The Impact of Antimalarial Drugs on the Evolution of Malaria Parasite

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Malaria parasites have a remarkable ability to develop resistance to antimalarial drugs. By mutating away from a presumed evolutionary-optimal biochemical structure, the drug resistant parasites are likely to be disadvantageous in the absence of drug. Therefore, the survival probability of parasites encoding drug resistance depends on both the intensive selective pressure by the antimalarials and the natural selection force acting against mutation. In this paper, a simplified model is developed to study the population genetics of malaria parasites. The impact of both monotherapy and combination therapy on parasite fitness are evaluated.

I. INTRODUCTION

As one of the most deadly parasitic diseases, malaria is responsible for over 200 million clinical cases and accounts for nearly one million deaths in 2009 [1]. The multistage life cycle of malaria parasite involves both asexual multiplications in the host blood stream as well as sexual reproduction within the vector mosquitoes. When a female Anopheles mosquito carrying malaria parasites feeds on a human, the parasites enter human blood stream in the form of sporozoites, which first invade liver cells producing thousands of haploid forms (merozoites) in the liver. As the merozoites re-enter the blood stream, they invade other red blood cells, initiating a 48 hour asexual reproduction cycle. Some merozoites, instead of continuing in the asexual replication phase, may develop into the sexual forms called gametocytes in the host red cells. When a mosquito bites an infected human, the gametocytes are then ingested and developed into mature sex cells (gametes) within the mosquito, beginning the sexual phase of parasite life cycle [2].

In the past 50 years, malaria parasites have been under intensive selection pressure to develop resistance to the prevailing antimalarial drugs including chloroquine (CQ), sulfadoxine-pyrimethamine (SP) and mefloquine (MQ). Antimalarial drug resistance has increasingly emerged to be a major public health problem which hinders the control of malaria [1]. Combination therapy with antimalarials of dissimilar mechanisms of action has now become the strategy to combat *plasmodium falciparum* malaria in the endemic areas.

Generally, malaria parasites develop drug resistance via two possible pathways: altered gene expression, or altered protein structure that reduces drug-binding efficacy [3]. Though these changes help parasites to survive under drug pressure, by mutating away from a presumed evolutionary-optimal biochemical structure, mutated parasites are also believed to be less fit genetically in absence of the drug [4]. Therefore, the survival probability of drug resistant malaria parasites depends on both the selective pressure by antimalarials as well as natural selection acting against mutation. In this paper, a simplified model is developed to study the population genetics of malaria parasites. Specifically, several questions are attempted to be addressed: first, how does natural selection act on the evolution of malaria parasites; second, how rapidly the frequency of drug resistant mutant can increase under constant drug pressure; third, what is the impact of combination therapy on the parasite fitness. For simplicity, this model discounts the effect of inbreeding and the population size is assumed to be fixed.

II. BASIC MODEL AND ASSUMPTIONS

(a) Estimating the effective population size N_e

In a diploid population with effective population size $2N_e$, the probability of two alleles sharing the same parent is $1/2N_e$. Conversely, the probability that they do not coalesce is $1 - 1/2N_e$. Based on coalescence theory, the probability that two alleles share the same ancestor t generations ago can be modeled as a geometric distribution as follows:

$$P_c(t) = \left(1 - \frac{1}{2N_e}\right)^{t-1} \left(\frac{1}{2N_e}\right), \tag{1}$$

where $p_c(t)$ is the probability of coalescence. From (1), we can estimate that the expected time of coalesce is $2N_e$ and the variance is $(1-1/2N_e)(2N_e)^2$. When N_e is large, variance can be approximate to be $(2N_e)^2$.

Based on a Bayesian coalescent approach as discussed by Lee KS *et al.* [5], the most probable time to coalesce for *p. knowelsi*, a primate malaria parasite, is approximately $257,000$ years ago. From this value, we could estimate the effective population size to be in the order of 10^5. Surprisingly, our crude estimation agrees very well with Lee KS *et al.* [5] and Hughes *et al.* [6]. For subsequent sections in the paper, $N_e = 10^5$ is used to estimate the effective population size, which is assumed to be constant.

