maximal energy accumulated in the sporozoites compartment (equation 11). The differences in fitness $\Delta F(E)$ (equation 12) and $\Delta F(S)$ (equation 13) are computed by subtracting the control scenarios (i.e., simulations without parasite and with only one infected blood meal for eggs and parasites, respectively) from the simulation of interest.

$$F(E) = \sum_{i=0}^{N-1} max(E(\tau_i)); \text{ with } iT_\sigma \leq \tau_i < (i+1)T_\sigma \tag{10}$$

$$F(S) = max(S(t)) \tag{11}$$

$$\Delta F(E) = F(E)_{iBM} - F(E)_{BM} \tag{12}$$

$$\Delta F(S) = F(S)_{N>1} - F(S)_{N=1} \tag{13}$$

Individual based model including mosquito metabolism

For simplicity, we include mosquito metabolism into the iBM by assuming that the energy accumulated into the sporozoites is translated to the mosquito-to-human transmission probability $p_{\text{M}\to\text{H}}$. Effectively, every parasite has an intrinsic basic probability of transmission ($p_0 = 0.2$) which grows after the acquisition of blood meals during oocyst development (i.e., 3 days after ingestion until T_{sp}) as described with a simple Hill function $p_{\text{M}\to\text{H}} = p_0 + \frac{n_{BM}}{n_{BM}+1}$. Note that with this description and our chosen parameters, a parasite requires $n_{BM} = 2$ to obtain the same transmission probability as in our control iBM. Similarly, the number of eggs developed per female will be dependent on the parasite 'strength', given by the same $p_{\text{M}\to\text{H}}$, as follows: $\hat{N_E} = 0.85N_E - 0.4p_{\text{M}\to\text{H}}$, where N_E is the number of eggs per batch, per individual female as described above. All other agents, events, and parameters are the same as in the model excluding metabolism (which we refer throughout this manuscript as control).

Model assumptions

The purpose of our theoretical framework is to explore the role of within-mosquito physiology on the evolutionary strategies of malaria parasites. Therefore, our models are necessarily an abstraction of complex metabolic and transmission processes.

Wherever possible, parameters were obtained by quantifying data generated in our laboratory, or adopted from the literature. In the individual-based model, we prioritized parameter choices that resulted in realistic infection dynamics. Therefore, the modeled human population is homogeneous, and remains constant throughout the simulations. Humans are assumed to have a short life span of one year. While unrealistic, we do this to prevent the infection from saturating, thereby ensuring that sufficient susceptible individuals are available. For simplicity, we model only one mosquito species, ignore climate seasonality, and do not consider super-infection in either of the hosts. This simplification allows us to focus on the role of mosquito metabolism on malaria transmission and evolution.

The ODE model of resource allocation simulates the energy flows induced by metabolic changes. Because there is currently no data available of these dynamic processes, parameter values were chosen to match the dynamics typically observed in the activation of 20E after a blood meal[23], and during parasite development. We assume that parasites scavenge resources at a constant rate β_P proportionally to their resources. As a result, less energy will be scavenged by the early-stages oocysts compared to the late-stages ones. we also assume that the energy scavenged by developing occysts will be completely transferred to sporozoites. While there is some experimental evidence that the relationship between oocyst and sporozoite numbers saturates at large parasite densities[54], we do this to keep the model simple. Importantly, we do not model parasite numbers, densities, or size, but only metabolic energy. A high accumulation of energy could be interpreted as either multiple small occysts, or few large ones. Similarly, a high amount of energy accumulated by sporozoites could translate into large numbers, and/or quality. Accordingly, we intuitively assume that the metabolic energy determines transmission probability in the evolutionary individual-based model. Future experimental studies are imperative to determine how the metabolic energy accumulated by oocysts would affect transmission potential, and validate our model implications.

Implementation and Code availability

The individual-based model was implemented in the C++ programming language. All analysis as well as the model of resources allocation were performed using R version 3.5. The source codes are available under https://gitlab.mpcdf.mpg.de/paca/resourceAllocation and https://gitlab.mpcdf.mpg.de/paca/vectorBorneModel.

