Requirements for effective malaria control with homing endonuclease genes

Anne Deredec, H. C. J. Godfray, A. Burt

Requirements for effective malaria control with homing endonuclease genes

Anne Deredec[a], H. Charles J. Godfray[b], and Austin Burt[a,1]

[a]Department of Life Sciences, Imperial College London, Silwood Park, Ascot, Berks SL5 7PY, United Kingdom; and [b]Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom

Edited by Anthony A. James, University of California, Irvine, CA, and approved August 18, 2011 (received for review July 25, 2011)

Malaria continues to impose a substantial burden on human health. We have previously proposed that biological approaches to control the mosquito vector of disease could be developed using homing endonuclease genes (HEGs), a class of selfish or parasitic gene that exists naturally in many microbes. Recent lab studies have demonstrated that HEGs can function in mosquitoes. We constructed and analyzed a model of mosquito population genetics and malaria epidemiology to determine how well HEGs need to function in order to have a significant effect on the burden of disease. Our model, combined with currently available data, indicates that populations of *Anopheles gambiae* could be eliminated by releasing 2–3 HEGs targeting female fertility genes, or a driving-Y chromosome that is transmitted to 75–96% of progeny. Combinations of fertility-targeting HEGs and Y drive may also be effective. It is possible to eliminate the disease without eliminating the vector, but the parameter space producing this outcome appears to be small. HEGs causing a quantitative reduction in adult survival can be more effective than those targeting female fertility, but the selection coefficients that need to be imposed are still large, unless many HEGs are to be released. Simulations show that HEG-based strategies can be effective over socially relevant time frames. Important limiting assumptions of the models are that there is only a single vector species, and we model a homogeneous population, not a landscape. Nevertheless, we conclude that HEG-based approaches could have a transformational effect on malaria control efforts.

biological control | genetic load | vector control

Malaria kills eight or nine hundred thousand people every year, mostly infants and children in tropical Africa (1). The best existing methods of control—artemisinin-based drug treatment and mosquito control with chemical sprays and treated bed nets—can reduce the burden of disease substantially if applied with some vigor, and can even eliminate the disease in some regions, but are not thought to be capable of global eradication (2). It is not even clear that current levels of efficacy can be maintained, given the likelihood of parasites and mosquitoes evolving further resistance, and immunity waning as a result of partial control (2, 3). Genetic approaches to vector control have been widely discussed as potentially powerful methods of augmenting existing malaria control measures (4, 5).

Homing endonuclease genes (HEGs) are a class of selfish genetic element found naturally in many microbes (6, 7). They spread through populations by encoding an endonuclease that recognizes an 18- to 30-bp sequence that typically exists only once per genome. The gene is inserted in the middle of its own recognition sequence, so that in heterozygous individuals only the chromosome not containing the gene gets cut. Recombinational repair of a broken chromosome will use the unbroken homolog (containing the HEG) as a template for repair, and so the HEG gets copied across to the chromosome where previously it was absent, converting a heterozygote into a homozygote. This process is called homing.

We have previously suggested two ways that HEGs could be used to control a vector population (8, 9). First, a HEG could be engineered to recognize a sequence in the middle of a native mosquito gene and then be inserted in the middle of its own recognition sequence. If such a HEG was active in the germ line, then it could increase in frequency in a population, knocking out the mosquito gene as it did so. If the target gene was important in survival or reproduction, then the number of mosquitoes may be reduced; if the gene was involved in the development or transmission of the parasite, then vector competence may be reduced. This approach uses the canonical homing reaction as it occurs in microbes. Alternatively, a HEG could be engineered to recognize a repeated sequence on the X chromosome and then be linked to meiosis-specific control sequences and inserted on the Y chromosome. Such a HEG would cleave the X chromosome at male meiosis, potentially resulting in a preponderance of functional sperm and zygotes carrying the Y chromosome. The HEG-bearing Y would spread through the population, biasing the sex ratio toward males, therefore reducing the number of mosquitoes and disease transmission. This driving-Y strategy does not rely on recombinational repair and homing, but merely on cleavage disrupting chromosomal transmission through meiosis.

Whether these strategies can be made to work effectively will depend on, among other things, the underlying mechanistic assumptions being correct and on the molecular processes occurring with sufficient frequency in mosquitoes as to have a significant epidemiological effect in a reasonable amount of time. Homing is not known to occur naturally in any animal, but two recent studies using a HEG and its recognition sequence from yeast have shown that homing can occur in both *Drosophila melanogaster* and *Anopheles gambiae* (10, 11). In male *An. gambiae* the homing rate (i.e., the fraction of potential recipient chromosomes in heterozygotes that acquire the HEG) was about 60% (11). Also in *An. gambiae*, a HEG targeting an X-linked repeat has been shown to result in the Y chromosome being transmitted to about 90% of progeny (12). The question then arises, what rates are needed to make HEGs useful for malaria control? The answer will be a key component of the "minimum product profile" for HEGs to be a viable control tool.

To address this question we have constructed and analyzed a model of mosquito population genetics and malaria epidemiology. Because these HEG-based strategies are meant to affect the vector, much of the detail in the model involves the mosquito portion of the disease transmission cycle, with the human side left deliberately simplified. We focus in particular on strategies that target aspects of mosquito demography (survival, reproduction, and the sex ratio). It is clear that a population-wide knockout

Author contributions: A.D., H.C.J.G., and A.B. designed research; A.D. performed research; and A.D. and A.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]To whom correspondence should be addressed. E-mail: a.burt@imperial.ac.uk.

See Author Summary on page 17575.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1110717108/-/DCSupplemental.

of a weakly selected mosquito gene that is essential for malaria transmission could be very effective; what is not clear is whether any such gene exists. By contrast, many genes with strong effects on survival and reproduction are known in *Drosophila* and are likely to be identifiable in *Anopheles* by homology and expression studies (13).