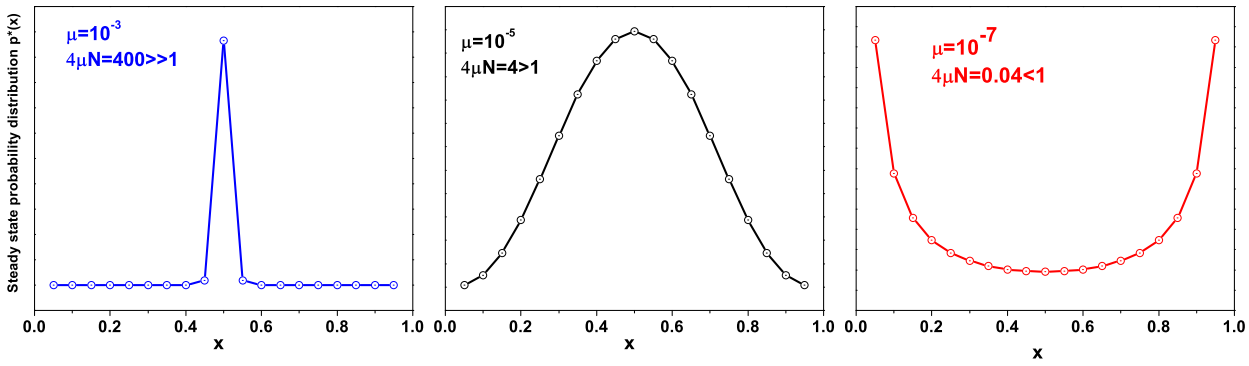

FIG. 1. Distribution of frequencies of a neutral allele in populations of fixed size at different mutation rates ($\mu = 10^3, 10^5, 10^7$). When $4\mu N > 1$, the distribution centres around $x = 0.5$, whereas when $4\mu N < 1$, genetic drift becomes significant (red curve).

(b) Mutation rate on the genetic variation of malaria parasite

In general, it is extremely difficult and inaccurate to measure parasite mutation rates due to their low frequency. In Hastings' model for the origins and spread of drug-resistant malaria [7], a fairly wide range of mutation rate from 10^{-3} to 10^{-7} per generation was assumed. In this section, the impact of mutation rate on the genetic variation of malaria parasite is investigated. In the simplest model, a constant population size $N = 10^5$ is assumed. The wild-type and mutant alleles are denoted as A and a respectively. The rate of mutation from a to A is denoted as μ_A and the reverse as μ_a.

From the *Forward Kolmogorov* equation, we can obtain that at steady state:

$$\frac{\partial p^*(x)}{\partial t} = -\frac{\partial}{\partial x}[v(x)p^*(x)] + \frac{\partial^2}{\partial x^2}[D(x)p^*(x)] = 0, \quad (2)$$

where the drift term $v(x)$ expresses the rate with which the position changes from x due to the transition rates and the diffusion coefficient $D(x)$ captures the probabilistic nature of the process. For a fixed population with mutation, reproduction and selection, $v(x)$ and $D(x)$ can be calculated as follows:

$$\begin{aligned} D_{\text{diploid}}(x) &\approx \frac{1}{4N}x(1-x) \\ v(x) &= \frac{s}{2}x(1-x) + \mu_A(1-x) + \mu_a(x) \end{aligned} \quad (3)$$

In the special case of no selection ($s = 0$), the steady state probability distribution of a wild type allele A after neutral mutation ($\mu_A = \mu_a$) can be derived from the following equation:

$$\begin{aligned} p^*(x) &\propto \frac{1}{x(1-x)} \cdot x^{4N\mu_1}(1-x)^{4N\mu_2} \cdot e^{2Nsx} \\ p^*(x) &\propto [x(1-x)]^{4\mu N-1} \end{aligned} \quad (4)$$

