A major issue in the control of malaria is the evolution of drug resistance. Ecological theory has demonstrated that pathogen superinfection and the resulting within-host competition influences the evolution of specific traits. Individuals infected with Plasmodium falciparum are consistently infected by multiple parasites; however, while this probably alters the dynamics of resistance evolution, there are few robust mathematical models examining this issue. We developed a general theory for modelling the evolution of resistance with host superinfection and examine: (i) the effect of transmission intensity on the rate of resistance evolution; (ii) the importance of different biological costs of resistance; and (iii) the best measure of the frequency of resistance. We find that within-host competition retards the ability and slows the rate at which drug-resistant parasites invade, particularly as the transmission rate increases. We also find that biological costs of resistance that reduce transmission are less important than reductions in the duration of drug-resistant infections. Lastly, we find that random sampling of the population for resistant parasites is likely to significantly underestimate the frequency of resistance. Considering superinfection in mathematical models of antimalarial drug resistance may thus be important for generating accurate predictions of interventions to contain resistance.

**Keywords:** Plasmodium falciparum; malaria; superinfection; drug resistance; evolution
sulphadoxine-pyrimethamine (SP) is of great interest for understanding the factors that are likely to be important for the evolution of resistance to artemisinin-class drugs and to the artemisinin combination therapies that are now the first-line treatment for *Plasmodium falciparum* malaria in much of the world.

The existing mathematical theory describing the evolution of resistance is poorly developed with respect to superinfection and within-host competition. Models of superinfection in other organisms [2,3] either have assumed that a dominant strain could displace another pathogen and take over the host, but not vice versa, or have not allowed for transmission from coinfected states [13,14]. Neither of these approaches is biologically relevant in malaria, where superinfection is the rule rather than the exception, and evidence from the field [15,16] and murine models [17–19] has demonstrated the impact of within-host competition on the survival and transmissibility of genetically distinct malaria clones.

In malaria, the mathematical theory of superinfection was developed by Walton, Macdonald, Irwin, Dietz and Bailey [5,20–24]. Their models allow for superinfection, meaning that multiple genetically distinct clones coinfect a host, and were developed to aid in matching assumptions of the transmission rate with clearance rates. Although the models generally fit the data on prevalence better than the assumption that additional infections have no effect on the clearance rate [20], epidemiological models of this type have not been applied to problems of within-host competition and drug resistance, where it is often assumed that individuals are infected by either drug-resistant or drug-sensitive parasites only [25–27].

In their model of antimalarial resistance, Koella & Antia [28] assume that individuals can be superinfected by both drug-resistant and drug-sensitive infections (heterotypic) but not by two drug-resistant or drug-sensitive types (homotypic). They assume that individuals with heterotypic infections transmit both parasites at the same rate regardless of the composition of drug-resistant and drug-sensitive parasites in the population. This significantly biases transmission, particularly when resistance is first emerging, and drug-resistant parasites are rare. As has been shown in species coexistence models [29,30], the only way that one species can exclude another is if there is significant competition in superinfected hosts, and in malaria, there is strong evidence that drug-resistant and drug-sensitive parasites compete in a host, and removing one will increase the fitness advantage of the other [31]. Because the full costs of competition are not embedded in the model (i.e. individuals cannot be multiply infected by the same type of parasite), when the fitness cost of resistance is low (but not zero), the model predicts coexistence even when there is no treatment in the population [28], a result that is not biologically plausible (see electronic supplementary material, appendix SI).

In this paper, using a general formulation of malaria superinfection, we present a general theory for the evolution of resistance when superinfection occurs. Building on population genetics models for malaria [32–34] as well as past epidemiological models [25], the approach presented here allows for an examination of some of the basic questions involved in how competition affects the spread of drug resistance in different environments.

Our model provides a new way of thinking about modelling antimalarial drug resistance, incorporating many of the concepts that are in common use in malaria epidemiology today [35], and suggests ways to better understand the important control points and identify new directions for future research.

