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DIFFERENTIAL IMPACT OF SICKLE CELL TRAIT ON SYMPTOMATIC AND ASYMPTOMATIC MALARIA

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Abstract. Individuals who carry the sickle cell trait (S-gene) have a greatly reduced risk of experiencing symptomatic malaria infections. However, previous studies suggest that the sickle cell trait does not protect against acquiring *asymptomatic* malaria infections, although the proportion of symptomatic infections is up to 50% in areas where malaria is endemic. To examine the differential impact of the sickle cell trait on symptomatic and asymptomatic malaria, we developed a mathematical model of malaria transmission that incorporates the evolutionary dynamics of S-gene frequency. Our model indicates that the fitness of sickle cell trait is likely to increase with the proportion of symptomatic malaria infections. Our model also shows that control efforts aimed at diminishing the burden of symptomatic malaria are not likely to eradicate malaria in endemic areas, due to the increase in the relative prevalence of asymptomatic infection, the reservoir of malaria. Furthermore, when the prevalence of symptomatic malaria is reduced, both the fitness and frequency of the S-gene may decrease. In turn, a decreased frequency of the S-gene may eventually increase the overall prevalence of both symptomatic and asymptomatic malaria. Therefore, the control of symptomatic malaria might result in evolutionary repercussions, despite short-term epidemiological benefits.

1. **Introduction.** Malaria is one of the worlds devastating and persistent diseases. Although the use of artemisinin has made a great progress in controlling malaria with deaths down 30% over the past decade ([41]), the annual incidence of malaria is approximately 300 to 500 million worldwide, resulting in 700,000 to 2.7 million malaria-associated deaths each year [31]. The majority of malaria infections are caused by either *Plasmodium falciparum* or *Plasmodium vivax* [35].

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Malaria transmission is highest in Oceania and Sub-Saharan Africa [35]. The most affected population are children [35]. Approximately 50% of malaria infections are asymptomatic in areas where malaria is endemic [10, 28]. In these areas, transmission is intense and consistent over time. As a result, most adults who live in these endemic areas possess partial immunity to malaria due to recurrent infections [10]. Such asymptomatic malarial infections are important impediment to malaria control, because asymptomatic patients are not likely to seek treatment. Instead, these individuals continue transmitting the disease to others and provide a long-lasting reservoir for the malaria vector [9, 39]. With the increased movement observed in human populations, the high prevalence of asymptomatic infection increases the risk of malaria, particularly in malaria-free zones.

Individuals who carry the S-gene (sickle cell trait) have a reduced risk of experiencing symptomatic malaria infections, although sickle cell traits do not seem to affect the course of *asymptomatic* infections [36, 38]. The sickle cell trait is carried by individuals who inherit a normal haemoglobin gene from one parent (HbA) and a sickle haemoglobin gene (HbS) from the other. Sickle-cell disease occurs when an individual inherits the autosomal recessive S-gene (HbS) from each parent. Sickle cell disease can cause multi-organ ischemic damage and deform red blood cells, which leads to anemia [11]. People with sickle cell disease who live in rural Africa rarely survive to reproductive age [3]. Even under this selective trade-off, the frequency of the S-gene is approximately 10% among populations in which malaria is endemic [7]. Malaria exerts substantial selective pressure on the human genome due to its high mortality and morbidity rates [26]. This selective pressure is believed to be responsible for the high prevalence of sickle cell disease in malaria-endemic regions. In fact, approximately one third of all aboriginal inhabitants of Sub-Saharan Africa carry the S-gene [7]. As a result, $200,000$ infants are born with sickle cell disease in Africa each year, and in some areas of Sub-Saharan Africa, up to 2% of all children are born with sickle cell disease [4].

Feng et al. [16] studied the influence of malaria on the selection of S-gene and the impact of genetic composition on the maintenance of malaria. Contrary to belief that the increased frequency of resistance to malaria would decrease the frequency of malaria, Feng et al. proposed that the sickle cell trait leads to longer-lasting parasitaemia, and therefore the presence of resistance may actually increase infection prevalence [16]. Later, Feng and Castillo-Chavez expanded the the model in [16] by including all three genotypes of individuals [15]. The expanded model in [15] resulted in periodic solutions, and was used to examine how human population genetics respond to the prevalence of malaria. However, existing mathematical models coupling the malaria epidemiology and the sickle-cell genetics ([15, 16, 17, 22]) have not incorporated asymptomatic infections of malaria.

Here, we developed a mathematical model that considers the interdependence between the dynamics of malaria and the sickle cell trait with consideration of both asymptomatic and symptomatic malaria infections. We analyzed the dynamics of the model using two temporal scales to capture the evolution of the S-gene within an epidemiological context. Our model has the components of fast and slow dynamics, and we apply singular perturbation techniques to deal with the complications of multiple temporal scales. We also examined the differential impact of symptomatic and asymptomatic malaria infections on the frequency of the S-gene as well as the potential role of malaria control in altering the evolutionary dynamics of the S-gene.

2. Compartmental model of malarial transmission and sickle cell trait with consideration of treatment.

2.1. **Mathematical model.** We present a mathematical model of malaria transmission in a human-mosquito system that incorporates changes in the frequency of the S-gene. In our model, the human population (N) living in malaria-endemic regions was divided into four classes: susceptible (u), asymptomatically infected (w), symptomatically infected but untreated (v), and symptomatically infected and treated (n) (Table 1). A two-allele single loci system was used to capture the dynamics of the S-gene in this population. The population was further subdivided by genotype. Specifically, we considered AA and AS individuals, where S denotes the S-gene and A the normal gene. Because SS homozygotes rarely survive to reproductive age in countries where transmission rates of falciparum malaria are high [3, 16], only the two genotypes, AA and AS, were considered, as denoted by the subscripts 1 and 2, respectively. We assumed that the proportion of infected mosquitoes in the total mosquito population (m) would depend on the density of infectious humans.

We defined $b(N)$ as the density-dependent per capita birth rate and assumed the Hardy-Weinberg principle to define the genotypes of newborns. The frequencies of the S and A genes are denoted by $q = g/2$ and $p = 1 - q$, respectively (Table 1). Therefore, the fractions of genotypes, AA and AS, that were born into the host population were denoted by $P_1 = p^2$ and $P_2 = 2pq$, respectively. Consistent with previous findings, we assumed that sickle cell trait would protect individuals against symptomatic, but not against asymptomatic infections [36, 38]. We let $1-\zeta$ denote the level of protection against symptomatic infections among the sub-population of individuals with the sickle cell trait.

To model transmission of malaria, we first defined β_h and β_m as the transmission coefficients from mosquitoes to humans and humans to mosquitoes, respectively. We also assumed that $\beta_h = a\theta c$, where a denotes the rate at which mosquitoes bite humans, θ denotes the probability that an individual of type 1 develops a parasitemia from a bite, and c denotes the ratio of mosquitoes to humans [16, 33]. For the transmission rate to a susceptible human from an infected mosquito bite, we let $\beta_m = a\phi$ where ϕ denotes the probability that a mosquito acquired plasmodium from biting an individual. Using these definitions, we assumed that a susceptible human could become infected via contacts with infective mosquitoes at a rate of $\beta_h u_i(t)m(t)$. Further, susceptible mosquitoes can become infected after biting an infected human host at a rate of $\beta_m\{1 - m(t)\}\{v_i(t) + w_i(t)\}/N$.