Figure 1 illustrates the impact of mutation rate on the distribution of steady state frequency in a fixed size population. When $\mu = \mu_A = \mu_a = 10^5, 4\mu N = 4 \sim 1$; steady state probability distribution $p^*(x)$ follows a Gaussian-like curve centered at $x = 0.5$. When the mutation rate is appreciably higher ($\mu = 10^3$), $p^*(x)$ becomes highly centralized around $x = 0.5$. However, when the mutation rate is significantly lower, $p^*(x)$ has peaks at either extreme– a situation where genetic drift is dominant. In the context of malaria parasites, the effect of genetic drift is assumed to be small due to the considerably large population size and wide genetic diversity. Therefore, a moderate mutation rate in the order of 10^5 (*i.e.*, $1/2N$) is used for subsequent analysis, where applicable.

(c) Selection force on the survival probability of malaria parasite

Malaria parasites have a remarkable ability to develop resistance to antimalarial drugs. As all mutations which deviate away from the presumed optimal condition are likely to be disadvantageous in the absence of drug, the cost of parasite mutation s against natural selection is an important parameter to consider in the modeling of parasite population genetics [3]. In the past, a number of studies have investigated the evolution of mutant malaria parasites, but the opinions on the impact of natural selection are divided. Some believes that the natural selection pressure against the cost of mutation is minor and can be safely ignored, whereas others report biochemical as well as laboratory evidence for the cost of resistance. In this section, the cost against natural selection is reviewed in relation to the *probability of loss* in a diploid population.

From the *Backward Kolmogorov* equation, we can obtain that at steady state:

$$\frac{\partial p(x,t|y)}{\partial t} = v(y)\frac{\partial p}{\partial y} + D(y)\frac{\partial^2 p}{\partial y^2} = 0 \quad (5)$$

Denote the fixation probability as $\Pi_1^*(y)$ that allele A

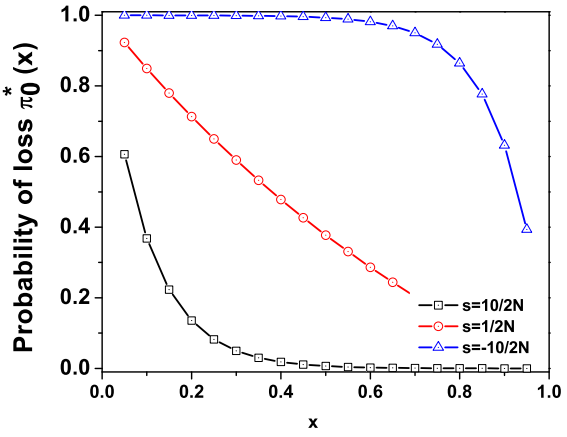

FIG. 2. The probability of losing an allele A is plotted against the allele frequency x. When the selection force is weak ($s = 1/2N$), the probability of loss decreases almost linear with x. When the selection force is significant ($s = \pm 10/2N$), advantageous selection leads to fixation and deleterious selection leads to loss.

with a starting composition y is fixed after a long time. Similarly, the probability of loss is denoted by $\Pi_0^*(y)$. By definition, $\Pi_0^*(y) = 1 - \Pi_1^*(y)$. To solve (5), we have the following relations:

$$\begin{aligned} v(y)\frac{d\Pi^*(y)}{dy} + D(y)\frac{d^2\Pi^*(y)}{dy^2} &= 0 \\ \Pi_1(y) &= \frac{1-e^{-2Nsy}}{1-e^{-2Ns}} \\ \Pi_0(y) &= 1 - \Pi_1(y) \end{aligned} \tag{6}$$

Based on (6), three specific cases are considered as shown in Figure 2. When the selection force is fairly weak ($s = 0$ or $s \ll 1/2N$), the probabilityprobability of loss decreases linearly with allele probability x. For example, when a new mutation encoding drug resistance first appear in a diploid population with a probability $x = 1/2N$, the probability of losing this mutant is $1 - 1/2N$, under weak selection force. However, if the cost of mutation is significant (*i.e.*, $s \ll -1/2N$), the probability of losing this mutant is almost certain even at the extreme case where majority of the population has mutated simultaneously. On the other hand, if the mutation is advantageous, the probability of losing the mutant is considerably lower and eventual fixation if likely with increasing x.

