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The evolution of drug-resistant malaria: the role of drug elimination half-life

Ian M. Hastings[1*], William M. Watkins[2] and Nicholas J. White[3,4]

[1]*Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*
[2]*Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L3 5QA, UK*
[3]*Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand*
[4]*Centre for Tropical Diseases, Nuffield Department of Clinical Medicine, University of Oxford, Oxford OX3 9DU, UK*

This paper seeks to define and quantify the influence of drug elimination half-life on the evolution of antimalarial drug resistance. There are assumed to be three general classes of susceptibility of the malaria parasite *Plasmodium falciparum* to a drug: *Res0*, the original, susceptible wildtype; *Res1*, a group of intermediate levels of susceptibility that are more tolerant of the drug but still cleared by treatment; and *Res2*, which is completely resistant to the drug. *Res1* and *Res2* resistance both evolve much faster if the antimalarial drug has a long half-life. We show that previous models have significantly underestimated the rate of evolution of *Res2* resistance by omitting the effects of drug half-life. The methodology has been extended to investigate (i) the effects of using drugs in combination, particularly when the components have differing half-lives, and (ii) the specific example of the development of resistance to the antimalarial pyrimethamine–sulphadoxine. An important detail of the model is the development of drug resistance in two separate phases. In phase A, *Res1* is spreading and replacing the original sensitive forms while *Res2* remains at a low level. Phase B starts once parasites are selected that can escape drug action (*Res1* genotypes with borderline chemosensitivity, and *Res2*): these parasites are rapidly selected, a process that leads to widespread clinical failure. Drug treatment is clinically successful during phase A, and health workers may be unaware of the substantial changes in parasite population genetic structure that predicate the onset of phase B. Surveillance programs are essential, following the introduction of a new drug, to monitor effectively changes in treatment efficacy and thus provide advance warning of drug failure. The model is also applicable to the evolution of antibiotic resistance in bacteria: in particular, the need for these models to incorporate drug pharmacokinetics to avoid potentially large errors in their predictions.

Keywords: malaria; *Plasmodium falciparum*; drug resistance; drug half-life; antibiotics

1. INTRODUCTION

Malaria is a potent and constant threat to public health in much of the developing world. In principle, it could be controlled by preventative public health measures, but in practice, most control is at the level of disease management through drug treatment. Even small decreases in drug efficacy can substantially increase mortality rates (Trape *et al.* 1998) and drug-resistant malaria carries serious cost implications for the developing world (Phillips & Phillips-Howard 1996; Goodman *et al.* 1999). There are relatively few classes of antimalarial drugs (Winstanley 2000) and the most effective means of using these drugs is still a subject for debate (White 1999; Hastings & D'Alessandro 2000). In particular, the definition of optimal patterns of use for drugs with very different pharmacokinetic properties remains unresolved. Drugs with a long elimination half-life have two valuable therapeutic properties. First, they can provide long-term protection against reinfection (up to two months, in the case of pyrimethamine–sulphadoxine (SP)). Prolonged antimalarial drug activity *in vivo* is an advantage to the patient who is recovering from malaria in an area of moderate or high transmission, because further disease episodes are prevented. This assists recovery from anaemia, a major cause of malaria morbidity and a contributor to mortality. Similarly, in epidemic malaria, where the population is highly susceptible and the clinical attack rate can approach 100%, but where the epidemic is of short duration and infrequent (Snow *et al.* 1999), long half-life drugs are of value. Second, long half-life drugs require a few (or only a single) administrations, which reduces the risk of underdosage and some of the problems of compliance. However, drugs that are eliminated slowly persist in the patient once the immediate therapeutic purpose has been achieved and the residual drug constitutes a potent selective force for the emergence of drug resistance (Watkins & Mosobo 1993). A conflict therefore arises: drugs with a long half-life are beneficial at an individual therapeutic level but are disadvantageous at a population level. The purpose of this study is to investigate and quantify the relationship between the half-life of a drug and the speed at which resistance spreads through a population. We concentrate on antimalarial drugs in this work, but many of the principles are equally applicable to antibiotics and other antimicrobials, and we briefly discuss the implications for antibiotic usage later.

* Author for correspondence (hastings@liverpool.ac.uk).

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Several previous models have examined the spread of antimalarial drug resistance (e.g. Curtis & Otoo 1986; Dye & Williams 1997; Hastings 1997). These studies have clarified the general features underlying the process (Hastings & D'Alessandro 2000) but contain two notable omissions. First, they ignore the effects of drug elimination half-life: essentially they assume it to be zero and we show later that this may lead to serious underestimates of the rate of spread of resistance. Second, they assume that there are only two gene classes in the population: fully sensitive and fully resistant. Field evidence suggests that this second assumption is often invalid, an exception being atovaquone resistance where a single point mutation in the *cyt b* gene produces complete resistance. Resistance increases either gradually (e.g. mefloquine resistance) or in a stepwise manner from the baseline sensitivity, through a series of increasingly less sensitive forms, to the fully resistant form (e.g. pyrimethamine resistance). We categorize these levels of resistance as types *Res0*, *Res1* and *Res2*. Type *Res0* is the original fully sensitive form, which predominated before drug introduction. Type *Res1* forms are less sensitive to the drug and can survive higher residual drug levels; critically, *Res1* genotypes are still killed by therapeutic concentrations of the drug and will be cleared by treatment, so the patient will be cured. Type *Res2* is fully resistant and parasite populations *in vivo* are not significantly affected by therapeutic drug concentrations. Previous models all assumed that only *Res0* and *Res2* types were present in the population, whereas we consider all three types in the present study.

These stringent definitions of resistance are necessary to make the mathematics soluble and have been employed in most previous population genetic models of antimalarial drug resistance (Hastings & D'Alessandro 2000). The mathematical results provide valuable generalized results and give insight into the general principles, factors and dynamics underlying the evolution of drug resistance. However, there is a need to equate the range of possible clinical response to malaria treatment with the different levels of parasite resistance employed in this model. In a successful clinical treatment, parasites disappear from the peripheral circulation and the patient improves. This response can be ascribed to the *Res0* and *Res1* parasite chemosensitivities. Similarly, in a case of clinical treatment failure, the parasitaemia remains high and the patient does not improve, which is the response expected from an infection that is primarily *Res2*. In cases of 'parasitological' resistance, while the patient improves clinically, a low parasitaemia persists after treatment because of 'borderline' chemosensitivity. Parasitological resistance is frequently observed in the early stages of drug resistance and is an important harbinger of future clinical resistance. The population model, used in the present work, does not permit this category; parasites must either escape treatment, in which case they are resistant (*Res2*), or succumb (*Res0* or *Res1*). Consequently, we have employed a device when dealing with the specific example of SP resistance: infections are permitted to exhibit degrees of *Res2* and *Res1* behaviour, such that in some circumstances the parasitaemia will be cleared while in others it will not.

Malaria is a blood infection. The response to treatment is determined by the blood concentrations of the drug. The interplay between elimination half-life, drug concentration and parasite chemosensitivity is illustrated in figure 1. This example explicitly considers SP pharmacokinetics (because it has the best-characterized resistance profile and it is later used as a specific example), but the general features are universal. All drugs, except some of the artemisinin derivatives, are absorbed more quickly than they are eliminated, which gives rise to the characteristic concentration/time profile: a maximum plasma concentration, achieved within a few hours of drug administration, followed by an exponential decline at a rate governed by the specific elimination rate constant. We do not deal specifically with variability in absorption and volume of distribution, which cause variability in blood concentrations and can also, therefore, contribute to the emergence of drug resistance. For a 'single compartment' model, drug elimination is governed by the first-order equation:

$$C_t = C_0 e^{-\lambda t},$$

where C_t is the drug concentration, *in vivo*, at time t; C_0 is the starting drug concentration, and λ is the elimination rate constant. Thus, the time taken for the drug concentration to decrease by half (elimination half-life: $t_{1/2}$) is

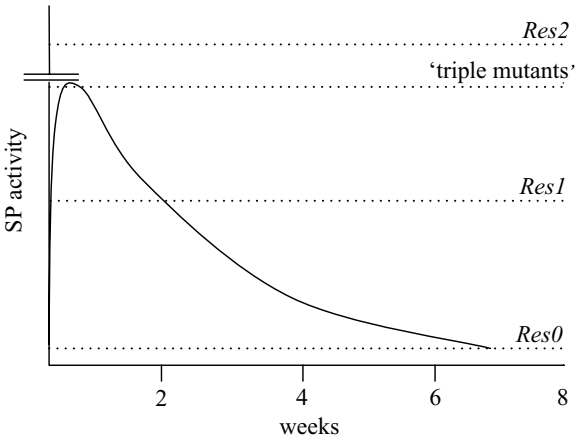

Figure 1. Pharmacodynamics and pharmacokinetics of SP (data adapted from Watkins *et al.* 1997). SP is administered orally at time zero and its concentration in the blood is represented by the solid line; it is rapidly absorbed, reaching peak concentrations within 12 h, after which both drugs are gradually eliminated at rates defined by the respective elimination rate constant (k) (from the first-order equation, 'half-life' is equal to $0.693/k$). Drug concentration is represented by 'SP activity' on the left-hand axis, rather than the more usual stochiometric concentration, because pyrimethamine and sulphadoxine exert synergistic activity against the parasite. Three classes of genotype are considered: *Res0*, *Res1* and *Res2*. *Res0* and all *Res1* subclasses except the triple mutant in dihydrofolate reductase (DHFR) are eliminated by drug treatment. This is because SP activity exceeds their respective minimum parasiticidal concentration (MPC; represented by the dotted lines) for a sufficient time-period. The DHFR 'triple mutant' is of borderline susceptibility. Given variability in SP activity profiles between treated individuals (see § 5) some of these infections will be eliminated, others will not. *Res0* can infect a host only after 52 days have elapsed since treatment (by which time SP activity has decayed to below the *Res0* MPC), while *Res1* can reinfect 15 days after treatment. The quadruple mutant (*Res2* resistance level) is completely unaffected by SP.

