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A theoretical approach to predicting the success of genetic manipulation of malaria mosquitoes in malaria control

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Abstract

Background: Mosquitoes that have been genetically modified to better encapsulate the malaria parasite *Plasmodium falciparum* are being considered as a possible tool in the control of malaria. Hopes for this have been raised with the identification of genes involved in the encapsulation response and with advances in the tools required to transform mosquitoes. However, we have only very little understanding of the conditions that would allow such genes to spread in natural populations.

Methods: We present here a theoretical model that combines population genetical and epidemiological processes, thereby allowing one to predict not only these conditions (intensity of transmission, evolutionary cost of resistance, tools used to drive the genes) but also the impact of the spread of refractoriness on the prevalence of the disease.

Results: The main conclusions are 1) that efficient transposons will generally be able to drive genes that confer refractoriness through populations even if there is a substantial (evolutionary) cost of refractoriness, but 2) that this will decrease malaria prevalence in the human population substantially only if refractoriness is close to 100% effective.

Conclusions: If refractoriness is less than 100% effective (because of, for example, environmentally induced variation in the effectiveness of the mosquito's immune response), control programmes based on genetic manipulation of mosquitoes will have very little impact on the epidemiology of malaria, at least in areas with intense transmission.

Background

Malaria is one of the most serious health problems facing the developing world, killing up to 2.5 million people every year [1] (i.e. about 5% of all deaths world-wide are directly caused by malaria). The problem is aggravated by the economic conditions in most malarious areas [2] and can only become more serious with the rapid spread of parasites that are resistant to antimalarial drugs and of mosquitoes that are resistant to insecticides. Thus novel methods of malaria control are desperately needed.

A potential method of malaria control, which is being developed in several laboratories world-wide, is the genetic manipulation of mosquitoes. The idea behind this is to transform mosquitoes with genes that render them refractory against infection by malaria, then release them into natural populations and thus make mosquito popula-

tions incapable of transmitting malaria. Hopes for this possibility were raised by the observation that there is some genetic variability for mosquito refractoriness against the malaria parasite [3,4]. This refractoriness is based on melanotic encapsulation of its early developmental stages [5]. First steps to achieve genetic manipulation have recently been accomplished with the identification of genes responsible for the encapsulation, melanization and death of early malarial infections in the mosquito [6,7] and with the identification of possible mechanisms of introducing these genes into mosquito genomes [8,9].

It is therefore time for discussions of how best to employ the tools that produce harmless strains of mosquitoes in attempts to control malaria [10]. There are two major questions on the ecological side of genetic manipulation. First, under what conditions can refractoriness be expected to spread in a natural population of mosquitoes? An answer to this question must take into account three parameters: the benefit of refractoriness that is due to avoiding the malaria parasite's detrimental effects on fecundity [11,12] and mortality [13], the cost of refractoriness associated with maintaining [14] and mounting an immune response in insects [15], and the efficiency of the transformation system. Second, if not all mosquitoes are completely refractory, what will be the impact of the release of transgenic mosquitoes on the malaria situation?

Answers to these questions can only be found with a combination of theoretical and empirical work on refractoriness in natural populations of mosquitoes. In a step towards this, we present a theoretical model that describes the spread of refractory genes in a population of mosquitoes. Though earlier theoretical models have approached this question [16,17], none have considered all three parameters mentioned above. Furthermore, we extended the models by combining a population genetical and an epidemiological approach. Not only can such a combination yield different conclusions than either approach on its own [18], but it also allows one to evaluate the effect of the release of transgenic mosquitoes on the prevalence of malaria in the human population.