In the context of malaria parasite, the force of natural selection is unlikely to be intensive given that drug resistant parasites could still survive for tens of years in the absence of the drug [8]. However, biochemical as well as laboratory evidence suggests that the cost of mutation is non-trivial [9]. Therefore a moderate estimation of $s = 0.005$ is assumed in subsequent sections, where applicable.

Genotype	A	a
Initial frequencies x_{initial}	x	$1-x$
Fitness coefficient	w_1	w_2
Relative fitness	$w_1/\bar{w}$	$w_2/\bar{w}$
After selection x_{after}	$w_1x/\bar{w}$	$w_2(1-x)/\bar{w}$
$\bar{w} = xw_1 + (1-x)w_2$		
$\Delta x = x_{\text{after}} - x_{\text{initial}} = \frac{d\bar{w}}{dx}(1-x)x/\bar{w}$		

TABLE I.

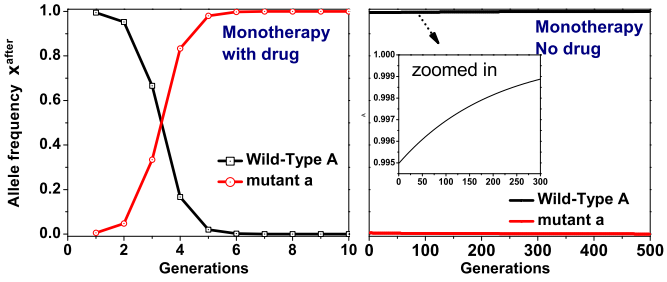

FIG. 3. Frequencies of wild type allele (black) and mutant allele (Red) after each selection generation. (haploid population)

III. ANTIMALARIAL DRUG EFFECT ON THE POPULATION GENETICS OF MALARIA PARASITE

In the past 50 years, a number of antimalarial drugs have been developed, clinically used, and then ceased to be effective due to the notable ability of malaria parasite to develop drug resistant mutants [1]. It is a question of how rapidly the drug resistant mutant can multiply under constant drug pressure and whether this mutant could survive for long enough against natural selection even in the absence of the drug. In this section, both monotherapy and combination therapy are considered in understanding the antimalarial treatment on the population genetics of malaria parasite.

(a) Monotherapy

Assume that the parasite has two loci at which there are two allelic forms A/a and B/b. Whereas drug α and β could selectively kill 90% of the wild-type alleles A and B, alleles a and b confer resistance to the antimalarial drug α and β respectively. For monotherapy, only A/a under the selection pressure of drug α are considered in the section for now.

Since malaria parasites are predominantly haploids in human blood cells, we first consider the effect impact of monotherapy on the genotype frequencies of a haploid population. Table I describes the calculation method.

At the absence of drug, natural selection act against drug resistant mutants. At $s = 0.005$, the fitness coefficient of mutant a (w_2) is estimated to be 0.995 whereas

Genotype	AA	Aa	aa
Initial frequencies x_{initial}	x^2	$2(1-x)x$	$(1-x)^2$
Fitness coefficient	w_{11}	w_{12}	w_{22}
Relative fitness	$w_{11}/\bar{w}$	$w_{12}/\bar{w}$	$w_{22}/\bar{w}$
After selection x_{after}	$\frac{w_{11}}{\bar{w}}x^2$	$\frac{w_{12}}{\bar{w}}2x(1-x)$	$\frac{w_{22}}{\bar{w}}(1-x)^2$
$\bar{w} = x^2 w_{11} + 2(1-x)x \cdot w_{12} + (1-x)^2 w_{22}$			
$\Delta x = x_{\text{after}} - x_{\text{initial}} = \frac{d\bar{w}}{dx}(1-x)x/\bar{w}$			

TABLE II.

w_1 is 1. When antimalarial drug α is applied, $w_1^{\text{drug}} = 0.1$ (*i.e.*, only 10% of wild type allele A could survive through drug after one generation) whereas $w_2^{\text{drug}} = 1$. Base on Table I, the relative frequencies of wild type allele A and mutant a after each generation are plotted in Figure 3. The black curve represents the frequency of A and the red curve represents the frequency of mutant a. The initial frequency for allele A is assumed to be 0.995 in both cases because mutants should be rare under natural selection. By natural selection, the wild-type allele exists predominantly. However, the selection happens very slow: more than 300 generations are required to increase x from 0.995 to 0.999. On the other hand, when drug is applied, the frequency of wild-type allele drops abruptly to 0 within 8 generations. Though starting at a very low frequency, drug resistant mutant a achieves population fixation in 8 generations.

