Survival probability of drug resistant mutants in malaria parasites

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SUMMARY

This study predicts the ultimate probability of survival of a newly arisen drug resistant mutant in a population of malaria parasites, with a view to understanding what conditions favour the evolution of drug resistance. Using branching process theory and a population genetics transmission model, the probabilities of survival of one- and two-locus new mutants are calculated as functions of the degree of drug pressure, the mean and variation in transmission rate, and the degree of natural selection against the mutant. Probability of survival increases approximately linearly with drug pressure, the slope of the line increasing with mean transmission rate. Thus increased drug pressure, especially in combination with high transmission rates, strongly favours the evolution of drug resistance. These conclusions also hold for the case of multiple drug resistance where it is coded for by two unlinked loci: the greater effective recombination breakdown in high transmission areas is counteracted by greater effective selection so that the net effect of higher transmission rates is to favour the evolution of multiple drug resistance. High variability in transmission rate and natural selection against the mutants are unfavourable to mutant survival, though these are relatively weak forces.

1. INTRODUCTION

Malaria parasites have a remarkable ability to develop resistance to drugs. This has created an urgent problem because resistance to all of the available drugs has arisen at least once (Bjorkman & Phillips-Howard 1990), and the development of new drugs has virtually stalled. Evidence from field and theoretical studies indicates that resistance continues to spread as long as there is any drug pressure, and that beyond a certain frequency, the rate of spread is very rapid (Curtis & Otoo 1986; Dye 1991; Wernsdorfer 1991). It therefore seems wise to try and prevent or delay the development of resistance in the first place.

The fate of a new drug resistance mutant, or set of mutants, in a malaria parasite depends on the relative forces of selection by drugs, natural selection (presumably unfavourable), recombination between resistance loci in the case of multi-locus resistance, and the probability that the mutant is transmitted. While it may be easy to predict the qualitative effect of each of these forces in isolation, it is their relative strengths and the interactions among these forces which determine the final outcome. Due to the structuring of the parasite population into hosts, and the heterogeneity of selection of the parasites by the hosts (i.e. hosts are either treated or untreated with drugs), prediction of the outcome of, say, a combination of drug pressure and transmission rate is not straightforward. For example, Paul *et al.* (1995) suggest that where drug resistance is coded for by more than one locus, higher levels of transmission will hinder the evolution of drug resistance because the associated increase in the degree of outbreeding will lead to greater effective recombination breakdown between the resistance loci. They do not examine this hypothesis in a quantitative way. On the other hand, simulations by Dye (1991) indicate that high transmission rates favour the spread of multi-locus drug resistance because when parasites are dispersed more widely at higher levels of transmission they encounter drugs more often, and so effective selection by drugs is stronger. Such contrasting conclusions demonstrate the need for studies which properly account for the two major effects of parasite population structure on gene flow, namely inbreeding due to limited numbers of genetically different haplotypes per host, and heterogeneous selection in the parasite population due to variable selection pressures by hosts. Dye & Williams (1997) account for the effects of inbreeding by relating it to the rate of recombination breakdown between resistance loci, but they ignore the effects of structuring on the intensity of selection by drugs. In the present study, a population genetics-transmission model is built which accounts for both factors.

Specifically, this study addresses the first of two relevant issues regarding the evolution of drug resistance. The first issue is how easily resistance can develop in the first place, i.e. from new mutations. Here the model is used to predict the probability that a newly arisen mutant survives the first few rounds of transmission until selection by drugs can bring it to a 'safe' frequency, i.e. beyond the risk of being lost to the population due to chance events during transmission.

Proc. R. Soc. Lond. B (1997) **264**, 53–59
Printed in Great Britain

Thus it addresses the issue of how readily a drug resistance problem can arise from a new mutation event, or from the arrival of a new migrant parasite into a resistance-free population, and therefore has implications for limiting the further evolution of drug resistance. The second issue is how rapidly the frequency of an established mutant will increase over generations of transmission under continuous drug pressure. This question will be addressed elsewhere.