### 2. METHODS

#### (a) Superinfection model

To model the evolution of resistance with superinfection, we modified a Markov chain model for superinfection and clearance to include clinical malaria and antimalarial drugs; the original model was developed for malaria [22]. MOI (the number of pathogens per host individual) increases as new infections occur, but decreases as they clear. The state variables in the model represent the fraction of the population that has a given MOI: \( X_i \) denotes the fraction of hosts with an MOI of \( i \). We have extended the model to track the MOI of sensitive and resistant types (figure 1). Let \( X_{ij} \) denote the fraction of the population with \( i \) sensitive and \( j \) resistant strains. The equations are formulated so that \( \sum_j X_{ij} = 1 \), and hence the sum of the time derivatives \( \sum_j \dot{X}_{ij} = 0 \). Thus, the values of the state variables describe the joint distribution of resistant and sensitive phenotypes in a population.

#### (b) Entomology

The dynamics of infection in the model follow a notation similar to that of single-infection models by Macdonald [36], as modified by Smith & McKenzie [37]. Vectorial capacity (\( V \)), the number of infectious bites by a mosquito over its lifetime, is given by the formula \( V = ma^2e^{-mr}g \), where \( m \) denotes the number of mosquitoes per human and \( a \) is the number of bites on humans per mosquito per
day. The instantaneous death rate is \( g \) (\( e^{-g} \) is the probability of a mosquito surviving 1 day) and \( n \) is the number of days required for sporogony.

The daily entomological inoculation rate (EIR), the number of infectious bites per person per day, is calculated as the product of vectorial capacity and the fraction of mosquitoes that are infectious \( (P(1 + aP/g)) \), where \( P \) is the proportion of the bites on the infected human population that infect mosquitoes, assuming transmission efficiency \( c \) from humans to mosquitoes \( (P = cX_{i,0} \) where \( i \neq 0 \) or \( j \neq 0 \)). The force of infection, or happenings rate \( (\dot{h}) \), is \( \delta \text{EIR} \), where \( \delta \), the infectivity rate, is the fraction of bites on humans that produce a patent infection.

(c) Competition and a biological cost of resistance

The model ignores fluctuations in the abundance of parasites within a host, but competition is naturally incorporated into the model as a transmission bottleneck at the mosquito. In the absence of any biases, the probability of a mosquito transmitting either a resistant or a sensitive parasite is a function of the proportion of each type ingested (assuming there is no preference for selfing). We assume that resistant genotypes in each gamete in the mosquito are lower than the proportion of each type ingested (assuming there is no mitting either a resistant or a sensitive parasite is a function of the model as a transmission bottleneck at the mosquito. In

The model ignores fluctuations in the abundance of parasites (primarily fever), which develop at rate \( \psi \). The rate at which clinical symptoms arise is independent of MOI. A fraction, \( \rho \), of symptomatic patients are assumed to use drugs and successfully clear all sensitive parasites. The drug usage rate is assumed to be constant over each simulation, but is varied among simulations as noted. Treatment of resistant parasites is assumed to be ineffective.

(d) Clinical infections and drug use

Drug use is assumed to be associated with clinical symptoms (primarily fever), which develop at rate \( \psi \). The rate at which clinical symptoms arise is independent of MOI. A fraction, \( \rho \), of symptomatic patients are assumed to use drugs and successfully clear all sensitive parasites. The drug usage rate is assumed to be constant over each simulation, but is varied among simulations as noted. Treatment of resistant parasites is assumed to be ineffective.

(e) Equations

The dynamics of the state variable, \( X_{i,0} \), which denotes the fraction of the population with \( i \) sensitive and \( j \) resistant strains, are thus described by the following set of coupled ordinary differential equations.

The equation describing the change in the proportion of uninfected hosts \((i = 0, j = 0)\) is

\[ \dot{X}_{i,0} = -hX_{i,0} + r_{i,0}X_{1,0} + r_{i,1}X_{i,1} + \sum_{i=1}^{N} \rho_{i}X_{i,0}, \]

where \( r \) is the recovery rate of an infection consisting of \( i \) sensitive and \( j \) resistant strains, \( h \) is the force of infection, \( \psi \) is the rate that clinical symptoms develop, and \( \rho \) is the fraction of those patients that are treated and clear the infection successfully.

