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The Impact of Transgenic Mosquitoes on Dengue Virulence to Humans and Mosquitoes

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Abstract

Dengue is a major public health concern in the tropics and subtropics. Innovative transgenic strategies to render *Aedes aegypti* mosquitoes, the primary vector of dengue, incompetent for dengue transmission are under development. We modeled the evolutionary impact of different transgenic mosquito strategies on dengue-induced mortality, that is, dengue virulence, to both humans and mosquitoes. This model incorporates various evolutionary trade-offs in dengue virus epidemiological traits, for example, a trade-off between dengue transmission rate and its virulence to humans. Our results indicate that strategies that block transmission or reduce mosquito biting impose selection on dengue virulence in humans. This selection can be for either higher or lower virulence, depending on the interaction between the effect of the transgene and the trade-offs in epidemiological traits, highlighting the need for detailed quantitative data to understand more fully the impact of mosquito transgenesis on dengue virulence. Dengue virulence in mosquitoes can be selected on by transgenic strategies of blocking transmission, decreased mosquito biting, increased mosquito background mortality, and increased mosquito infection-induced mortality. Our results suggest that dengue control strategies that raise mosquito background mortality or mosquito infection-induced mortality pose less risk of causing increased virulence to humans than strategies that block transmission or reduce mosquito biting.

Keywords

dengue; mosquito transgenesis; virulence evolution

Introduction

Dengue is a growing public health problem in the tropics and subtropics for which there is no vaccine or cure (Gubler 2002; Kyle and Harris 2008). Fifty million dengue infections are estimated to occur annually, resulting in 500,000 hospitalizations (Calisher 2005) and a few thousand deaths per year (Guzmán and Kouri 2002). The dengue virus is transmitted by mosquitoes, and although other primates are a reservoir where human density is low, urban centers with high human and mosquito densities can suffer from severe epidemics (Gubler 1998; Kyle and Harris 2008). In these urban centers, there is no nonhuman reservoir host for dengue, and *Aedes aegypti* is the primary vector because of its preference for human blood

meals and adaptation to domestic environments (Christophers 1960; Gubler and Clark 1996; Gubler 1998; Kyle and Harris 2008). Current control strategies rely heavily on mosquito control using insecticides (Gubler 1998; Kyle and Harris 2008), which are susceptible to the emergence of insecticide-resistant mosquito populations (Hemingway and Ranson 2000). Transgenesis of *A. aegypti* mosquitoes is an alternative dengue control strategy that offers potentially minimal ecological and environmental impact (Scott et al. 2002).

Dengue is an RNA virus in the family Flaviviridae, which also includes yellow fever virus and West Nile virus. Dengue is divided into four serotypes, each of which induces a specific antigenic response in humans. Transmission is by female mosquitoes, which require blood meals in order to produce eggs; male mosquitoes do not feed on blood. During a blood meal, a mosquito may acquire dengue when biting an infected human. The incubation period in mosquitoes corresponds roughly to the 7 days between bouts of oviposition and therefore to the time between blood meals. In these subsequent blood meals, an infected mosquito can transmit dengue to susceptible human hosts. After approximately 7 days of incubation, an infected person enters the acute phase of infection for about 5 days, with symptoms ranging the full spectrum from asymptomatic, mild fever, high fever with severe headache and joint pain, and internal hemorrhaging to circulatory failure and death. Cases are classified, in order of increasing severity, as dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. A female mosquito may become infected by taking a blood meal on a human who is in this acute phase of infection, beginning the cycle anew. After the acute phase in humans, the infection is cleared: the person has lifelong immunity to that dengue serotype and temporary cross-immunity to the other dengue serotypes that lasts less than a year. Mosquitoes, on the other hand, never clear the virus, remaining infected for life. Vertical transmission, that is, transmission from mother to offspring, is thought to be rare in both humans and mosquitoes (Siler et al. 1926; Sabin 1952; Gubler 1998; Guzmán and Kouri 2002; Luz et al. 2003; Kyle and Harris 2008).

The possibility of transgenesis of mosquitoes to be less effective disease vectors was hypothesized more than 4 decades ago (Curtis 1968), and new advances make implementation seem attainable. Genes that have the potential to reduce pathogen transmission have been identified, which we survey below. However, these genes are at best selectively neutral and would not maintain themselves in wild populations, let alone spread from a small release into a large population. To overcome this problem, disease-control genes must be coupled with a driver gene that will facilitate spread and maintenance of the disease-control genes in the population. Most of the gene drivers subvert Mendelian inheritance to facilitate spread despite providing no selective advantage in the host. Transposable elements, meiotic drive, cytoplasmic incompatibility, and underdominance have been suggested as potential gene drivers (Sinkins and Gould 2006). A disease-control program using transgenic mosquitoes may rely on either a single initial release and fixation of the transgene into the mosquito population or periodic releases to maintain the transgene.

Dengue control using transgenic mosquitoes could be achieved through four possible mechanisms: blocking transmission, either from humans to mosquitoes or from mosquitoes to humans; reducing mosquito biting; increasing mosquito background mortality; and increasing mosquito infection-induced mortality. Blocking transmission is the most developed of these transgenic strategies. Experimental strains of *A. aegypti* have been engineered that inhibit dengue replication in the midgut and migration to the salivary glands (Olson et al. 1996, 2002; Kokoza et al. 2000; Adelman et al. 2001; Franz et al. 2006), thereby impeding infection of mosquitoes and subsequent transmission to humans. Another gene has been identified that reduces host-seeking behavior in *A. aegypti* (Brown et al. 1994; Stracker et al. 2002). A transgene might be developed that causes mosquitoes to bite humans less frequently, perhaps only after the first blood meal or, optimally, on dengue