2. THE BASIC MODEL AND ASSUMPTIONS

In the following section the assumptions made in the model about the epidemiology, transmission cycle, drug selection and relative fitness of mutants are given.

(*a*) *Distribution of number of transmissions per host*

Transmission of malaria is characterized by its variability due to a multitude of host, vector and parasite-related factors. Though transmission rate is usually described in terms of a single parameter, R_0, which is often thought of as a constant because it represents the average number of transmissions from one infected host to other hosts, it is recognized that there is variability around R_0, i.e. in the number of transmissions from individual hosts (Koella 1991). It is this variability which makes new mutants vulnerable to loss during the first few rounds of transmission. If the average R_0 is one, then on average the total parasite population replaces itself each generation. However, individual parasites with distinct multi-locus haplotypes may not always replace themselves due to sampling variation in the transmission process. Thus when considering the population genetics of parasites, it is necessary to account for the uneven redistribution of genes from one generation to the next.

In the model presented here the transmission rate is modelled by a variable, R, which is distributed as a negative binomial with a mean of $\bar{R}$ and variance of σ_R^2. The parameters of the negative binomial distribution, p, and k, are related to the mean and variance in the following way:

$$\bar{R} = \frac{k(1-p)}{p}, \quad \sigma_R^2 = \frac{k(1-p)}{p^2}. \tag{1}$$

The parameter k can be thought of as the aggregation or shape parameter: as k increases, the less 'clumped' are the data and when $k = \infty$, the distribution is Poisson. The parameter p can be thought of as the zero probability parameter: the frequency of the zero class is given by p^k so that the mean increases as p decreases.

(*b*) *Number of clones per host*

It is assumed that a host carries an average of c independent infections. It is also assumed that c is the number of haplotypes which simultaneously have sexual forms of the parasite (gametocytes) in the blood and therefore are potential mates during fertilization after the mosquito has taken a blood meal. Here c is assumed to be constant even though these c infections come from a variable number of transmissions. In this study c is defined as a function of $\bar{R}$ in the following way, although such a relationship has not yet been properly explored in the field. The conditional mean number of successful (i.e. non-zero) transmissions from one host to others is $\bar{R}/(1-p^k)$. If malaria is stable it follows that the average rate of loss of infections is equal to the average rate of acquisition of infections. However, depending on levels of immunity, the average number of infections per host could be between zero and $\bar{R}/(1-p^k)$. In this study it is assumed that the average number of infections is halfway between these extremes, i.e.

$$c = \frac{1}{2}\frac{\bar{R}}{1-p^k} \tag{2}$$

though in the remaining theory c is modelled independently of $\bar{R}$ and σ_R^2 to keep the model general. Values of c from the field have been inferred from the observed number of one or two-locus haplotypes in the blood (Carter & McGregor 1973; Conway *et al*. 1991; Babiker *et al*. 1994; Hill & Babiker 1995; Hill *et al*. 1995; Ntoumi *et al*. 1995; Paul *et al*. 1995), or from studies on the amount of heterozygosity among oocysts formed in mosquitoes. In a high transmission area in Tanzania the estimated number of clones per host was 3.5 (Hill *et al*. 1995; Hill & Babiker 1995) and in a low transmission area in New Guinea was 1.1 (Paul *et al*. 1995). As an example, some realistic values of $\bar{R}$ and σ_R^2 to correspond to $c = 3.5$ and $c = 1.1$ are

$$(\bar{R} = 5; \quad \sigma_R^2 = 50) \quad \text{and} \quad (\bar{R} = 1.5; \quad \sigma_R^2 = 3.0).$$