For individuals infected only with drug-sensitive clones \((i > 0, j = 0)\),

\[ \dot{X}_{i,j} = -hX_{i,j} - r_{i,j}X_{i,j} + r_{i,j}X_{i,j-1} + \rho_{i}X_{i,j}, \]

where \( g \) is the reduction in the duration of infection owing to the biological cost of resistance, and \( h_{w} \) is the force of infection for drug-sensitive parasites.

For individuals infected only with drug-resistant clones \((i = 0, j > 0)\),

\[ \dot{X}_{0,j} = -hX_{0,j} - r_{i,j}X_{0,j} + r_{i,j}X_{0,j-1} + \rho_{i}X_{i,j}, \]

where \( h_{r} \) is the force of infection for drug-resistant parasites.

For individuals infected with both drug-sensitive and drug-resistant clones \((i > 0, j > 0)\),

\[ \dot{X}_{i,j} = -hX_{i,j} - r_{i,j}X_{i,j} + r_{i,j}X_{i,j-1} + \rho_{i}X_{i,j}, \]

The equations are constrained such that the model has a 'triangular' formulation (as in figure 1).
Triangularity and the neutrality condition

The model presupposes coexistence in the parasite population of a large number of distinct genotypes, but it considers competition solely between drug-sensitive and drug-resistant parasites, which differ both genetically and phenotypically. To describe the effect of competition on the evolution of resistance, it was necessary to establish that the model satisfied general principles of ecological and population genetic neutrality as described by Lipsitch et al. [43]. These principles note that the models of competition between genotypically different but phenotypically similar strains should meet two criteria: (i) the relative fraction of infected and uninfected hosts should not depend on the frequency of either strain; and (ii) the relative frequency of both strains should remain stable for all time greater than zero. In other words, the structure of the model should not, in and of itself, generate coexistence of indistinguishable strains, but mechanisms that could induce coexistence should be introduced explicitly. These principles suggest that the prior superinfection model published by Koella & Antia [28] needs modification because, as formulated, the model structure itself promotes coexistence. In the electronic supplementary material, appendix SI, we describe a model with similar properties to the Koella & Antia superinfection model, in which homotypic superinfection was not allowed, and rigorously demonstrate that coexistence is always an outcome of the model, even when the resistant strain has a fitness cost.

To ensure that coexistence in our model of superinfection, described earlier, is not an artefact of the model formulation, we analysed a special case of the general form of the model, in which the maximum MOI was two. In this case, the model allows for both heterotypic superinfection and homotypic superinfection. Numerical simulations confirm that in the absence of drug treatment, drug-resistant parasites can neither invade nor persist when they have a cost of resistance. We also found that in the absence of either drug pressure or a biological cost of resistance, the model is neutrally stable, provided the transmission rate and the recovery rate of each type in a heterotypic superinfection are exactly equal to each type in a homotypic superinfection. In other words, the rate that individuals become doubly infected with either the same type or a different type must be equal, and the transmission rate of heterotypic and homotypic infections must be the same regardless of strain composition, with an equal rate of transmission of each type from heterotypic infections. Thus, the model in this form does not predict coexistence when there is no specific mechanism promoting its spread.

Both conditions remain true as MOI increases, provided that the model structure maintains a triangular formulation in which every possible combination of the maximum MOI is included (i.e. in a model with a maximum MOI of three, all possible states where the MOI is equal to three must be possible: \(X_{1,0}\) and \(X_{2,1}\) and \(X_{1,2}\) and \(X_{0,3}\)). Otherwise, the model structurally creates a niche that allows for coexistence. Our numerical simulations were done using a general form of the model with the triangularity condition in place and in which the maximum MOI was 30. Parameters used in simulations were consistent with malaria epidemiological literature [35,37,44], and are listed in the electronic supplementary material, table S1.

Frequency of resistance

We employ two measures of the frequency of resistance in complex infections: (i) the fraction of the population that is infected by at least one resistant strain, \(1 - \sum X_{i,0}\); and (ii) the fraction of the parasite load that is resistant, \(\sum j(i + j)X_{i,j}\). The former is important because it tracks nearly exactly with the frequency that a treated clinical infection is resistant, which is the measure by which resistance is often tracked in a population [45,46]. On the other hand, the latter is particularly useful when discussing the role that asymptomatic infections play in the spread of resistance.