We begin by modeling HEGs that knock out specific mosquito genes and spread by homing, deriving the requirements for mosquito elimination. Because the main goal is to eliminate malaria, not mosquitoes, we then derive requirements for disease elimination, under two scenarios: first, when the only effect of the HEG is to reduce (but not necessarily eliminate) the number of mosquitoes, and second, when the effect of the HEG is to reduce adult longevity. Because malaria parasites require many days to develop within a mosquito before they can be transmitted to a human, increasing adult mosquito mortality has long been considered a particularly effective form of control (14, 15). We also consider what will be required to prevent cleavage-resistant alleles from spreading, and the possibility of a HEG being lost due to chance as a population approaches elimination. We then model the alternative approach of using Y-linked HEGs that target the X chromosome at male meiosis to bias the sex ratio. Finally, for selected scenarios, we use computer simulations to analyze the dynamics of HEG-based malaria control, to assess whether it can occur over a socially useful time frame.

Results

Homing and Mosquito Elimination. To analyze what is required to eliminate a mosquito population, we begin with a simple model of mosquito population dynamics in a homogeneous, constant environment using a discrete-time lumped age–class formulation adapted from ref. 16. The life cycle is divided into three juvenile stages (egg, larva, and pupa) and one adult stage. The three juvenile stages are each assumed to be of fixed duration, during which individuals experience a constant rate of density-independent mortality. Little is known about population regulation in mosquitoes, except that density-dependent mortality is thought to occur during the larval stage (17, 18). We therefore assume that larvae experience an additional rate of mortality that increases monotonically with larval density. In this model the maximum or intrinsic growth rate of the population occurs at low density and is represented by R_m.

For an isolated mosquito population to persist, R_m must be greater than 1, and for a HEG to eliminate a population, it must reduce R_m below 1. If we let R'_m be the intrinsic rate of population increase in the presence of the HEG and define the "HEG load" as $\mathscr{L} = 1 - R'_m/R_m$, then to eliminate a vector population (i.e., have $R'_m < 1$), it is necessary that $\mathscr{L} > 1 - 1/R_m$ (9). We have been unable to find any previous estimate of R_m for *An. gambiae* and therefore have derived an estimate from the maximum rate of increase of a population at the beginning of the wet season. As part of the Garki project in Northern Nigeria (19), *An. gambiae* densities in a village (Kwaru) were measured every 2 wk for over 3 y by indoor pyrethrum spray collections. Mosquito densities showed strong seasonal fluctuations, increasing exponentially at the beginning of the wet season and crashing in the dry season (Fig. S1). The average rate of increase at the beginning of three different wet seasons (1971–1973) was 1.096 ± 0.0056 (se) d^{-1}. With an estimated generation time of 24.2 d (see *SI Appendix*), this daily rate of increase translates into a per-generation rate of increase of $R_m = 1.096^{24.2} = 9.2$. As there are a number of uncertainties associated with this estimate (see *Discussion*), and as R_m is expected to vary across the species range, falling to $R_m \sim 1$ near the edge of its distribution (assuming the boundaries are at equilibrium), we have used $R_m = 12$, $R_m = 6$, and $R_m = 2$ as representing high, medium, and low values, and illustrate the implications of these different values for HEG-based control.

We have previously derived expressions for the HEG load as a function of the heterozygous and homozygous effects of knocking out the target gene and the homing rate (8, 9). For example, a HEG targeting an essential gene for which the knockout is completely recessive will impose an equilibrium load of $\mathscr{L} = e^2$, where e is the rate of homing. Therefore, if $R_m = 12$, the homing rate must be greater than 0.96 to eliminate the population; if $R_m = 6$, the condition is $e \geq 0.91$; and if $R_m = 2$, it is $e \geq 0.71$. Targeting a female fertility gene is somewhat more efficient, as the equilibrium load is $\mathscr{L} = 4e^2/(1 + 3e^2)$, and the conditions for elimination are $e \geq 0.86 (R_m = 12)$, $e \geq 0.75 (R_m = 6)$, and $e \geq 0.45$ $(R_m = 2)$.

Homing rates as high as these may not be easily achievable in mosquitoes; the recent study of Windbichler et al. (11) recorded homing rates of about 0.6 in males (see *SI Appendix*). One approach to increasing the load is to target more than one gene (8). Homing acts analogously to recombination in breaking up linkage disequilibrium, and therefore if we assume that homing rates and fitness effects are independent across loci, then HEGs at different loci will be in linkage equilibrium in the population even if the loci are close together on the same chromosome (8). Therefore, if n different loci are targeted, and the load imposed at the ith locus is $\mathscr{L}_{[i]}$, the combined load imposed on the population will be

$$\mathscr{L}_n = 1 - \Pi_{i=1}^{n}[1 - \mathscr{L}_{[i]}]. \quad [1]$$

Fig. 1 shows the number of HEGs that need to be released as a function of the homing rate (assumed to be equal across loci) for the case of homing in both sexes and target genes that affect female fecundity. For any given homing rate, more HEGs are needed the higher the R_m. Similar calculations for different classes of target gene, and according to whether homing occurs in one or both sexes, are fully consistent with the results of Deredec et al. (9): (*i*) it is more effective to target a fertility gene than an essential gene; (*ii*) it is better that homing occurs in both sexes than only in one; and (*iii*) if homing can occur only in one sex, it is better to target fertility in that sex than in the other sex (Fig. S3).

Homing and Malaria Elimination (1): Reducing the Number of Mosquitoes. If a mosquito population is not eliminated, it may nonetheless be reduced sufficiently in size that it can no longer support a parasite population and the disease is eliminated. For the parasite, the quantity that is analogous to the intrinsic rate of increase is the basic reproductive rate, or the number of secondary cases induced by a single primary case in a naive population, typically represented by R_0. In the same way that we defined $\mathscr{L}$ as the proportional reduction in the mosquito's intrinsic rate of increase due to a HEG, we now define Λ as the proportional reduction in the parasite's basic reproductive rate due to a HEG:

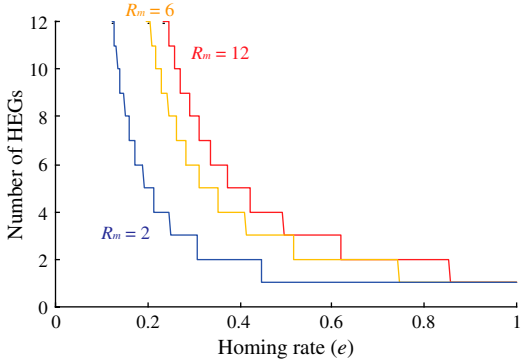

Fig. 1. Number of HEGs required to eliminate a population as a function of the homing rate when homing occurs in both sexes and the knockouts are recessive female sterile, for different values of R_m.