(*c*) *Drug resistance genes*

Now assume that the parasite has two loci at which there are two allelic forms – one the wild-type allele, and the other allele encoding resistance to a given drug which would otherwise kill all parasites not carrying the mutant allele. Denote these alleles as A and a for mutant and wild-type alleles, repectively, for the locus encoding resistance against drug α and similarly B and b for alleles for the locus encoding resistance against drug β. Thus there are four relevant genotypes, AB, Ab, aB and ab. The fitnesses, which determine the relative frequencies of haplotypes within the host, in the presence of both drugs, is represented by a vector, $\boldsymbol{W}_T = [1 \quad 0 \quad 0 \quad 0]$, and in the absence of drugs by the vector $\boldsymbol{W}_U = [w^2 \quad w \quad w \quad 1]$. These reduce to $\boldsymbol{W}_T = [1 \quad 0]$ and $\boldsymbol{W}_U = [w \quad 1]$ when only one drug is in use, in which case the second locus is irrelevant. It is important to note that fitness is defined here as the relative number of gametocytes with the two-locus haplotype at the time of transmission, rather than the number of distinct clones in the host's blood. If a double mutation has arisen at the beginning of an infection in a host which carries c independent infections, the relative frequencies of the two-locus haplotypes will be

$$\boldsymbol{W}_U = \frac{1}{w^2+2c-1}[w^2 \quad 0 \quad 0 \quad 2c-1]. \tag{3a}$$

For example, if a host has one infection ($c = 1$) and a double mutation (AB) occurs which has equal fitness to the wild-type haplotype (ab), then its frequency in the host is $\frac{1}{2}$. If the host is infected twice more ($c = 3$) with non-mutant parasites, this frequency is reduced to $\frac{1}{6}$. If the recombinant meiotic products (Ab and aB) are also in the host, as may occur in subsequent generations, the relative frequencies in the host will be

$$\boldsymbol{W}_{\mathrm{U}} = \frac{1}{w^2+2w+4c-3}[w^2 \quad w \quad w \quad 4c-3]. \tag{3b}$$

(*d*) *Transmission-genetics cycle*

The basic model of transmission assumes that each host receives c independent infections from separate transmission events and that the parasites from these infections co-exist in the blood of the host. The relative frequencies of the haplotypes in these infections are adjusted during the course of the infection according to their relative natural fitnesses by multiplying with $\boldsymbol{W}_{\mathrm{U}}$ and re-scaling. In a proportion of the host population, T, the frequencies are adjusted for drug selection by multiplying with $\boldsymbol{W}_{\mathrm{T}}$ and rescaling i.e. the frequency of mutant parasites in treated hosts are set at unity, and non-mutant parasites at zero. The parasites then form gametocytes which are taken up by a mosquito in a blood meal and undergo self or cross fertilization during the zygote stage. The frequencies of the diploid genotypes in the mosquito are assumed to reflect random mating among the gametocytes within the host from which the mosquito took the blood meal. These diploid genotypes then undergo recombination and the frequencies among the haploid meiotic products are adjusted accordingly. The meiotic products, or a subset of them are transmitted to a new host to initiate a new infection.

3. SURVIVAL PROBABILITY OF A NEW MUTANT

(*a*) *General introduction to branching process theory*

The following section describes the branching process theory used to predict the ultimate survival probability of a single copy of a mutant. This theory enables prediction of the probability that a single replicating particle (in this case a mutant allele) which is subject to stochastic processes during its replicative cycle ultimately survives in the population, i.e. it is still in the population after many generations. This probability can be predicted from the distribution of the number of 'offspring' each particle produces each generation. The distribution of the number of offspring can be summarized by a single function called the probability generating function (pgf, denoted $\phi(s)$ where s is a dummy variable). For example, the pgf for a Poisson variable is represented by

$$f(s) = \mathrm{e}^{-\lambda(1-s)} = \sum_{k\,0}^{\infty} \mathrm{e}^{-\lambda}(\lambda s)^k/k!.$$

Probability generating functions are useful because the value of s which solves the equation

$$\phi(s) = s \tag{4}$$

gives the ultimate extinction probability, and hence the ultimate survival probability (denoted usp) is given by $1-s$. If the mean of the distribution described by $\phi(s)$ is less than one, the solution to equation (4) is $1-s = 0$. Thus the particle will not survive if the mean number of offspring is less than one (because it does not on average replace itself). If the mean number of offspring is greater than one, the particle has a finite, but less than perfect probability of surviving, as given in equation (4). A further property of branching process theory is that if there are several processes during the replication cycle, each with different pgfs, then the pgf used in equation (4) is the compounded distribution of the individual pgfs involved in the process. Note that equation (4) only holds if the distribution of the number of offspring is the same in all generations. If, however, environmental fluctuations cause a change in the pgf over time, it is necessary to compound over the different distributions from the generations to obtain the overall pgf. Note also that it is assumed that the population size is infinite or very large such that there is a zero probability that two identical mutants will meet and produce offspring together. A digestible review of branching process theory is given by Schaffer (1970).

