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The fixation of malaria refractoriness in mosquitoes

Matthew W. Hahn and Sergey V. Nuzhdin*

Malaria is the deadliest human parasitic disease, causing over one million deaths every year. Because insecticide-based programs have failed to control the main vector of malaria, the mosquito *Anopheles gambiae*, hope for the eradication of malaria has turned to genetically modified mosquitoes that are refractory to infection [1,2]. Plans to use engineered transposable elements or other drive mechanisms to enable fixation of malaria refractoriness [3] come up against two major obstacles: the best engineered refractoriness gene is not very effective [4], and transposable elements or other vectors in which the refractoriness gene is deleted or inactivated will preferentially spread in populations [2,5]. So far, no drive mechanism has been proposed that would result in the fixation of a refractoriness gene, rather than of itself, in a population. Here we propose an evolutionary mechanism that will lead to the fixation of refractory alleles segregating in nature with any type of driver. We illustrate this mechanism in detail for a model involving drive by transposable elements.

Mosquitoes infected with the malaria parasite *Plasmodium falciparum* suffer quantifiable reductions in fitness, such as reduced longevity, fecundity, and flight distance [2]. Although the immune system of mosquitoes has the potential to kill malaria parasites at several stages of development, and refractory alleles are found in nature [6], refractoriness has not spread due to high maintenance and activation costs [2]. Because the refractory allele will not spread on its own,

several researchers have proposed using a drive mechanism, such as bacteria, meiotic drive, or transposable elements, to aid the fixation of an engineered refractory gene [1–3]. These proposals involve the insertion of a refractory 'effector' gene into a driver, whereby infection of the whole population will lead to the fixation of the refractory gene and the loss of malaria infection. Analysis of the genome sequence of *A. gambiae* has uncovered many active transposable elements [7] and stable transformation of *A. gambiae* has been achieved [8]. Given that most of the basic engineering requirements for genetic modification of mosquitoes have been met, how do we succeed in overcoming the inefficiency of engineered refractory genes and the tendency for drivers to lose the linked genes that are unnecessary for transmission?

We propose to take advantage of highly efficient refractory alleles found in nature, and to drive them to fixation without a linked effector gene. This goal is achieved by making individuals susceptible to infection have a lower fitness. We propose to use differences in gene expression between refractory and susceptible individuals to design a driver that is activated by expression of susceptible-specific genes. Recent analyses of gene expression in mosquitoes have revealed several genes that are strongly differentially expressed between susceptible and refractory strains [9] and between infected and uninfected individuals [10]. We suggest putting expression of a transposable element under the control of a promoter or enhancer sequence that promotes strong expression in susceptible or infected individuals, but not in refractory or uninfected ones; in order for transposition to be heritable, the transposable element must be mobilised in the germline. Activity of this modified transposable element is, therefore, limited to susceptible mosquitoes, though the copy number of the transposable element will equilibrate between

susceptible and refractory mosquitoes due to random mating. The fitness cost of transposable element activity, however, is restricted to susceptible individuals. Thus, susceptible alleles incur fitness costs that outweigh the cost of refractoriness when transposable elements are sufficient in number. Because no effector gene is needed to lower the fitness of susceptible individuals, and because naturally occurring refractory alleles can be highly effective [2,6], this mechanism should drive refractory alleles to fixation in a mosquito population.

To see whether this proposed mechanism would work in a natural population upon introduction of an engineered mosquito, we studied a model of transposable element dynamics. We modeled the above scenario in a way that is conceptually similar to many models developed previously (see Supplemental Data for a description of the model). Transposable elements spread through transposition in susceptible individuals and subsequent mating; they are selected against because of the reduction in fitness they impose on susceptible hosts. As the average copy number of transposable elements per individual rises, individuals carrying the refractory allele gain relative fitness. Over a large range of parameters, the refractory allele goes to fixation (Figure 1). Many parameters did not appear to influence the final outcome of the simulations, as refractory alleles were always driven to fixation, but they did affect the speed of fixation. Fixation was faster when transposition events were more frequent (Figure 1A), when the initial proportion of susceptible individuals was higher (Figure 1B), or when the initial number of transposable elements introduced into the population was higher (Figure 1C). The refractory allele became fixed when transposable elements were introduced at frequencies as low as 10^{-6} (Figure 1C) or when the transposition rate was only 10% (Figure 1A). The effect of the infection rate on time to fixation was very small (not
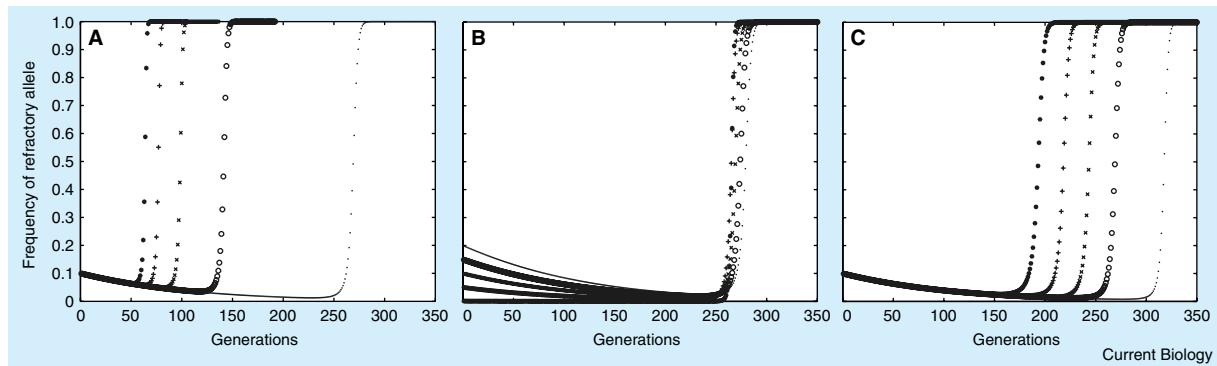
Figure 1. Fixation of a refractory allele after introduction of a transposable element decreasing fitness of susceptible individuals.
All panels use standard values taken from the literature (Supplemental Data): copy number per individual (P=0.00001), frequency of the refractory allele (R=0.10), transposition rate per transposable element (u=0.10), deleterious effect of each transposition (d=0.00001), infection rate (I=0.01), cost of the refractory allele (k_R=0.01), and cost of infection (k_S=0.001).
(A) Relationship between time to fixation of the refractory allele and the transposition rate, u. Different values of u are represented by •=0.10, o=0.20, x=0.30, +=0.40, *=0.50. (B) Relationship between time to fixation of the refractory allele and the initial frequency, R, of the refractory allele in nature. Different values of R are represented by •=0.20, o=0.15, x=0.10, +=0.05, and *=0.001. (C) Relationship between time to fixation of the refractory allele and the initial average number of transposable elements, P. Different values of P are represented by •=0.000001, o=0.00001, x=0.0001, +=0.001, and *=0.01.