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Supplementary Material

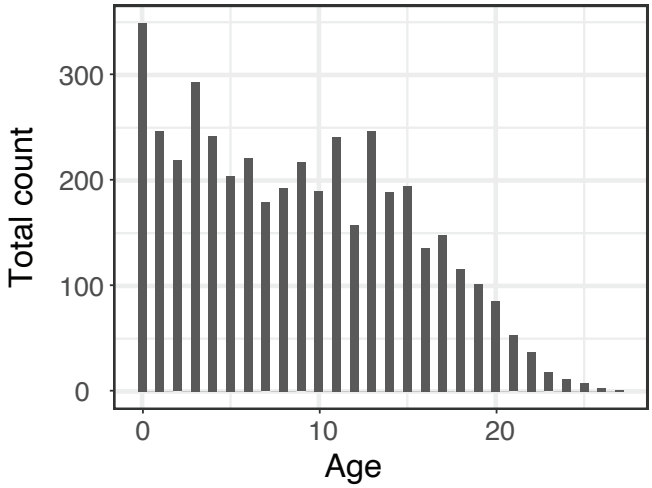

Figure S1. Mosquito age-distribution in the individual-based model We model the mosquito death event as an age-dependent process described with a gamma distribution with shape $k = 3.07$ and scale $\theta = 0.261$. Although this death rate function was fitted to mosquitoes reared under laboratory conditions, the emerging age-distribution is remarkably similar to mosquitoes sampled in the field[25]. Representative example of one mosquito population after reaching steady-state,. i.e., after 300 days.

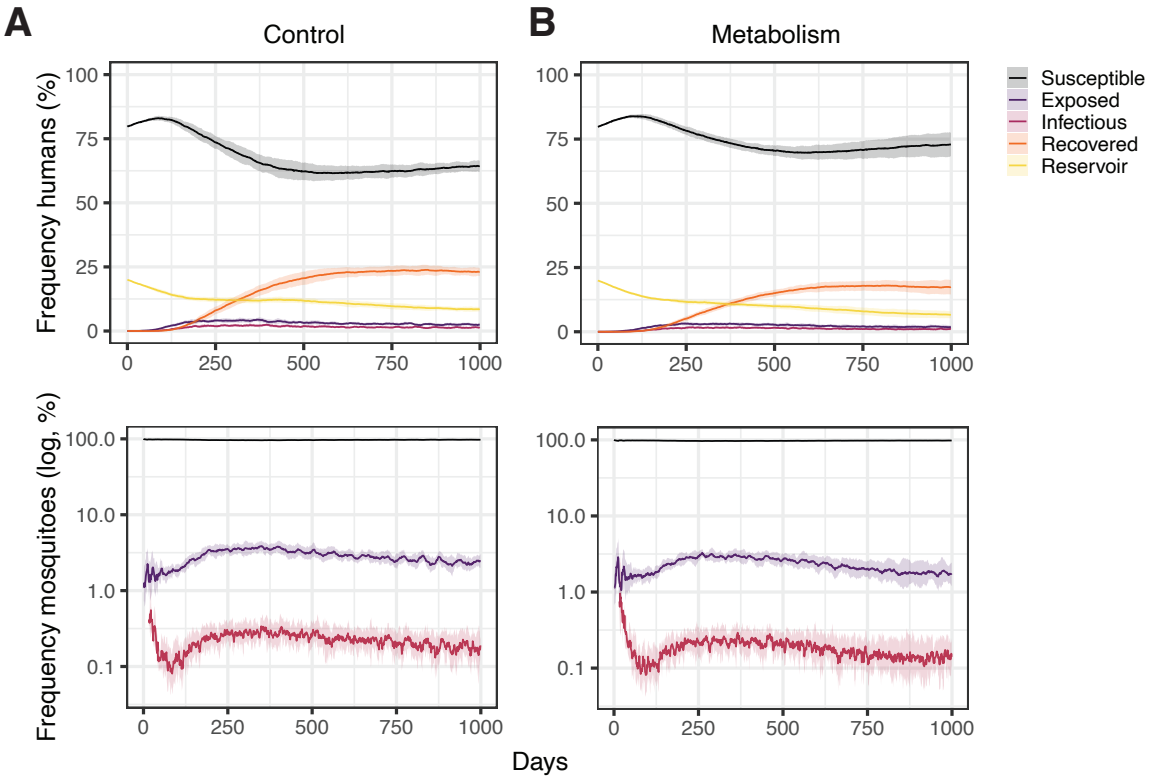

Figure S2. Infection dynamics of humans and mosquitoes. Proportion of susceptible (black), exposed (purple), infectious (pink), recovered (orange), and initial reservoir (yellow) humans (top rows), and susceptible, exposed, and infectious mosquitoes (bottom rows). The chosen parameters result in stable infection dynamics. Importantly, including mosquito metabolism (right column) does not cause a substantial change in these infection dynamics.