Gametocytes are the sexual phase of the malaria parasite and are essential for disease transmission via mosquito. Some antimalarial drugs are reported to affect gametocytes production in vivo leading to a potential increase in transmission. In this section, we try to understand drug effect on gametocytes from the perspect of population genetics.

In the diploid population model, the effect of monotherapy on the genotype frequencies can be summarized as Table II.

Assume w_{11}, w_{12} and w_{22} are 1, 0.995 and 0.995^2 respectively in the absence of the drug, and $w_{11}^{\text{drug}} = w_{12}^{\text{drug}} = 0.1$ and $w_{22}^{\text{drug}} = 1$ when antimalarial drug α is applied. x is assumed to be 0.995 initially. Figure 4 illustrates the drug effect on genotype frequencies. Under the force of natural selection, wild-type AA exists predominantly. However, when drug α applied, the frequency of mutant aa rapidly rise and achieves population fixation in 18 generations.

(b) Combination Drug therapy

As more clinical cases report monotherapy failure, combination therapy with antimalarials of dissimilar mechanisms of action has now become the strategy to combat *plasmodium falciparum* malaria in the endemic areas. In the following section, the impact of combination therapy on both haploid and diploid parasite population

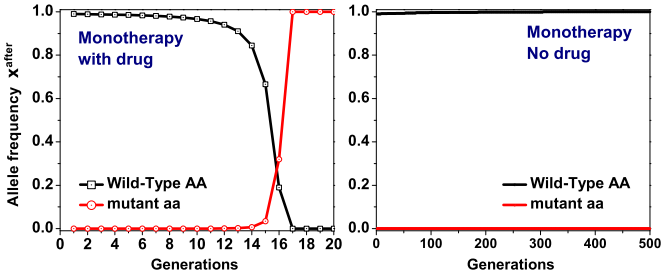

FIG. 4. Frequencies of wild type parasite containing AA (black) and mutant parasite containing aa (Red) after each selection generation. (diploid population)

Genotype	AB	Ab/aB	ab
Initial frequencies x_{initial}	x^2	$2(1-x)x$	$(1-x)^2$
Fitness coefficient	w_{11}	w_{12}	w_{22}
Relative fitness	$w_{11}/\bar{w}$	$w_{12}/\bar{w}$	$w_{22}/\bar{w}$
After selection x_{after}	$\frac{w_{11}}{\bar{w}}x^2$	$\frac{w_{12}}{\bar{w}}2x(1-x)$	$\frac{w_{22}}{\bar{w}}(1-x)^2$
$\bar{w} = x^2 w_{11} + 2(1-x)x \cdot w_{12} + (1-x)^2 w_{22}$			
$\Delta x = x_{\text{after}} - x_{\text{initial}} = \frac{d\bar{w}}{dx}(1-x)x/\bar{w}$			

TABLE III.

is evaluated. Assuming independent pathway and wild-type allele A/B occur at same frequency of x, we can calculate the effect of simultaneous treatment of drug α and β in a haploid population based on the Table III.

The fitness coefficients w_{11}, w_{12} and w_{22} are assumed to be 1, 0.995 and 0.995^2 respectively in the absence of the drug. When antimalarial drug α is applied, $w_{11}^{\text{drug}} = 0.01, w_{12}^{\text{drug}} = 0.1$ and $w_{22}^{\text{drug}} = 1$.

Similarly, in a diploid parasite population, the effect of combination therapy on the genotype frequencies can be summarized as Table IV. The fitness coefficients under drug pressure are shown in red. Figure 5 illustrates the combination drug effect.