Upon infection, the proportion, k, of infected individuals was assumed to be symptomatic, whereas $1 - k$ was assumed to be asymptomatic. We also assumed that the proportion, f, of individuals who experience symptomatic infections would receive antimalarial therapy. The relative infectiousness of treated individuals was reduced to δ when compared to the corresponding rate for untreated individuals (β_h). Additionally, $1/\gamma$ denotes the average infectious period of symptomatic patients. We denoted the malaria-induced death rate as α_i ($\alpha_1 \gg \alpha_2$). In addition, μ_i denotes the per-capita human mortality where $\mu_i = \mu_h + \nu_i$; μ_h is the per capita natural mortality and ν_i is the mortality rate associated with S-gene related death among humans of type i. For treated individuals (n_i), the average infectious period of symptomatic patients was shortened to $1/\gamma_T$ and disease-induced mortality was reduced to α_{Ti}. With or without treatment, it was assumed that prior infections would not confer permanent immunity, allowing repeated infections. A description

Notation	Description	Notes
$i=1$	AA individuals	
$i=2$	AS individuals	
u_i	Number of uninfected humans of genotype i	
v_i	Number of symptomatically infected and untreated humans of genotype i	
n_i	Number of symptomatically infected and treated humans of genotype i	
w_i	Number of asymptomatically infected humans of genotype i	
N	Population size	$\Sigma_{i=1}^{2}(u_i+v_i+w_i)$
x_i	Fraction of humans that are uninfected and have genotype i	$\frac{u_i}{N}$
y_i	Fraction of humans that are symptomatically infected and have genotype i	$\frac{v_i}{N}$
z_i	Fraction of humans that are asymptomatically infected and and have genotype i	$\frac{w_i}{N}$
m	Proportion of infected mosquitoes in the total mosquito population	
g	Frequency of AS individuals	$x_2+y_2+z_2$
q	Frequency of S-gene	$\frac{g}{2}$
P_1	Fraction of total births of genotype AA	$(1-\frac{g}{2})^2$
P_2	Fraction of total births of genotype AS	$g(1-\frac{g}{2})$

TABLE 1. Definition of variables

of variables and parameters that reflect the epidemiology of malaria and sickle cell diseases are presented in Tables 1 and 2, respectively.

Using these assumptions and definitions, we generated the following model of malaria transmission with treatment that considers the sickle cell trait:

$$\frac{du_1}{dt} = P_1 b(N)N - \beta_h u_1 m + \gamma(v_1+w_1) + \gamma_T n_1 - \mu_1 u_1, \tag{1a}$$

$$\frac{du_2}{dt} = P_2 b(N)N - \{\zeta k + (1-k)\}\beta_h u_2 m + \gamma(v_2+w_2) + \gamma_T n_2 - \mu_2 u_2, \tag{1b}$$

$$\frac{dv_1}{dt} = k(1-f)\beta_h u_1 m - (\gamma+\mu_1+\alpha_1)v_1, \tag{1c}$$

$$\frac{dv_2}{dt} = \zeta k(1-f)\beta_h u_2 m - (\gamma+\mu_2+\alpha_2)v_2, \tag{1d}$$

$$\frac{dn_1}{dt} = kf\beta_h u_1 m - (\gamma_T+\mu_1+\alpha_{T1})n_1, \tag{1e}$$

$$\frac{dn_2}{dt} = \zeta kf\beta_h u_2 m - (\gamma_T+\mu_2+\alpha_{T2})n_2, \tag{1f}$$

$$\frac{dw_1}{dt} = (1-k)\beta_h u_1 m - (\gamma+\mu_1)w_1, \tag{1g}$$

$$\frac{dw_2}{dt} = (1-k)\beta_h u_2 m - (\gamma+\mu_2)w_2, \tag{1h}$$

$$\frac{dm}{dt} = \frac{\beta_m(v_1+v_2+w_1+w_2+\delta n_1+\delta n_2)(1-m)}{N} - \mu_m m \tag{1i}$$

where $N = \sum_{i=1}^{2} u_i + v_i + n_i + w_i$ and $b(N) = b(1 - N/K)$. Here K is defined as a carrying capacity. We normalized Eqs.(1a)-(1i) by introducing the new variables, $x_i = u_i/N$, $y_i = v_i/N$, $j_i = n_i/N$, and $z_i = w_i/N$. Hence, it follows that $\sum_{i=1}^{2}(x_i + y_i + j_i + z_i) = 1$, and that $g = (u_2 + v_2 + n_2 + w_2)/N = x_2 + y_2 + j_2 + z_2$. Using the chain rule leads to the following system of equations in the re-scaled variables.

$$\frac{dy_1}{dt} = k\beta_h(1-f)(1-g-y_1-j_1-z_1)m - (\gamma+\mu_1+\alpha_1)y_1 - y_1N'/N, \tag{2a}$$
$$\frac{dy_2}{dt} = \zeta k(1-f)\beta_h(g-y_2-j_2-z_2)m - (\gamma+\mu_2+\alpha_2)y_2 - y_2N'/N, \tag{2b}$$
$$\frac{dj_1}{dt} = kf\beta_h(1-g-y_1-j_1-z_1)m - (\gamma_T+\mu_1+\alpha_{T1})j_1 - j_1N'/N, \tag{2c}$$
$$\frac{dj_2}{dt} = \zeta kf\beta_h(g-y_2-j_2-z_2)m - (\gamma_T+\mu_2+\alpha_{T2})j_2 - j_2N'/N, \tag{2d}$$
$$\frac{dz_1}{dt} = (1-k)\beta_h(1-g-y_1-j_1-z_1)m - (\gamma+\mu_1)z_1 - z_1N'/N, \tag{2e}$$
$$\frac{dz_2}{dt} = (1-k)\beta_h(g-y_2-j_2-z_2)m - (\gamma+\mu_2)z_2 - z_2N'/N, \tag{2f}$$
$$\frac{dm}{dt} = \beta_m(1-m)(y_1+y_2+\delta j_1+\delta j_2+z_1+z_2) - \mu_m m, \tag{2g}$$
$$\frac{dg}{dt} = P_2b(N) - \mu_2 g - \alpha_2 y_2 - \alpha_{T2}j_2 - gN'/N, \tag{2h}$$
$$\frac{dN}{dt} = N\{(P_1+P_2)b(N) - \mu_1(1-g) - \mu_2 g - \alpha_1 y_1 - \alpha_2 y_2 - \alpha_{T1}j_1 - \alpha_{T2}j_2\} \tag{2i}$$

where $b(N) = b(1 - N/K)$.

2.2. **Fast dynamics of epidemics.** Eqs. (2a)-(2i) include parameters that are tied into human demographics and vector life cycles. As a result, the values of these parameters vary across many orders of magnitude. That is, the dynamics of malaria disease evolve over a much faster time scale than human demographics and the evolutionary changes of sickle cell trait. Specifically, the reciprocals of the human demographic parameters (b, μ_i, and α_i) are expressed in decades, whereas the reciprocals of the malaria disease parameters (β_h, β_m, γ, and μ_m) are expressed in days. To use slow and fast dynamics in the analyses of Eqs. (2a)-(2i) effectively, a new variable, ϵ, was introduced, where $\epsilon > 0$ is small. Specifically, ϵ was used to rescale parameters as follows: $b = \epsilon\tilde{b}$, $\mu_i = \epsilon\tilde{\mu}_i$, $\alpha_i = \epsilon\tilde{\alpha}_i$, and $\alpha_{Ti} = \epsilon\tilde{\alpha}_{Ti}$.

Setting $\epsilon = 0$ allowed us to study the fast dynamics of our model and focus on the disease dynamics separately from the evolution of S-gene:

$$\begin{aligned}
\frac{dy_1}{dt} &= k(1-f)\beta_h(1-g-y_1-j_1-z_1)m - \gamma y_1,\\
\frac{dy_2}{dt} &= \zeta k(1-f)\beta_h(g-y_2-j_2-z_2)m - \gamma y_2,\\
\frac{dj_1}{dt} &= kf\beta_h(1-g-y_1-j_1-z_1)m - \gamma_T p_1,\\
\frac{dj_2}{dt} &= \zeta kf\beta_h(g-y_2-j_2-z_2)m - \gamma_T p_2,
\end{aligned} \tag{3}$$

$$\frac{dz_1}{dt} = (1-k)\beta_h(1-g-y_1-j_1-z_1)m - \gamma z_1,$$
$$\frac{dz_2}{dt} = (1-k)\beta_h(g-y_2-j_2-z_2)m - \gamma z_2,$$
$$\frac{dm}{dt} = \beta_m(1-m)(y_1+y_2+\delta j_1+\delta j_2+z_1+z_2) - \mu_m m,$$

The effective reproductive ratio can be computed at the disease-free equilibrium,