(*b*) *Application to malaria mutants*

In malaria, the replicative cycle is a transmission from one host to the next which involves the transfer of parasites to mosquitoes, meiosis in the mosquito and then transfer to a new host. This process can be broken down into two stochastic processes each with their probability distributions. One distribution is for the number of transmissions from one infected host to a number of new hosts, assumed to have a negative binomial form. The second distribution is for segregation during meiosis which has a binomial distribution. The combination of these distributions will determine how many copies of a single mutant are left in the population after one transmission cycle. The parameters of these distributions depend on whether the host has been treated with drugs or not. The case of no drug treatment is given first, and the case of drug treatment is then derived from it.

If the host is not treated with drugs the pgf for the negative binomial distribution, assumed here to represent the number of transmissions to new hosts, is given by

$$\boldsymbol{\Phi}_{\mathrm{NB}}(s) = \left[\frac{p}{1-(1-p)\,s}\right]^k. \tag{5}$$

The binomial pgf for the segregation during meiosis is:

$$\boldsymbol{\Phi}_{\mathrm{B}}(s) = 1-\pi+\pi s, \tag{6}$$

with mean π and variance $\pi(1-\pi)$. Here the parameter π represents the average probability that in each of $\bar{R}$ independent successful transmissions, the mutant haplotype AB is represented among the

transmitted parasites. This probability is worked out from the overall self replacement rate, ρ, of the AB haplotype over $\bar{R}$ transmissions as

$$\pi = \frac{\rho}{\bar{R}}. \tag{7}$$

If malaria is stable, individual alleles with equal fitness should on average replace themselves. Rare two-locus haplotypes should have replacement rates of $\frac{1}{2}$ if the loci are unlinked and the parasites are mating randomly. In the case of unequal fitness, and some inbreeding due to a limited number of genotypes per host, the replacement rate is calculated from the relative fitnesses among the gametocytes in the host (3*a*) as

$$\rho = \frac{2c[w^2+\frac{1}{2}w^2(2c-1)]}{(w^2+2c-1)^2}. \tag{8a}$$

The first and second terms within the brackets of the numerator represent the probabilities of getting an AB meiotic product from $AB \times AB$ and $AB \times ab$ matings, respectively, and the denominator is the appropriate scaling factor. The factor of $2c$ in the numerator ensures that the replacement rate for equally fit alleles is unity and reflects the fact that for each transmission and meiosis, two gametes are sampled. Note that equation (8*a*) reduces to $\rho = 1/2+1/4c$ when the mutant has equal fitness to the wild-type allele. This is greater than the value of $\frac{1}{2}$ expected from random mating (where $c = \infty$) because sometimes the parasites self-fertilize. In subsequent generations, when the recombinant meiotic products may also be within a host (3*b*), the replacement rate is

$$\rho = \frac{4c[2w^2(w+c)]}{(w^2+2w+4c-3)^2}, \tag{8b}$$

and the replacement rate when $w = 1$ is

$$\rho = \frac{1}{2}+\frac{1}{2c}.$$

Now that the two component probability distributions determining the overall probability of the mutant surviving the transmission have been defined, they can be compounded into a single probability distribution by using their pgfs. The compounded pgf is denoted as $\Phi_{\mathrm{B,NB}} = \Phi_{\mathrm{NB}}(\Phi_{\mathrm{B}}(s))$ and can be shown to be distributed as negative binomial with a mean of $\pi\bar{R}$ and a variance of $\pi\bar{R}(1-\pi+\pi\sigma_R^2/\bar{R})$ (Kojima & Kelleher 1962). This means that the pgf of the compounded distribution is given by:

$$\Phi_{\mathrm{B,NB}} = \left[\frac{p^*}{1-(1-p^*)\,x}\right]^{k^*}, \tag{9a}$$

where

$$p^* = \frac{p}{p-\pi p+\pi}, \mathrm{k}^* = \mathrm{k}. \tag{9b}$$

If a host is treated with drugs, the probability that the double mutant is among the meiotic products is $\pi = 1$. Thus the pgf, if the mutant arises in a drug treated host, is negative binomial with mean $\bar{R}$ and variance σ_R^2, i.e. the same as the transmission distribution (5).