$\Lambda = 1 - R_0'/R_0$, where R_0 and R_0' are, respectively, the basic reproductive rate of the parasite before and after vector control by the HEG. R_0 is a linear function of the number of adult mosquitoes (20), and therefore if the only effect of a HEG is to reduce the density of mosquitoes, then Λ will be equal to the proportional reduction in that density. This reduction in adult density will in turn depend upon the precise form of the density-dependence function regulating mosquito populations. We modeled this effect by assuming that the probability of surviving the larval period is

$$p_{\text{survive}} = \theta_L \times \alpha/(\alpha + L),$$

where θ_L is the probability of larval survival at low densities, α is a constant (which can be interpreted as the larval density at which survival is half the maximum attainable), and L is the number of larvae. This form of density dependence is the same as that modeled by Beverton and Holt (21) and can be thought of as a discrete-time version of a continuous logistic growth model (22). The mortality implied by this function is assumed to be spread evenly over each day of the larval period. This function has a number of desirable features: (*i*) it has only a single parameter (α), (*ii*) the resulting equilibrium density of adults is a linear function of α (and therefore the proportional change in adult density is independent of α), and (iii) adult density is a monotonically increasing function of larval density [i.e., the function does not show "overcompensation" (23)].

The effect of introducing a HEG that imposes a load upon the population will be to reduce the density of all life stages. Because density dependence is assumed to act on larvae, the equilibrium larval density will depend on the magnitude of the load and will be independent of whether the load is expressed before or after the larval stage (e.g., whether the HEG causes death of embryos or adults). But parasite transmission depends on the number of adult female mosquitoes, not the number of larvae, and the equilibrium number of adult females will depend on when the load is expressed. All else being equal, HEGs that target genes that are only essential to pupae or adults will be more effective than those targeting genes essential to embryos or early larvae, because deaths of these early life stages will relax density-dependent larval mortality, which will compensate in part for the HEG-associated deaths (24). The difference is shown in Fig. 2*A*, which plots Λ as a function of $\mathscr{L}$. If the HEG acts after density dependence, then the effect on parasite transmission increases nearly linearly with the effect on the mosquito rate of reproduction, and with a slope greater than 1. However, if the HEG acts before density dependence, it is less effective, though the timing of HEG action does not affect the criterion for completely eliminating the vector, and the curves eventually converge. The graph also shows that for any given load $\mathscr{L}$, there will be larger decrements in parasite transmission when R_m is small than when it is large, because low R_m corresponds to relatively little density-dependent compensation for the mortality imposed by the HEGs. As a result, if R_m has already been reduced by releasing a HEG, a second HEG will have a greater effect on disease transmission than if the first one had not been released. That is, there is positive synergy between multiple HEGs, evident from a greater-than-linear decline in $\log(\Lambda)$ with increasing numbers of HEGs (Fig. 2*B*). This relationship occurs because of the nonlinear relationship between HEG load and equilibrium mosquito abundance.

HEGs can impose a load not only by causing mosquitoes to die, but also by reducing female fertility or by skewing the sex ratio toward males. What is the relationship between $\mathscr{L}$ and Λ in these cases? It can be shown that for HEGs that reduce female fecundity, and thereby the number of eggs laid, the relationship is the same as for HEGs that kill mosquitoes before density dependence, whereas for HEGs that distort the sex ratio (whether by altering sex chromosome inheritance or targeting a gene in the