Name	Description	Values	Ref
g	Frequency of AS individuals	0.25	[43]
θ	Probability of a human acquiring a parasitemia per bite	0.06	[16]
ζ	Relative susceptibility of individuals of type 2 to symptomatic infection relative to individuals of type 1	0.4	[1, 2]
k	Proportion of symptomatic infection	0.5	
f	Probability of seeking treatment upon symptomatic infection	0.5	[18]
δ	Relative infectiousness of treated individuals compared to non-treated ones	0.2	[29, 30]
γ_0	Rate of recovery from malaria infection without treatment	$1/80$ day^{-1}	[32]
γ_T	Rate of recovery from symptomatic malaria infection with treatment	$1/80$ day^{-1}	[32]
a	Biting rate per human per mosquito	0.67 day^{-1}	[18, 19]
c	Number of mosquitoes per human	2	[20, 21, 34]
ϕ	Probability that a mosquito acquires plasmodium from biting a human	0.05	[16]
μ_m	Mosquito death rate	0.1 day^{-1}	[18, 19]
μ_h	Per-capita natural human death rate	0.000054 day^{-1}	[42]
ν_i	Per-capita S-gene related death rate of humans of type i, ($\nu_1 = 0$, $\nu_2 > 0$)	$\nu_1 = 0$ day^{-1} $\nu_2 = 0.00002$ day^{-1}	[16]
α_i	Per-capita malaria-induced death rate of humans of type i, ($\alpha_1 \gg \alpha_2$)	$\alpha_1 = 0.00034$ day^{-1} $\alpha_2 = 0.00005$ day^{-1}	[9] [16]
α_{Ti}	Per-capita malaria-induced death rate of treated humans of type i	$\alpha_{T1} \le \alpha_1$ $\alpha_{T2} \le \alpha_2$	
$b(N)$	Per-capita birth rate of humans, $b(N) = b(1-N/K)$		

TABLE 2. Definition of parameters and baseline values

$E_0 = (0,0,0,0,0,0,0)$ of the fast system (3), using a next generation matrix (Appendix A). The effective reproductive ratio is given by

$$\Re_c = (1-g)\Re_1\{1-fk(1-\delta)\} + g\Re_1\{1-k+k\zeta(1-f(1-\delta))\} \tag{4}$$

where $\Re_1 = \frac{\beta_m}{\mu_m}\frac{\beta_h}{\gamma}$. The expression $\Re_c$ in Eq (4) represents the contribution of non-carriers and carriers of sickle cell disease to the reproductive ratio of malaria. Furthermore, $\Re_c < 1$ provides the threshold condition for the eradication of malaria.

Using $\Re_c$, we can calculate the followings:

$$\frac{\partial \Re_c}{\partial f} = -k\Re_1(1-\delta)\{1-(1-\zeta)g\} \leq 0,$$

$$\frac{\partial \Re_c}{\partial g} = -k\Re_1(1-\zeta)\{1-(1-\delta)f\} \leq 0,$$

$$\text{and } \frac{\partial \Re_c}{\partial k} = -\Re_1[f(1-\delta)+g(1-\zeta)\{1-f(1-\delta)\}] \leq 0.$$

Therefore, we conclude that the burden of malaria is likely to be reduced not only when we increase the treatment rate, but also when the proportion of symptomatic infections becomes higher, or if the number of sickle cell carriers increases. Fig 1 shows how $\Re_c$ changes as the probability of treatment (f) or the proportion of symptomatic infection (k) varies. It was found that the value of $\Re_c$ was more sensitive to the changes in the proportion of symptomatic infection (k) than to changes in the treatment probability (f). Furthermore, when the proportion of symptomatic infection (k) is relatively low, increasing treatment rate is unlikely to be effective in lowering the burden of malaria (Fig 1). Given that the asymptomatic infection of malaria is common in malaria-endemic regions, our result indicates that the effect of treatment might be limited. In addition, strong selection of sickle-cell traits (i.e. lower value of ζ) is likely to reduce the impact of treatment on reducing the prevalence of malaria.

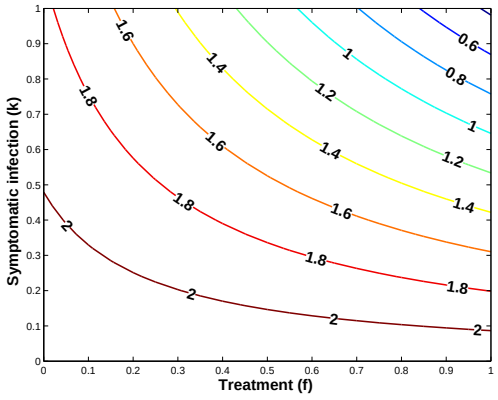

FIGURE 1. Level curve of a control reproductive ratio ($\Re_c$) when a proportion of symptomatic infection (k) and a proportion of treatment (f) are varied.

Theorem 2.1. *The disease-free equilibrium, $E_0 = (0,0,0,0,0,0,0)$ of the fast system (3) is locally asymptotically stable if $\Re_c < 1$. Furthermore, E_0 is locally asymptotically stable if $\Re_0 < 1$ and unstable if $\Re_0 > 1$.*

Proof. To prove the stability of E_0, it must be shown that all eigenvalues of the Jacobian matrix of the system (3) have negative real parts. The characteristic equation is

$$(\lambda+\gamma)^3(\lambda+\gamma_T)(\lambda^3+p_1\lambda^2+p_2\lambda+p_3) = 0 \tag{5}$$

where

$$\begin{aligned} p_1 &= \gamma + \gamma_T + \mu_m, \\ p_2 &= -\delta\beta_m(\mathbf{C}+\mathbf{D}) - \beta_m(\mathbf{A}+\mathbf{B}+\mathbf{F}+\mathbf{G}) + \mu_m(\gamma+\gamma_T) + \gamma\gamma_T, \\ p_3 &= -\gamma\delta\beta_m(\mathbf{C}+\mathbf{D}) - \beta_m\gamma_T(\mathbf{A}+\mathbf{B}+\mathbf{F}+\mathbf{G}) + \mu_m\gamma\gamma_T. \end{aligned} \tag{6}$$

For ease of notation, we introduced $\mathbf{A} = k(1-f)\beta_h(1-g)$, $\mathbf{B} = \zeta k(1-f)\beta_h g$, $\mathbf{C} = kf\beta_h(1-g)$, $\mathbf{D} = \zeta kf\beta_h g$, $\mathbf{F} = (1-k)\beta_h(1-g)$, and $\mathbf{G} = (1-k)\beta_h g$. According to Routh-Hurwitz conditions, all roots of Eq (5) have negative real parts if and only if $p_1 > 0$, $p_3 > 0$, and $p_1p_2 > p_3$. For $\Re_c < 1$, by Eq (4), it follows that

$$\gamma\mu_m > (\mathbf{A}+\mathbf{B}+\mathbf{F}+\mathbf{G})\beta_m \tag{7}$$

and

$$\gamma_T\mu_m > (\mathbf{C}+\mathbf{D})\delta\beta_m. \tag{8}$$

From Eq (6) we see that $p_1 > 0$. Using Eq (4), we show that $p_3 > 0$ if and only if $\Re_c < 1$. Lastly,

$$\begin{aligned} p_1p_2 - p_3 =& -\delta\beta_m(\mathbf{C}+\mathbf{D})(\gamma_T+\mu_m) \\ &+ (\gamma+\mu_m)\{\gamma\mu_m - \beta_m(\mathbf{A}+\mathbf{B}+\mathbf{F}+\mathbf{G})\} \\ &+ \gamma_T(\gamma+\mu_m)(\gamma+\gamma_T+\mu_m) \\ >& -\delta\beta_m(\mathbf{C}+\mathbf{D})(\gamma_T+\mu_m) + \gamma_T(\gamma+\mu_m)(\gamma+\gamma_T+\mu_m) \\ >& \gamma_T\{(\gamma_T+\mu_m)\gamma + (\gamma+\mu_m)\gamma_T\} > 0. \end{aligned} \tag{9}$$

The last two inequalities in (9) can be shown using (7) and (8), respectively. Thus we conclude that all roots of Eq (5) have negative real parts if $\Re_c < 1$, which proves that E_0 is l.a.s. when $\Re_c < 1$.