related to λ by the relationship $t_{1/2} = \ln 2/\lambda = 0.693/\lambda$. For many drugs, it may be necessary to model their blood concentration profiles with two or three compartment models, although in general the majority of the elimination profile is contributed by the terminal phase that has a terminal rate constant β and a terminal half-life $t_{1/2}\beta$. The residual drug concentration in the blood of the host will be sufficient to prevent reinfection until its concentration falls below the minimum parasiticidal concentration (MPC) for a particular parasite genotype (figure 1). This period of time, during which residual drug levels protect against reinfection, will be referred to as the 'period of chemoprophylaxis' (PC). As less susceptible parasites become more frequent, the average PC decreases. For pyrimethamine–sulphadoxine, the average PC has been estimated to be 52 days for the *Res0* genotype and 15 days for the first *Res1* genotypes to appear (figure 1 and Watkins & Mosobo 1993). For the *Res2* genotype this period is, by definition, zero (figure 1). Drugs with a shorter half-life exhibit the same dynamics but the time-scale (plotted along the *x*-axis of figure 1) is obviously much shorter with correspondingly shorter PC. The very rapid elimination of artemisinin, its derivatives and their active metabolites (Teja-Isavadharm *et al.* 1996) means that even fully susceptible parasites are affected by residual drug for times only marginally greater than the period of treatment. Thus, no 'partial activity' occurs. The drug effect is either maximal or zero. These differing times of chemoprophylaxis are critical pharmacokinetic factors determining the rate at which resistance evolves and they play a crucial role in determining the 'useful therapeutic life' of drugs in operational use.

2. THE DYNAMICS OF PARTIAL (*Res1*) RESISTANCE

In this section, we deal with the selective pressure exerted by residual drug concentrations of slowly eliminated drugs on newly acquired infections. We do not deal with emergence from the original infecting biomass (for a brief discussion of the difference between such 'biomass' models, and the population genetic model considered here, see Hastings & D'Alessandro (2000)). The period of chemoprophylaxis is n_0 days for *Res0* and n_1 days for *Res1*. If the average number of drug treatments per patient is d per year (d/365 per day), then the fitnesses of *Res0* and *Res1* (r_0 and r_1, respectively) are:

$$r_0 = k(1 - d/365)^{n_0}$$
$$r_1 = k(1 - d/365)^{n_1}.$$

The factor $(1 - d/365)^n$ is simply the chance that a randomly chosen host had not been treated during the period of chemoprophylaxis (which lasts n days) and k is a constant representing all the other biological factors affecting transmission, such as mosquito biting rate, loss of infection resulting from host immunity, probability of the infection being terminated by drug treatment and so on. The relative fitness of the *Res1* genotype, r, is therefore:

$$r = \frac{r_1}{r_0} = \frac{k(1 - d/365)^{n_1}}{k(1 - d/365)^{n_0}} = (1 - d/365)^{n_1 - n_0}. \qquad (2.1)$$

The relative fitness describes the rate of increase of *Res1* resistance in a population initially composed almost entirely of the susceptible *Res0* genotype (because, in the absence of drug pressure, the *Res1* genotypes are very rare). As the *Res1* genotypes are rare in the initial stages of selection, this easily translates into percentage increase per parasite generation: for example, $r = 1.05$ is equivalent to a 5% increase per generation, 1.17 to a 17% increase, and so on.

The rates of increase of *Res1* resistance to three antimalarial drugs, chosen for their differences in elimination half-life, are shown in figure 2. Elimination half-life acts indirectly to increase the rate of evolution because, all other things being equal, an increase in half-life increases the difference in periods of chemoprophylaxis (which is the direct cause). The principal reason for comparing SP and chlorproguanil–dapsone (CPG–DDS or 'Lapdap') is that resistance to both drugs is encoded by the same mutations in the dihydrofolate reductase (DHFR) gene. The original wild-type allele is, by definition, *Res0*, while the 108, 108 + 51 and 108 + 59 mutations in DHFR encode increased *Res1* tolerance to the drugs. The periods of chemoprophylaxis for SP can be measured directly in the field as 52 and 15 days, respectively (Watkins & Mosobo 1993). The tolerance of DHFR mutations to therapeutic regimens of CPG–DDS (three daily doses of chlorproguanil at 2 mg kg^{-1} body weight plus dapsone at 2.5 mg kg^{-1} body weight, designed to reach maximum, unbound *in vivo* plasma concentrations of *ca.* 60 nM for chlorcycloguanil and 650 nM for dapsone) can be investigated using an *in vitro* isobologram and analogous periods of chemoprophylaxis calculated based on the dosage and elimination half-life. These were estimated as 6 days after termination of treatment for the wild-type and a minimum of 1.2 days after termination of treatment for the *Res1* 108, 108 + 15, 108 + 59 and 108 + 51 + 59 mutations (the triple 108 + 51 + 59 DHFR mutation acts as *Res1* to CPG–DDS unlike its relationship to SP, where it may act partially as *Res2*; see § 5). These figures are taken from Table 1 of Watkins *et al.* (1997) noting that their estimates for the duration of inhibitory concentration includes the two-day treatment regime. No resistance has been noted to artesunate so we assumed the worst-case scenario i.e. *Res1* can invade immediately after the artesunate dose (i.e. no period of chemoprophylaxis), while *Res0* can invade after 2 days, a generous difference in the periods of chemoprophylaxis given the rapid elimination of artesunate. This putative *Res1* allele for artesunate is much more resistant than that for SP or CPG–DDS as it can invade immediately after treatment. However, figure 2 shows that despite this much higher level of *Res1* resistance, it evolves much more slowly than for SP or CPG–DDS as a consequence of the latters' longer half-lives (and hence bigger difference in periods of chemoprophylaxis).

The relationship between elimination half-life and periods of chemoprophylaxis is not necessarily as straightforward as illustrated in figure 1 for SP. The antimalarial chloroquine is a good counter-example because its elimination is multiphase. The final elimination phase is very long (much longer then SP) but for the most part occurs at sub-therapeutic concentrations, while its elimination at therapeutic concentrations is more rapid than SP. Its *effective* therapeutic half-life is therefore much shorter than SP, its period of chemoprophylaxis is shorter, selection for *Res1* resistance will consequently be less intense, and this

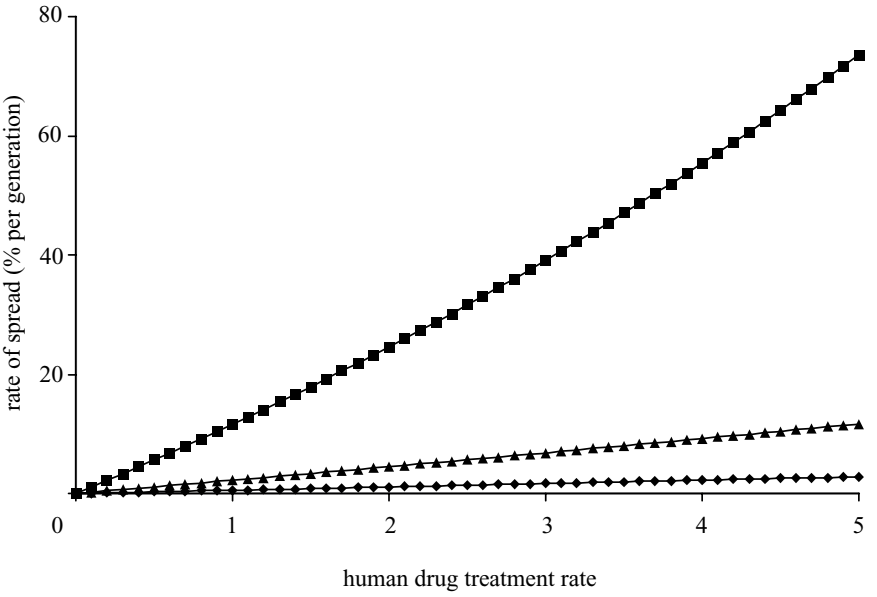

Figure 2. Selection of *Res1* resistance to three current antimalarial drugs (from equation (2.1)). The critical parameters are the periods of chemoprophylaxis for the fully sensitive and *Res1* resistance forms, denoted n_0 and n_1, respectively. Filled squares represent pyrimethamine–sulphadoxine (SP): $n_0 = 52$ days and $n_1 = 15$; data from Watkins & Mosobo (1993). Filled triangles represent chlorproguanil–dapsone (CPG–DDS): $n_0 = 6$ and $n_1 = 1.2$ days. Filled diamonds represent artesunate: n_0 is *ca.* 2 days. No *Res1* resistance to artesunate has yet been observed so the worst case scenario is investigated: infection by *Res1* resistant malaria is assumed to be possible immediately after treatment is finished, i.e. $n_1 = 0$, which predicts the maximum possible rate of increase. Human drug treatment rate is the mean number of treatments taken per person per year.

may be one of the reasons why it has had a relatively long useful therapeutic lifespan.

Figure 2 shows the extent to which drugs with long half-lives increase the selection pressure driving resistance through the population. The differences between drugs become even larger when compounded over generations: for example, after ten parasite 'generations', *Res1* genotypes with relative fitness of 1.2 or 1.5 result in $1.2^{10} = 6.2$-fold increase compared to a $1.5^{10} = 58$ fold increase.