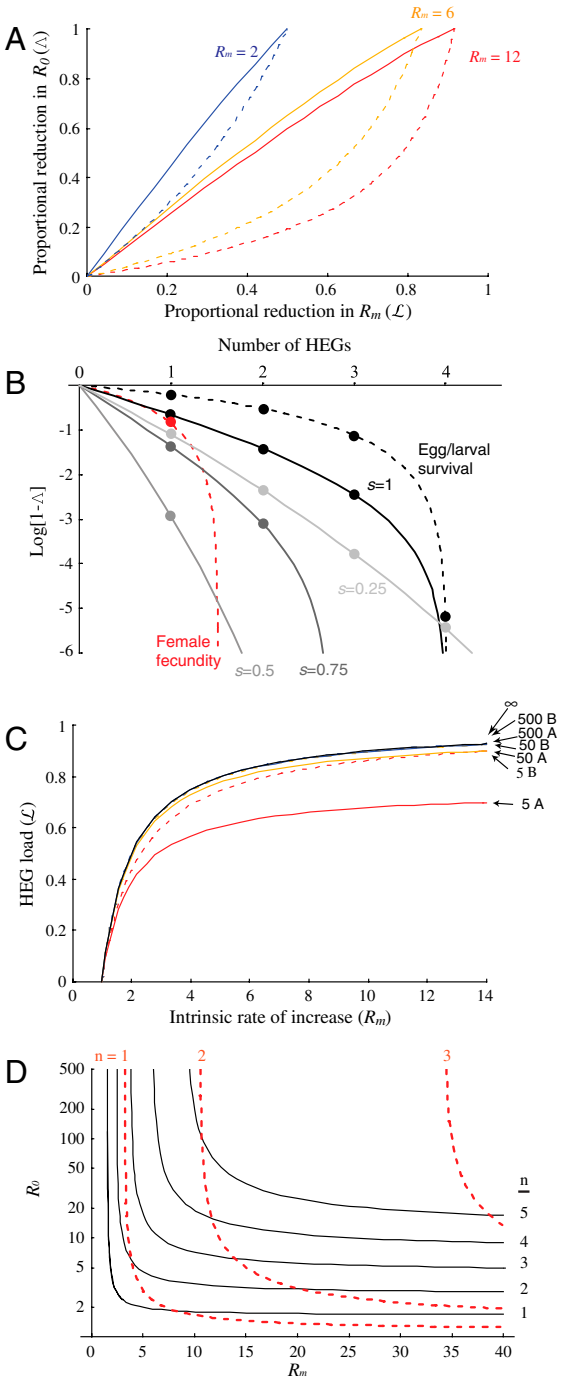

Fig. 2. (*A*) Proportional reduction in the basic rate of increase of the parasite as a function of the reduction in the intrinsic rate of increase of the vector when mortality is imposed before (dashed line) or after (solid line) the density-dependent larval stage for different values of R_m. HEGs that affect female fecundity will follow the dashed lines, and HEGs that alter the sex ratio will follow the solid lines. (*B*) Synergistic effect of releasing multiple HEGs on the proportional reduction in R_0 for HEGs that target genes essential for female fecundity (red), prelarval survival (dashed black), postlarval survival (solid black), or causing quantitative reductions in adult survival (dark, medium, and light gray for $s = 0.75$, 0.5, and 0.25, respectively). (*C*) HEG load required to eliminate the disease when the load is imposed before (B, dashed lines) or after (A, solid lines) density dependence for $R_0 = 5$ (red), 50 (orange), or 500 (blue). For comparison the load needed to eliminate the vector is also shown (black line). (*d*) Contour plots showing combinations of R_m and R_0 (log scale) for which the disease is eliminated using the specified number of HEGs (*n*), for HEGs targeting genes essential for postlarval survival (black) or female fecundity (red), with a homing rate of $e = 0.6$. Malaria is eliminated in populations below and to the left of each line. All plots derived from Eq. S5 (*SI Appendix*).

sex determination pathway), the relationship is the same as for HEGs that kill mosquitoes after density dependence. This is because the number of eggs per female does not change, and males are assumed to contribute as much as females to the competition among larvae, so sex ratio distortion does not lead to an immediate relaxation of density-dependent larval mortality.

Having calculated the relationship between $\mathscr{L}$ and Λ, we are now able to compare the HEG load required to eliminate the disease with that required to eliminate the mosquito (Fig. 2*C*). Only if R_0 is relatively small and the load is imposed by killing adults or skewing the sex ratio is it significantly easier to eliminate the disease than to eliminate the mosquito.

Finally, it is interesting to ask whether the goal of eliminating the disease rather than the vector changes the relative efficacy of targeting genes affecting viability versus fertility. As we have seen, for any given homing rate, the equilibrium load is higher when a female fertility gene is targeted than a gene needed for adult survival. But for any given load, targeting female fertility is less effective than targeting adult viability, because it reduces density-dependent mortality. To compare the overall efficacy of targeting female fertility and adult viability, we calculated the number of HEGs of each type needed to eliminate the disease for varying values of R_m, R_0, and homing rate (Fig. 2*D* and Fig. S4). For most parameter values it is more effective to target female fertility genes, and only in populations with very large R_m and very small R_0 do strategies targeting adult mortality become more effective.

Homing and Malaria Elimination (2): Reducing Adult Longevity. The viability targets we have considered thus far are essential genes, with homozygous knockouts being lethal. The only epidemiological effect of targeting such genes is via a reduction in the numbers of mosquitoes. We now consider HEGs that target genes that are useful but not essential to the adults, and in particular genes for which homozygous knockouts have a daily survival rate that is reduced by a fixed factor compared to wild type. In this case the effect of HEG-based control is not only to reduce the numbers of mosquitoes, but also to reduce their average lifspan, and therefore the individual vectorial capacity of the mosquitoes [i.e., the expected number of infectious bites by a single mosquito after feeding on an infected host (25)]. In this case the effect of a HEG cannot be summarized in a single metric (load), and instead we need to keep track of its two constituents separately: the homing rate and the effect on adult longevity (here measured by the selection coefficient s against the homozygote). Consistent with expectations, the parameter space for eliminating the disease can be significantly larger than that for eliminating the vector, particularly for low R_0 (Fig. 3). Also as expected, there is little to be gained by imposing quantitative reductions in female fecundity as compared to complete sterility. The greater effectiveness of reducing adult longevity is also manifest in some cases as a reduction in the number of HEGs that need to be released to eliminate the disease (Fig. S5).

In principle, one of the potential advantages of targeting adult longevity is that it will select less strongly for resistance (15, 26). To assess this possibility, we have investigated the relationship between the number of HEGs required to eliminate the disease and the selection coefficient against the knockout. Numerical simulations suggest that Λ is maximized if the selection coefficient against the knockout is equal to the homing rate (i.e., $s = e$) and decreases for either higher or lower values of the selection coefficient (e.g., Fig. S6). If the homing rate is 0.6, targeting a gene with $s = 0.6$ is not dissimilar to targeting an essential female fertility gene (which is expressed only in one sex). To have a significantly smaller selection coefficient (e.g., 0.1) would in many environments require up to 10 HEGs be released, even with homing rate as high as 0.9.

Cleavage-Resistant Mutants. In modeling HEGs that induce the canonical homing reaction, we have thus far made the simplifying assumption that cleavage of the target site always leads to successful homing. In fact, a significant proportion of cleavage events can be repaired in other ways, notably by nonhomologous end joining (11, 27). This does not lead to insertion of the HEG and instead often leads to some change at the target site that would make it no longer recognized by the enzyme—it would be resistant to cleavage. The fate of such alleles will depend upon their fitness effects: If they have normal fitness, then they will be strongly selected for and spread to fixation, and the HEG will disappear (9). At the other extreme, if the alleles are nonfunctional and have the same selection coefficient as the HEG-bearing allele, then they will not spread and the equilibrium load is unchanged (*SI Appendix*). For intermediate cases and for rates of homing and misrepair in keeping with results of experimental studies (11), the load increases more or less linearly with the selection coefficient against the mutant (Fig. S7).