infection. For increased mosquito background mortality, there are a variety of strategies, including the release of engineered males homozygous for a dominant female-killing gene (i.e., population reduction by elimination of female offspring; Thomas et al. 2000; Alphey and Andreasen 2002), the introduction of mosquito-specific lethal *Densovirus* into the environment (Carlson et al. 2006; Suchman et al. 2006), the release of mosquitoes infected with life-shortening *Wolbachia* (McMeniman et al. 2009; Read and Thomas 2009), and the use of insecticides. Viruses of the genus *Densovirus* are known to infect only insects, and several have been found that are environmentally and vertically transmitted in mosquitoes and cause high mortality (Kittayapong et al. 1999; Ledermann et al. 2004; Carlson et al. 2006; Suchman et al. 2006). A release of a mosquito-specific *Densovirus* has the advantage over insecticides because it replicates and spreads itself into the mosquito population. Similarly, a strain of *Wolbachia* bacteria has been shown to cause both increased mortality and cytoplasmic incompatibility in *A. aegypti*, the latter of which can facilitate the spread of *Wolbachia* into the mosquito population (McMeniman et al. 2009; Read and Thomas 2009). *Densovirus*, *Wolbachia*, and insecticides are not transgenic strategies per se, but we consider them as well in our analysis (Gubler 2002; Guzmán and Kouri 2002). In contrast, lethal genes expressed only in the presence of dengue virus would increase mortality only in dengue-infected mosquitoes (Chow et al. 1998; Ritchie et al. 2004; Mammen et al. 2008).

Despite the promise of these new control strategies, their impact on the evolution of virulence to both the human and the mosquito hosts must be considered (Chevillon and Failloux 2003; Struchiner et al. 2006). Here we develop a mathematical model that takes into account trade-offs between virulence and epidemiological traits, virulence within serotypes, and the increased risk of severe disease due to secondary infection. Virulence is defined as the extent to which a pathogen exploits its host (Lipsitch and Moxon 1997; Galvani 2003), measured in our model as increasing mortality. Elevated mortality reduces the duration of the infectious period. Simultaneously, higher exploitation could lead to increases in transmission rate (Anderson and May 1982; Lipsitch and Moxon 1997; Mackinnon and Read 1999, 2004), and so a trade-off is assumed between the rate and the duration of transmission, such that an increase in one leads to a decrease in the other (Levin and Pimentel 1981; Anderson and May 1982; Frank 1996; Gandon et al. 2001; Galvani 2003). Consequently, these dynamics of pathogen transmission may select for intermediate rate and duration of transmission: if one is too high and the other too low, a strain of a pathogen may not spread as quickly as other strains of the same pathogen (Levin and Pimentel 1981; Ewald 1983; Dieckmann 2002; Galvani 2003). Trade-offs between virulence and epidemiological traits other than transmission have also been considered (Ewald 1983; Lipsitch and Moxon 1997; Ewald and De Leo 2002; Galvani 2003); we discuss evidence for evolutionary trade-offs in general and for specific trade-offs between pathogen traits in “Methods.” We then apply techniques from the theory of virulence evolution, which was developed to understand the impact of the evolution of pathogens on infection dynamics under these trade-offs in pathogen traits (Levin and Pimentel 1981; Ewald 1983; May and Anderson 1983; Dieckmann 2002; Galvani 2003).

Host and virus characteristics, as well as epidemiological risk factors, interact to produce dengue hemorrhagic fever and dengue shock syndrome in a fraction of individuals (Guzmán and Kouri 2002). When an individual who has been infected with one dengue serotype acquires an infection with one of the three remaining serotypes, the presence of heterotypic antibodies appears to enhance viral replication and the severity of disease (Halstead et al. 1970; Guzmán and Kouri 2002; Gibbons et al. 2007). However, in addition to secondary infection as a determinant of severe disease, different dengue genotypes within single serotypes vary in risk of causing severe cases (Gubler et al. 1978; Morens et al. 1991; Rico-Hesse et al. 1997; Watts et al. 1999; Vaughn et al. 2000; Guzmán and Kouri 2002; Holmes and Twiddy 2003; Messer et al. 2003; Rico-Hesse 2003; Chen et al. 2008; Halstead 2008;

Kyle and Harris 2008). For example, within dengue serotype 2, the American genotype leads to less severe disease than does the Asian genotype (Leitmeyer et al. 1999). We focus our analysis on virulence within a single serotype, not on virulence caused by the interaction of serotypes in subsequent infections. Despite considering only a single serotype, the definition of virulence in our model incorporates the increased risk of severe disease due to secondary infection by including all severe disease caused by a specific genotype within a single serotype, regardless of whether that severe disease occurred during a primary or secondary infection.

Both the human and the mosquito hosts impose selection on dengue viruses (Chevillon and Failloux 2003; Gandon 2004; Cologna et al. 2005). Therefore, we developed a model that includes human and mosquito populations and incorporates virulence to both hosts. Although transgenesis for control of dengue modifies the mosquito, our model shows that such an intervention might result in altered dengue virulence not just to mosquitoes but also to humans.

We demonstrate that transgenic strategies based on blocking transmission or reducing mosquito biting can select for increased virulence to humans, whereas strategies that increase mosquito background or infection-induced mortality do not select for changes in virulence to humans. In some situations, whether selection increases or decreases virulence depends on the form of the function relating the effect of the transgene to the trade-off between virulence and other epidemiological traits: choosing a particular functional form in the absence of empirical evidence may result in spurious conclusions about the direction of selection on virulence.

A previous model was developed for analyzing various vaccination mechanisms against malaria, and we generally follow those methods (Gandon et al. 2001). However, these authors made the assumption of one particular functional form describing the effect of the intervention on trade-offs between pathogen traits. We show that this assumption can lead to the conclusion that there is no selection on virulence in humans imposed by the introduction of a transgene, whereas more general assumptions result in selection for either increasing or decreasing virulence in humans. We find that the direction of selection for different strategies depends on the impact of the transgene on trade-offs between epidemiological traits. Specifically, dengue control strategies that raise mosquito background mortality or mosquito infection-induced mortality pose less risk of causing increased virulence to humans than do strategies that block transmission or reduce mosquito biting.