3. THE DYNAMICS OF COMPLETE (*Res2*) RESISTANCE

If it is assumed that the maximum duration, or 'lifespan', of a malaria infection in an untreated host is g days (it is then eliminated by host immunity, or death), then if drug treatment is now provided, the expected lifespan $E(l)$ of susceptible genotypes would be

$$E(l) = \sum_{n=1}^{g} (1 - d/365)^n, \tag{3.1a}$$

if the drugs were taken randomly over the course of an infection. However, patients clearly take antimalarials at the beginning of infections. This reduces E(l). An alternative and more flexible approach is to recalculate $E(l)$ as

$$E(l) = \sum_{n=1}^{g} {}^{n}\Pi[1 - d(n)], \tag{3.1b}$$

where ${}^{n}\Pi[1 - d(n)] = [1 - d(n)] \times [1 - d(n-1)] \times [1 - d(n-2)].....[1 - d(0)]$ and $d(n)$ is the probability of being drug-treated on day n of infection. This flexible approach is more complicated because it requires knowledge of the probability of being treated on each day of infection, so equation (3.1*a*) will be used in subsequent calculations in the interest of algebraic clarity.

The reduction in length of infection of susceptible genotypes (expressed as the proportion of total expected lifetime lost as a result of being cleared by drugs), x, is the selection pressure acting against the susceptible forms. It can be calculated (using equation (3.1*a*) to simplify the mathematics) as

$$\begin{aligned} x &= 1 - \frac{\sum_{n=1}^{g} (1 - d/365)^n}{g} \\ &= 1 - \frac{1 - d/365 - (1 - d/365)^{g+1}}{g(d/365)}. \end{aligned} \tag{3.2}$$

It is customary to use a time-scale of generations in the construction of the type of population genetic models used here (e.g. Falconer & Mackay 1996; Hartl & Clark 1997). A generation in malaria is the average time taken for it to complete its life cycle, for example the mean time between being inoculated into a human host and its subsequent successful inoculation into the next human host. The fitness of a genotype over a malaria generation is a product of (i) its expected lifespan before it is cleared by drugs, or the host's immune system; (ii) the number of potentially successful secondary transmissions, denoted k as before; and (iii) the proportion of these secondary inoculations that are not introduced into patients who have been treated and still harbour the drug. The fitnesses of *Res0*, *Res1* and *Res2* resistance (denoted r_0, r_1 and r_2, respectively) are:

$$r_0 = \frac{(1 - x)k(1 - d/365)^{n_0}}{\overline{W}}, \tag{3.3a}$$

$$r_1 = \frac{(1 - x)k(1 - d/365)^{n_1}}{\overline{W}}, \tag{3.3b}$$

$$r_2 = \frac{k[(1 - x) + xv(t)]}{\overline{W}}, \tag{3.3c}$$

where $\overline{W}$ is the mean fitness of genes in the populations i.e. $\overline{W} = f_0 r_0 + f_1 r_1 + f_2 r_2$ and f_0, f_1 and f_2 are the frequencies of *Res0*, *Res1* and *Res2* genes, respectively. The parameter $v(t)$ represents assumptions made about intrahost dynamics and subsequent transmission. If co-infecting clones are transmitted independently, then $v(t) = 1$. If a resistant clone in a treated individual 'expands' to fill the void left by those sensitive co-infecting clones eradicated by the drug, then $v(t)$ equals the mean number of clones in a host (see Hastings (1997) and Hastings & D'Alessandro (2000) for further discussion). In the initial stages of invasion, f_1 and/or f_2 are extremely small so we can calculate r_2, assuming that the population is initially all of genotype *Res0*, as

$$r_2 = \frac{k[(1 - x) + xv(t)]}{(1 - x)k(1 - d/365)^{n_0}}. \tag{3.4a}$$

Alternatively, if initial selection has spread *Res1* resistance through the population, the fitness of an invading *Res2* is

$$r_2 = \frac{k[(1 - x) + xv(t)]}{(1 - x)k(1 - d/365)^{n_1}}. \tag{3.4b}$$

In particular, if we assume that *Res2* is replacing *Res0* and that no chemoprophylaxis occurs ($n_0 = 0$), equation (3.4*a*) reduces to

$$r_2 = \frac{(1 - x) + xv(t)}{(1 - x)}. \tag{3.5}$$

This equation recovers the equations obtained in previous studies (eqns (10) and (11) in Hastings (1997)), noting that (i) x, the selection pressure against the susceptible forms is equivalent to d, the proportion of infections treated per generation in his equations; (ii) that $c\tau = 1$ for a single locus as considered here; and (iii) $v(t) = c$, the number of co-infecting clones for a generalized immunity model (his eqn (10)) and $v(t) = 1$ for a specific immunity (SI) model (his eqn (11)). This equivalence demonstrates that it is straightforward to incorporate the effects of drug elimination half-life into previous modelling work. The use of expected lifespans in *Res2* resistance is more efficient when investigating factors such as sub-therapeutic dosages, variation in host pharmacokinetics, etc. (I. M. Hastings, unpublished data). The derivation developed is simplified in several important respects: (i) it ignores the incubation period during which the infection may be susceptible to treatment with the drug, but is not infective; (ii) it assumes that intrahost dynamics $v(t)$ act immediately after susceptible forms are cleared by the drugs; and (iii) it assumes that the drug has gametocytocidal activity so that treatment immediately kills the transmission stages. Equation (3.1*a*) can be modified in this light to

$$E(l) = \sum_{n=1}^{g} {}^{n}\Pi[(1 - d(n)] - h.$$

$E(l)$ is now the expected infective lifespan, g is the maximum infective lifespan before host immunity prevents further transmission (transmission blocking immunity) and h is the incubation period where the infection is present but cannot be transmitted (the hepatic and early blood stages in Plasmodium). It is the third assumption listed above, that of gametocytocidal action, which is the least plausible for antimalarials (although artesunate, for example, has this property). Many antimalarials kill the asexual forms that ultimately give rise to gametes. These drugs therefore cut off the supply of gamete precursors and there may be a lag of 10–14 days before transmission is stopped. This can be incorporated into the methodology simply by replacing $d(n)$ with $d(n - 10)$, which is the probability that the infection had been treated up to a time-point 10 days ago (assuming that the drug takes 10 days to cut off the supply of gametes). Another observation is that drug treatment of a resistant infection may increase its infectivity to its mosquito vectors (e.g. Robert *et al.* 2000). This raises the interesting evolutionary conundrum of why malaria has not evolved to maximize its own intrinsic infectivity rather than 'waiting' for a drug to stimulate it, but if we take the observations at face value and assume that the drug increases the overall (rather than just short-term) infectivity, this can be easily incorporated simply by increasing the value of $v(t)$ in equation (3.3*c*). These complications are not considered further as they do not affect the general qualitative conclusions of this study, but are included to illustrate how a more flexible approach may be developed in subsequent studies.

As transmission intensity increases, so does the acquisition of immunity, with symptomatic disease confined to childhood. In areas of intense transmission, the majority of hosts will be immune and this effective host defence will cut the expected lifespan of an average infection. The precise relationship between transmission intensity, drug resistance and human infectivity is complex; however, in high transmission areas, resistant forms generally have less time for transmission between treatment and their eventual eradication by host immunity, and so have a lower selective advantage compared to resistant forms in low transition areas. The value of g in equations (3.1*a*,*b*) and (3.2) will therefore be lower in areas of intense transmission and inspection of equation (3.4*a*,*b*) shows that as g decreases, so does the rate of evolution of resistance. This therefore favours the evolution of *Res2* resistance in areas of low transmission and low immunity.

The rates of increase of *Res2* resistance to three antimalarial drugs, chosen for their differences in elimination half-life, are shown in figure 3. Once again, the differences between drugs become even larger when compounded over generations: for example, after ten parasite 'generations', *Res2* genotypes with relative fitness of 1.2 or 1.5 result in $1.2^{10} = 6.2$-fold increase compared to a $1.5^{10} = 58$-fold increase.

The standard simulations and calculations on the evolution of drug resistance (e.g. Curtis & Otoo 1986; Dye & Williams 1997; Hastings 1997) all assume that 'resistant' parasites are completely unaffected by the treatment drug. Hosts are treated, the infecting malaria parasites either survive or die according to their genotypes and, if they survive, are then able to be transmitted to secondary hosts. Importantly, these secondary hosts are assumed to be drug