Possible Stochastic Loss of HEGs. Because a HEG targeting an essential female fertility gene does not go to fixation, it is conceivable that, as the population is reduced to very low numbers, the HEG might be lost by chance, allowing the population then to recover. To investigate this possibility, we performed stochastic simulations. For simplicity, we considered only a single HEG, under conditions in which that was sufficient for deterministic elimination (i.e., $\mathscr{L} > 1 - 1/R_m$). In simulations with $e = 0.8$

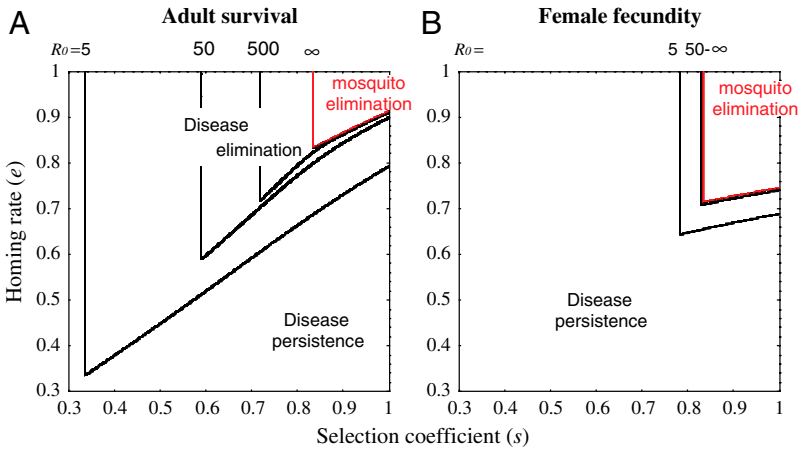

Fig. 3. Fate of the mosquito and the disease as a function of the homing rate and selection coefficient against homozygous knockouts for HEGs causing quantitative reductions in (*A*) adult survival and (*B*) female fecundity. Red lines show combinations of parameters in which the mosquito is eliminated, and black lines show combinations of parameters in which the disease is eliminated, for different initial values of R_0.

and $R_m = 6$, the population was eliminated in every one of 500 runs. For $e = 0.5$ and $R_m = 2$, there were 486 runs in which the population was eliminated; 2 in which the HEG was lost and the population had recovered its original density, and 12 in which both the mosquitoes and the HEGs still persisted at the end of the simulations (5 y). These simulations are for HEGs that have no heterozygous fitness effects. In *Drosophila*, homozygous lethal mutations typically reduce fitness by a few percent when heterozygous (28). We are not aware of any similar generalization for female sterile mutations, but introducing a 5% heterozygous fitness cost for HEG-bearing females had no significant effect on the outcome of our simulations.

Driving-Y Chromosomes. If a HEG is used to create a driving-Y chromosome and a male-biased sex ratio, the equilibrium load imposed on the population will be $2m$-1, where m is the proportion of males in the population, or the proportion of sperm that inherit the Y chromosome [assuming the HEG has no effect on male fertility (9)]. Combining this result with our range of estimates for R_m, the conditions for eliminating a mosquito population will be $m = 96$, 92, and 75% for $R_m = 12$, 6, and 2, respectively. If the mosquito is not eliminated, then the effect on malaria epidemiology will be the same as for a HEG that affects survival after density dependence (i.e., the solid lines in Fig. 2 *A* and *C*).

It is also possible to release simultaneously both a driving-Y chromosome and a HEG that targets some important mosquito gene. The effect of a combined release will depend upon the gene targeted by the HEG. If, for example, it is a female fertility gene, then the effects of the two constructs are independent, each will spread as if the other was absent, and the equilibrium load will again be given by Eq. **1**. However, if the HEG targets a gene involved in sex determination, then the effects of it and the driving Y are not independent, and the presence of one will affect the spread of the other. For example, in the medfly (*Ceratitis capitata*), homozygous knockouts for the *tra* gene are male regardless of their sex chromosome constitution (29). If a HEG targeting such a gene is released simultaneously with a driving Y, the two constructs would act antagonistically, and the equilibrium proportion of males (and therefore the load) would only be equal to the higher of the values expected from each construct separately (Fig. S8).

Other combinations are also possible, such as putting a HEG that targets a female fertility gene onto a driving-Y chromosome. The HEG will create mutations in the female fertility gene, but would not home; instead, it would spread along with the driving Y, which will not be counterselected by the mutations it causes because it never occurs in females. If the target gene is essential for female fertility and knockouts are recessive, then once the Y has gone to fixation the additional load due to these mutations will be $\mathscr{L} = j/(1+j)$ if the target gene is on an autosome, or $j/2$ if it is on the X chromosome, where j is the rate of mutation caused by the HEG. The maximum load is therefore ½ for each gene targeted. This is in addition to the load caused by the sex ratio distortion. The load can be increased if the mutations have some heterozygous effects; if fully dominant, the additional load will be $\mathscr{L} = 4j/(1+3j)$ for autosomal targets and $2j/(1+j)$ for X-linked targets. The maximum additional load in these cases is 1.

Time Course to Elimination. To determine whether HEG-based control can occur over a socially useful time frame, we have used deterministic computer simulations to investigate the length of time between release of HEG-bearing mosquitoes into a population and reductions in disease, assuming a single release of males equal to 1% of the wild adult male population (Fig. 4). For all three scenarios modeled (HEGs targeting female fertility, HEGs targeting adult longevity, and a HEG-based driving-Y chromosome), there is little change in vector abundance or disease prevalence for more than a year, as the HEGs spread through the population. After a year, vector abundance and disease prevalence each begins to decline. Decreasing the number of released males by a factor of 10 postpones the effect of the HEG only by 4–5 mo. There are some quantitative differences in timing among strategies, but the differences are relatively small (<2 y) compared to the time scales of product development or of public health interventions more generally. Although more detailed tactical models will be needed to make precise predictions about HEG spread and malaria control in any particular population, these simple models suggest that the time from release to positive impact will not be unreasonably long.