Methods

We will first present a transmission model of a single dengue serotype and show that the evolutionarily stable strategy for a dengue genotype is the strategy that maximizes the basic reproductive number. We will then discuss seven evolutionary trade-offs between dengue epidemiological traits to be considered in our analysis of transgenic mosquitoes.

Dengue Transmission Model

Our model separates human hosts into susceptible (H_S), exposed (H_E), infectious (H_I), and recovered (H_R) compartments, whereas mosquitoes are divided into susceptible (V_S), exposed (V_E), and infectious (V_I) compartments. Mosquitoes do not recover from infection (Siler et al. 1926). Infectious humans include all the many mild cases of dengue (Guzmán and Kouri 2002), not just infections that lead to clinical symptoms. Our model accounts for birth and death of both humans and mosquitoes, including dengue-induced mortality in both hosts, which we use as our definition of virulence (Levin and Pimentel 1981; Anderson and May 1982; Lipsitch and Moxon 1997; Gandon et al. 2001; Galvani 2003). In humans,

virulence corresponds to increased risk of severe clinical manifestations of dengue, dengue hemorrhagic fever and dengue shock syndrome, which have heightened case fatality over dengue fever. Although we do not model multiple serotypes, this definition of virulence incorporates the increased risk of severe disease due to previous infection with a different serotype by not distinguishing whether a person suffering severe disease due to the modeled serotype has been infected with another serotype. In this way, human virulence is a sort of weighted average over people for whom the modeled serotype is their first dengue infection and people for whom the modeled serotype is a second infection. The model is given by the system of differential equations

$$\begin{aligned}
 \frac{dH_S}{dt} &= B_H - a_1 \beta_H V_1 \frac{H_S}{H} - \mu_H H_S, \\
 \frac{dH_E}{dt} &= a_1 \beta_H V_1 \frac{H_S}{H} - (\mu_H + \tau_H) H_E, \\
 \frac{dH_I}{dt} &= \tau_H H_E - (\gamma_H + \mu_H + \nu_H) H_I, \\
 \frac{dH_R}{dt} &= \gamma_H H_I - \mu_H H_R, \\
 \frac{dV_S}{dt} &= B_V - a_s \beta_V \frac{H_I}{H} V_S - \mu_V V_S, \\
 \frac{dV_E}{dt} &= a_s \beta_V \frac{H_I}{H} V_S - (\mu_V + \tau_V) V_E, \\
 \frac{dV_I}{dt} &= \tau_V V_E - (\mu_V + \nu_V) V_I,
 \end{aligned} \tag{1}$$

where $H = H_S + H_E + H_I + H_R$ and $V = V_S + V_E + V_I$ are the human and mosquito population sizes, respectively. For simplicity, we use birth rates B_H and B_V that maintain constant population sizes: $B_H = \mu_H H + \nu_H H_I$ and $B_V = \mu_V V + \nu_V V_I$. (The assumption that the mosquito population size is constant is relaxed using a more complex model in “Diffusion Model” in the appendix in the online edition of the *American Naturalist*.) We also consider transmission to occur only between humans and mosquitoes, neglecting any intraspecies transmission (e.g., vertical transmission from mother to offspring). Table 1 describes the model parameters.

The basic reproductive number, R_0 , is defined as the number of secondary dengue infections arising from a single dengue infection in a wholly susceptible population (Anderson and May 1991). The next-generation matrix technique (van den Driessche and Watmough 2002)¹ gives the basic reproductive number for dengue from model (1):

$$R_0 = \frac{a_1 \beta_H \tau_H a_s \beta_V \tau_V m}{(\mu_H + \tau_H)(\gamma_H + \mu_H + \nu_H)(\mu_V + \tau_V)(\mu_V + \nu_V)}, \tag{2}$$

where $m = V/H$ is the ratio of mosquitoes to human hosts.²

A phenotype that is resistant to invasion by other genotypes is called an evolutionarily stable strategy (ESS; Maynard Smith 1982), while a genotype that can invade all other genotypes is referred to as convergence stable (Eshel 1983). In “Invasion Analysis” in the appendix, we use invasion analysis to show that in the absence of humans or mosquitoes being simultaneously infected with multiple virus genotypes, the dengue genotype with maximal R_0 is the ESS and is convergence stable (Anderson and May 1982; Bremermann and Thieme 1989; Frank 1996). Thus, the genotype with maximal R_0 will dominate all other dengue genotypes. The maximal R_0 under the constraint of an evolutionary trade-off, in turn, gives

¹The next-generation matrix technique converts from a model like system (1), where the unit of analysis is transitions of hosts between epidemiological states, into a model that projects “offspring” between “generations,” where here offspring are new infections and a generation is the infectious period of a host (van den Driessche and Watmough 2002).

²The R_0 in equation (2) defines human-mosquito-human transmission as one generation of new infections. If human-mosquito transmission is used as one generation, R_0 is the square root of the right-hand side of equation (2) because human-mosquito-human transmission is now two generations. Our results are the same in either case.

the optimal virulence. This framework allows us to predict the potential evolution of dengue epidemiological traits in response to transgenic mosquito strategies.

Evolutionary Trade-Offs

Evolutionary trade-offs describe relationships between genetic traits where a change in one trait that increases fitness is linked to a change in another trait that decreases fitness. As Stearns (1992, p. 75) argues, “If such trade-offs did not exist, selection would have driven all traits correlated with fitness to limits imposed by history and design. The fact that many life history traits are maintained well within those limits suggests that microevolutionary trade-offs must exist.” (See Stearns 1992, chap. 4, for a full discussion of evolutionary trade-offs.) With this general motivation, we consider seven evolutionary trade-offs between dengue virulence to humans and mosquitoes (v_H and v_V , respectively) and epidemiological parameters for transmission, mosquito biting, incubation, and recovery (table 2). These parameters represent quantitative traits of dengue virus that are subject to selection. We assume that the function that links an epidemiological parameter with virulence to either humans or mosquitoes is smooth and monotone and displays “diminishing returns” as virulence increases (i.e., an increasing function is concave, and a decreasing function is convex).