Because the mutant may arise in a treated or untreated host and will be transmitted to either treated or untreated hosts in each subsequent generation, the overall probability of survival will depend on the sequence of treatment in the hosts encountered by the mutant during the first few generations. For example, the pgf for the number of offspring for a mutant which arises in a treated host and then is transmitted to an untreated host is not the same as if the mutant arose in an untreated host and then transmitted to a treated host, i.e. $\Phi^T(\Phi^U(s))$ is not the same as $\Phi^U(\Phi^T(s))$ where Φ^U and Φ^T denote the pgfs in the cases where the host is untreated and drug treated respectively. Thus to obtain the average probability of survival over all possible sequences of treatment, the usps for all of the 32 possible combinations of sequence of treatments in the first five generations (i.e. $\{U, U, U, U, U\}$, $\{U, U, U, U, T\}, \ldots \{T, T, T, T, T\}$) were found after compounding five times and then solving the treated and untreated generating functions in sequence (i.e. $\Phi^{UUUUU}(s) = s, \quad \Phi^{UUUUT}(s) = s, \ldots \Phi^{TTTTT}(s) = s$). These usps were weighted by their binomial probabilities of occurrence assuming that all possible sequences are equally likely (i.e. $(1-T)^5, (1-T)^4 T, \ldots, T^5$) and then summed to obtain the average usp. For the first generation of each series, it was assumed that there were no recombinant haplotypes (Ab and aB) in the host and so equation (8*a*) was used, though for subsequent generations they were assumed to be present and so equation (8*b*) was used.

(*c*) *Numerical evaluation*

The procedure described above was used to evaluate survival probabilities of single-locus and two-locus mutants as functions of T for three levels of natural selection ($w = 1, 0.9$ and 0.5) and three levels of transmission – one with low mean transmission number and low variability ($\bar{R} = 1.5; c = 1.1; \sigma_R^2 = 3$), one with high mean transmission and high variability ($\bar{R} = 5; c = 3.5; \sigma_R^2 = 50$), and one with high mean and low variability ($\bar{R} = 5; c = 2.6; \sigma_R^2 = 10$). The corresponding parameters for the negative binomial in these three cases are ($p = 0.5; k = 1.5$), ($p = 0.1; k = 0.56$) and ($p = 0.5, k = 5$), respectively. The same calculations were also performed over a wide range of values of all the parameters ($T = 0$ to 1, $w = 0.1$ to 1, $\bar{R} = 1$ to 15, $p = 0.1$ to 0.9) with the restriction that $1 \leqslant \sigma_R^2 \leqslant 50$ (considered to be the bounds of reality). This was done in order to generate data to which a linear regression model was fitted, to enable a first order approximation of the influence of the main factors affecting survival probability to be obtained. The regression terms fitted were the interactions between T and $\bar{R}$, σ_R^2 and w (or w^2 for the two-locus case). By fitting interactions with T rather than main effects, it was ensured that the intercepts at $T = 0$ were always zero since mutants are only expected to survive if they have above average

fitness. Quadratic terms were also fitted to test whether there was an improvement in fit of the model by allowing for some curvilinearity in the relationship.

4. RESULTS

Figure 1 shows the probability distributions for the three transmission levels. It illustrates that more hosts produce zero transmissions when the mean level of transmission is low, and for the same mean level, when the variability is high. Thus a new mutant is at higher risk in more variable transmission environments.

Figure 2 shows the survival probabilities as a function of drug treatment rate. It illustrates the following five qualitative conclusions demonstrated by the model.