Discussion

Genetic approaches to vector control have rightly attracted considerable attention for their potential to reduce disease transmission (4, 5). Formal modeling is required to assess the various possibilities and guide future development. The main conclusions from our modeling of HEG-based malaria control strategies are as follows:

i. The best available data on homing rates and R_m indicate that populations of *An. gambiae* could be eliminated by releasing 2–3 HEGs targeting female fertility genes. Alternatively, populations could be eliminated with a driving-Y chromosome that is transmitted to 75–96% of progeny. Combinations of fertility-targeting HEGs and Y drive may also be effective.

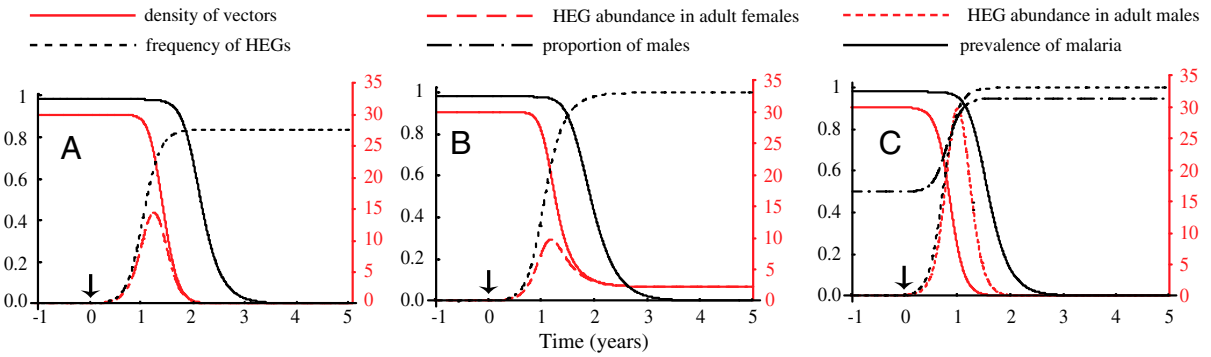

Fig. 4. Example time courses after a single release of heterozygous HEG-bearing mosquitoes into a population at initial frequencies of 1% (arrows). $R_m = 6$; $R_0 = 162$. (*A*) The introduced mosquitoes carry two independent HEGs each of which targets a gene essential for female fertility (homing rate $e = 0.6$). (*B*) The introduced mosquitoes carry two independent HEGs each of which targets a gene involved in adult survival, with homozygous knockouts having $s = 0.5$ (homing rate $e = 0.6$). (*C*) The introduced mosquitoes carry on their Y chromosome a HEG that targets a repeated sequence on the X chromosome, resulting in transmission of the Y to $m = 91\%$ of sperm. The black curves refer to the left Y axis, whereas the red curves can be read on the right Y axis. In *A* and *B*, the abundance of the HEG among adult females is calculated as the number of homozygotes plus half the number of heterozygotes, whereas in *C* the abundance of the HEG is calculated as the number of adult males carrying it.

ii. It is possible to impose a load sufficient to eliminate the disease without eliminating the vector. In this context, targeting genes essential to the adult (i.e., after density dependence) is more effective than targeting genes essential to the egg or early juvenile stages (i.e., before density dependence), and targeting genes essential for female fertility is usually more effective than either one. Moreover, there is positive synergy between multiple HEGs in the reductions they cause in R_0. However, if the only effect of the HEG is to reduce the number of vectors, the parameter space in which the disease is eliminated but not the vector is very small, the exception being when R_0 is small and R_m is large.
iii. HEGs causing a quantitative reduction in adult survival can in some circumstances be more effective than those targeting female fertility. However, the selection coefficients that need to be imposed are still large, unless many HEGs are to be released.
iv. To prevent the spread of resistant alleles, it will be necessary to target genomic sites that cannot tolerate changes.
v. Stochastic loss of a HEG targeting a female fertility gene can occur before population elimination, but it does not appear to be a common outcome.
vi. HEG-based strategies can be effective over socially relevant time frames.

Our conclusion that eliminating the disease is not much easier than eliminating the vector derives in part from the fact that *An. gambiae* is such an efficient vector and even low densities can maintain the disease. The high levels of anthropophily of at least some variants make it much more efficient than vectors in other locations, where malaria has been eliminated without eliminating the vector (30). When vectors are inefficient, R_m can be large but R_0 small, putting the population in our zone of exception (Fig. 2*C*). Another contributing factor may be in the form of the relationship between density and survival of larvae. Because little is known about this relationship, we have used a particularly simple relationship that has some mechanistic justification and gives simple dynamics (22). We have also investigated alternative relationships, and for some density-dependent functions even a small load will give a dramatic reduction in equilibrium density and eliminate the disease (*SI Appendix*). Yet other forms of density dependence can make it more difficult to eliminate the disease than modeled here, though obviously in no case does it become more difficult than eliminating the vector, the requirements for which are independent of the precise density-dependent function (assuming R_m is the maximum rate of increase of the population). Some of these alternative density-dependent functions can also show overcompensation, in which a reduction in egg or larval density can lead to an increase in the number of adults, and therefore imposing a small load can increase disease transmission [i.e., $\Lambda < 0$; (31)]. Such behavior occurs only if the load is imposed before density dependence; if the load is imposed by killing adults or distorting the sex ratio, then Λ remains a monotonically increasing function of the load.

As we have noted, the requirements for eliminating the vector are independent of the density-dependent function, and instead the critical parameter is R_m. We have been unable to find any previous estimate of this key parameter for *An. gambiae* and therefore have derived an estimate from the maximum rate of increase of a population at the beginning of the wet season. This estimate should be considered as only roughly indicative of the true value: It will be an underestimate to the extent that there continues to be density-dependent processes at this time, and an overestimate to the extent that the population increase is due to mosquitoes coming out of aestivation rather than reproducing (32). In addition, R_m is expected to be lower during the dry season, and it is the geometric mean of values throughout the year (assuming equal generation times) that is relevant for our purposes. Moreover, we have made a single estimate of this quantity from a single population, but also recognize that it will vary across the species range. In light of these uncertainties, we have used a range of estimates for our calculations. By comparison, R_m for *Aedes aegyptii* has been estimated to be in the range 3.1–11.2 (17).