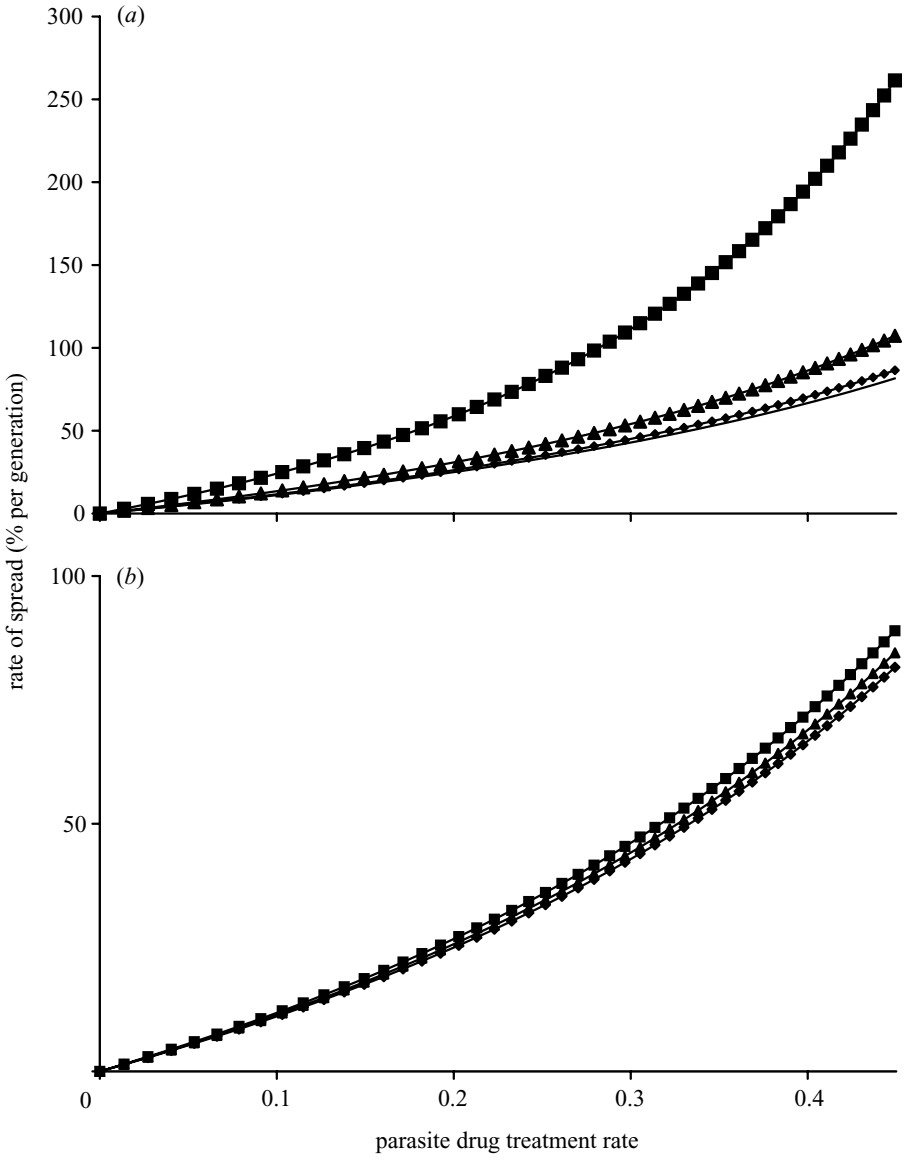

Figure 3. The spread of *Res2* resistance (from equations (3.4*a*,*b*)). Symbols are as in figure 2. The same values of n_0 and n_1 were used as in figure 2. A malaria generation is assumed to last for 100 days and independent transmission of clones was assumed (i.e. $v(t) = 1$). Parasite drug treatment rate is measured as the expected reduction in duration of the infection (x in equation (3.5)). (*a*) The spread of *Res2* into a population initially of type *Res0*. This is included for comparison with previous models that assumed only *Res0* and *Res2* genotypes could exist (*op. cit.*). Limit (solid line) is the limit as n_0 tends to zero: in this case the drug is assumed to have zero half-life, no period of chemoprophylaxis occurs and previous analytic results (Hastings 1997) are recovered. (*b*) The spread of *Res2* into a population initially of type *Res1*; note the change in scale on the y-axis.

free i.e. that they are not harbouring residual drug from a previous treatment. In effect, an implicit assumption of these models was that the drug had a half-life of zero (i.e. was eliminated instantaneously). The effect of this is shown in figure 3*a*, where the 'limit' curve was derived making this assumption of instantaneous drug elimination. Comparison of this result with those obtained when half-life is incorporated (i.e. the artesunate, CPG–DDS and sulphadoxine–pyrimethamine lines) shows that previous models, in failing to incorporate the influence of drug elimination, may have greatly underestimated the rate of evolution of *Res2* resistance, the bias increasing as half-life becomes longer (equation 3.4*a* and figure 3*a*). The quantitative results can also be understood intuitively: when generating figure 3*a*, it was assumed that a malaria generation is 100 days and that period of chemoprophylaxis (for *Res0*) in SP is 52 days. Drugs kill susceptible infections but also prevent the establishment of subsequent sensitive infections for a period of 52 days, or about 50% of a generation. Thus, effective drug pressure is probably 50% greater than expected under the standard models and the rate of evolution is correspondingly much faster. The magnitude of the bias diminishes with half-life until, in the case of artesunate, with a period of chemoprophylaxis of *ca.* 2 days (or 2% of the generation time), the bias becomes negligible.

It is important to realize that different aspects of drug use drive *Res1* and *Res2* resistance through the population. *Res1* replaces *Res0* simply because it can establish itself in people harbouring higher residual levels of the drug; the

selection pressure driving the evolution of *Res1* resistance is thus simply the proportion of *people* treated in the population and who will therefore subsequently harbour residual drug levels. By contrast, there are two factors pushing *Res2* through a population. The first factor is, as above, its ability to establish itself in people with high residual drug levels. The second factor is its ability to survive the therapeutic drug treatments that kill *Res0* and *Res1*. The first factor is determined by the proportion of *people* treated and the amount of time during which the drug persists in sub-therapeutic doses (i.e. its half-life), whereas the second factor is determined only by the proportion of *infections* treated. If *Res2* is directly replacing *Res0* then both factors will be driving *Res2* resistance, which will subsequently be rapid (figure 3*a*). Conversely, if resistance evolves through a series of cumulative mutations encoding *Res1* resistance, then *Res2* will be replacing *Res1* rather than *Res0* (figure 3*b*). In this case, the advantage of *Res2* in invading hosts with high residual drug levels becomes much less important (because *Res1* can invade drug-treated people almost as quickly as *Res2*; see figure 1) and the first factor becomes relatively unimportant. Consequently, the dynamics are determined overwhelmingly by the second factor, i.e. the ability of *Res2* to survive treatment, which is not affected by drug half-life. The dominant role of this second factor explains why drugs with very different half-lives have very similar dynamics once *Res1* has evolved, i.e. figure 3*b* versus figure 3*a*. It is to emphasize this fact that different scales were chosen for the *x*-axis when displaying the results in figures 2 and 3.

4. THE EFFECTS OF HOST HETEROGENEITY

The sources of host heterogeneity are varied: immunity (as a function of age), human behaviour, drug access, drug absorption, distribution and clearance, disease severity and the size and distribution *in vivo* of the parasite biomass. In developing these models the PC was assumed to be a constant and the effects of host heterogeneity were ignored. In reality, there are significant differences in the rates at which individual hosts absorb and eliminate drugs, which will entail similar differences in individual values of PC. This is easily incorporated, as the factor $(1 - d/365)^{n_0}$ in equation (2.1) represents the proportion of susceptible hosts in the population. Replacing that term with the following can incorporate host heterogeneity:

$$\sum_h f(h)(1 - d/365)^{n(h)},$$

where summation is over all the host types, *h*. $f(h)$ is the frequency of that particular host type in the population and $n(h)$ is the period of chemoprophylaxis for that host type. This function can incorporate heterogeneity in drug absorption, distribution, metabolism and elimination, and can also be used to investigate variability in host behaviour and to incorporate the effect of incorrect dosage or poor compliance (failure to complete a course of treatment). These are important considerations, because the differences in drug concentration between individuals can be considerable. For the lipophilic compounds atovaquone, halofantrine and lumefantrine absorption can vary by a factor of ten or more. Metabolic differences can also be considerable. An example is the biguanide antimalarials (chlorproguanil, proguanil), where metabolism to the active triazine metabolite is subject to a genetic polymorphism; some individuals are 'poor metabolizers' and others are 'extensive metabolizers' (Watkins *et al.* 1987, 1990; Ward *et al.* 1989; Helsby *et al.* 1991). Dosage varies considerably; no patient ever receives the 'right' dose, only an approximation. When dosage depends on breaking tablets, or taking spoonfuls of liquid, and especially when the dosage regimen extends over several days, this can be a major source of variation in the *in vivo* drug concentration. These many secondary effects can be incorporated into our model framework but are not considered further here, as our primary purpose is to investigate the influence of a major pharmacokinetic variable on the evolution of resistance. Note, however, that these two factors are not unrelated: drugs with long half-lives tend to be given as a single dose, while those with short half-lives usually require repeated dosage, with the attendant compliance problems.

5. A REAL EXAMPLE: THE CASE OF MUTATIONS IN THE GENE ENCODING *Pf* DHFR LEADING TO SP RESISTANCE

The dynamics have been presented separately for the evolution of *Res1* and *Res2* resistance. In practice, it may be that both types of resistance spread simultaneously, or at least sequentially, through the population. We now examine a consequence of considerable practical importance in the design of current antimalarial drug programmes: how these individual processes of *Res1* and *Res2* evolution interact. We consider the example of the antifolate antimalarial combination SP, for which there is a considerable volume of field and clinical data describing the evolution of resistance. There is still, however, some uncertainty over the precise mechanism by which the parasite becomes resistant. Two enzyme targets in the endogenous folate pathway of the parasite are involved: DHFR (classically, the site of pyrimethamine action) and dihydropteroate synthase (DHPS; classically, the site of sulphonamide action). Parasites that have been selected by SP, over time, tend to have mutations in the genes encoding both enzymes (Plowe *et al.* 1997; Wang *et al.* 1997), although there is evidence that the DHFR mutations are of central importance to the resistance mechanism and that the DHPS mutations augment the resistance level (Nzila *et al.* 2000*a*,*b*). For this reason, we describe a model that addresses mutations in DHFR only, although the basic model may easily be adapted to incorporate the effects of DHPS mutations. There appear to be three classes of DHFR genotype involved in the evolution of resistance to SP (figure 1). *Res0* is the original wild-type that predominated in Africa prior to the use of antifolate drugs, either as antibacterial or antiparasitic agents. *Res0* is wild-type at DHFR codons 108, 51, 59 and 164. The *Res1* class contains four DHFR genotypes; a mutation at codon 108 alone, or with a second mutation at either codon 51 or 59 and a 'triple mutant' with mutations at all three codons. These genotypes have all been identified in the field (Mberu *et al.* 2000; Nzila *et al.* 1998). The 'triple mutant' has the lowest chemosensitivity of the *Res1* class, and SP concentrations are inhibitory to triple mutants for only about 5 days (Watkins *et al.* 1997). The triple mutants are of borderline susceptibility to SP treatment (Watkins *et al.* 1997): some