Furthermore, if we assume no treatment, and consider the fast system (10), the disease-free equilibrium is reduced to $E_0 = (0,0,0,0,0)$. The Jacobian matrix of system (10) at the disease-free equilibrium has a leading eigenvalue,

$$\sqrt{(1-g)\left(\frac{\beta_m\beta_h}{\mu_m\gamma}\right) + g\left(\frac{\beta_m\beta_h(1-k(1-\zeta))}{\mu_m\gamma}\right)}.$$

By defining the basic reproductive ratio as $\Re_0 = \Re_c(f=0)$, we conclude that E_0 is locally asymptotically stable if $\Re_0 < 1$ and unstable if $\Re_0 > 1$. □

The conditions for the existence of a disease-free equilibrium (E_0) and a non-trivial equilibrium (E^*) are presented in Theorem 3.1.

3. Mathematical model of malarial transmission and sickle cell trait.

In this section, we present a simplified model of malarial transmission and sickle cell trait in the absence of control strategies.

3.1. Mathematical model.

If the effects of treatment are not included, we can consider Eqs. (1a)-(1d) and (1g)-(1i), and assume $f = 0$, $\delta = 0$, and $\gamma_T = 0$. We then normalize the system using the new set of variables: $x_i = u_i/N$, $y_i = v_i/N$, and $z_i = w_i/N$. It follows that $\sum_{i=1}^{2} x_i + y_i + z_i = 1$. The fraction of the AS individuals in the population is denoted by $g = (u_2+v_2+w_2)/N = x_2+y_2+z_2$. The use of the chain rule leads to the set of equations using genotype-specific re-scaled variables, resulting in Eqs. (2a)-(2b) and (2e)-(2i) where we assume $j_i = 0$, $f = 0$, $\alpha_{Ti} = 0$ and $\delta = 0$ $(i = 1, 2)$.

3.2. **Fast dynamics.** To analyze the impact of control measures on the slow and fast dynamics in Eqs. (2a)-(2b) and (2e)-(2i) where $j_i = 0$, $f = 0$, $\alpha_{Ti} = 0$ and $\delta = 0$ $(i = 1, 2)$, we used the scaling parameter $\epsilon > 0$, with ϵ being small. The slow variables are defined using the scaled parameters: $b = \epsilon\tilde{b}$, $\mu_i = \epsilon\tilde{\mu}_i$, and $\alpha_i = \epsilon\tilde{\alpha}_i$. Letting $\epsilon = 0$ leads to the relevant fast dynamics described by the following system:

$$\begin{aligned}
\frac{dy_1}{dt} &= k\beta_h(1-g-y_1-z_1)m - \gamma y_1,\\
\frac{dy_2}{dt} &= \zeta k\beta_h(g-y_2-z_2)m - \gamma y_2,\\
\frac{dz_1}{dt} &= (1-k)\beta_h(1-g-y_1-z_1)m - \gamma z_1,\\
\frac{dz_2}{dt} &= (1-k)\beta_h(g-y_2-z_2)m - \gamma z_2,\\
\frac{dm}{dt} &= \beta_m(y_1+y_2+z_1+z_2)(1-m) - \mu_m m.
\end{aligned} \tag{10}$$

On the fast time scale, the basic reproductive number of malaria disease can be calculated as the leading eigenvalue of the next generation matrix (Appendix A):

$$\begin{aligned}
\Re_0 =& (1-g)\left(\frac{\beta_m\beta_h}{\mu_m\gamma}\right) + g\left(\frac{\beta_m\beta_h(1-k(1-\zeta))}{\mu_m\gamma}\right)\\
=& (1-g)\Re_1 + g\Re_2.
\end{aligned} \tag{11}$$

Here $\Re_i$ $(i = 1, 2)$ is defined as

$$\Re_i = T_v T_{hi} \tag{12}$$

where

$$\begin{aligned}
T_v =& \frac{\beta_m}{\mu_m},\\
T_{h1} =& \frac{\beta_h}{\gamma},\\
\text{and } T_{h2} =& \frac{\beta_h(1-k(1-\zeta))}{\gamma}.
\end{aligned} \tag{13}$$

To evaluate the impact of S-gene frequencies on the prevalence of malaria infection, numerical simulations of the fast system (10) were performed (Fig 2). We observed that increases in the fraction of sickle cell trait lower the prevalence of symptomatic infection, but increase the relative prevalence of asymptomatic infections (Fig 2). Thus, the protective effect of the sickle cell trait on symptomatic malaria infection is captured by our model; however, the detrimental effect of the sickle cell trait on asymptomatic malaria infection was also confirmed using numerical simulations of the fast system (10).

Additional analyses were performed using the equivalent system derived from the introduction of the new variables, s_1 and s_2, where $s_1 = y_1 + z_1$ and $s_2 = y_2 + z_2$. The system (10) is equivalent to the following nonlinear system:

$$\begin{aligned}
\frac{ds_1}{dt} &= \beta_h(1-g-s_1)m - \gamma s_1,\\
\frac{ds_2}{dt} &= (1-k(1-\zeta))\beta_h(g-s_2)m - \gamma s_2,\\
\frac{dm}{dt} &= (1-m)\{\beta_m(s_1+s_2)\} - \mu_m m.
\end{aligned} \tag{14}$$

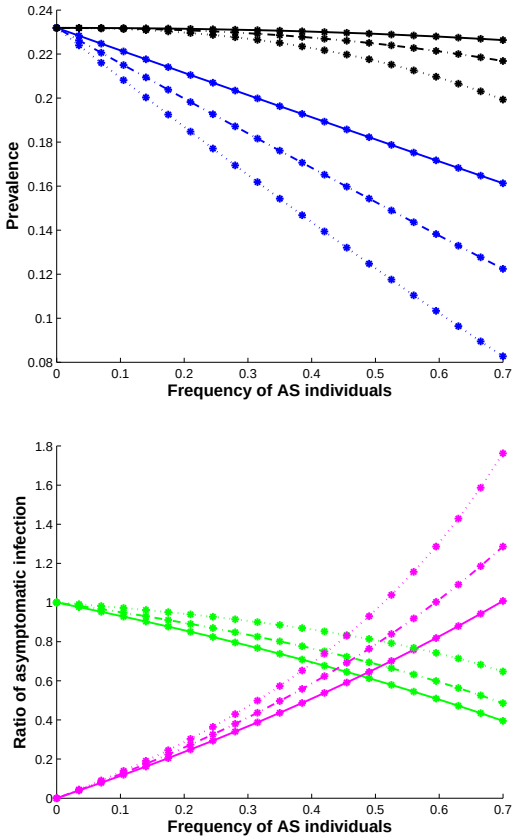

FIGURE 2. Impact of sickle cell trait on the epidemiology of malaria as the frequency of AS individuals (g) was varied (solid: ζ=0.6; dashed: ζ=0.4; dotted: $\zeta = 0.2$). The non-trivial equilibrium, $E^* = (y_1^*, y_2^*, z_1^*, z_2^*, m^*)$, of the fast system (10) was solved as a function of g where other parameters were fixed at their baseline values. (A) The prevalence of symptomatic ($y_1^* + y_2^*$, blue) and asymptomatic ($z_1^* + z_2^*$, black) malaria infection for different values of ζ. (B) The relative prevalence of asymptomatic AA individuals and asymptomatic AS individuals compared to all symptomatic individuals are shown as $z_1^*/(y_1^* + y_2^*)$ (green) and $z_2^*/(y_1^* + y_2^*)$ (magenta), respectively.