(c) Discussions

Based on our model, the evolution of drug resistant malaria parasite can be better understood. Three specific observations were made by comparing x_{after} under the selection force by nature, monotherapy and combination therapy.

1. Weak force by natural selection in malaria parasites. Figure 3 shows that in a haploid parasite population, more than 300 generations are required to

		x^2		$2x(1-x)$		$(1-x)^2$	
		AA		Aa		aa	
x^2	BB	x^4	0.01	$2x^3(1-x)$	0.01	$x^2(1-x)^2$	0.1
$2x(1-x)$	Bb	$2x^3(1-x)$	0.01	$4x^2(1-x)^2$	0.01	$2x^3(1-x)$	0.1
$(1-x)^2$	bb	$x^2(1-x)^2$	0.1	$2x^3(1-x)$	0.1	$(1-x)^4$	1

TABLE IV.

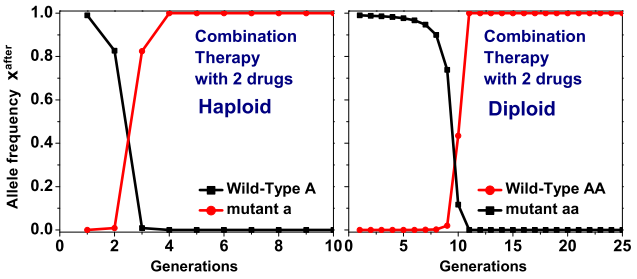

FIG. 5. Frequencies of wild type parasite (Black) and mutant parasite (Red) after combination drug treatment.

increase the frequency of wild-type parasite from 0.995 to 0.999. On the other hand, when drug is applied, the frequency of wild-type allele drops abruptly to zero in less than 10 generations. Similar trend is presented in Figure 4 for a diploid parasite population. That means at current situation, it is close to impossible to have a drug resistant parasite to go extinction. This is consistent with the persistent drug resistance described by Escalante *et al.* [10]. Instead of relying on the weak force of natural selection, we should rather take active measures to reduce the rise of more drug resistant parasites including reduce the transmission via controlling the vector mosquito and avoid the misuse and exploitation of current antimalarial drugs.

2. Combination therapy as preferred treatment to monotherapy. Under the intensive selection pressure of antimalarial drugs, the frequency of wild-type parasite drops rapidly in both monotherapy and combination therapy. However, based on the speed of extinction, combination therapy appears to be a more efficient treatment than monotherapy. For the haploid parasite population in human blood, the wild type parasite dies off after 8 generations under monotherapy whereas only 4 generations are required when combination therapy is applied. Similarly, in a diploid population (gametocytes), the expected time to extinction reduces from 17 to 11 when combination therapy is used instead of monotherapy. The result suggests that in terms of efficient killing parasite, combination therapy is preferred. However, there is a risk of developing multigenic drug resistance. It is hoped that multigenic drug resistant parasites are significantly disadvantaged in the absence of drug due to the considerable disruption of metabolism and can be cleared out by the force of natural selection.

3. Haploid populations are more adversely affected after drug treatment. Compare the haploid population with the diploid population, the time to extinction is significantly shorter in haploids under both monotherapy and combination therapy. This observation may provide indirect support to some *in vivo* study that drug treatment may lead a potential increase in transmission. As merozoites invade human red blood cells, majority undergoes the asexual reproduction phase while a few develop to gametocytes. Under the intensive drug pressure, the haploid population being more adversely affected, may lead to a effectively larger fraction of gametocytes. Therefore, it is possible to explain the potential rise in transmission after drug treatment based on the model we propose.

IV. CONCLUSION

In conclusion, our model attempts to study the evolution of drug resistant malaria parasite by looking at the relative selection force by nature and by antimalarial drugs. Both monotherapy and combination therapy are analyzed and combination therapy is proven to be a more efficient treatment, though at the risk of developing multigenic drug resistant parasites. Both asexual phase and sexual phase are considered to gain a more complete view of the evolution of malaria parasite.

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