Two other assumptions of the model are worth noting. Most obviously, our model has only a single vector species, and if there are multiple noninterbreeding vectors, then HEGs released into only one of them may eliminate that vector but not the disease. In sub-Saharan Africa, where about 90% of malaria cases and fatalities occur (1), there is a relatively limited number of vector species, the most important being the two sibling species *An. gambiae s.s.* and *Anopheles arabiensis*, plus the more distantly related *Anopheles funestus* (33). Second, we have modeled the spread of HEGs within a homogenous vector population, not over a geographical landscape. *An. gambiae* can disperse a kilometer or more every generation (34), and more modeling will be required to determine what this means for HEG spread and to determine the optimal scale of deployment. It will also be interesting to determine the frequency of stochastic HEG loss in a landscape model. Seasonality could also be incorporated into such a model, though unfortunately even less information is available on population biological parameter values during the dry season (35). Further information on density-dependent and stage-specific mortality rates and fecundity schedules throughout the annual cycle should be a priority for future ecological fieldwork. Our model also does not explicitly consider any other control measure. HEGs are not likely to be deployed in a vacuum, but rather in the presence of bed nets, insecticides, etc. HEG-based strategies should be fully compatible with these other control measures, and there should be no need to interrupt them in order for HEGs to be effective, though modeling would be required to investigate this issue in more detail.

As with any other form of pest control, the use of HEGs will generate selection for resistance, and steps should be taken to reduce the likelihood of resistance evolving. We have modeled the most obvious form of resistance against fertility-targeting HEGs, a change in the recognition site, and this indicates that it will be important to target sites at which such changes cannot be tolerated. Active sites of proteins are obvious candidates. Other forms of resistance management should also be considered. For example, naturally occurring HEGs that target protein-coding genes tend not to recognize variation at silent sites (36, 37), and ideally this feature could be maintained in the engineered variants. Also, combination therapy is well known to retard the evolution of resistance to drugs, and the same principle could be applied to HEGs, targeting multiple sites per gene and/or multiple genes. For the X-shredding strategy, the rDNA repeat, found only on the X chomosome in *An. gambiae*, is an attractive target, and changes in the recognition sequence are probably unlikely, as hundreds of copies would have to change simultaneously (12). Other forms of resistance could conceivably evolve and ought to be investigated in large lab populations before release. As the history of chemical interventions has proven, even if resistance does eventually evolve, transient suppression can be socially useful, particularly if there are follow-up interventions with no cross-resistance.

The strength of selection for resistance will be critically dependent on the choice of target gene. For example, targeting adult longevity can substantially reduce disease transmission while imposing little selection for resistance (15, 26). However, our modeling shows that this effect cannot be achieved by a simple increase in the hazard of adult living. If the increased mortality could be restricted to females, or, even better, to old females, then selection for resistance would be reduced. The main practical difficulty with these approaches in the context of HEG-based control is in identifying suitable target genes that will have the desired effects when knocked out. The situation is not helped by the uncertainty in extrapolating lab- or cage-based fitness

estimates to the field. By contrast, genotypes causing complete lethality or sterility in the lab are more likely to have these effects in the field. More generally, although we have focused in this paper on targeting aspects of vector demography, with obvious (and calculable) fitness effects, it should also be possible to use HEGs to target other aspects of vectorial capacity, including host seeking and feeding behavior and the ability of the mosquito to support parasite development. The difficulty in modeling such strategies at this point is that in the absence of any candidate genes, it is not clear what fitness effect to ascribe to the knockouts. If there is little fitness effect on the mosquito but the effect on the parasite is substantial, then they may be very attractive, and when such candidate genes are found it will be straightforward to adapt the model to assess them.

HEG-based strategies of reducing or eliminating a pest population have not previously been deployed, and are not to be done lightly. Conventional biological control programs—which involve the release of a self-sustaining control agent and which have been used to suppress more than 200 species of invasive insects in many countries around the world (38)—are perhaps the closest precedent. Much work will need to be done on risk assessments, community engagement, regulatory protocols, and associated issues before any release could be performed, not to mention the further work on molecular biology and entomology. Potential direct and indirect effects on the abundance of other species and on ecosystem services should be considered. Nevertheless, the conclusion from this study, combined with recent estimates of the critical parameters in the lab (11, 12), is that such approaches could have a transformational effect on malaria control efforts.

Materials and Methods

Mosquito population dynamics are modeled using an overlapping-generation, discrete-time lumped age–class formulation adapted from ref. 16, with time intervals of 1 d. The three juvenile stages (egg, larva, and pupa) are each assumed to be of fixed duration, and individuals in these stages experience a constant rate of density-independent mortality. Larval mosquitoes also experience density-dependent mortality, and it is this that regulates population densities. When adult female mosquitoes emerge, they start feeding, mate a single time with a randomly chosen adult male, and begin to oviposit. We assume that when mosquitoes feed on infectious humans, they take up the malaria pathogen with a fixed probability and then enter a disease maturation phase of fixed duration, after which they can transmit the pathogen. We include the dynamics of malaria in humans but in a deliberately simplified manner. Humans, whose density is assumed to be constant, are infected with a fixed probability after being fed upon by an infectious mosquito and then immediately become infectious until they recover from the disease or die. Adult mosquitoes have a fixed probability of dying on any particular day, independent of their infection status.

The population genetics of the mosquitoes are modeled by considering the frequencies of each genotype at each stage (9). Autosomal HEGs can have a variety of fitness effects, and in heterozygotes they home with probability e [and therefore are transmitted to the next generation with probability $(e+1)/2$]. X-shredding HEGs inserted on the Y chromosome are transmitted along with the Y to a fraction m of the sperm.

The model considers only a single vector species. Its parameterization is mostly based on estimates from the literature for *An. gambiae* (see Table S1), in particular from Molineaux and Gramiccia (19). To estimate the frequency of stochastic loss and the time course for control, and to confirm that the theoretical results match the equilibrium behavior of the model, simulations were run in Mathematica 5.2 using a time step of 1 d. For more details, see *SI Appendix*.