Let $E^* = (s_1^*, s_2^*, m^*)$ be a non-trivial equilibrium of (14) under the assumption that N is constant. Setting the right hand side of (14) equal to zero gives

$$\begin{aligned} s_1^* &= \frac{T_{h1} m^*}{1 + m^* T_{h1}}(1 - g), \\ s_2^* &= \frac{T_{h2} m^*}{1 + m^* T_{h2}} g. \end{aligned} \tag{15}$$

In Eq (15), m^* is the unique positive solution of a quadratic equation,

$$h(m) := p_0 m^2 + p_1 m + p_2 = 0, \tag{16}$$

with

$$\begin{aligned} p_0 &= T_{h2}(\Re_1 + T_{h1}), \\ p_1 &= T_{h1} + T_{h2}(1-\Re_1) + \Re_1(1-g) + \Re_2 g, \\ p_2 &= 1 - \Re_1(1-g) - \Re_2 g. \end{aligned} \tag{17}$$

This gives the following result:

Theorem 3.1. *Let* $\Re_0 = (1-g)\Re_1 + g\Re_2$. *For any* $g \in [0,1]$, *if* $\Re_0 < 1$, *the fast system has only a trivial equilibrium; and if* $\Re_0 > 1$, *the fast system has a unique positive equilibrium* $E^* = (y_1^*, y_2^*, z_1^*, z_2^*, m^*)$ *where* $m^* \in [0,1]$ *is the unique positive solution of Eq. (16).*

Proof. If $\Re_0 = (1-g)\Re_1 + g\Re_2 < 1$, then $p_2 = 1 - \Re_0 > 0$ and both solutions of Eq. (16) are negative. Therefore, E^* is not biologically feasible.

Assuming that $\Re_0 > 1$ implies that $p_2 = 1 - \Re_0 < 0$. Therefore, Eq (16) has a unique positive solution m^*, because $p_0 > 0$. If we observe that $h(0) = p_2 < 0$, $h(1) = (T_{h1}+1)(T_{h2}+1)$, and $h(m^*) = 0$, then from Eq (15), we conclude that $0 < s_1^* < 1$ and $0 < s_2^* < 1$. The non-trivial equilibrium of Eqs. (10) corresponds to $y_1^* = ks_1^*$, $z_1^* = (1-k)s_1^*$, $y_2^* = \frac{\zeta k}{1-k(1-\zeta)}s_2^*$, and $z_2^* = \frac{1-k}{1-k(1-\zeta)}s_2^*$. It follows that $E^* = (y_1^*, y_2^*, z_1^*, z_2^*, m^*)$ exists and is unique. □

Using the results in [16], it can be shown that E^* is locally asymptotically stable (Appendix A). Therefore, the system (21) with $\epsilon = 0$ contains a two-dimensional stable manifold of steady states $U_0(g,N) = (y_1^*, y_2^*, z_1^*, z_2^*, m^*, g, N)^T$.

3.3. **Slow dynamics of population genetics.** By using the re-scaled time $\tau = \epsilon t$, we can re-write the full system, Eqs. (2a)-(2b) and (2e)-(2i) (with $j_i = 0$, $f = 0$, $\alpha_{Ti} = 0$ and $\delta = 0$; $i = 1, 2$) which has a two-dimensional slow manifold (Appendix B):

$$M = \{(y_1, y_2, z_1, z_2, m, g, N) : y_i = y_i^*(g,N), z_i = z_i^*(g,N), m = m^*(g,N), i = 1, 2.\}$$

The slow manifold M would be hyperbolically stable as it consists of a set of hyperbolic equilibria of the fast system (10). Here y_i^* and z_i^* $(i = 1, 2)$ are given in the Eqs (15).

The slow dynamics on M is described by the equations

$$\begin{aligned} \frac{dg}{d\tau} =& \{(1-g)P_2 - gP_1\}\tilde{b}(N) + (\tilde{\mu}_1 - \tilde{\mu}_2)g(1-g) + \tilde{\alpha}_1 g y_1^* \\ & - \tilde{\alpha}_2(1-g)y_2^*, \\ \frac{dN}{d\tau} =& N\{(P_1+P_2)\tilde{b}(N) - \tilde{\mu}_1(1-g) - \tilde{\mu}_2 g - \tilde{\alpha}_1 y_1^* - \tilde{\alpha}_2 y_2^*\}, \end{aligned} \tag{18}$$

where $P_1 = (1-g/2)^2$ and $P_2 = g(1-g/2)$.

We define the fitness of the S-gene [15, 16], $\mathcal{F}$, as

$$\begin{aligned} \mathcal{F} =& \left(\frac{1}{g}\frac{dg}{d\tau}\right)|_{g=0} \\ =& \tilde{\mu}_1 - \tilde{\mu}_2 + \tilde{\alpha}_1 \frac{k(\Re_1 - 1)T_{h1}}{\Re_1(1+T_{h1})} - \tilde{\alpha}_2\left(\frac{\zeta k}{1-k(1-\zeta)}\right)\frac{(\Re_1-1)T_{h2}}{\Re_1(1+T_{h1}) + T_{h1} - T_{h2}} \\ =& \sigma_1 - \sigma_2, \end{aligned} \tag{19}$$

where

$$\sigma_1 = \tilde{\mu}_1 + \tilde{\alpha}_1 \frac{k(\Re_1 - 1)T_{h1}}{\Re_1(1 + T_{h1})},$$
$$\sigma_2 = \tilde{\mu}_2 + \tilde{\alpha}_2 \left(\frac{\zeta k}{1 - k(1 - \zeta)} \right) \frac{(\Re_1 - 1)T_{h2}}{\Re_1(1 + T_{h1}) + T_{h1} - T_{h2}}. \tag{20}$$

Here g represents the abundance of S-gene, and thus, $\mathcal{F}$ is the growth rate of sickle-cell genes per-capita upon initial introduction of the gene into a population. A weighted sum of human death rates, μ_i and α_i, gives σ_i. Notably, $\Re_i = T_v T_{hi}$ where T_{hi} is a function of the parameters related to genotype i human malaria infections, which are generated by mosquitoes. Similarly, T_v involves parameters related to malaria infections of mosquitoes generated by humans.

4. **Influence of S-gene frequency on the malaria epidemic.** To assess how the frequency of the S-gene, g, influences the overall endemic level of malaria, we examined how the basic reproductive ratio of malaria ($\Re_0$) changes when g is varied (Figs. 3 and 4). In general, the disease prevalence increases with $\Re_0$. It is also worth noting that $\Re_0 = (1 - g)\Re_1 + g\Re_2$ where $\Re_1$ and $\Re_2$ are contributions from non-carriers and carriers of sickle cell disease, respectively (Eq. 11). As a result, $\Re_1$ is greater than $\Re_2$ in the presence of the S-gene, and $\Re_0$ decreases with the frequency of the S-gene (g). This proves that the overall prevalence of malaria infection decreases with an increasing number of individuals who have the sickle cell trait. However, this effect is stronger on symptomatic infections than on asymptomatic infections (Fig 2A).

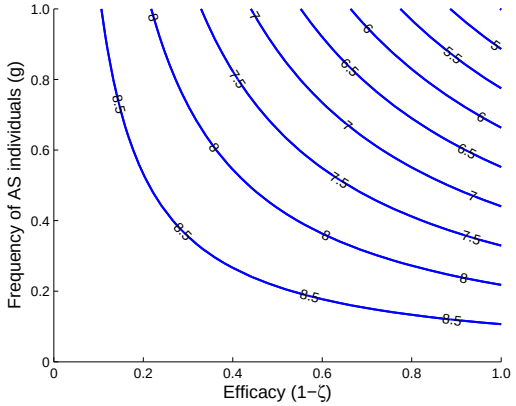

FIGURE 3. Level curve of a basic reproductive ratio ($\Re_0$) when relative efficacy against symptomatic infection among individuals of type 2 compared to individuals of type 1 and a proportion of sickle trait are varied.

Nevertheless, the relative prevalence of asymptomatic infections among sickle-cell carriers increased with the frequency of the S-gene (Fig 2B). Specifically, the relative prevalence of asymptomatic infections with sickle cell trait, compared to symptomatic infections among both the carriers and non-carriers of sickle cell disease, increased from 0.12 to 0.59 as the frequency of the S-gene increased from 0.10

to 0.40. This increase occurred partially because the sickle cell trait does not provide protection against asymptomatic malaria. This pattern was more pronounced when the relative susceptibility of sickle cell carriers to malaria (ζ) decreased (Fig 2B). Similarly, the role of sickle cell trait in reducing the burden of malaria was abrogated when the protection of the S-gene against symptomatic malaria infections was not highly effective (Fig 3).