Because the ESS for dengue virus is the one with maximal R_0 and because the mosquito-to-human (β_H) and human-to-mosquito (β_V) transmission rates appear as a single composite parameter in R_0 , we need consider only trade-offs involving the product $\beta = \beta_H\beta_V$. Consequently, the ESS is insensitive to whether virulence affects transmission from mosquito to human or from human to mosquito. Likewise, the biting rates for susceptible (a_S) and infectious (a_I) mosquitoes appear only as a composite parameter in R_0 : we consider their product, $a = a_S a_I$, instead of separate rates.

There is empirical support for a trade-off in dengue virus between transmission and virulence in humans. Laboratory studies on dengue serotype 2 have found that genotypes that are more virulent to humans also more readily infect mosquitoes (Armstrong and Rico-Hesse 2001, 2003; Cologna et al. 2005) and human cells (Cologna et al. 2005). Additionally, dengue severity was positively correlated with viremia titer in infected children (Vaughn et al. 2000; Libraty et al. 2002). Elevated human viremia is then supposed to increase the chance of transmission to mosquitoes (Rico-Hesse 2003). For a trade-off between human incubation rate and virulence to humans, more virulent strains of dengue serotype 2 were found to replicate and shed more quickly in human cells than were less virulent strains (Cologna et al. 2005), suggesting that humans infected with virulent strains become infectious sooner.

The remaining trade-offs are based on theoretical arguments, some of which have been empirically confirmed in other host-pathogen systems. Much work on virulence evolution hypothesizes a trade-off between transmission and virulence (Levin and Pimentel 1981; Anderson and May 1982; Frank 1996; Gandon et al. 2001; Galvani 2003). Consistent with this hypothesis, we assume that the transmission rate increases with virulence to mosquitoes. For biting rate, it has been suggested that people harboring more virulent parasites may be easier prey for mosquitoes, thereby increasing the frequency of being bitten (Ewald 1983; Ewald and De Leo 2002). Following this, we assume that the biting rate increases with virulence to humans. Dengue infection of *Aedes aegypti* increases biting behaviors that might lead to increased transmission (Platt et al. 1997); thus, we allow biting rate to increase with the virulence of a dengue strain to mosquitoes. We also consider that incubation rate in mosquitoes increases with virulence to mosquitoes, so that more virulent strains replicate more quickly, causing the mosquito to become infectious earlier (Sasaki and Iwasa 1991). (Note that if a mosquito is unable to take a full blood meal, it may feed more often than the

interval between bouts of oviposition.) Finally, we are not aware of any evidence for a relationship between recovery rate and virulence. Therefore, we make no assumption about the direction of a trade-off between human recovery rate and virulence to humans, such that humans infected with more virulent strains may recover either more quickly or more slowly (Frank 1992, 1996; Gandon et al. 2001).

Results

We first analyze the selection on dengue virulence imposed by the four transgenic strategies. Second, we explore the sensitivity of the direction of selection to the functional form that describes the impact of the transgene on an evolutionary trade-off.

Analysis of Transgenic Strategies

Transgenic strategies modify mosquito biology, reducing their competence as dengue vectors. We quantified the effect of a transgene using the parameter “refractoriness,” r , with vector competence decreasing as r increases. (Wild-type mosquitoes correspond to $r = 0$.) We considered four transgenic strategies that increase r : blocking transmission, reducing mosquito biting, increasing mosquito background mortality, and increasing mosquito infection-induced mortality. For each strategy, the appropriate model parameter was assumed to be a function of r (Anderson and May 1982). Under an evolutionary trade-off, the resulting maximal R_0 and optimal virulence are also functions of r . The transgenic strategies were analyzed by examining the response of maximal R_0 and optimal virulence to increasing r . Here we assessed selective pressure on dengue virus in response to these four transgenic mosquito strategies under each of the seven evolutionary tradeoffs (table 2). The combination of a transmission-blocking transgene under the trade-off between transmission and human virulence is examined in detail to illustrate the analysis, generally following the methods of Gandon et al. (2001). The results for the other transgenic strategies and trade-offs are then described in less detail (table 3).

Blocking Transmission—An infection-blocking human vaccine prevents susceptible people from becoming infected during exposure to the pathogen (Molineaux et al. 1985; Struchiner et al. 2006). Similarly, a transgene that reduces dengue replication within the mosquito midgut prevents susceptible mosquitoes from becoming infected after they have taken an infected blood meal. Under the trade-off between transmission and virulence to humans, transmission is a function of both virulence to humans and transgenic refractoriness, $\beta_H \beta_V = \beta(v_H, r)$, with β decreasing in r and increasing and concave in v_H . Because R_0 is now a function of both v_H and r , the optimal level of virulence to mosquitoes for a particular value of r , $v_H^*(r)$, is given by

$$\frac{\partial R_0}{\partial v_H} = R_0 \left(\frac{1}{\beta} \frac{\partial \beta}{\partial v_H} - \frac{1}{\gamma_H + \mu_H + v_H^*} \right) = 0, \quad (3)$$

where β and its derivative are evaluated at $v_H = v_H^*(r)$ (Anderson and May 1982). The second derivative

$$\frac{\partial^2 R_0}{\partial v_H^2} = R_0 \frac{1}{\beta} \frac{\partial^2 \beta}{\partial v_H^2} < 0 \quad (4)$$

because we assumed that β is concave, which guarantees that v_H^* gives the maximal R_0 . Given the optimal virulence, the change in the maximal R_0 with respect to r is

$$\frac{d}{dr}R_0(v_H^*(r), r) = R_0 \frac{1}{\beta} \frac{\partial \beta}{\partial r} < 0, \quad (5)$$

such that increasing r decreases the maximal R_0 . The derivative of equation (3) with respect to r gives the selective pressure on virulence to humans:

$$\frac{dv_H^*}{dr} = \left(\frac{1}{\beta} \frac{\partial \beta}{\partial v_H} \frac{\partial \beta}{\partial r} - \frac{\partial^2 \beta}{\partial v_H \partial r} \right) \left(\frac{\partial^2 \beta}{\partial v_H^2} \right)^{-1} = -\beta \frac{\partial^2 \ln \beta}{\partial v_H \partial r} \left(\frac{\partial^2 \beta}{\partial v_H^2} \right)^{-1}. \quad (6)$$