1. The strongest influence is that of drug pressure: survival probability approximately linearly increases with drug treatment rate (figures 2*a–f*). Even though the probability that an individual mutant survives is low when drug pressure is low (figures 2*c–f*), if there are ten separate mutation events, then the probability that at least one survives (which is all that is required for drug resistance to become established when drug pressure continues) is moderate (figures 2*a*, *b*).

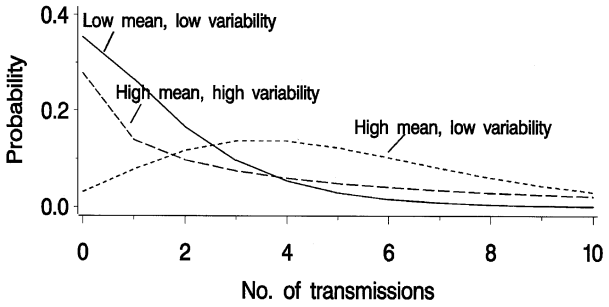

Figure 1. Probability distribution of number of transmissions per host for transmission areas with low mean and low variability (solid line, $\bar{R} = 1.5$; $c = 1.1$; $\sigma_R^2 = 3$), high mean with high variability (long dash, $\bar{R} = 5$; $c = 3.5$; $\sigma_R^2 = 50$) and high mean with low variability (short dash, $\bar{R} = 5$; $c = 2.6$; $\sigma_R^2 = 10$).