ACKNOWLEDGMENTS. This work is funded by a grant from the Foundation for the National Institutes of Health through the Vector-Based Control of Transmission: Discovery Research program of the Grand Challenges in Global Health initiative. H.C.J.G. is part of the Research and Policy for Infectious Disease Dynamics program of the Science and Technology Directorate, Department of Homeland Security, and the Fogarty International Center.

1. WHO-Global-Malaria-Programme (2010) *World Malaria Report* (World Health Organisation, Geneva).
2. Mendis K, et al. (2009) From malaria control to eradication: The WHO perspective. *Trop Med Int Health* 14:802–809.
3. O'Meara WP, et al. (2008) Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet* 372:1555–1562.
4. James AA (2005) Gene drive systems in mosquitoes: Rules of the road. *Trends Parasitol* 21:64–67.
5. Marshall JM, Taylor CE (2009) Malaria control with transgenic mosquitoes. *PLoS Med* 6:e1000020.
6. Burt A, Koufopanou V (2004) Homing endonuclease genes: The rise and fall and rise again of a selfish element. *Curr Opin Genet Dev* 14:609–615.
7. Stoddard BL (2005) Homing endonuclease structure and function. *Q Rev Biophys* 38:49–95.
8. Burt A (2003) Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proc R Soc Lond B Biol Sci* 270:921–928.
9. Deredec A, Burt A, Godfray HCJ (2008) The population genetics of using homing endonuclease genes in vector and pest management. *Genetics* 179:2013–2026.
10. Chan Y-S, Huen DS, Russell S (2011) Characterisation of a homing endonuclease-based drive system in *Drosophila melanogaster*. *Genetics* 188:33–44.
11. Windbichler N, et al. (2011) A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. *Nature* 473:212–215.
12. Windbichler N, Papathanos PA, Crisanti A (2008) Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles gambiae*. *PloS Genet* 4:e1000291.
13. Baker DA, et al. (2011) A comprehensive gene expression atlas of sex- and tissue-specificity in the malaria vector, *Anopheles gambiae*. *BMC Genomics* 12:296.
14. MacDonald G (1957) *The Epidemiology and Control of Malaria* (Oxford Univ Press, London).
15. Read AF, Lynch PA, Thomas MB (2009) How to make evolution-proof insecticides for malaria control. *PloS Biol* 7:e1000058.
16. Hancock PA, Godfray HCJ (2007) Application of the lumped age-class technique to studying the dynamics of malaria-mosquito-human interactions. *Malar J* 6:98.
17. Dye C (1984) Models for the population dynamics of the yellow fever mosquito, *Aedes aegypti*. *J Anim Ecol* 53:247–268.
18. Legros M, Lloyd AL, Huang YX, Gould F (2009) Density-dependent intraspecific competition in the larval stage of *Aedes aegypti* (Diptera: Culicidae): Revisiting the current paradigm. *J Med Entomol* 46:409–419.
19. Molineaux L, Gramiccia G (1980) *The Garki Project. Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa* (World Health Organization, Geneva).
20. Smith DL, McKenzie FE (2004) Statics and dynamics of malaria infection in *Anopheles* mosquitoes. *Malar J* 3:13.
21. Beverton RJH, Holt SJ (1957) *On the Dynamics of Exploited Fish Populations*, Fishery Investigations Series II (Ministry of Agriculture, Fisheries and Food, London, UK), Vol 19.
22. Prout T (1978) Joint effects of release of sterile males and immigration of fertilized females on a density regulated population. *Theor Pop Biol* 13:40–71.
23. Abrams PA (2009) When does greater mortality increase population size? The long history and diverse mechanisms underlying the hydra effect. *Ecol Lett* 12:462–474.
24. Phuc HK, et al. (2007) Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol* 5:11.
25. Saul AJ, Graves PM, Kay BH (1990) A cyclical feeding model for pathogen transmission and its application to determine vectorial capacity from vector infection rates. *J Appl Ecol* 27:123–133.
26. Koella JC, Lynch PA, Thomas MB, Read AF (2009) Towards evolution-proof malaria control with insecticides. *Evol Appl* 2:469–480.
27. Preston CR, Flores CC, Engels WR (2006) Differential usage of alternative pathways of double-strand break repair in *Drosophila*. *Genetics* 172:1055–1068.
28. Simmons MJ, Crow JJ (1977) Mutations affecting fitness in *Drosophila* populations. *Ann Rev Genet* 11:49–78.
29. Pane A, De Simone A, Saccone G, Polito C (2005) Evolutionary conservation of *Ceratitis capitata* transformer gene function. *Genetics* 171:615–624.
30. Kuhn KG, Campbell-Lendrum DH, Davies CR (2002) A continental risk map for malaria mosquito (Diptera: Culicidae) vectors in Europe. *J Med Entomol* 39:621–630.
31. Yakob L, Alphey L, Bonsall MB (2008) *Aedes aegypti* control: The concomitant role of competition, space and transgenic technologies. *J Appl Ecol* 45:1258–1265.
32. Lehmann T, et al. (2010) Aestivation of the African malaria mosquito, *Anopheles gambiaein* the Sahel. *Am J Trop Med Hyg* 83:601–606.
33. Hay SI, Rogers DJ, Toomer JF, Snow RW (2000) Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: Literature survey, internet access and review. *Trans R Soc Trop Med Hyg* 94:113–127.
34. Killeen GF, Knols BGJ, Gu WD (2003) Taking malaria transmission out of the bottle: Implications of mosquito dispersal for vector-control interventions. *Lancet Infect Dis* 3:297–303.
35. Lehmann T, et al. (2010) Aestivation of the African malaria mosquito, *Anopheles gambiae* in the Sahel. *Am J Trop Med Hyg* 83:601–606.
36. Koufopanou V, Goddard MR, Burt A (2002) Adaptation for horizontal transfer in a homing endonuclease. *Mol Biol Evol* 19:239–246.
37. Kurokawa S, et al. (2005) Adaptation of intronic homing endonuclease for successful horizontal transmission. *FEBS J* 272:2487–2496.
38. van Driesche R, Hoddle M, Center T (2008) *Control of Pests and Weeds by Natural Enemies: An Introduction to Biological Control* (Blackwell, Malden, MA).