Because β is concave in v_H ($\partial^2 \beta / \partial v_H^2 < 0$), selective pressure on virulence (eq. [6]) has the same sign as

$$\frac{\partial^2 \ln \beta}{\partial v_H \partial r}, \quad (7)$$

which depends on the change in β caused by simultaneous changes in both virulence and refractoriness. Therefore, virulence to humans can either increase or decrease, depending on the functional form of $\beta(v_H, r)$.

Figure 1 shows two example functions $\beta(v_H, r)$ for the interaction of the transgene with the trade-off and the resulting R_0 as r increases. For both interaction functions, the maximal R_0 decreases as r increases (fig. 1B, 1D, *dashed lines*). For the trade-off Michaelis-Menten-type function (fig. 1A), optimal virulence to humans decreases as r increases (the dashed line goes down and to the left in fig. 1B). Conversely, the scaled trade-off function (i.e., v_H is scaled by $1 - \beta_2 r$; fig. 1C) leads to optimal virulence to humans increasing as r increases (the dashed line goes down and to the right in fig. 1D).

If selective pressure on virulence (eq. [6]) is positive, the derivative

$$\frac{d}{dr}\beta(v_H^*(r), r) = \frac{\partial \beta}{\partial v_H} \frac{dv_H^*}{dr} + \frac{\partial \beta}{\partial r} \quad (8)$$

shows that while the transgene directly reduces transmission ($\partial \beta / \partial r < 0$), selective pressure on transmission is positive through the trade-off with virulence ($[\partial \beta / \partial v_H][dv_H^* / dr] > 0$), which may at least partially counteract the effect of the transgene. In this case, a virus genotype that offsets the decrease in transmission by increasing virulence to humans is favored. Overall, the transgene reduces the maximal R_0 (as shown in eq. [5]). In contrast, if selective pressure on virulence (eq. [6]) is negative, then the selective pressure on transmission is negative. In this scenario, reducing virulence to humans—and thereby reducing transmission further below the direct effect of the transgene—is advantageous to dengue virus. Repeating the above analysis with trade-offs between virulence to humans and human incubation rate, human recovery rate, or mosquito biting rate results in no selection for virulence, independent of the functional form representing the trade-off between parameters.

In humans, a vaccine that prevents an infected person from transmitting infection to others is referred to as transmission blocking (Molineaux et al. 1985; Struchiner et al. 2006). A transgene that blocks virus migration to the mosquito salivary glands would have a similar effect, preventing mosquitoes from infecting humans. Both human vaccine mechanisms, transmission blocking and infection blocking, disrupt the transmission cycle, acting on either infectious or susceptible people. Henceforth, we will refer to the analogous transgenic

mosquito strategies together as “transmission blocking” for simplicity, regardless of whether the effect is to reduce infection of susceptible mosquitoes or to reduce transmission by infectious mosquitoes. Analyzing the model with transmission as a function of mosquito virulence, $\beta(v_v, r)$, shows that transgenic transmission blocking and the trade-off between transmission and virulence to mosquitoes cause a reduction in the maximal R_0 and select for changes in virulence, the direction of which is given by the sign of

$$\frac{\partial^2 \ln \beta}{\partial v_v \partial r}. \quad (9)$$

As before, virulence can either increase or decrease depending on the form of $\beta(v_v, r)$. Trade-offs between virulence to mosquitoes and mosquito incubation rate or mosquito biting rate do not impose selection on dengue virulence to humans.

Reducing Mosquito Biting—In our analysis, the role of biting ($a = a_1 a_S$) is the same as the role of transmission ($\beta = \beta_H \beta_V$). For a transgene that reduces mosquito biting, two trade-offs impose selection, the trade-offs between biting rate and virulence in either humans or mosquitoes. Under both trade-offs, the maximal R_0 is reduced as r increases. For the trade-off between mosquito biting and virulence to humans, the direction of selective pressure on virulence is given by the sign of

$$\frac{\partial^2 \ln a}{\partial v_H \partial r}, \quad (10)$$

whereas under the trade-off between mosquito biting and virulence to mosquitoes, selective pressure on virulence has the same sign as

$$\frac{\partial^2 \ln a}{\partial v_v \partial r}. \quad (11)$$

The other trade-offs lead to no selection for virulence to either humans or mosquitoes.

Increasing Mosquito Background Mortality—A strategy that increases mosquito background mortality has two benefits: it reduces the number of mosquitoes and reduces the mean lifetime of the mosquitoes. Model (1) assumes a constant mosquito population size, incorporating only the reduction in mean lifetime of mosquitoes, which is important because the age at which a mosquito can transmit dengue is of the same magnitude as the mean lifetime. (We consider a model that does not assume a constant mosquito population size in “Diffusion Model” in the appendix.)

For a strategy that increases mosquito background mortality, the trade-offs between virulence to mosquitoes and transmission, mosquito incubation, or biting all decrease the maximal R_0 and select for increased virulence to mosquitoes. Increased virulence to mosquitoes implies that transmission increases, the mosquito incubation period decreases, and biting increases under their respective tradeoffs. These results are consistent with previous theoretical studies showing that virulence increases with increasing background mortality (May and Anderson 1983; Kakehashi and Yoshinaga 1992; Lenski and May 1994; Ebert and Mangin 1997). More important, there is no selection on virulence to humans under any trade-off for this strategy.