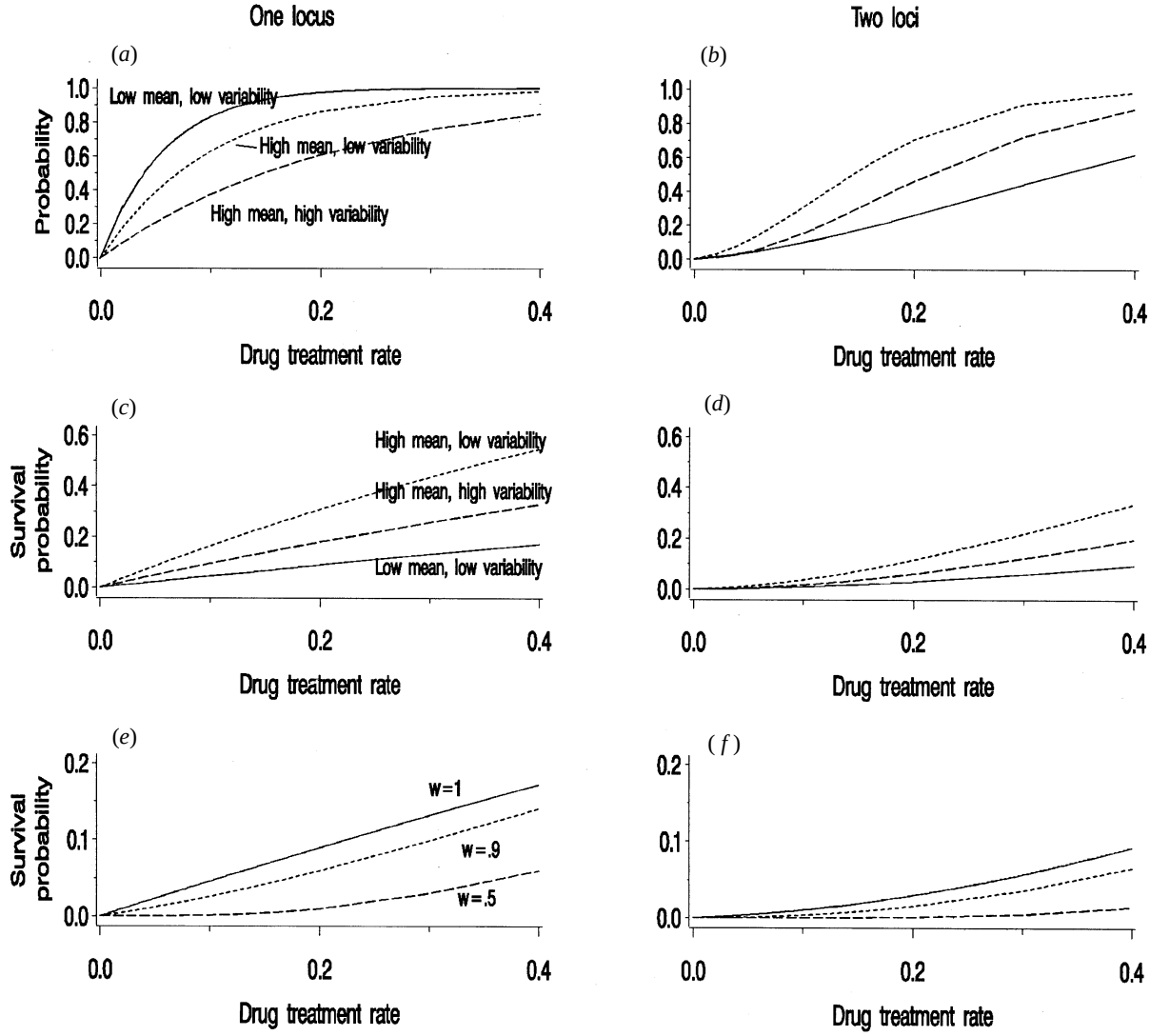

Figure 2. Survival probabilities as functions of drug treatment rates for one (left) and two-locus mutants (right). (*a*) and (*b*) Probability that at least one out of ten survives for three different transmission intensities. (*c*) and (*d*) Probability of survival of an individual mutant for three different transmission intensities. (*e*) and (*f*) Probability of survival of an individual mutant for three different levels of natural selection. Labels of line types are consistent within each row of the figure.

2. One-locus mutants have a higher chance of survival than two-locus mutants (figures 2*a*, *c*, *e* vs figures 2*b*, *d*, *f*). This is because recombination breaks down the *AB* haplotypes at each meiosis if the parasite does not mate with the same haplotype as itself.

3. The mean transmission rate increases the probability that a new mutant survives. The effect of increasing mean transmission rate from 1.5 to 5 while keeping the mean to variance ratio the same ($p = 0.5$) is shown by comparing the 'low mean, low variability' vs 'high mean, low variability' lines in figures 2*c*, *d*.

4. An increase in the variance of transmission while keeping the mean the same decreases survival probability. This is shown by comparing the 'high mean, low variability' and 'high mean, high variability' lines in figures 2*c*, *d*. Much of this difference is due to the greater proportion of hosts which produce zero transmissions (figure 1).

5. Natural selection against the mutant decreases survival probability (figures 2*e*, *f*).

The above qualitative conclusions can be described more generally and quantitatively using results from the regression analysis. The best fit to the data were obtained using the following equations in the one-locus and two-locus cases,

$$\begin{aligned} \mathrm{usp}_1 &= T(0.196\bar{R} - 0.008\bar{R}^2 - 0.008\sigma_R^2 + 0.217w), \\ \mathrm{usp}_2 &= T(0.191\bar{R} - 0.008\bar{R}^2 - 0.007\sigma_R^2 + 0.127w^2), \end{aligned} \quad (10)$$

where the subscripts on usp denote the number of loci involved. Thus while T had a linear effect on usp, the slope of this line increased with increasing $\bar{R}$ with a plateau effect at values of around $\bar{R} > 10$. The slope also increased with increasing w (or w^2 in the two-locus case) and with decreasing σ_R^2. As $\bar{R}$ accounted for 52% and 58% of the variation in usp_1 and usp_2 respectively, and $\bar{R}^2$ for a further 22% and 23%, transmission rate, over and above the effect of drug pressure, was the major influence on survival probability. The influences of natural selection and variation in transmission rate were much weaker, with w and σ_R^2 each explaining less than 5% of the variation in *usp*.

5. DISCUSSION

This study demonstrates that the fate of a newly arisen drug resistant mutant is primarily determined by whether drugs are in use in the host population, and the rate of transmission of the parasite. The reason why these two factors jointly determine whether a mutant survives is because the mutants only have a selective advantage when they encounter the drug. If transmission rates are high, the new mutant has a greater chance of being transmitted to at least one host which is treated with drugs and therefore subjected to selection by drugs. Low transmission rates reduce the probability that a copy of the mutant is exposed to the drug.