The model

Population genetics

We assumed that refractoriness is determined by a single gene and that the dynamics can be described by discrete generations. As the costs and benefits of the refractory gene will differ between males and females, we based our model, describing the spread of a given allele in a population, on the standard population genetics equations for sex-dependent fitness [19]. We further assumed that genetic manipulation is achieved by linking the refractory gene to transposons, which have the tendency to infect the offspring of transfected parents and can therefore spread rapidly through populations, even if they are associated with reduced fitness [20,21]. We did not take into account the dynamics of the copy number of the transposon within a genome, but simply assumed that the transposon increases the probability that the refractory gene in a heterozyote parent is transmitted to the offspring by the factor ∂, the efficiency of the genetic drive [22]. Thus, if $p_{f,t}$ describes the frequency of the refractory gene in female gametes at generation t, $p_{m,t}$ describes the frequency of the refractory gene in male gametes, $W_{f,RR}$ describes the fitness of females that are homozygous for the refractory gene, $W_{f,RS}$ describes the fitness of females that are heterozygous for the refractory gene, and $\bar{W}_f$ describes the mean fitness of females, then the frequency of the refractory allele in female gametes at generation t+1 can be described by

$$p_{f,t+1} = \frac{p_{f,t}p_{m,t}W_{f,RR} + 0.5(1+\partial)\left[p_{f,t}(1-p_{m,t}) + (1-p_{f,t})p_{m,t}\right]W_{f,RS}}{\bar{W}_f}$$

where

$$\bar{W}_f = p_{f,t}p_{m,t}W_{f,RR} + \left[p_{f,t}(1-p_{m,t}) + (1-p_{f,t})p_{m,t}\right]W_{f,RS} + (1-p_{f,t})(1-p_{m,t})W_{f,SS}.$$

An analogous equation describes the spread of the allele for the males.

Fitness

The fitness of males and females is determined by the cost of maintaining refractoriness. The fitness of females is additionally determined by the burden of the parasite. In both sexes, we assumed that the transposon reduces fitness.

The fitness of sensitive males (harbouring neither transposon nor, obviously, parasite) was assumed to be 1. The fitness of sensitive females is thus 1 - (the burden of the parasite). We assumed that infection by malaria reduces fitness by the parasite's virulence α. The probability that a mosquito is infected when it bites a human host is proportional to the prevalence γ of malaria in the human population and to the probability, b, that a mosquitoes will become infected on a single infectious bloodmeal. If a mosquito bites a human, on average, k times during its life, the probability that it will become infected at least once during its life-time is thus proportional to the nonzero-term of the Poisson distribution 1 - exp(- byk). Note that this equation is based on several assumptions, e.g. that infections occur independently of the current infection status of the mosquito. Changing the details of these assumptions, however, will not alter the qualitative conclusions of the model; what is important is that the probability that a mosquito will become infected increases with the prevalence in humans. For the rest of the paper,

we assumed that b = 1, without changing the general conclusions. The fitness of sensitive females is then given by $w_{f,SS} = 1 - [1 - \exp(-\gamma k)]\alpha$.

Mosquitoes that are homozygous for the refractory allele are protected from infection with the effectiveness of protection *s*; the probability that they will become infected is therefore reduced to $1 - \exp(-\gamma k(1 - s))$. However, they must pay the cost of harbouring two refractory alleles, assumed to be *c*. Furthermore, as they carry the transposon along with the refractory alleles, their fitness is further reduced by the cost c_T of the transposon. Overall, the fitness of females homozygous for refractoriness is thus $w_{f,RR} = 1 - [1 - \exp(-\gamma k(1 - s))]\alpha - c - c_T$, while the males suffer from the costs of harbouring the refractory alleles and the transposons and thus have fitness $w_{m,RR} = 1 - c - c_T$.

Finally, heterozygotes were assumed to fall between the refractory and sensitive homozygotes, with the effectiveness of protection and the cost of refractoriness being reduced by *h*, the level of dominance. Thus, the fitness of heterozygous females is $w_{f,RS} = 1 - [1 - \exp(-\gamma k(1 - hs))]\alpha - hc - c_T$ and that of males is $w_{m,RS} = 1 - hc - c_T$.