Increasing Mosquito Infection-Induced Mortality—Another alternative transgenic strategy is based on increasing mosquito infection-induced mortality without directly impacting the interaction between dengue virus and mosquito. Such a transgene causes infected mosquitoes to die more quickly, although without increasing the replication of dengue virus. For example, infection with dengue virus may trigger the expression of a lethal transgene. Therefore, r does not directly affect dengue virus traits through evolutionary trade-offs. Consequently, the mosquito infection-induced mortality rate $\nu_V(\hat{\nu}_V, r)$ must be separated into a function of both intrinsic virulence ($\hat{\nu}_V$), which interacts through trade-offs with epidemiological parameters, and r . A similar mechanism in human vaccination is referred to as disease modification (Molineaux et al. 1985; Struchiner et al. 2006), although the goal of vaccination is to reduce virulence to humans by vaccination, whereas the goal of transgenesis is to increase virulence to mosquitoes.

For all the trade-offs with virulence to mosquitoes, increasing infection-induced mortality imposes selection to alter virulence, subject to additional constraints. For the other transgenic strategies, parameter function assumptions ensure that R_0 is concave in virulence, so the first derivative of R_0 with respect to virulence gives the maximal R_0 . Here, additional constraints are required to ensure that R_0 is concave. For example, for the trade-off between transmission and virulence, $\partial^2 R_0 / \partial \nu_V^2 < 0$ requires

$$\frac{1}{\beta} \frac{d^2 \beta}{d \hat{\nu}_V^2} < \frac{1}{\mu_V + \nu_V} \frac{\partial^2 \nu_V}{\partial \hat{\nu}_V^2}. \quad (12)$$

The trade-offs for virulence and mosquito incubation or biting rate lead to similar second-derivative conditions. For all three trade-offs with virulence to mosquitoes, the maximal R_0 decreases, and the sign of

$$-\frac{\partial^2 \ln(\mu_V + \nu_V)}{\partial \hat{\nu}_V \partial r} \quad (13)$$

gives the direction of selective pressure on virulence to mosquitoes as r increases. Again, there is no selection on virulence to humans under any of the trade-offs for this strategy.

Functions of Both Virulence and Refractoriness—When an epidemiological parameter is a function of both virulence and refractoriness, the direction of selection on virulence depends on the response of the parameter to simultaneous changes in both variables, that is, how refractoriness impacts the shape of the trade-off between the parameter and virulence (as given by the sign of eqq. [7], [9]–[11], and [13]). The interaction between refractoriness and epidemiological trade-offs has often been assumed to be multiplicative (e.g., Gandon et al. 2001). However, considering the current understanding of interactions among dengue virus, mosquitoes, and humans, no simple assumption about the form of a function of both virulence and refractoriness is justified. Accordingly, for the results presented thus far, we have not assumed any form for the relationship between the two dependent variables.

Some simple relationships can be explored. For example, for the transmission-blocking strategy and the trade-off between transmission and virulence to humans, consider three different forms of $\beta(\nu_H, r)$: multiplicative, $\beta = f(\nu_H)g(r)$; the Michaelis-Menten-type function, $\beta = \beta_0 \nu_H / [\beta_1 + (1 + r)\nu_H]$, where increasing r reduces the asymptotic value of β for large ν_H (fig. 1A); and scaled, $\beta = f(g(r)\nu_H)$ (fig. 1C), where virulence is scaled by a function of r . These forms for β result in no selection on virulence, selection for decreasing

virulence, and selection for increasing virulence, respectively. (In “Functional Form and Direction of Virulence Evolution” in the appendix, we derive for a simple, one-species susceptible-infected-recovered model these same results on the direction of selection imposed by different functional forms for the impact of refractoriness on the trade-off between transmission and virulence.)

Similarly, the effect of a transmission-blocking transgene and the trade-off between transmission and virulence to mosquitoes is expressed by the function $\beta(v_V, r)$, while a bite-reducing transgene and the trade-offs between mosquito biting and virulence to either humans or mosquitoes are given by $a(v_H, r)$ and $a(v_V, r)$, respectively. The same result as above holds for the functions $\beta(v_V, r)$, $a(v_H, r)$, and $a(v_V, r)$: the multiplicative form, $f(v)g(r)$, gives no selection; the Michaelis-Menten-type function, $c_0v/[c_1 + (1 + r)v]$, leads to selection for decreasing virulence; and the scaled form, $f(g(r)v)$, results in selection for increasing virulence. Finally, a transgene that increases infection-induced mortality leads to the function $v_V(\hat{v}_V, r)$. The multiplicative form, $v_V = f(\hat{v}_V)g(r)$, selects for decreasing virulence, whereas the scaled form, $v_V = f(g(r)\hat{v}_V)$, selects for increasing virulence. Thus, alternate forms of the functions of both virulence and refractoriness give qualitatively different results for the direction of selection on virulence.

Discussion

Mosquito transgenesis is currently being developed as a control strategy for dengue. To determine the impact of transgenic mosquitoes on dengue evolution, we analyzed the effect of transgenic refractoriness using a model that explicitly incorporates virulence in both human and mosquito hosts. We evaluated four transgenic strategies for dengue control: blocking transmission, decreasing mosquito biting, increasing mosquito background mortality, and increasing mosquito infection-induced mortality. We identified both the short-term and the long-term effects of the four strategies under consideration. In the short term, all of these control strategies reduce R_0 , thereby decreasing disease incidence. Over the longer term, the optimal evolutionary response to a transgene that alters a particular epidemiological trait may offset, at least to some extent, the benefit of the intervention strategy.

Dengue virulence to mosquitoes is predicted to evolve in response to all four strategies. Increased background mortality selects for elevated virulence to mosquitoes (May and Anderson 1983; Kakehashi and Yoshinaga 1992; Lenski and May 1994; Ebert and Mangin 1997), whereas the other three strategies can select for either increasing or decreasing virulence to mosquitoes. For virulence to humans, transmission blocking and reduced mosquito biting can select for either increasing or decreasing virulence. In contrast, increasing mosquito natural or infection-induced mortality does not affect virulence to humans.