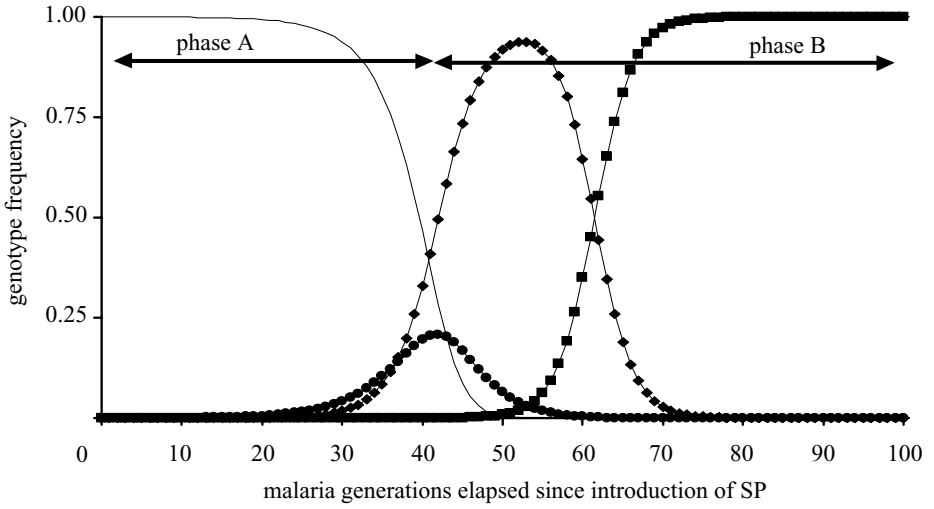

Figure 4. The evolution of resistance to SP. The method and parameter values are given in Appendix A. The genotypes tracked are the original wild-type, fully susceptible genotype (solid line), and the single, double (filled circles), triple (filled diamonds) and quadruple (filled squares) mutants; see § 5 and Appendix A for more details. The single mutant genotype never reaches appreciable frequencies under the selected parameter set, so is imperceptible in the figure. The evolution of resistance can be regarded as arising in two phases: phase A, where large changes in the genetic makeup of the population occur, but overt therapeutic drug resistance is rare, and phase B, where clinical resistance to SP emerges very rapidly. Transition between phases is arbitrarily assumed to occur when the frequency of triples reaches 25%.

of these infections are eliminated by SP treatment but many are not. Where parasitaemia remains patent one week after treatment, this genotype is predominant (Nzila *et al.* 2000*a*,*b*). We account for this borderline behaviour by the assumption that the 'triple mutants' sometimes display *Res1* resistance and sometimes *Res2* (Appendix A). Mutations at all four codons in DHFR result in *Res2*, i.e. complete drug resistance. To our knowledge, no parasites of this type have been found in Africa to date, although they are common in other parts of the world with established SP resistance (Plowe *et al.* 1997; Wang *et al.* 1997). Interestingly, the 164 mutation occurs only with the other three DHFR mutations, not alone. Figure 4 shows the interaction between the different DHFR genotypes in determining the pattern of resistance to SP, as described in Appendix A. Although the rate of spread of *Res1* resistance is much slower than *Res2* (figure 2 versus figure 3), the former, partially resistant, genotypes are probably present initially at much higher frequencies. Figure 4 shows a series of successive waves of emerging genotypes, under drug pressure, in which there is a progressive replacement of sensitive by more resistant genotypes. Phase A selection is characterized by the elimination of the wild-type, *Res0* parasites, and their replacement by *Res1* chemosensitivities: initially by double DHFR mutants and then by an increasing frequency of the triple mutants. There is evidence that the frequency of the single mutation at codon 108 in field isolates is comparatively low in Africa (Nzila *et al.* 1998). The reasons for this are unknown, but may imply a reduced metabolic efficiacy. Thus, in effect, the progression is from *Res0* to the double mutant *Res1* genotype and then to the triple mutant *Res1* genotype (Nzila *et al.* 1998). Once the frequency of triple mutants reaches the point where a sizeable fraction of infections can escape SP treatment, then phase B selection becomes apparent, clinical resistance becoming overt rather than covert (the transition between phases is arbitrarily illustrated on figure 4 as occurring when the frequency of triples reaches 25% so that 2.5% of all infections escape SP treatment; see Appendix A, noting that $p = 0.9$). Phase B is characterized by a progressive increase in the frequency of the triple mutants, initially in residual parasitaemia surviving at day seven after treatment, and, eventually, in pre-treatment parasitaemias. The number of recrudescent infections increases significantly. When the *Res2* genotype (in our model this is represented by the mutation at codon 164 in DHFR) eventually emerges, it is selected rapidly, sweeps out all susceptible and *Res1* levels of resistance and dominates the malaria population.

As parasites progressively escape drug action, phase B becomes the significant selection process and, from this point onwards, phase A selection ceases to be important as a contributory factor to the future 'useful therapeutic life' (UTL) of the treatment. Again, this important and fundamental change in treatment efficacy is not immediately obvious to health workers. The main characteristic of phase B is that it progresses very swiftly: a parasite population within a single human host containing 0.001% *Res2* parasites before treatment may be converted to 100% *Res2* parasites within 7 days of SP treatment, and these resistance alleles will be the genes transferred to a new host by gametocyte carriage. In Tanzania, the development of overt SP resistance was rapid; from undetectable levels in 1991 to 15% of treatments in 1997 (Msuya & Curtis 1991; Trigg *et al.* 1997). When the proportion of triple mutants in the infecting population is low, parasitaemia may appear to clear (blood slide negative at day seven, patient improves clinically), but in many cases, the small number of triple mutants remaining in the body will increase, rather than decrease, eventually producing another malaria episode several weeks or months later. In areas of moderate to high malaria transmission, these cases will be indistinguishable from new infections.

We argue that these processes may be characteristic of resistance to other antimicrobial drugs: an evolution

through a series of increasingly drug-tolerant stages to complete insensitivity. The results can be generalized from the current model (which is strongly supported by field and molecular data on the resistance to SP): that the selection process may conveniently be split into two phases. During phase A, *Res1* genotypes gradually replace the original *Res0* wild-type. Importantly, therapeutic efficacy remains high, because the frequency of the fully drug-tolerant *Res2* is imperceptibly low. Thus, an invidious characteristic of phase A selection is the essentially 'hidden' or 'creeping' nature of the phenomenon. Patients continue to respond to treatment. The massive changes in parasite genotype frequency will pass undetected by routine treatment monitoring, unless this includes molecular technology. This clearly indicates the importance of these new techniques in monitoring drug treatment efficacy effectively, and in providing the necessary early warning of drug failure. In clinical evaluations, no sign of increasing resistance will be detected unless (i) the period of chemoprophylaxis for the wild-type gene (the 'resistance selection period', RSP, of Watkins & Mosobo (1993)) has been computed and follow-up is adequate to detect a decreasing interval between the initial and subsequent malaria episodes, or (ii) the drug sensitivity of new infections that occur during the RSP is measured *in vitro*, or (iii) the molecular basis of resistance is known and a screening programme is in place.

6. DRUG COMBINATIONS WITH COMPONENTS OF DIFFERING HALF-LIVES: GENERAL PRINCIPLES BUT WITH SPECIAL APPLICATION TO THE ANTIMALARIAL DRUG COMBINATION ARTESUNATE/SP

There are, at present, three groups of generally affordable antimalarial drugs: (i) chloroquine, although widespread resistance has made this unusable in many regions, and amodiaquine, which is more effective; (ii) SP, although resistance is rapidly spreading, threatening to make it unusable, and CPG–DDS that is more effective, and (iii) the artemisinin derivatives to which resistance has not yet developed. It has been proposed that antimalarial drugs should be used in combination to delay the evolution of resistance (Peters 1990; White & Olliaro 1996; White 1998, 1999; White *et al.* 1999), and a natural development is to propose the introduction of artesunate as a therapeutic agent in combination with SP. Artesunate, however, has a very short half-life, while SP has a very long half-life. Since pharmacokinetic considerations have been omitted from previous models of the evolution of resistance, it is informative to consider their effects here.