3. RESULTS

The model is based on the ideas of malaria superinfection as first laid out by Walton [24] and Macdonald [5] and written down in equation form by Bailey [22]. However, unlike previous models of drug resistance in malaria [25,28], we explicitly model the effect of within-host competition between drug-resistant and drug-sensitive parasites. This allows for an examination of the importance of different types of fitness costs as well as how competition affects the rate at which resistance spreads across differential transmission rates.

To evaluate the model, we ran the system to equilibrium without resistance and then introduced resistance at a low frequency \((10^{-5})\). At low transmission rates (an annual EIR of approximately one or less), the model predicts that drug-resistant parasites will invade and spread, and eventually dominate the drug-sensitive parasites. However, as the transmission rate increases, the ability of drug-resistant parasites to competitively exclude drug-sensitive parasites decreases (see electronic supplementary material, figure S1). This is because the mean MOI increases with the intensity of transmission, measured in terms of either vectorial capacity or EIR, and so competition increases. Thus, at low transmission rates, most individuals are infected with approximately one infection only, and the influence of within-host competition is limited. As the mean MOI increases, it becomes more difficult for resistant parasites to invade because competition within the host increases, overcoming the ability of resistant parasites to spread when they are rare. This is consistent with historical suggestions that resistance to the former first-line malaria drugs, CQ and SP, both emerged from areas of low or unstable transmission [47,48]. Changing the drug treatment rate for any particular transmission level increases the competitive ability of resistant parasites, allowing them to invade even when within-host competition increases at higher transmission levels.

The model also predicts that superinfection modulates coexistence. This result depends on the biological fitness costs as well as the treatment rate. At high treatment rates (80% of clinical infections treated), the drug-resistant parasite competitively excludes the drug-sensitive parasite at all fitness costs at low transmission. As the transmission rate increases (annual EIR approx. 7), coexistence can occur over a large range of fitness costs—even where the fitness cost of clearance is zero (figure 2). At higher transmission rates (annual EIR approx. 25), the range over which coexistence is possible contracts. Lowering the treatment rate (20% of clinical cases treated) shifts the results and allows for coexistence at lower transmission rates, and shrinks the parameter range over which coexistence can occur at higher transmission rates (see electronic supplementary material, figure S2).
was assumed to be 80%. An annual EIR of approximately 25. The treatment rate of clearance is measured as the reduction in the rate of clearance of a resistant parasite relative to a sensitive parasite when in competition. Thus, when there is little or no cost of clearance but a significant cost of transmission, the result is coexistence. As the transmission rate is increased (b), the parameter space over which coexistence can occur is abrogated. In addition, there are significant differences in the range of each parameter over which coexistence can occur. Areas of coexistence are marked by low to moderate fitness costs of clearance and high costs of transmission. In fact, in low-transmission areas, resistant parasites can coexist with sensitive parasites even when there is no fitness cost of clearance but a significant cost of transmission, the result is coexistence. As the transmission rate is increased (b), the parameter space over which coexistence can occur is abrogated. In addition, there are significant differences in the range of each parameter over which coexistence can occur. Areas of coexistence are marked by low to moderate fitness costs of clearance and high costs of transmission. In fact, in low-transmission areas, resistant parasites can coexist with sensitive parasites even when there is no fitness cost of clearance. (a) Moderate transmission is defined as an annual EIR of approximately 7, and (b) high transmission is an annual EIR of approximately 25. The treatment rate was assumed to be 80%.

The fitness of drug-resistant parasites is proportional to both the average duration of an infection and the ability to transmit. The former is a function only of within-host competition, whereas the latter is a function of both within-host and among-host competition, because transmission potential is a function of both transmissibility to a susceptible vector (a function of competition between clones within the host) and the propensity for infectious vectors to infect a new host (the relative fraction of each type). Results from the model suggest that resistant parasites can invade even with significant fitness costs of transmission, but not when the fitness cost of clearance increases significantly. This suggests that the ability to remain competitively infectious within hosts is a stronger determinant of the invasion capacity of drug-resistant parasites than their probability of transmission at any single event.