Transgenes that block transmission or reduce mosquito biting can select for changes in virulence to humans because transmission and biting can themselves be altered by virulence to humans. Conversely, in our model there is no connection between virulence to humans and either mosquito background mortality or mosquito infection-induced mortality. Thus, strategies that increase either of these mosquito mortality rates do not select for changes in virulence to humans.

Only for increased mosquito background mortality is the direction of selection determined by qualitative features of the trade-off functions. For the other three transgene strategies (blocking transmission, increasing mosquito infection-induced mortality, and reducing mosquito biting), the direction of selective pressure on virulence to mosquitoes depends on

quantitative features of the effect of the transgene on the trade-off between dengue epidemiological traits. Moreover, assuming a particular form of trade-off without biological justification can lead to spurious conclusions. This sensitivity to functional form prevents direct biological interpretation of the refractoriness variable, which cannot be interpreted as either the absolute or the relative reduction in the epidemiological trait in question (e.g., transmission rate). The detailed quantitative information about the trade-off functions necessary to predict the direction of selection pressure is not available for dengue. As a result of this uncertainty, we caution that the risk of increasing virulence to humans, that is, case fatality, is expected to be greater for strategies that block transmission or reduce mosquito biting than for strategies that increase mosquito background or infection-induced mortality. Previous studies assumed a multiplicative effect of refractoriness on the trade-offs between pathogen traits (Gandon et al. 2001), which limits possible selective pressures on virulence.

Our results also apply to control strategies that are not based on transgenesis. In particular, the use of *DENV* (Kittayapong et al. 1999; Ledermann et al. 2004; Carlson et al. 2006; Suchman et al. 2006), *Wolbachia* (McMeniman et al. 2009; Read and Thomas 2009), or insecticide to increase mosquito background mortality has the same effect in our model as does the introduction of a dominant female-killing gene (Thomas et al. 2000; Alphey and Andreasen 2002): these control strategies select for increased dengue virulence to mosquitoes (May and Anderson 1983; Kakehashi and Yoshinaga 1992; Lenski and May 1994; Ebert and Mangin 1997) and impose no selection on virulence to humans.

Under a given trade-off in epidemiological traits, the methods we used are based on optimizing virus dissemination to subsequent generations (R_0), resulting in a balance between the two traits. If transgenic refractoriness does not alter any of the terms that define this balance, transgenesis does not impose selection to alter the balance between traits, in particular, virulence. For example, the balance under a trade-off between transmission rate (β) and virulence to humans (v_H) is given by the term in parentheses in equation (3). If a transgene alters biting rate (a) under this trade-off, there is no change to the balance between transmission rate and virulence and thus no selection caused by the transgene to change this balance.

A transgene may be effective at reducing infections with dengue, at the expense of causing an increase in virulence to humans. In this situation, a crucial question is whether the reduction in number of infections is greater, in proportional terms, than the increase in virulence; if so, the total number of severe cases would decrease. Much more experimental data is needed to finely quantify the trade-offs between epidemiological traits before such questions can be answered.

Transgene fixation in the mosquito population is expected to be much quicker than the evolution of virulence in the dengue virus. However, if the timescales of the two processes were similar, the evolutionary effects of transgenesis on the dengue virus might affect the spread of the transgene itself. Increased virulence to mosquitoes would increase the life span of dengue-resistant mosquitoes relative to those nonresistant. A transgene that increases mosquito resistance (e.g., transmission blocking) would then spread more quickly, while a transgene that decreases mosquito resistance (e.g., increasing infection-induced mortality) would spread more slowly. Similarly, if virulence to mosquitoes decreased, a resistance-increasing transgene would spread more slowly, while a resistance-decreasing transgene would spread more quickly.

Our model assumed constant mosquito and human population sizes for simplicity and used the resulting endemic equilibrium to analyze the model. While changing human population

size probably plays little role in dengue evolution (Kawaguchi et al. 2003; Rohani et al. 2003), seasonal periodicity in mosquito numbers might significantly complicate the evolutionary trajectory of dengue virulence. In “Diffusion Model” in the appendix, this seasonality is incorporated into a more complex model to examine the evolution of virulence when the mosquito population size is allowed to decrease as mortality rates increase. Simulation results for this more complex model are consistent with those from the simple model (eq. [1]), with seasonally fluctuating mosquito populations leading to fluctuations in optimal virulence. This more complex model was not amenable to analytic results, but the simulation results suggest that our results for the simple model (eq. [1]) are insensitive to the assumption of fixed mosquito population size.

In many settings where dengue is endemic, multiple serotypes and genotypes circulate. Here we have explored how genotype dynamics within a single serotype affect the evolution of virulence. We assumed no coinfection or superinfection, which have both been shown to increase virulence due to competition within a single host (May and Nowak 1994, 1995; Nowak and May 1994; Frank 1996; Read and Taylor 2001; Galvani 2003). Because of possible synergistic interactions between dengue serotypes leading to more severe disease (Halstead et al. 1970; Guzmán and Kouri 2002; Gibbons et al. 2007), reducing the prevalence of a single serotype by transgenesis could reduce the incidence of severe disease caused by the other serotypes. Moreover, if a transgenic strategy affects multiple dengue serotypes, then an amplified reduction in secondary infections and, thus, severe disease might occur. However, for any transgenic strategy, the magnitude of the reduction in disease likely depends on the exact mechanism of the synergy between serotypes (Cummings et al. 2005; Wearing and Rohani 2006), making the impact of transgenesis on multiple serotypes difficult to predict. For example, increased susceptibility to and transmission by secondary infections would amplify the impact on severe disease of transgenesis, while the exclusion of third or fourth infections would decrease the impact because of reduced competition between serotypes for hosts.