It is now possible to construct a qualitative argument for combination therapy (CT) incorporating both *Res1* and *Res2* resistance and pharmacokinetics (Appendix B). The drug with the longest half-life determines the dynamics by masking selection on the other drug(s) in the mixture. This can be illustrated by the example of a mixture of SP and artesunate. *Res0* genes, for SP alone, have a period of chemoprophylaxis of *ca.* 52 days, while those encoding partial resistance (the *Res1* types) have a period of chemoprophylaxis of *ca.* 15 days. We use the arbitrary but equivalent figures for artesunate of 3 and 1 days. (This is based on the fact that the hydrolytic conversion from artesunate to dihydroartemisinin is rapid *in vivo* and little if any artesunate crosses the intestinal barrier unchanged (Price *et al.* 1995): on this basis, the elimination half-life of artesunate equals that of dihydroartemisinin; a mean of 1.90 h, 95% CI 1.4–2.4 h (Teja-Isavadharm *et al.* 1996)). No selection acts on the theoretical, potential difference (3 days versus 1 day) in artesunate susceptibility because both periods are substantially shorter than the 15 day protection afforded by the second drug. In effect, SP completely protects artesunate from the evolution of phase A resistance, if used in combination from the start. The converse, however, does not apply. The period of chemoprophylaxis of artesunate is so short that it has disappeared long before differences in SP CP occur, so its inclusion cannot slow the evolution of *Res1* (and hence, phase A) SP resistance. Co-administration of artesunate will, however, retard *Res2* (and hence phase B) selection in SP, as it kills both *Res1* and *Res2* genotypes and removes the selective difference between them. This difference is perhaps more easily understood by recalling the differing selective forces that drive *Res1* and *Res2* resistance. It is the residual levels of SP within hosts that drive *Res1* resistance through the population: artesunate is rapidly eliminated from the hosts and so cannot affect this selection on SP, or the subsequent speed of phase A. Conversely, it is the ability of *Res2* resistance to withstand *therapeutic* doses that drives it through the population: co-administration of artesunate kills both SP-resistant and SP-susceptible genotypes at the time of treatment and thus removes this selection pressure on SP.

This argument assumes that there is no overlap in the period of chemoprophylaxis i.e. *Res0* and *Res1* periods of chemoprophylaxis for one drug both exceed *Res0* and *Res1* for the second drug. This assumption is easily relaxed (Appendix B) to investigate the proposed combinations of CPG-DS with artesunate and SP with artesunate. Figure 5*a* shows the rate at which *Res1* resistance evolves to CPG–DDS and how the presence of artesunate slows this rate. For example, if human drug treatment rate is three per year then the increase slows from 4.04% to 3.35% per generation, a proportionate decrease in the rate of evolution of 17%. This has a large impact once it is compounded across the generations, greatly extending the useful therapeutic lifespan. Figure 5*a* also illustrates how this protective effect of artesunate declines if *Res1* resistance evolves to artesunate. SP has replaced chloroquine as the first-line antimalarial in several African countries. Resistance evolved very rapidly, its useful therapeutic lifespan has been short and it has subsequently been proposed that it be deployed as a combination with artesunate to increase its therapeutic lifespan. One particularly important facet of this strategy is that as *Res2* resistance evolves to SP, then its ability to protect the artesunate against selection gradually falls. In practical terms, this means that to achieve optimal mutual protection, CT would be implemented from treatment inception prior to *Res2* resistance evolving in SP (figure 5*b*). Equations (A 1) and (A 2) reveal how this will minimize pressure on both resistance selection processes. From the same line of argument, CT should be implemented for second-line treatment in areas (e.g. West Africa) where chloroquine is still the first-line treatment and SP has not been widely employed. Conversely, in other areas of Africa (e.g. East

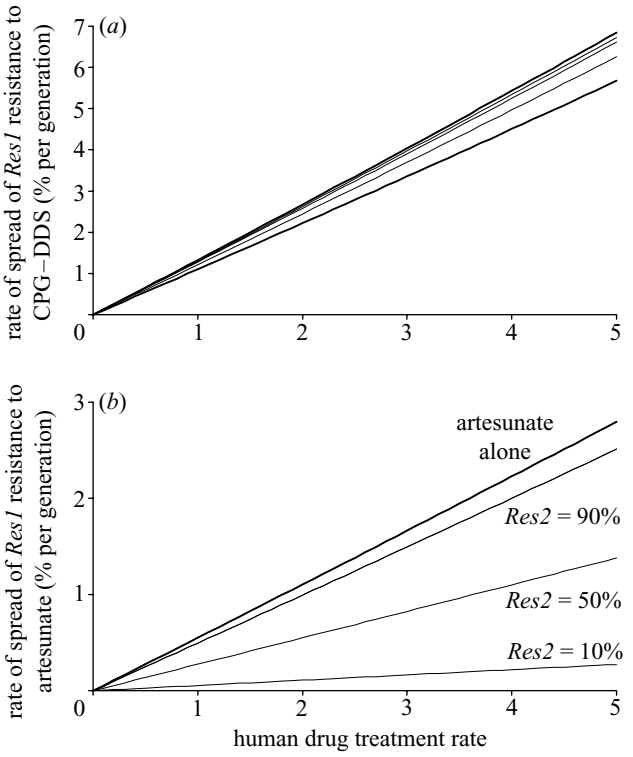

Figure 5. How combination therapy initially provides mutual protection against the evolution of resistance to each component. This protection starts to break down as resistance evolves to the individual components. The evolution of *Res1* resistance is plotted for comparison with figure 2. (*a*) CPG–DDS plus artesunate. The upper heavy line indicates the rate of *Res1* evolution (percentage change per generation) of CPG–DDS alone, while the lower heavy line shows the rate when used in combination with artesunate and no resistance is present to the latter. As *Res1* resistance evolves to artesunate then more selection is thrown onto CPG–DDS, with corresponding increases in the rate of evolution of resistance. This figure shows the rate of evolution of *Res1* resistance to CPG–DDS when *Res1* resistance to artesunate is (from lowest to highest lines in diagram) 0, 10, 50 or 90% or CPG–DDS alone (from equation (A 2)). (*b*) SP plus artesunate. As noted in Appendix B § (b), artesunate is eliminated so rapidly compared with SP that it cannot protect against the evolution of resistance to SP. Conversely, the long half-life of SP initially provides complete protection against the evolution of resistance to artesunate (the initial frequency of *Res2* resistance to SP is 0% so the rate of evolution of resistance to artesunate is 0%, i.e. the line lies along the *x*-axis in this figure). However, once resistance evolves to SP then the declining efficacy of SP throws much more selective pressure onto artesunate. This figure shows the rate of evolution of *Res1* resistance to artesunate when *Res2* resistance to SP is (from lowest line to highest) 10, 50 or 90% or artesunate alone (equation (A 2)).

Africa), phase B selection of SP is well advanced (Nzila *et al.* 1998, 2000*b*). If CT is not implemented before the emergence of the *Res2* SP genotype, it will in reality not be 'CT' at all, because high-level resistance will have emerged to one of the components, allowing direct selection for resistance to the second component. A different combination of drugs would need to be considered under these circumstances.

7. THE RELATIONSHIP BETWEEN INTENSITY OF TRANSMISSION AND RATE OF EVOLUTION

This is a question often posed by policy makers. It is important for two reasons. First, because it is most effective to set up surveillance programmes in areas where resistance is most likely to arise. Second, because it is vital to understand whether controls to decrease transmission (vector control, bednets, gametocytocidal drugs, transmission-blocking vaccines, etc.) will increase or decrease the rate at which resistance evolves. The rate of evolution of *Res1* resistance is independent of clone multiplicity (i.e. the mean number of malaria clones per individual; Arnot (1998)), so factors to reduce transmission rate such as bednets and vaccination programmes, which also decrease clonal multiplicity, will not affect selection of *Res1* resistance (and hence phase A). It is simply the level of drug use, and hence the *proportion of people* harbouring residual levels of drug, in the population that determines phase A evolution, so intensity of transmission is immaterial. By contrast, the dynamics of *Res2* resistance are primarily determined by the *proportion of infections* treated because *Res2* spreads by surviving therapeutic drug doses that eliminate *Res1* (equation (3.4*b*) and figure 3*b*).

The assertion that the rate of evolution of *Res1* resistance is independent of clone multiplicity may need further clarification as it has been an important factor in previous models (e.g. Curtis & Otoo 1986; Dye & Williams 1997; Hastings 1997). Clone multiplicity played two roles in these previous models. First, sexual recombination between different clones from the same host results in genetic recombination and shuffling of genes: the higher the level of clone multiplicity, the greater the extent of genetic recombination. If two or more genes are required to encode drug resistance then recombination affects the dynamics by bringing together, or breaking apart, the genes required to encode resistance. However, we have assumed that mutations in a single gene (e.g. DHFR) are sufficient to encode resistance, so recombination cannot break down the resistant genotype and hence has no effect on the dynamics (see discussion of fig. 1 in Hastings & D'Alessandro (2000)). The second effect of clone multiplicity arises as a consequence of intrahost dynamics. Assume, for the sake of argument, that a host contains three parasite clones (i.e. a clone multiplicity of three) and that one of these clones is *completely* resistant to the drug (i.e. *Res2*), while the other two clones are sensitive (i.e. *Res0* or *Res1*). If the host is drug treated then the resistant clone may 'expand' to take the place of the two sensitive clones, which have been eradicated. The resistant clone has been amplified by 200%: an effect that has major implications for the rate at which resistance spreads (see discussion in Box 2 of Hastings & D'Alessandro (2000)). However, in the current context of *Res1* resistance, both *Res1* and *Res0* genotypes are eradicated by drug treatment, so there is no opportunity for the *Res1* genotype to amplify itself through intrahost dynamics. In summary, clone multiplicity has no effect on the evolution of *Res1* resistance because *Res1* is encoded by a single gene and because the *Res1* genotype is eradicated by therapeutic drug treatment.

The calculations rest on the assumption that a human host is equally susceptible to infection irrespective of his/her current level of clonal multiplicity. If successful

infection becomes increasingly more difficult as clonal multiplicity increases (because pre-established clones tend to 'exclude' incomers) then transmission intensity may affect the rate of *Res1* resistance. *Res1* would spread faster in areas of intense transmission (if all other factors were equal), because not only can they invade hosts more rapidly following drug treatment, but they would also face less competition from pre-established clones. We do not consider this model of intrahost dynamics to be particularly plausible, but its existence needs to be acknowledged. Once again this emphasizes how even qualitative predictions of the evolution of resistance are hampered by ignorance of basic intrahost dynamics.