Cost of refractoriness

The cost of refractoriness can be expressed in two ways. On the one hand, there may be a fixed cost involved in maintaining the physiological machinery necessary for the immune encapsulation response. Such a maintenance cost would be expressed as a genetic correlation between the effectiveness of the immune response and other traits that determine fitness. This type of cost has been demonstrated for several insects (e.g. *Drosophila*[14]), including the mosquito *Aedes aegypti*, where the effectiveness of encapsulating Sephadex beads is genetically correlated with the mosquito's age at pupation (Koella and Boëte, submitted). On the other hand, the cost may be expressed when the immune response is mounted, so that it is expressed only in infected individuals. Such a conditional cost has also been observed in several insects (e.g. bumble-bees [15]), including *Anopheles gambiae*, where mosquitoes with an effective immune response have lower fecundity (Schwartz and Koella, submitted).

To allow for these types of cost, we modelled the cost in two ways. The fixed maintenance cost assumes that the cost *c* is constant, i.e. $c = c_0$. The conditional cost, in contrast, depends on the rate of infection, and was thus modelled as $c = c_0[1 - \exp(-\gamma k)]$.

Epidemiology

Finally, the prevalence of infection in the human population is determined by the classical Macdonald-Ross equations describing the epidemiology of malaria [23,24], and is thus given as

$$\gamma = \frac{R_0 - 1}{R_0 + \frac{a}{\mu}}$$

where R_0 is the basic reproductive number of malaria, *a* is the mosquito's biting rate and μ is its mortality rate (and thus the number of bites per life-time is $k = a/\mu$).

The basic reproductive number, on the other hand, is determined by the number of sensitive mosquitoes [23]. Assuming that the population of mosquitoes is at Hardy-Weinberg equilibrium, we therefore write

$$R_{0,t} = R_0^* \left\{ p_{f,t} p_{m,t} (1 - s) + \left[p_{f,t} (1 - p_{m,t}) + (1 - p_{f,t}) p_{m,t} \right] (1 - hs) + (1 - p_{f,t})(1 - p_{m,t}) \right\}$$

where R_0^* is the basic reproductive number when all mosquitoes are sensitive, i.e. before the introduction of the transgenic mosquitoes.

Results

Spread of refractoriness

We first describe the spread of the allele coding for refractoriness in the absence of a transposon-mediated drive mechanism. As we are interested in the end-result of the evolutionary (control) process, we only show and discuss the predictions of the model at equilibrium.

Fixed maintenance cost

If the cost of refractoriness is fixed, a threshold value of the cost, which depends on the intensity of transmission, determines whether refractoriness can spread in a population of mosquitoes. Below this cost, the proportion of mosquitoes that harbours the allele coding for refractoriness increases with the intensity of transmission up to an asymptotic value, and decreases slightly with the cost of refractoriness (Fig. 1a). Concomitantly, the prevalence of disease in the human population decreases to low values when the cost of refractoriness is low and, paradoxically, in epidemiological situations with intense transmission (Fig. 1b). Note that refractoriness (if it has complete efficacy) cannot be fixed in the population and thus that the disease cannot be eradicated due to the epidemiological feed-back on the selection pressure on the parasite.

There is a similar threshold for the efficacy of refractoriness. If the efficacy is lower than this threshold, refractoriness cannot spread. Above this threshold, refractoriness will generally spread to a high proportion of the mosquitoes and may reach fixation (Fig. 1c). As the efficacy approaches 100%, the proportion of refractoriness decreases because of the epidemiological feed-back: if refractoriness