A further consideration is the timing of events in the transmission cycle (Day 2003; Day et al. 2007). If the risk of infection-induced mortality to humans occurs after viremia, for example, because of an overactive immune response (Day et al. 2007), then there is no selection on virulence at all. In this scenario, virulence can be understood as a by-product of infection that does not affect dengue transmission. However, in addition to empirical support for a correlation between dengue transmission and virulence to humans (Armstrong and Rico-Hesse 2001, 2003; Rico-Hesse 2003; Cologna et al. 2005), fatal cases of dengue have shown significant viral load at the time of death (Gubler et al. 1981), suggesting that the period of high viremia and high transmission seems to coincide at least partially with the risk of mortality so that there is selection on dengue virulence to humans.

In summary, we modeled the evolutionary impact of different transgenic-mosquito strategies on dengue virulence to both humans and mosquitoes. We found that all four transgenic strategies evaluated for dengue control (blocking transmission, decreasing mosquito biting, increasing mosquito background mortality, and increasing mosquito infection-induced mortality) reduce disease incidence. However, although transgenic strategies have a short-term epidemiological benefit, the evolutionary repercussions may be detrimental for some strategies. Our results suggest that transgenic strategies that increase mosquito background or infection-induced mortality pose a smaller risk of increasing dengue virulence to humans than blocking transmission or reducing mosquito biting. Thus, more experimental research is needed to facilitate prediction of the direction of selection on virulence caused by the introduction of transgenic mosquitoes. In addition, the effect of an introduced transgene may be mitigated by an evolutionary response in dengue virus. Therefore, monitoring of the effectiveness of transgenic strategies should be conducted with measures of the entire

epidemic cycle, such as incidence, prevalence, or basic reproductive number, not specific epidemiological traits.

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Figure 1.

Transmission (β) and basic reproductive number (R_0) versus virulence to humans (ν_H) for a transmission-blocking transgene and a trade-off between transmission and virulence. The lines in A and C show the effect of increasing refractoriness on the transmission–virulence trade-off, given by the Michaelis-Menten-type function, $\beta = \beta_0 \nu_H / [\beta_1 + (1 + r) \nu_H]$ (A), and the scaled function, $\beta = [\beta_0 (1 - \beta_2 r) \nu_H] / [\beta_1 + (1 - \beta_2 r) \nu_H]$ (C; i.e., ν_H is scaled by $1 - \beta_2 r$). (Note that these two functions are identical for $r = 0$.) The solid lines in B and D show the resulting R_0 under these trade-off functions. For the solid lines, the upper line is for $r = 0$, the next lower line is for $r = 0.2$, and so on, with $r = 1$ for the lowest line, where increasing r indicates increasing effectiveness of the transgene. The dashed lines in B and D show the optimal virulence ν_H^* versus the maximal R_0 for different values of r . Parameter values are $a_S = a_I = 1 \text{ day}^{-1}$, $\mu_H = 1/70 \text{ year}^{-1}$, $\mu_V = 1/25 \text{ day}^{-1}$, $\nu_V = 3.65 \text{ day}^{-1}$, $\tau_H = \tau_V = 1/7 \text{ day}^{-1}$, $\gamma_H = 1/5 \text{ day}^{-1}$, $m = 10$, $\beta_0 = 1 \text{ day}^{-1}$, $\beta_1 = 0.001 \text{ day}^{-1}$, and $\beta_2 = 0.9$ (Luz et al. 2003).

Table 1

Model parameters

Parameter	Definition
a_S	Biting rate of susceptible mosquitoes
a_I	Biting rate of infectious mosquitoes
β_H	Mosquito-to-human transmission probability
β_V	Human-to-mosquito transmission probability
μ_H	Human natural mortality rate
μ_V	Mosquito natural mortality rate
ν_H	Human infection-induced mortality rate (virulence)
ν_V	Mosquito infection-induced mortality rate (virulence)
τ_H	Human incubation rate
τ_V	Mosquito incubation rate
γ_H	Human recovery rate
$m = V/H$	Mosquitoes per person
r	Refractoriness

Table 2

Possible evolutionary trade-offs between epidemiological parameters and virulence

	Virulence to humans, ν_H	Virulence to mosquitoes, ν_V
Transmission	β increases with ν_H	β increases with ν_V
Mosquito biting	a increases with ν_H	a increases with ν_V
Incubation	τ_H increases with ν_H	τ_V increases with ν_V
Recovery	γ_H is a function of ν_H	...

Note: See “Methods” for a discussion of the theoretical and empirical support for these trade-offs.

Table 3

Summary of selective pressures due to transgenic strategies and trade-offs

Trade-off	Transgenic strategy			
	Blocking transmission	Reducing mosquito biting	Increasing mosquito background mortality	Increasing mosquito infection-induced mortality
β vs. v_H	$v_H \uparrow \downarrow, \beta \uparrow \downarrow$	...	...	...
β vs. v_V	$v_V \uparrow \downarrow, \beta \uparrow \downarrow$	...	$v_V \uparrow, \beta \uparrow$	$v_V \uparrow \downarrow, \beta \uparrow \downarrow$
a vs. v_H	...	$v_H \uparrow \downarrow, a \uparrow \downarrow$	...	...
a vs. v_V	...	$v_V \uparrow \downarrow, a \uparrow \downarrow$	$v_V \uparrow, a \uparrow$	$v_V \uparrow \downarrow, a \uparrow \downarrow$
τ_H vs. v_H	...	...	...	...
τ_V vs. v_V	...	...	$v_V \uparrow, \tau_V \uparrow$	$v_V \uparrow \downarrow, \tau_V \uparrow \downarrow$
γ_H vs. v_H	...	...	...	...

Note: An ellipsis denotes no selection. An upward arrow denotes selection for increasing the parameter. A downward arrow denotes selection for decreasing the parameter. An upward arrow and a downward arrow together denote selection for either increasing or decreasing the parameter, depending on quantitative features of the effect of the transgene on the trade-off between virulence and the other epidemiological trait.