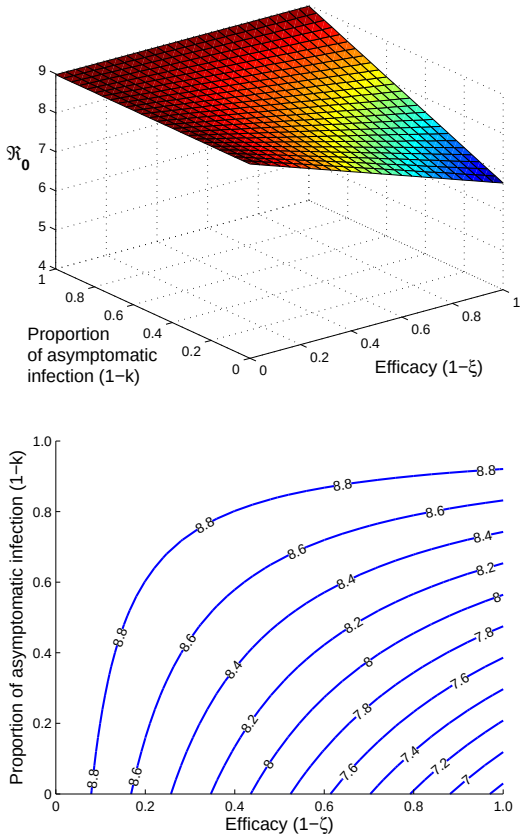

FIGURE 4. (a) Plot of $\Re_0$ vs. relative efficacy against symptomatic infection among individuals of type 2 compared to individuals of type 1, and the proportion of asymptomatic infection. (b) Level curve of a basic reproductive ratio.

5. **Sensitivity analysis of a basic reproductive ratio.** The magnitude of a basic reproductive ratio ($\Re_0$) depends on parameters that are associated with malarial epidemiology and sickle cell trait. To study the sensitivity of $\Re_0$ to key parameters, we performed a sensitivity analysis on $\Re_0$ with respect to three parameters: relative susceptibility of sickle cell carriers to malaria (ζ), proportion of symptomatic infection of malaria (k), and S-gene frequency (g) (Fig 5). The sensitivity index S_I is defined as

$$S_I = \frac{\partial \Re_0}{\partial P} \cdot \frac{P}{\Re_0},$$

where P is the parameter of interest [5]. The larger the magnitude of the sensitivity index, the more sensitive $\Re_0$ is with respect to that parameter.

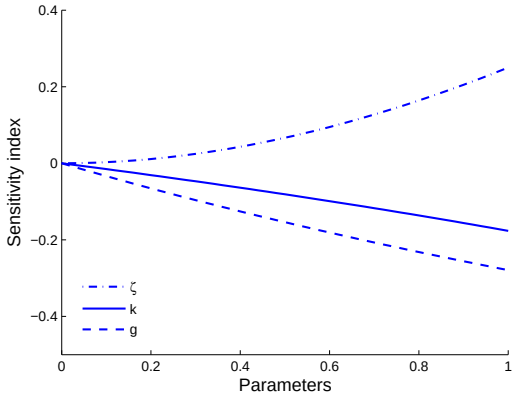

FIGURE 5. Sensitivity analysis of the basic reproductive number. The sensitivity indices of the model parameters (ζ, k and g) were computed through local derivatives.

For sickle cell carriers, the sensitivity of susceptibility to symptomatic malaria (ζ) was positive for all values of ζ between 0 and 1; that is, $\Re_0$ increased as malaria protection conferred by the sickle cell trait became less effective. Confirming this finding, the lower protective efficacy of the sickle cell trait, $1-\zeta$, corresponded to a higher prevalence of symptomatic malaria infection among the AS-individuals (Fig. 6). Furthermore, this dependence of $\Re_0$ on protective efficacy was more pronounced when the frequency of the S-gene was relatively high (Fig 3).

In contrast to the protective efficacy of the S-gene (ζ), the sensitivity indices for the proportion of symptomatic malaria infection (k) and for the frequency of the S-gene (g) were negative. The former means that, as indicated by $\Re_0$, the epidemiological burden in the presence of the S-gene is likely to be less when individuals infected with malaria are more likely to become symptomatic (Fig 4). This occurs because the sickle cell trait can reduce the prevalence of symptomatic malaria infections more effectively. Furthermore, in general, the value of $\Re_0$ was more sensitive to changes in the frequency of the S-gene than to changes in the proportion of symptomatic infection.

Specifically, the sensitivity indices for ζ, k and g were found to be 0.04, -0.08 and -0.08, respectively, when all parameters were fixed at their baseline values (Table 2 and Fig 5). For example, if the relative susceptibility of AS-individuals to symptomatic malarial infection increased by 1%, then the value of $\Re_0$ would increase by 0.04%. Similarly, a 1% increase in the proportion of symptomatic infections or a 1% increase in S-gene frequency would correspond to a 0.08% decrease in the value of $\Re_0$.

6. **Fitness of the sickle-cell trait with consideration of malaria epidemiology.** The fitness coefficient of the S-gene, $\mathcal{F}$, is defined as the per-capita growth rate of the sickle-cell gene when the gene is initially introduced into a population

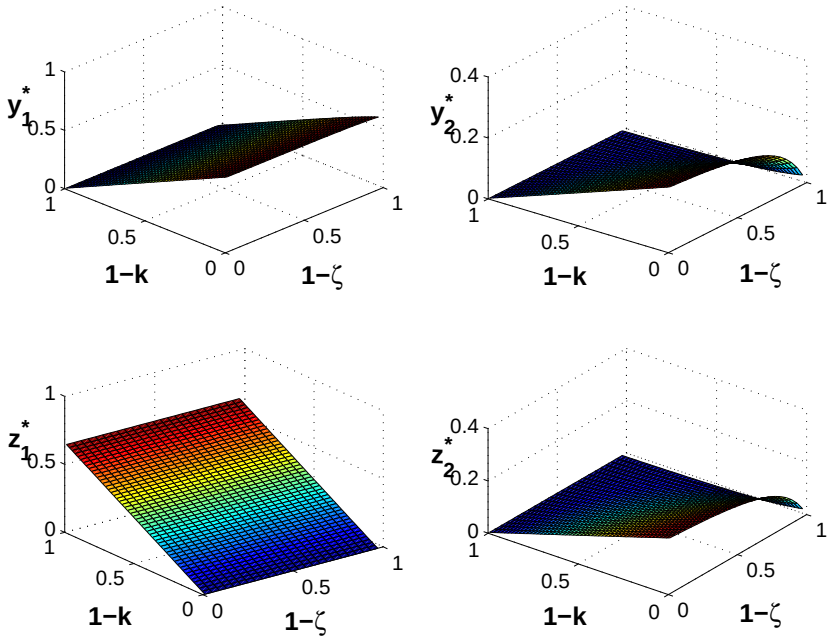

FIGURE 6. Sensitivity analysis of endemic equilibrium of malaria infection. Two parameters, a proportion of asymptomatic infection $(1-k)$ and the efficacy of sickle cell trait in reducing symptomatic infection $(1-\zeta)$, were varied.

(see Eq (19)) [16]. Therefore, whether the S-gene can invade a particular population is determined by the fitness of the S-gene. The S-gene cannot invade if its fitness coefficient $(\mathcal{F})$ is negative, but invasion is possible if the fitness coefficient is positive [15, 16, 17]. Fitness of the S-gene is determined by the parameters that are associated with malaria epidemiology and with the sickle cell trait. For example, we show that an increment in either the duration of malaria infection $(1/\gamma)$ (Fig 7) or transmission rate (β_h) would increase fitness of the S-gene.

Interestingly, the relationship between the fitness of the S-gene and the proportion of symptomatic malaria infections depended on the recovery rate of the malaria infection, γ (Fig 7). Specifically, fitness, $\mathcal{F}$, would increase with the proportion of symptomatic malaria infections when the infectious period of malaria is long. However, such a positive correlation between fitness of the S-gene and the proportion of symptomatic infections could be reversed if, for example, the average infectious period of the host was shortened to 10 days or fewer. This is because the persistence of the S-gene requires the endemicity of malaria, and malaria can be eradicated when the infectious period of the host is greatly shortened, potentially reducing $\Re_0$ to below one.