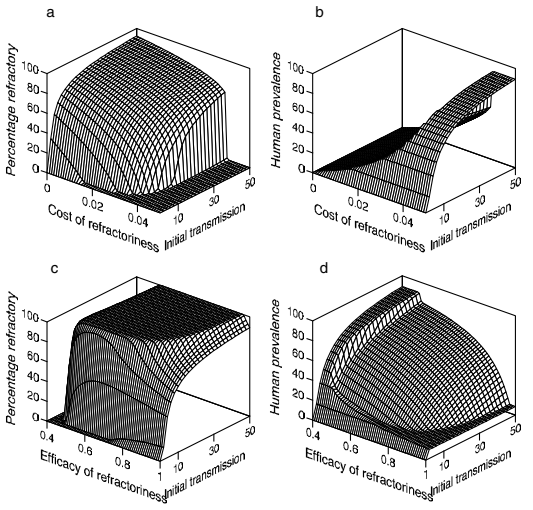

Figure 1
Spread of refractoriness in the absence of a transposon as a genetic drive mechanism, and its effect on the prevalence of malaria in the human population at equilibrium under the assumption of a fixed maintenance cost of refractoriness. The parameters used to simulate the equations were: biting rate a = 0.5 day^{-1}, mortality μ = 0.1 day^{-1}, virulence = 0.1 and dominance h = 0.9. In panels (a) and (b) the efficacy of refractoriness was held at s = 1, and the cost of refractoriness and the initial intensity of transmission (i.e. before the spread of refractoriness) were varied. In panels (c) and (d) the cost of refractoriness was held at c = 0.01, and the efficacy of refractoriness and the initial intensity of transmission were varied.

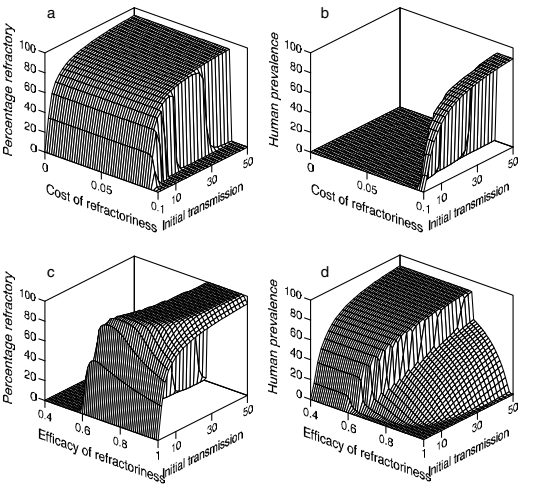

Figure 2
Spread of refractoriness in the absence of a transposon as a genetic drive mechanism, and its effect on the prevalence of malaria in the human population at equilibrium under the assumption of a conditional cost of refractoriness that is induced by the response to parasite infection. The parameters used to simulate the equations were: biting rate a = 0.5 day^{-1}, mortality μ = 0.1 day^{-1}, virulence = 0.1 and dominance h = 0.9. In panels (a) and (b) the efficacy of refractoriness was held at s = 1, and the cost of refractoriness and the initial intensity of transmission (i.e. before the spread of refractoriness) were varied. In panels (c) and (d) the cost of refractoriness was held at c = 0.05, and the efficacy of refractoriness and the initial intensity of transmission were varied.

becomes too effective, the proportion of infected mosquitoes drops to a level where there is little selection pressure for the mosquito to increase its refractoriness. Accordingly, prevalence decreases with the efficacy of refractoriness, but again does not reach 0 (Fig. 1d).

Conditional cost

When the cost is conditional, i.e. is paid only after infection, the simulations showed two main differences to the results described above (Fig. 2). First, the threshold cost that prevents the spread of refractoriness is higher than when the cost is fixed. Second, the spread of refractoriness can lead to the eradication of the parasite. Both of these differences are due to the lower cost paid as refractoriness spreads, and thus to a decreasing probability of a mosquito becoming infected.

Spread of the tandem refractoriness allele & transposable element

If the allele coding for refractoriness is linked to a transposon, this tandem either spreads to fixation or disappears from the population, with no parameters allowing intermediate prevalence. The three main parameters determining the spread of the tandem are (a) the efficacy of the drive mechanism (i.e. the probability that heterozygotes turn homozygous), (b) the cost of the transposon and (c) the cost of refractoriness. When the cost of refractoriness is fixed, the efficacy of drive that is necessary to enforce the spread of refractoriness increases more or less linearly with both the cost of refractoriness and the cost of the transposon (Fig. 3a). When, however, the cost of refractoriness is conditional, it has no influence on the conditions for the spread of refractoriness (Fig 3b). In either case – with a fixed maintenance cost (Fig. 3c) or a conditional cost of refractoriness (Fig. 3d) – the efficacy of refractoriness and the intensity of transmission have only a slight effect on the conditions for the spread of refractoriness. Thus, when the cost of refractoriness is fixed, the efficacy of the transposon required to drive refractoriness into a population decreases slightly with increasing intensity of transmission if the efficacy of refractoriness is high, but increases with increasing intensity of transmission if the efficacy of refractoriness is low (Fig. 3c). When the cost of refractoriness is conditional upon infection, the required efficacy of the genetic drive mechanism increases with in-