Competition also affects the rate that resistance spreads in a population. At low transmission rates, increasing the transmission intensity increases the rate that resistance spreads in a population because, as noted earlier, most infected individuals have an MOI of one and there is no competition. Thus, the ability of drug-resistant parasites to competitively exclude drug-sensitive parasites increases as transmission increases. However, as the average MOI increases and competition begins to inhibit the ability of the drug-resistant parasite to exclude drug-sensitive parasites, the rate at which resistance spreads in a population decreases—or, stated another way, the waiting time for resistance to reach a certain threshold value grows longer (electronic supplementary material, figure S3). This change is rapid at first, but as the transmission rate continues to increase, the rate of increase slows.

Superinfection also affects how the frequency of resistance is calculated. Although the prevalence of resistance (the fraction of infected people that harbour at least one resistant parasite) increases concomitantly with the fraction of the population that harbours at least one resistant parasite, the resistance load (the proportion of types that are resistant) changes at a different rate (figure 3). As the transmission intensity increases, the resistance load increases at a rate that is similar to the other measures. However, the rate of increase slows after a point and then falls, while
the prevalence of resistance continues to increase. At high transmission levels, this spread can be more than five percentage points different when the fraction of clinical infections reaches 10 per cent.

4. DISCUSSION
A prior model of superinfection in malaria with drug-resistant and drug-sensitive parasites assumes that heterotypic superinfection is possible but homotypic superinfection is not [28]. However, this assumption means that coexistence will always occur (see electronic supplementary material, appendix SI). The structure of this prior model [28] is also similar to earlier models of species coexistence [29,30], which always predict coexistence unless there is some type of competition in individuals heterotypically superinfected. Thus, coexistence is an artefact of the mathematical model, not a generic property of the underlying biological process.

In this paper, we replace those assumptions with a model that implements a more robust competition framework that allows for a thorough examination of the effect of competition on the spread of drug-resistant malaria parasites in an epidemiological context. We found general conditions for the evolution of resistance as the outcome of within- and among-host competition between two classes of parasite types with differing fitness; the conditions depend on competition, drug pressure and the biological cost of resistance.

Results from a general model demonstrate that the estimate of disease prevalence as a function of the transmission rate was qualitatively similar to that found in other models [5] and similar to estimates of the same relationship in the field [49–51]. Our results also support earlier studies showing a strong relationship between the fitness cost of resistance and transmission [25,33]. When the fitness cost of resistance is low, drug-resistant parasites are able to invade and spread across all transmission levels. However, as the fitness cost of resistance increases, the ability of drug-resistant parasites to invade and spread is reduced. One possible reason previously suggested for this relationship is that the parasite is exposed to a higher level of drug pressure per infection in low-transmission areas because a higher fraction of infections in these areas result in clinical symptoms [33]. Our results suggest that the low force of infection when recolonization of infected individuals is rare plays a significant role in low-transmission areas as well. After drug use eliminates drug-sensitive parasites, individuals harbouring resistant parasites are less likely to be recolonized by drug-sensitive parasites, reducing within-host competition.

Although a biological cost of resistance has been measured in in vitro experiments [52,53] and estimated from the field [11,12], reductions in the competitive ability of the parasite are not expected to be equal across different axes of competition. This has significant biological and epidemiological importance. On the basis of in vitro experiments in which CQ-resistant parasites had an estimated 25 per cent loss of fitness per generation [52], as well as murine models demonstrating slower growth of drug-resistant parasites in the mouse [54,55], it has been suggested that mutations conferring resistance decrease the reproductive efficiency of the parasite and slow growth [10]. The end result of the lower growth rate is assumed to be a decrease in the probability of a parasite being transmitted when a mosquito feeds on blood because of the lower relative numbers of gametocytes. A similar mechanism is also presumed to affect the duration of infection; however, evidence on the relative cost of resistance on clearance is lacking, particular in relation to transmissibility. We found that although the reductions in transmission probability were important, the ability to persist in an infection may be a more important measure of the resistant parasite’s competitive ability, particularly in lower-transmission settings. These results can be partly explained by the vast timescale differences between these processes. Because the time from infection of a susceptible mosquito to infection of a human is an order of magnitude faster than the duration of infection, the benefit of increasing the duration of infection is significantly greater than the benefit of increasing infectiousness within an infection. The result is that resistant parasites can invade even with significant fitness costs of transmission, but not when the fitness cost of clearance increases significantly.