The study also shows that even when resistance is coded for by two mutant alleles at unlinked loci, the frequent breakdown of the double mutant haplotype under conditions of high transmission is not sufficient to prevent the double mutant surviving because selection by drugs is a more powerful force to keep the mutant in the population. Thus the suggestion by Paul *et al.* (1995) that high transmission rates are unfavourable for the evolution of multi-locus drug resistance because of greater recombination breakdown is not supported, and the results of Dye (1991) are. In other words, this study shows that effective selection and effective recombination are both increased by high transmission rates, but selection wins. This conclusion was reached by taking a population genetics approach in which the two major effects of population structuring of the parasite into hosts were delineated – namely, mating structure (or degree of inbreeding), and selection structure (heterogeneous selection by hosts). As both of these factors determine the rate of gene flow through populations in compartmentalized populations typical of many parasites, the model developed here is likely to have wider applicability to theories on parasite evolution.

In the present study it was assumed that the drug was completely effective in killing parasites. In practice, improper administration of the drug (e.g. through underdosing) will mean that selection pressure on drug resistance mutants is not as strong as assumed here and, therefore, that drug resistance in the field will evolve less easily than predicted here. This is not true, however, in the case when drug resistance (to one or more drugs) is controlled by more than one locus: partial killing will cause more resistant alleles to be maintained in the population so that the chances of formation of a doubly resistant haplotype are greater. Thus for the same proportion of hosts treated with drugs, incomplete efficacy will favour faster spread of multiple drug resistance, but not single drug resistance. This does not mean, however, that intermediate drug treatment levels (proportion of hosts treated) will increase the rate of spread of resistance.

It was also assumed that drugs are taken randomly by the infected host population and that the frequency of treatment is independent of transmission rate. This allowed the conclusion to be reached that effective selection on the mutant is directly related to both drug pressure and transmission rate. However, if hosts only take drugs when they are sick and the frequency of being sick depends on transmission rates (e.g. due to the number of previous infections), then the effect of transmission rate in the field may be somewhat less than predicted here.

A key assumption made in this study is that malaria is stable, i.e. the net change in the average number of parasite infections per host does not change over the generations. Clearly this is unlikely to be true in malaria parasite populations which typically fluctuate in size due to seasonal factors. During expansion phases, most mutants, even neutral ones, will survive because the mean number of offspring is greater than one. During contraction phases, few will survive. Thus the fate of new mutations under selection very much depends on the prevailing population dynamics when the mutant arises. However, such fluctuations are only likely to affect the conclusions from this study in a quantitative, but not qualitative way because com-

parisons of the effects of drug pressure, transmission rate and natural selection are performed under the same conditions (i.e. stable malaria).

As the model described here shows, variation in transmission rate can have considerable consequences to the fate of new mutants, and probably any low frequency allele or multi-locus haplotype under selection. If variation in transmission rate is high, unique genetic entities have low probabilities of surviving because stochastic forces can easily eliminate them before they can multiply. In the case of drug resistance, high variability in transmission rate is detrimental to the mutants' survival because of the greater risk involved in transmission. Thus if the stability of transmission rate, as well as the mean transmission rate, is reduced by control strategies such as bednets, vaccines or drugs, such interventions may further inhibit the parasite's adaptation to these strategies. The importance of variation in transmission rate on the persistence of 'strains' (multi-locus haplotypes) in a population has been illustrated by Gupta *et al*. (1994) and has implications as to how rapidly hosts acquire specific immunity. More generally, the role of variation among parasites and hosts in transmissibility is relatively unexplored, although there is a growing awareness that its effect on the epidemiology of parasitic diseases can be profound (Anderson & May 1991; Read *et al*. 1995).

This work was supported by the Medical Research Council, UK, Bill Hill, Andrew Read, Phillipe Baret, Armando Caballero, Ian Hastings and two anonymous referees are thanked for their enlightening comments.

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Received 11 September 1996; accepted 24 September 1996