shown). Transposable elements spread in the population only when the transposition rate outweighed the loss in host fitness due to transposable element load; this is the standard condition that must be fulfilled for any transposable element to survive [3]. Importantly, as a modified transposable element can only multiply when under an active promoter, it is selected to maintain its susceptible-specific promoter intact. The dynamics presented here are unchanged if we imagine a transposase that is up-regulated only upon infection of susceptible individuals, although the time to fixation is greatly increased as infection rates in nature are generally low (results not shown).

The idea of fixing a refractory allele by making the susceptible allele too costly should work similarly with other drivers that may be natural or engineered pests, as long as they are up-regulated by an honest signal of susceptibility or malaria infection. In fact, a combination of several drivers might be the most efficient and robust plan against *Plasmodium* counter-adaptation. The model proposed here is both effective and fast: refractory alleles can fix in as quickly as 60 mosquito generations (~4 years). Once the ethical and economic implications of genetically modified mosquito introduction have been settled, we may be able

to rid the world of malaria in a short period of time.

Supplemental Data
Supplemental Data are available at http://www.current-biology.com/supplemental

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Center for Population Biology and Section of Evolution and Ecology, University of California, Davis, California 95616, USA. *E-mail: svnuzhdin@ucdavis.edu

Correspondence

Supplemental Data: The fixation of malaria refractoriness in mosquitoes

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Our model considers change over time in the average transposable element copy number per individual (P), and the coupled change in the frequency of the refractory allele (R). Parameters in the model include: u, the transposition rate per transposable element; d, the deleterious effect of each transposition; I, the infection rate among susceptible individuals; k_R, the cost of the refractory allele when there is no infection; and, k_S, the cost of infection. Change in transposable element copy number over time (t) is then (cf. [S1,S2])

$$dP/dt = uP(1-R) + (\partial \ln f_S/\partial P)(1-R)$$

where f_S is the relative fitness of susceptible individuals, defined below. The change in the frequency of the refractory allele is

$$dR/dt = R(1-R)(f_R - f_S)$$

where f_R is the relative fitness of refractory individuals.

The relative fitness of refractory and susceptible individuals is defined as

$$f_R = (e^{-k_R})/[(e^{-k_R})R + (e^{-uPd-k_S I})(1-R)]$$

and

$$f_S = (e^{-uPd-k_S I})/[(e^{-k_R})R + (e^{-uPd-k_S I})(1-R)]$$

Here the fitness of refractory individuals is a function of the cost of the refractory allele (k_R). The fitness of susceptible individuals is a function of the product of transposition rate (u),

the number of transposable elements per individual (P), and the deleterious effect of each transposition (d); susceptible individuals also suffer a cost when infected (k_S). The relative fitness of each genotype is then the individual fitness divided by the average fitness of the population. Note that the choice of fitness functions does not qualitatively affect the evolutionary outcome (data not shown). While we have considered a single panmictic population here, it is almost certainly true that mosquito populations in Africa do not meet this ideal [S3,S4]. However, this means that it may only be the local frequency of introduced transposable elements and refractory alleles that is important; migration of mosquitoes and the use of multiple introduction sites will then both help to fix refractoriness more quickly [S5].

We investigated the behavior of this model over a range of values for each of the parameters. Results shown in Figure 1 use the following values taken from the literature [S6–S11]: P=0.00001, R=0.10, u=0.10, d=0.00001, I=0.01, k_R=0.01, and k_S=0.001.

Drosophila has served as a model species for transposable element dynamics, and the concepts developed in this species have successfully been applied to a wide spectrum of organisms from *Daphnia* [S12] to humans [S13]. Analogously, we have built our mathematical model using concepts first proven in *Drosophila*. One question our model does not test can only be clarified in experiments: Will transposable elements multiply to a frequency sufficient to create a decrease in the fitness of susceptibles? In *A. gambiae*, the transposable element copy number varies between families from a few to approximately 2000 [S14]. In many species, including our own, the most abundant transposable element families can reach numbers of up to hundreds of thousands of copies. In our model, multiplication of the element to 400 copies per genome sufficed to reverse the relative fitness of susceptible and refractory alleles — well within the

limit of natural variation of copy number. This number is highly dependent on the deleterious effects of each transposition; the stronger the effect, the fewer transposable elements are eventually needed per genome. To increase the likelihood of sufficient multiplication, we recommend avoiding elements that self-regulate copy number (e.g. mariner [S15]), are inactivated by RNAi-related mechanisms [S16], or produce many harmless aborted copies (e.g., P [S17]).

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