Because the parasite has evolved an extremely long duration of infection to maximize transmission opportunities and can transmit efficiently at very low densities [56], it is not surprising that the effect of changes in the clearance rate is more pronounced on the viability of drug-resistant parasites than on reductions in transmission. However, the effect on attempts to control the spread of resistance is important. Recent evidence has shown the emergence of a delayed clearance phenotype to the drug artemisinin in western Cambodia [57], which has led to calls for implementing a resistance containment strategy. Our results suggest that interventions that can shorten the duration of infection for resistant parasites, such as mass drug administration or mass screening and treating, may be more beneficial than has been recognized by earlier models that ignored superinfection [58].

Within-host competition can also produce coexistence of the two different parasite phenotypes at the population level. Coexistence can occur when the relative advantage of the sensitive parasite, measured as transmission potential over recovery rate, is greater in mixed infections. However, the parameter range over which coexistence can occur is altered by the transmission rate. At low transmission rates, competition is lessened between different phenotypes as well as with the same phenotypes. Individuals with drug-resistant parasites can then be easily reinfected because they have only a few parasites. Thus, if drug-sensitive parasites have an advantage in mixed infections, the result for a fixed treatment rate will be a greater tendency to coexist. On the other hand, as the transmission rate increases, individuals who are treated with drugs are likely to harbour multiple drug-resistant parasite clones. In this case, the invasion capability of drug-sensitive parasites is reduced, making the range over which coexistence can occur smaller.

In terms of designing control strategies, the time until resistance reaches a critical level, which is critically influenced by the transmission and treatment rates, may be a more important measure than the final equilibrium level of resistance. Currently, there is no standardized methodology for assessing the frequency of resistance, though it is generally measured as the proportion of a type specimen
isolated from a clinical infection that did not adequately respond to treatment [46]. However, screening surveys of the human population using in vitro tests to determine resistance of a type specimen has also been suggested as a means to ascertain the frequency of resistance in the population [59]. These sampling mechanisms are not identical when individuals have complex infections, and it is important to understand how these sampling differences may end up measuring radically different quantities. In high-transmission areas, when the frequency of resistance is low, complex infections will primarily consist of drug-sensitive parasites. Because sampling is imperfect, the probability of false negatives (i.e. at least one resistant parasite is present but was not detected) is high [45], which suggests that random screening surveys as a measure of resistance in higher-transmission areas may result in biased results. This is probably also true when withdrawing a drug. For instance, even though the detection of CQ-resistant mutations has dropped to undetectable levels in Malawi [11,60], the complexity of malaria infections suggests that drug-resistant parasites may still be present at low frequencies and that the re-introduction of CQ may be followed by a rapid resurgence in resistant infections.

In this model of malaria superinfection, which was extended to incorporate competition between drug-sensitive and drug-resistant parasites, our results are limited to examining only the axis of competition between drug-resistant and drug-sensitive phenotypes. The evolution of antimalarial drug resistance is obviously a complicated process involving superinfection and many interacting processes, including immunity [25], the patterns of drug use and heterogeneous biting [49]. The results of this simple model clearly demonstrate that within-host competition is a significant component in the emergence and spread of drug resistance even at lower transmission rates. Models that examine the best ways to control or contain the emergence of resistance must take account of superinfection and a variable degree of within-host competition across the spectrum of transmission. In particular, the assumption of a blanket fitness cost of resistance is less reasonable when the duration of infection dominates the probability that resistance will spread. Future studies in this area will include how heterogeneous biting by the mosquito vector changes the dynamics of competition, particularly in low-transmission areas, and how host immunity interacts with virulence to change the dynamics of the emergence of drug resistance.

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