Transmission intensity *per se* therefore has no effect on the rate of evolution of resistance (at least in the simplest assumptions of intrahost dynamics), but may have indirect effects since intensity of transmission and patterns of drug use may be correlated. It is possible to construct arguments based on differing patterns of drug use, although caveats are necessary and are discussed below. Areas of low transmission have lower overall levels of *per capita* drug use (because there are few infections), but a higher proportion of infections are treated (because they are more likely to be symptomatic). This leads to an interesting prediction: that *Res1* resistance may evolve more slowly in areas of low transmission (lower background drug usage) but *Res2* resistance will be established more quickly (high proportion of infections treated). Since only *Res2* leads to clinical failure, the observation will be that clinical resistance emerges rapidly in areas of low transmission. Conversely, in areas of high transmission, *Res1* will evolve more quickly but *Res2* more slowly than in areas of low transmission. This appears to be born out by patterns of evolution of resistance to SP. Resistance was first noted in areas of low transmission and initial accumulation of the *Res1*-type single, double and triple mutations was relatively slow, but the fully protective *Res2* quadruple mutant was noted soon afterwards. Conversely, in areas of high transmission in Africa, the single, double and triple mutants have emerged rapidly, but interestingly the quadruple mutation still appears to be absent. The useful therapeutic lifespan of SP has been estimated at *ca*. 5 yr in both southeast Asia (White & Olliaro 1996) and South America (Peters 1987), and it is feared that a similarly short UTL will occur in sub-Saharan Africa, a supposition supported by evidence that alleles conferring *Res1* resistance are spreading rapidly. However, the results presented here suggest that such an extrapolation of UTL may be unduly pessimistic in areas of intense transmission and provides hope that a little more breathing space may occur before widespread clinical *Res2* resistance spreads through this region.

There are, inevitably, a couple of caveats in this argument. The observation that many infections are asymptomatic in areas of intense transmission ignores host heterogeneity. Many of the asymptomatic infections occur in older people who will not subsequently transmit the infection (due to transmission blocking immunity), while non-immune children are responsible for most of the symptomatic infections and malaria transmission. The former type of infection can be ignored (these hosts are 'dead ends' for malaria) and treatment rate of the latter may be much higher than predicted simply by a crude consideration of drug usage in the area. The second caveat concerns the relationship between level of transmission and drug use: people in areas of low transmission still take antimalarial drugs fairly frequently, but in a presumptive manner (i.e. they treat fevers caused by other factors, such as viral infections, with antimalarials); obviously this level of presumptive treatment depends on the cost and availability of the antimalarial. Unfortunately, we know of no data quantifying these effects. The argument outlined above is nevertheless valuable as it emphasizes that the evolution of resistance must be split into two phases and that the relative speeds of the two phases will vary according to differing patterns of drug use.

8. GENERAL DISCUSSION AND CONCLUSIONS

First a caveat: figures 2 and 3 show the rates at which drug resistance can increase, as relative fitnesses of the *Res1* and *Res2* genotypes, respectively, over the *Res0* genotype, assuming equivalent transmission characteristics and a constant annual treatment frequency. What is actually observed depends on the initial frequencies of resistance genes in the population. Obviously, if no resistance to artesunate exists, then a predicted 5% increase will still result in a frequency at the next generation of $0 \times 1.05 = 0\%$ to $0 \times 105\% = 0\%$. The frequencies of mutations conferring resistance in populations before drug use are a function of the forward and reverse mutation rates, the extent to which other drugs with the same mechanism of action have been employed in the locality (e.g. trimethoprim–sulphamethoxazole may select for mutations in the DHFR gene which is important in relation to SP resistance) and any biological deficit associated with the mutation in the absence of a drug. The subject is too complex to be discussed here (see Hastings 1997; Koella 1998; Hastings & D'Alessandro 2000). The same effect is seen in the dynamics of SP resistance. The triple mutants will be spreading faster than the single and doubles, but the latter are initially present at much higher frequencies and so dominate the dynamics during the early phase of resistance (figure 4).

Several previous papers have discussed the effects of dosage and/or drug half-life in the context of drug resistance (Cross & Singer 1991; Lipsitch & Levin 1997; Austin *et al.* 1998). However, these studies all investigated their effects in determining whether an infection would actually be cleared by the therapeutic treatment. We have investigated an entirely different aspect of drug half-life: it is assumed that therapeutic treatment always clears the current infection and focuses on the consequences of the drug's subsequent persistence, in chemoprophylactic concentrations, as a selective pressure for the evolution of drug resistance. The addition of this aspect of pharmacokinetics to models of drug resistance has resulted in several novel findings, as detailed earlier. Since this manuscript is long, we feel it appropriate to reiterate the most important findings, as follows. (i) A long elimination half-life at therapeutic concentrations results in long periods of chemoprophylaxis (n_0 and n_1 in equations (2.1) and (3.4*a*,*b*), so is a potent selective force increasing the rate at which resistance evolves. The absence of elimination half-lives from previous models is a significant omission resulting in underestimated rates of evolution of resist-

ance. (ii) Resistance encoded by multiple mutations at a single locus may occur in two phases, phase A: where the drug is better tolerated by the parasites but therapeutic doses still usually clear the infection, and phase B, when clinical failures start to occur. (iii) Phase B is very rapid and it is essential that surveillance programmes are in place and capable of monitoring the change from phase A to B. (iv) Phase A may occur more quickly, but the subsequent phase B more slowly, in areas of high transmission. (v) These general principles are strongly supported by field evidence for the antimalarial SP. (vi) Combination therapy significantly slows the rate of evolution of resistance but it should be instigated before significant resistance to either component is present in the population.

Given that the model has incorporated two new factors (drug elimination half-life and partial resistance) into models of antimalarial drug resistance, its seems appropriate to consider the implications for practical drug usage. The results are consistent with all previous models in suggesting that drug use should be minimized. This brings into stark contrast the effect on drug policy of the differences between the needs of the individual versus those of public health. Malaria is a disease that can develop within days from the mildly unpleasant to the fatal, so that rapid access to inexpensive drugs is important, and is a major aim of the WHO 'Roll Back Malaria' initiative. The downside of this policy is that wide access to antimalarial drugs leads to excessive use for non-malarial fever and illness: a scenario that, by all models, will drive the evolution of resistance. There is no obvious solution to this dilemma that would restrict antimalarial drug use to malaria cases only, while retaining generalized access, although the advent of cheap rapid diagnostic tests would be a step in the right direction. The model does allow evaluation of other strategies in a more qualitative manner. It is known that malaria infection during pregnancy is damaging for both mother and baby, and prophylactic use of SP (which, in a fully sensitive malaria population, provides protection for almost two months) is extremely useful. Again, there are dangers for resistance selection in this degree of routine use, since not all pregnant women will necessarily either be at risk, or actually contract malaria. The effect is an increase in the proportion of individuals who harbour residual chemoprophylactic drug levels, which, if significant, will accelerate the evolution of *Res1*, phase A, resistance (figure 2) limiting its subsequent therapeutic use. Similarly, although we conclude that drugs with short half-lives are beneficial, there are still problems with their practical application. Artesunate alone is highly effective but must be given for at least 5, or preferably 7 days to achieve a guaranteed cure. In most African settings, compliance with such a long dosage regimen will be poor: with a drug of acceptable efficacy, three consecutive daily doses is probably the longest practicable regimen. As a result, artesunate should always be combined with another antimalarial to achieve an acceptably short regimen, and thus reasonable compliance. The new antifolate antimalarial CPG–DDS ('Lapdap') is used in a 3 day regimen and is therefore a candidate for combination with artesunate. Compliance will remain a problem and the development of methods of modelling partial resistance, described in § 7, will provide a basis for further investigation of this. If compliance is a problem, a resistance allele may behave as *Res1* in patients who complete the course, but may survive and recrudesce (i.e. behave as *Res2*) in a proportion of patients who do not complete it. A similar situation occurred in the triple mutations of DHFR and the ability for this allele to have both *Res1* and *Res2* properties (developed in Appendix A) means non-compliance can now be investigated.

The model applications have been illustrated with regard to antimalarial drugs, although there are more general pharmacological implications, which apply to all infective agents and the drugs used to treat them. There have been numerous theoretical studies of drug resistance in bacteria (review in Levin & Anderson 1999). A consistent and intuitive prediction of these models was that drug usage should be minimized to reduce resistance selection. The results developed above are in agreement with this principle and further suggest that the half-lives of antibiotics may also play a significant role in determining the rate at which resistance evolves. In retrospect, this is intuitively obvious because it is not only the degree of drug usage, but also the time over which these drugs persist *in vivo* that constitutes the 'drug pressure' driving resistance through the population. Most models of antibiotic resistance have investigated resistance in the context of developed countries where the prevalence of symptomatic bacterial infections is low and consequently few people harbour residual chemoprophylactic levels of antibiotics (in contrast to widespread symptomatic malaria and resulting high antimalarial drug use in many tropical areas). At low levels of drug use, the effects of pharmacokinetics are less pronounced (figures 2 and 3). However, a major factor in the appearance and selection of multiple resistant bacteria is the transmission of nosocomial infections in hospitals. Here, antibiotic use is higher, which increases drug pressure accordingly (defined as a function of the frequency with which drug and microorganism come into contact) and may approach the extremely high pressures that characterize malaria chemotherapy. Under these circumstances, the same pitfalls may occur as in malaria, i.e. (i) large biases and underestimates of the speed of evolution of resistance will occur if the drugs have long half-lives and pharmacokinetic impact is not addressed. (ii) If resistance evolves through a series of intermediate levels of chemosensitivity, then the process may occur in two phases, a slow imperceptible phase A not associated with clinical failure, but leading to phase B, a rapid build up of fully resistant types with associated widespread clinical failures.