7. **Discussion.** We developed a mathematical model that couples the transmission dynamics of malaria with the evolution of the sickle cell trait in order to examine

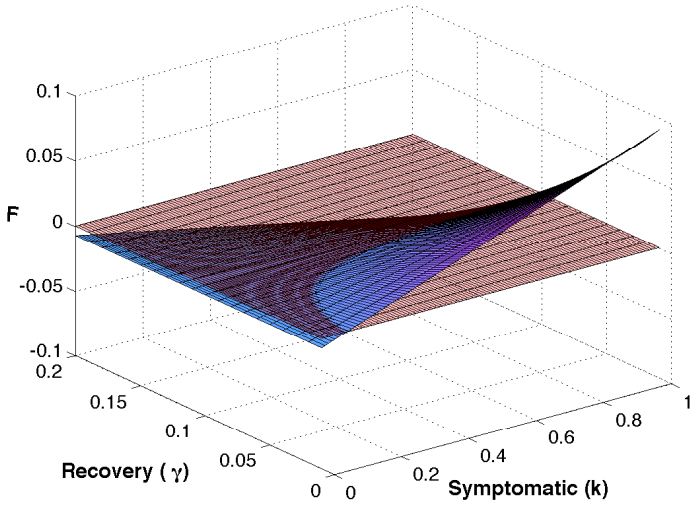

FIGURE 7. Plot of fitness $\mathcal{F}$ of S-gene against recovery rate (γ) and the proportion of symptomatic infection (k). This shows that $\mathcal{F}$ increases with a proportion of symptomatic infection, and changes from negative to positive, when a baseline parameter value of the recovery rate (γ), 1/80 per day, is used. However, such a positive correlation is reversed if the recovery rate of a host (γ) is increased to 0.1 per day or higher.

the interdependence of the evolution of sickle cell trait and the prevalence of malaria infections. Given that the presence of sickle cell does not affect immunity against asymptomatic malaria infections [36, 38], we expanded previous malaria transmission modeling efforts ([15, 16, 17]) by incorporating asymptomatic malaria infections and their role in modifying the fitness of the sickle cell trait in malaria endemic areas. To facilitate the analysis of our model, we separated malaria transmission (on a fast time scale) and S-gene dynamics (on a slow time scale). We also investigated the dynamics of malaria prevalence as a function of a slowly changing genetic composition of the host population.

Using our model, we computed the threshold treatment levels that are required to eradicate malaria. In malaria-endemic regions, the proportion of asymptomatic infections is relatively high. For instance, in Senegal, the prevalence of asymptomatic carriage was between 23% and 32% in 2002-2003 [27]. Furthermore, asymptomatic P. falciparum carriers had a significantly higher mean parasite density than did non-carriers. Thus, our study indicates that the required treatment level to eradicate malaria in such regions is much higher than what is normally expected (Fig 1). In fact, it was suggested that community screening and treatment of asymptomatic carriers would be necessary to result in significant reduction in the prevalence of malaria [25]. In addition, treatment of asymptomatic individuals, regardless of

their malaria infection status, with regularly spaced therapeutic doses of antimalarial drugs has been proposed as a method to reduce malaria-associated morbidity and mortality [27].

Using our model, we computed the threshold conditions that are required to maintain malaria in a human population and identified the conditions that facilitate increases in the frequency of the S-gene. These two threshold conditions proved to be interdependent; the epidemic threshold condition, $\Re_0$, depended on the proportion of symptomatic infection and S-gene frequency. Furthermore, we found that the fitness of the S-gene depends on parameters that are linked to the genetics of the S-gene and on parameters that are related to malaria epidemiology. For instance, the fitness of the S-gene decreases when the proportion of symptomatic malaria infections decreases. This could become possible with treatment efforts of malaria that decrease the symptomatic cases. Potential mechanisms for reducing the fitness of S-gene heterozygotes also include a shortened infectious period. Our methodologies that incorporates the interdependence between genetics and disease transmission will be applicable to other genetic polymorphism more generally. For instance, G6PDH (Glucose-6-phosphate dehydrogenase) defficiency, the most common human enzyme defect, is known to confer protection against malaria [24]. Thus, this phenomenon might give G6PDH deficiency carriers an evolutionary advantage in malarial endemic environments. Consequently, malaria transmission is known to select for G6PDH deficiency [24].

When only limited control efforts are applied, with occasionally intense efforts (such as the Garki Project), malaria is not likely to be eradicated because of the existing reservoir of asymptomatic infections in a host population. With lowered prevalence of malaria, the heterozygote advantage of the S-gene decreases, reducing the frequency of the S-gene. However, asymptomatic infections will serve as the reservoir of malaria and won't be affected by treatment efforts. This, in turn, will increase the prevalence of symptomatic malaria if control efforts are unsuccessful or temporary. Such detrimental effects of malaria control efforts can be pronounced, particularly in endemic areas where the prevalence of asymptomatic infections is relatively high.

In population genetics parlance, heterozygotes in sickle cells have a higher fitness than either of the homozygotes, and as a consequence, the sickle cell gene has not been eliminated in malaria-endemic areas. Using Prices equation, it has been proposed that the sickle cell gene could be eliminated from the gene pool in the absence of malaria [12, 37]. However, given the current state of affairs, the sickle cell gene is likely to persist due to the differential impacts of the sick-cell trait on symptomatic and asymptomatic malaria infections.

In the Asia Pacific sites with low and unstable transmission, malaria was eliminated in 1991 after implementation of a combined intervention strategies, including mass drug administration (MDA) and insecticide-treated bed nets (ITNs) [23]. The success of this strategy is especially remarkable when contrasted with observations from different islands in the same archipelago, where interrupted control efforts transiently led to larger epidemics [8]. Although the World Health Organization (WHO) submitted a proposal for the eradication of malaria worldwide in 1955 [40], malaria has not been eradicated in countries such as India and Sri Lanka [6, 13]. In general, the implementation of limited treatment efforts aimed at diminishing the burden of symptomatic malaria might increase the proportion of asymptomatic

infections (reservoir for malaria) and decrease the frequency of the S-gene. Therefore, efforts to control malaria should be examined carefully, because their massive implementation without vector control and/or treatment of patients with asymptomatic long-term parasitemia may have negative consequences over evolutionary time scales [27].

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Appendix A. Making use of the fact that the demographic parameters (b, μ_i, and α) are much smaller than the malaria-related disease parameters (β_h, β_m, γ, and μ_m), we introduced new parameters built from the "old" after re-scaling them with ϵ. Specifically, $b = \epsilon\tilde{b}$, $\mu_h = \epsilon\tilde{\mu}$, and $\alpha = \epsilon\tilde{\alpha}$ where $\epsilon > 0$ is small. By assuming $j_i = 0$, $f = 0$, $\alpha_{Ti} = 0$ and $\delta = 0$ $(i = 1, 2)$, we can re-write Eqs. (2a)-(2b) and (2e)-(2i) using the new parameters as follows:

$$\frac{dQ}{dt} = G(Q) + \epsilon F(Q), \tag{21}$$

where

$$Q = \begin{pmatrix} y_1 \\ y_2 \\ z_1 \\ z_2 \\ m \\ g \\ N \end{pmatrix}, \quad G(Q) = \begin{pmatrix} k\beta_h(1-g-y_1-z_1)m - \gamma y_1 \\ \zeta k\beta_h(g-y_2-z_2)m - \gamma y_2 \\ (1-k)\beta_h(1-g-y_1-z_1)m - \gamma z_1 \\ (1-k)\beta_h(g-y_2-z_2)m - \gamma z_2 \\ \beta_m(y_1+y_2+z_1+z_2)(1-m) - \mu_m m \\ 0 \\ 0 \end{pmatrix}, \tag{22}$$

and

$$F(Q) = \begin{pmatrix} F_1 \\ F_2 \\ F_3 \\ F_4 \\ F_5 \\ F_6 \end{pmatrix} = \begin{pmatrix} -(\tilde{\mu}_1+\tilde{\alpha}_1)y_1 - y_1\tilde{f}(N) \\ -(\tilde{\mu}_2+\tilde{\alpha}_2)y_2 - y_2\tilde{f}(N) \\ -\tilde{\mu}_1 z_1 - z_1\tilde{f}(N) \\ -\tilde{\mu}_2 z_2 - z_2\tilde{f}(N) \\ 0 \\ P_2\tilde{b}(N) - \tilde{\mu}_2 g - \tilde{\alpha}_2 y_2 - g\tilde{f}(N) \\ N\{(P_1+P_2)\tilde{b}(N) - \tilde{\mu}_1(1-g) - \tilde{\mu}_2 g - \tilde{\alpha}_1 y_1 - \tilde{\alpha}_2 y_2\} \end{pmatrix}. \tag{23}$$