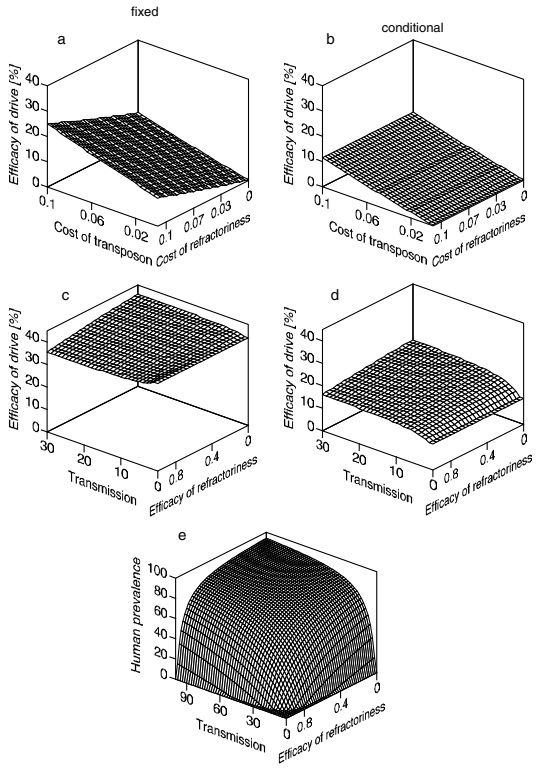

Figure 3
Spread of refractoriness linked to a transposon as a genetic drive mechanism and its effect on the prevalence of malaria in the human population at equilibrium. Panels (a)-(d) show the efficacy of the genetic drive mechanism (i.e. the proportion of heterozygotes that turn homozygous) that is required for refractoriness to spread to fixation. Panels (a) and (c) assume that the cost refractoriness is fixed, panels (b) and (d) assume a conditional cost induced by parasite infection. Panel (e) shows the effect of fixation of the allele coding for refractoriness on the prevalence of malaria. The parameters used to simulate the equations were: biting rate a = 0.5 day^{-1}, mortality μ = 0.1 day^{-1}, virulence = 0.1 and dominance h = 0.9. In panels (a) and (c) the efficacy of refractoriness was held at s = 1, the initial intensity of was set to $R_{0,init}$ = 30 and the cost of refractoriness and the cost of the transposon (i.e. before the spread of refractoriness) were varied. In panels (b) and (d) the cost of refractoriness was held at c = 0.2, the cost of the transposon was held at c_T = 0.05, and the efficacy of refractoriness and the initial intensity of transmission were varied. In panel (e) the parameters not mentioned above were chosen to allow fixation of refractoriness.

creasing intensity of transmission at any efficacy of refractoriness (Fig. 3d).

The efficacy of refractoriness and the intensity of transmission, however, have a large effect on the effect of the spread of refractoriness on the prevalence of disease in the human population (Fig. 3e). Even in conditions that allow the allele conferring refractoriness to spread to fixation, the impact on prevalence in humans is slight unless the efficacy of refractoriness is considerable, particularly in areas with intense transmission.

Discussion

Summary of results

In the absence of the genetic drive provided by a transposon (Figs. 1 & 2), the spread of an allele conferring refractoriness is determined by the balance of evolutionary costs (reduced reproductive success of refractory mosquitoes) and benefits (reduced detrimental effect of the parasite) of refractoriness. These are partly determined by the probability that mosquitoes are infected, i.e. by the prevalence of malaria in the human population, which, in turn, is determined by the level of refractoriness in the mosquito population. This epidemiological feedback is the cause of frequency-dependent selection, which prevents the eradication of the parasite.