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APPENDIX A: INCORPORATING PHARMACODYNAMICS INTO THE EVOLUTION OF RESISTANCE TO SP

Resistance to SP appears to evolve through the sequential accumulation of mutations in the DHFR (and DHPS) gene. There are five DHFR alleles, with either 0, 1, 2, 3 or 4 mutations. The parasite with no mutations is sensitive, while up to three mutations encode *Res1* resistance.

The triple mutant is of intermediate type: in some individuals it is eradicated by therapeutic doses (i.e. exhibits *Res1* resistance), while in other individuals it is not eradicated (i.e. confers *Res2* resistance). The proportion of hosts in which it acts as *Res1* is p, and hence it exhibits *Res2* resistance in a proportion $1 - p$ of hosts. The quadruple mutant encodes complete, *Res2*, resistance. We denote the frequencies of these different alleles as m_0, m_1, m_2, m_3 and m_4, respectively. The mutation rates at the four codons is μ (we assume, purely for mathematical brevity, that the forward and backwards mutation rates at each of the four codons are the same; we further assume that the rates are so small that the probability of getting two spontaneous mutations in the same individual parasite is so small as to be negligible). The frequencies of the alleles after one generation can be calculated using the fitnesses derived in the main text (equations (3.3*a–c*) as:

$$m_0 = \frac{m_0 r_0(1 - 4u) + m_1\mu}{\overline{W}}$$

$$m_1 = \frac{m_1 r_1(1 - 4u) + m_0 4\mu + m_2 2\mu}{\overline{W}}$$

$$m_2 = \frac{m_2 r_1(1 - 4u) + m_1 3\mu + m_3 3\mu}{\overline{W}}$$

$$m_3 = \frac{m_3[pr_1 + (1 - p)r_2](1 - 4u) + m_2 2\mu + m_4\mu}{\overline{W}}$$

$$m_4 = \frac{m 4 r_2(1 - 4u) + m_3\mu}{\overline{W}}, \qquad \text{(A 1)}$$

where $\overline{W}$ is the normalizing factor equal to the sum of the numerators. We ignore natural selection against the mutations by assuming that its magnitude will be negligible compared with the selection pressures generated by drug use. The length of a malaria generation is assumed to be 100 days, $n_0 = 52$ and $n_1 = 15$ days (as already stated) and independent transmission of clones is assumed (see Hastings & D'Alessandro (2000) for discussion of intrahost dynamics; this is an SI model in their nomenclature) so that clonal multiplicity is unimportant. For illustrative purposes (figure 4), the initial frequency of the single mutant is 10^{-6}, that of the double is 10^{-4}, that of the triple is 10^{-6} and that of the quadruple is 10^{-15}. Mutation rate is 10^{-8}. The triple mutant is assumed to have *Res1* properties 90% of the time and *Res2* for the remaining 10%. Since the presence of triple and quadruple mutations results in clinical failures, drug treatment rates are assumed to rise from an average of two per person per year to four per person per year according to the formula $2 + f(4 - 2)$, where f is the frequency of triples plus quadruples. The exact pattern and timing of events shown in figure 4 depends on the values of these parameters but are qualitatively robust.

APPENDIX B: DYNAMICS OF DRUG COMBINATIONS

The main text investigated the evolution of resistance to drugs used on their own. It has been suggested that drugs be used in combination (for treatment of diseases as disparate as malaria, TB and HIV) as a strategy for reducing the rate at which resistance evolves. The methodology of the main text can be easily extended to examine the evolution of resistance to drug mixtures. The case of two components will be examined but the same procedure can be used to examine drug combinations with more than two components. It is has been pointed out, at least in malaria research, that the drug with the longest half-life will help to protect the other component against the evolution of resistance. The drug with the longer half-life will be referred to as the 'protective partner'. The PCs of drug X are represented as X_0 and X_1 for the *Res0* and *Res1* genotypes, respectively. Similarly, the *Res0* and *Res1* PCs for drug Y are represented as Y_0 and Y_1, respectively.

(a) *PCs of the protective partner do not overlap those of the second drug*

We use the real example of SP (the protective partner) and artesunate:

	drug X (SP)	drug Y (artesunate)
PC for *Res0* genotype	$X_0 = 52$ days	$Y_0 = 2$ days
PC for *Res1* genotype	$X_1 = 15$ days	$Y_1 = 0$ days

Simple consideration of this table reveals that the evolution of *Res1* resistance to SP is completely unaffected by the presence of artesunate in the mixture, as the latter will have decayed to ineffective residual levels in hosts still carrying prophylactic levels of SP. The evolution of resistance to SP therefore still proceeds as described by equation (2.1). Artesunate, however, is completely protected by SP in the combination since the PCs (3 and 1 days) are less than the minimum PC for SP. The ability of SP to protect artesunate from the evolution of resistance only fails as *Res2* resistance evolves (see § (c) of this Appendix).

(b) *PCs of the protective partner overlap those of the second drug*

CPG–DDS (or 'Lapdap') and artesunate are used as real examples but the results can be generalized to other drug combinations where PCs overlap.

	drug X (CPG–DDS)	drug Y (artesunate)
PC for *Res0* genotype	$X_0 = 6$ days	$Y_0 = 2$ days
PC for *Res1* genotype	$X_1 = 1.2$ days	$Y_1 = 0$ days

The frequency of *Res0* to drug X is $f(x)$ and that of *Res1* is therefore $1 - f(x)$. Similarly, for drug Y where the frequency of *Res0* is $f(y)$ and that of *Res1* is therefore $1 - f(y)$. Consider first the evolution of resistance to drug X. For the *Res0* gene, chemoprophylaxis is always 6 days. For the *Res1*, it depends on the allele at the other locus: it is 2 days if the Y gene is type *Res0* (frequency $f(y)$) and 1.2 days if it is *Res1* (frequency $1 - f(y)$), thus the mean PC for the *Res1* gene is

$f(y)(1 - d/365)^2 + (1 - f(y))(1 - d/365)^{1.2}$.

Thus we can rederive equation (2.1) for drug X as

$$r = \frac{r_1}{r_0} = \frac{k[f(y)(1 - d/365)^{Y_0} + (1 - f(y))(1 - d/365)^{X_1}]}{k(1 - d/365)^{X_0}}, \quad \text{(A 1)}$$

and similarly for drug Y. This is the initial selection pressure for *Res1* resistance. It is the minimum value and will steadily increase as linkage disequilibrium (LD) builds up between the loci encoding resistance to the separate components of the mixture. The rate at which LD builds up, and its steady state, depends predominantly on the level of drug use in the population (Dye & Williams, 1997).

(c) *PCs of the protective partner bracket those of the second drug*

Bracketing occurs when both the shortest and longest PCs occur in the protective partner. This is most likely to arise as a consequence of *Res2* resistance evolving in the protective partner. To use the SP + artesunate example, then if the population is fixed for *Res1* for SP (with PC of 15 days) but is being replaced with *Res2* for SP (with zero PC), then the protection provided by SP against *Res1* resistance to artesunate starts to break down:

	drug X (SP)	drug Y (artesunate)
PC for *Res0* genotype	n.a.	$Y_0 = 2$ days
PC for *Res1* genotype	$X_1 = 15$ days	$Y_1 = 0$ days
PC for *Res2* genotype	$X_2 = 0$ days	n.a.

If $f(x)$ is the frequency of *Res1* for SP then $1 - f(x)$ is the frequency of *Res2* and it is straightforward to rederive equation (2.1) for artesunate under these circumstances as

$$r = \frac{r_1}{r_0} = \frac{k[f(x)(1 - d/365)^{X_1} + (1 - f(x))(1 - d/365)^{Y_1}]}{k[f(x)(1 - d/365)^{X_1} + (1 - f(x))(1 - d/365)^{Y_0}]}. \quad \text{(A 2)}$$

When *Res2* for SP is absent, $f(x) = 1$ and there is no selection for *Res1* resistance in artesunate. As the frequency of *Res2* in SP increases, selection pressure on artesunate increases, until when *Res2* in SP is fixed, $1 - f(x) = 1$ and SP no longer provides any protection. This equation provides a simple prediction of how the magnitude of protection depends on the frequency of the SP *Res2* allele.

(d) *PCs of both partners identical*

This is the ideal scenario because a malaria parasite can only survive contact with the mixture by being resistant to both components. This is the two-gene model of resistance analysed in previous models (Curtis & Otoo 1986; Dye & Williams 1997; Hastings 1997; Hastings & D'Alessandro 2000) (assuming resistance to each component is encoded by separate, single genes). Mixtures with components of differing PC are less effective because as one component decays, selection then acts directly on the single gene encoding resistance to the remaining component and the appropriate model is partially a double- and partially a single-gene model. Selection on a single gene is much more rapid than on two genes (*op cit.*) so a combination with differing PCs increases the rate at which resistance evolves. It is therefore desirable that the components have PCs as closely matched as possible. This recommendation will, in practice, be very difficult to achieve, but this argument suggests that, for example, a combination of CPG–DDS with artesunate would be preferable to one of SP–artesunate. As a caveat to this recommendation of equality of half-lives, note that the speed of action of the drugs also needs to be considered: if a drug takes a long time (relative to its half-life) to kill the parasites, then its partner in the CT may need to have longer half-life to eradicate the residue of parasites escaping the first drug action. This is certainly the case in artesunate, where recrudescent rates are high unless combined with a drug with a longer acting half-life, such as mefloquine. Drugs with short half-lives typically require multiple doses so the benefits of reduced rate of evolution of resistance have to be considered against the possible problems of compliance.

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