Here, it is assumed that

$$\tilde{f}(N) = (P_1+P_2)\tilde{b}(N) - \tilde{\mu}_1(1-g) - \tilde{\mu}_2 g - \tilde{\alpha}_1 y_1 - \tilde{\alpha}_2 y_2.$$

The fast dynamics at the disease scale are approximated by Eq (21) when $\epsilon = 0$, in other words by the system

$$\frac{dQ}{dt} = G(Q), \tag{24}$$

which can be reduced to System (14).

Let $E^* = (s_1^*, s_2^*, m^*)$ be a non-trivial equilibrium of (14) under the assumption that N is constant. The stability of E^* is determined by the eigenvalues of the following matrix H:

$$H = \begin{pmatrix} -(\beta_h m^* + \gamma) & 0 & \beta_h(1-g-s_1^*) \\ 0 & -(1-k(1-\zeta))\beta_h m^* - \gamma & (1-k(1-\zeta))\beta_h(g-s_2^*) \\ \beta_m(1-m^*) & \beta_m(1-m^*) & -\beta_m(s_1^*+s_2^*) - \mu_m \end{pmatrix}$$

The matrix H is equivalent to the matrix $M - D$ where

$$M = \begin{pmatrix} 0 & 0 & \beta_h(1-g-s_1^*) \\ 0 & 0 & (1-k(1-\zeta))\beta_h(g-s_2^*) \\ \beta_m(1-m^*) & \beta_m(1-m^*) & 0 \end{pmatrix}$$

and

$$D = \begin{pmatrix} \beta_h m^* + \gamma & 0 & 0 \\ 0 & (1-k(1-\zeta))\beta_h m^* + \gamma & 0 \\ 0 & 0 & \beta_m(s_1^*+s_2^*) + \mu_m \end{pmatrix}.$$

Because all the elements of M are non-negative ($1-g-s_1 = x_1 > 0$ and $g - s_2 = x_2 > 0$) and D is a diagonal matrix with positive diagonal elements, it follows that all eigenvalues of H have negative real parts only if the dominant eigenvalue of the matrix MD^{-1} is less than one [14]. Using the results in [16], it can be shown that the dominant eigenvalue of the matrix MD^{-1} is less than one. Thus, E^* is locally asymptotically stable. Therefore, the system (21) with $\epsilon = 0$ contains a two-dimensional stable manifold of steady states $U_0(g, N) = (y_1^*, y_2^*, z_1^*, z_2^*, m^*, g, N)^T$.

Appendix B. Using the re-scaled time $\tau = \epsilon t$, we can re-write the full system, Eqs. (2a)-(2b) and (2e)-(2i) with $j_i = 0$, $f = 0$, $\alpha_{Ti} = 0$ and $\delta = 0$ $(i = 1, 2)$, as follows:

$$\begin{aligned}
\epsilon\frac{dy_1}{d\tau} =& k\beta_h(1-g-y_1-z_1)m - \gamma y_1 - \epsilon y_1\{(\tilde{\mu}_1-\tilde{\mu}_2)g + \tilde{\alpha}_1(1-y_1) \\
& - \tilde{\alpha}_2 y_2 + (P_1+P_2)\tilde{b}(N)\}, \\
\epsilon\frac{dy_2}{d\tau} =& \zeta k\beta_h(g-y_2-z_2)m - \gamma y_2 - \epsilon y_2\{(\tilde{\mu}_1-\tilde{\mu}_2)(g-1) \\
& - \tilde{\alpha}_1 y_1 - \tilde{\alpha}_2(1-y_2) + (P_1+P_2)\tilde{b}(N)\}, \\
\epsilon\frac{dz_1}{d\tau} =& (1-k)\beta_h(1-g-y_1-z_1)m - \gamma z_1 \\
& - \epsilon z_1\{(\tilde{\mu}_1-\tilde{\mu}_2)g - \tilde{\alpha}_1 y_1 - \tilde{\alpha}_2 y_2 + (P_1+P_2)\tilde{b}(N)\}, \\
\epsilon\frac{dz_2}{d\tau} =& (1-k)\beta_h(g-y_2-z_2)m - \gamma z_2 \\
& - \epsilon z_2\{(\tilde{\mu}_1-\tilde{\mu}_2)(g-1) - \tilde{\alpha}_1 y_1 - \tilde{\alpha}_2 y_2 + (P_1+P_2)\tilde{b}(N)\}, \\
\epsilon\frac{dm}{d\tau} =& (1-m)\{\beta_m(y_1+\rho z_1) + \beta_m(y_2+\rho z_2)\} - \mu_m m, \\
\frac{dg}{d\tau} =& \{(1-g)P_2 - gP_1\}\tilde{b}(N) + (\tilde{\mu}_1-\tilde{\mu}_2)g(1-g) + \tilde{\alpha}_1 g y_1 \\
& - \tilde{\alpha}_2(1-g)y_2, \\
\frac{dN}{d\tau} =& N\{(P_1+P_2)\tilde{b}(N) - \tilde{\mu}_1(1-g) - \tilde{\mu}_2 g - \tilde{\alpha}_1 y_1 - \tilde{\alpha}_2 y_2\}.
\end{aligned} \tag{25}$$

This system has a two-dimensional slow manifold :

$$M = \{(y_1, y_2, z_1, z_2, m, g, N) : y_i = y_i^*(g, N), z_i = z_i^*(g, N), m = m^*(g, N), i = 1, 2\}$$

which normally would be hyperbolically stable as it consists of a set of hyperbolic equilibria of the fast system (10). Here y_i^* and z_i^* $(i = 1, 2)$ are given in Eqs (15).

The slow dynamics on M is described by the equations

$$\begin{aligned} \frac{dg}{d\tau} &= \{(1-g)P_2 - gP_1\}\tilde{b}(N) + (\tilde{\mu}_1 - \tilde{\mu}_2)g(1-g) + \tilde{\alpha}_1 g y_1^* - \tilde{\alpha}_2(1-g)y_2^*, \\ \frac{dN}{d\tau} &= N\{(P_1 + P_2)\tilde{b}(N) - \tilde{\mu}_1(1-g) - \tilde{\mu}_2 g - \tilde{\alpha}_1 y_1^* - \tilde{\alpha}_2 y_2^*\}, \end{aligned} \tag{26}$$

where $P_1 = (1 - g/2)^2$ and $P_2 = g(1 - g/2)$. Beause $P_1 + P_2 = 1 - g^2/4$ and that $(1-g)P_2 - gP_1 = -\frac{g^2}{2}(1 - \frac{g}{2})$, we can rewrite the slow system (26) as follows:

$$\begin{aligned} \frac{dg}{d\tau} &= -\frac{1}{2}\tilde{b}(N)g^2(1 - g/2) + r_1(g), \\ \frac{dN}{d\tau} &= N\{\tilde{b}(N)(1 - g^2/4) - r_2(g)\}, \end{aligned} \tag{27}$$

where

$$\begin{aligned} r_1(g) =& (\tilde{\mu}_1 - \tilde{\mu}_2)g(1-g) + \tilde{\alpha}_1 g y_1^* - \tilde{\alpha}_2(1-g)y_2^*, \\ r_2(g) =& \tilde{\mu}_1(1-g) + \tilde{\mu}_2 g + \tilde{\alpha}_1 y_1^* + \tilde{\alpha}_2 y_2^*. \end{aligned}$$

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