If a transposon is linked to the gene responsible for refractoriness (Fig. 3), the epidemiological feedback is largely lacking, so that refractoriness is either lost from the population or spreads to fixation. It is reassuring that fairly low efficacy of the genetic drive mechanism ensures the fixation of refractoriness in many situations. The predictions of our equations, which combine population genetical and epidemiological processes, thus corroborate results from previous population genetical [21] or spatially explicit individual-based [17] models.

More importantly from the perspective of malaria control, if the efficacy of refractoriness is 100%, genetic manipulation can eradicate malaria from the population. If, however, the refractoriness is less than complete, the impact on the malaria situation is negligible in areas of intermediate to high transmission. This should come as no surprise, as the Macdonald-Ross model of malaria epidemiology shows that reducing the number of (susceptible) mosquitoes is an inefficient way of reducing malaria transmission [23].

Thus, from the model described here, it is clear that knowledge about three parameters is critical for our understanding of the spread of refractoriness: the benefit of refractoriness, its cost, and its efficacy.

Any data?

Although there are no quantitative data on any of these parameters, recent studies have started to provide at least some qualitative estimates. The benefit of refractoriness is due to the advantage of reducing the deleterious effects of infection. Though the virulence of the parasite is still being questioned (Ferguson, under review), recent studies have shown that malaria infection reduces the survival of

mosquitoes in the field [13] and their fecundity [11,12]. A possible cost of refractoriness is due to the physiological cost of mounting an encapsulation immune response. Though studies on mosquitoes have only just started (Koella & Boëte, submitted, Schwartz & Koella, submitted), other insects provide ample evidence of such a cost [14,15,25]. Overall, however, the cost of refractoriness appears to outweigh the benefits, as the proportion of refractory mosquitoes in natural populations is negligible [26].

The efficacy of refractoriness depends not only on its genetic determination, but also on environmentally induced variation. And indeed, the encapsulation response of mosquitoes decreases substantially if larvae are reared in bad conditions [27], if adults do not obtain a blood meal [28] and as they age [26]. Thus, it seems unlikely that refractoriness would be completely effective in natural situations.

Criticism of model

Obviously, like any other mathematical (or non-mathematical) model our equations are simple caricatures of the epidemiological and genetic processes being considered. The success of a model will depend on the inclusion of the relevant processes.

There are several reasons for believing that the epidemiological aspect of our model captures some aspect of reality. Compartment models of infectious diseases in general have had considerable success in epidemiology [29]. In particular, compartment models similar to the ones used here have had considerable success in describing epidemiological patterns of malaria [30]. Similar models are considered to be helpful in evaluating the sensitivity of malaria transmission to different control measures [23] and in predicting the effectiveness of vaccine programs [31–33].

In the population genetical parts of the model, we have made three critical assumptions. First, by following standard ideas of population genetics we have assumed an infinite population size. However, other models have shown that finite populations that take into account density-dependence [21] change the general conclusions only slightly. Furthermore, as the effective population sizes of mosquitoes are often large [34], the stochasticity of genetic drift will only affect the initial spread of very rare resistance alleles and have little effect on the outcome of the simulations [17]. Second, refractoriness is determined by an allele at a single locus. Although it is known that several genes are involved in the immune response [4,35], the main difference between susceptible and refractory mosquitoes appears to be determined by one or very few major genes [6,7]. Third, our modelling of the transposon neglected several aspects of the biology of transposons, e.g. the regulatory process that determines their copy number [36,37] and the fact that the refractory gene could disassociate from the transposon, making the system ineffective [38,39]. Modifying these assumptions will generally make the likelihood that refractoriness spreads to fixation less likely, so that our results are optimistic predictions about the success of a control programme.

Conclusions

The approach offered here can provide insights into the use of genetically engineered mosquitoes for malaria control. The most important one is that, while refractoriness may be driven into a population of mosquitoes despite considerable costs, less than complete refractoriness of mosquitoes harbouring the allele will only slightly decrease the prevalence of infection in the human population, in particular in areas with intense transmission. But clearly, what is needed is more detailed knowledge of the critical parameters involved: costs and benefits of refractoriness and in particular the efficacy of refractoriness. Only this will allow one to give advice about rational malaria control using genetically manipulated mosquitoes.

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