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Temporal dynamics and spatial analysis of competing dengue 2 virus strains in the Americas

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THESIS

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Abstract

The dengue virus is the causative agent of an important re-emerging infectious disease that has become increasingly significant in tropical America and the Caribbean due to the infiltration of a more pathogenic Asian/American strain of dengue serotype 2 into the population. This invading strain is responsible for epidemics of dengue hemorrhagic fever, a life-threatening disease that was not previously a large public health concern in the region. Here, I create a historical map of the invasion and replacement of the endemic American strain of dengue 2 by the Asian/American strain, showing that the timing of invasion spans 25 years, and is highly variable in the region. In addition, I model the competitive dynamics of the two strains using differential equation models. By calculating and comparing the basic reproductive ratio (R0) for the Asian/American and American strain of dengue 2, I identify potential evolutionary trade-offs between the two strains and the ecological circumstances that benefit one trade-off over another. Numerically solving my models help to understand possible mechanisms behind variable timing of invasion by the Asian/American strain. The fitness gain resulting from the Asian/American strains shorter latency period increases as the adult vector mortality rate increases, indicating that regions where adult mosquito death rate is high will select for the more virulent strain of dengue 2.

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Introduction

The emergence and re-emergence of infectious diseases pose significant challenges to human health. In particular, novel viruses, or changes in the epidemiology of extant viruses have recently been at the center of many public-health threats, including influenza, SARS, Hantavirus, and dengue virus (Morens et al. 2004 and Sim and Mackie 2009). Therefore, understanding the transmission and spread of infectious diseases remains a focus of public health research.

Upon the emergence of a novel pathogen, researchers study key aspects of its epidemiology such as mode of transmission and incubation period. These studies are the first step toward reducing transmission by educating the public and informing public health policy. Once enough genetic and molecular information is obtained about the new pathogen, pharmaceutical interventions may be developed. Though this approach is an important step in decreasing epidemic spread and suffering, it can be costly and time-consuming. Vaccines, especially for viruses, are a key component in mitigating the effects of new diseases because they directly reduce the susceptible pool, and have the proven potential to eradicate a virus from a population (Fenner 1982).

Implementation of both preventative and reactive treatment programs on a large scale can be very expensive and logistically difficult; therefore, it is important to estimate the risk of an emerging infectious disease as well as the effectiveness of prevention and treatment measures as quickly as possible. Mathematical modeling of infectious disease dynamics has recently become a popular approach for this purpose (Hethcote 2000; Sattenspiel and Lloyd 2009). Models can be used to gain an understanding of the underlying dynamics of

an emerging disease and to predict the efficacy and efficiency of disease control programs.

Here, I use mathematical modeling techniques to gain insight into the competition between the more pathogenic Asian/American strain of dengue serotype 2, which has recently emerged in tropical America and the Caribbean, and the endemic American strain of dengue 2. I also map the presence of the two strains of Dengue 2 in tropical America and the Caribbean using phylogenetic techniques. My results indicate that the Asian/American strain is replacing the endemic strain throughout the region due to the Asian/American strain's decreased latency period in the mosquito.

Dengue Virus

The dengue virus is the causative agent of an important re-emerging infectious disease that has become increasingly significant in tropical America and the Caribbean. Dengue is a single-stranded, positive sense RNA virus made of approximately 10,000 bases and belonging to the family *Flaviviridae*, which also includes West Nile virus and Yellow Fever virus (Gubler 1998). There are both sylvatic (generally with non-human primate hosts) and urban dengue groups (with human hosts), which do not generally cause disease in non-primary hosts, but may cause the build-up of antibodies (Kyle and Harris 2008). I plan to study only the urban forms of dengue virus, because the sylvatic and urban viruses do not generally interact with one another and do not depend on each other for persistence, and because the urban form of the dengue virus is the only one known to cause epidemics in humans (Wang et al. 2000). The dengue virus includes four antigenically distinct serotypes: Dengue 1, 2, 3, and 4. Generally, when a host becomes infected with one serotype, it

gains life-long immunity from that serotype upon recovery, but only gains transient immunity from other serotypes (Kliks et al. 1989). In addition, within each serotype family are various genotypes that may have different epidemiological characteristics but are not considered antigenically distinct (Gubler 1998).

Dengue virus is vector-borne, meaning that it has a mostly asymptomatic vector host as well as symptomatic hosts. The urban form of dengue virus is transmitted from human host to mosquito vector (most commonly *Aedes aegypti*) and from mosquito to human (Gubler and Clark 1995). Like many vector-borne diseases, dengue is not transmissible from human to human or mosquito to mosquito. The virus must be able to live and reproduce in both the human host and the mosquito vector. This poses an interesting problem for the virus, due to the large variance in size, lifespan, and immune system between mosquitoes and humans. It also limits the location of the virus to where *Aedes* sp. and humans live in contact with one another, which is generally in tropical regions of the Americas, Asia, the South Pacific, the Caribbean, and Africa (Gubler 1998).

The most common human disease associated with dengue virus is dengue fever (DF). Dengue fever is similar to influenza, with symptoms including fever, severe muscle and joint pain, and rash (WHO 2009; Gubler 1998). Approximately 50 million people experience dengue fever each year, and it is generally not fatal. A more severe manifestation of the Dengue virus in humans is dengue hemorrhagic fever (DHF). Symptoms of DHF include the symptoms of DF as well as high fever, hemorrhaging, liver enlargement, and circulatory failure (WHO 2009; Gubler 1998). Dengue hemorrhagic fever has a death rate of greater than 20% without hospitalization and less than 1% with hospitalization, and an average death rate globally of approximately 5% (WHO 2009). It is generally believed

that DHF is caused by antibody-dependent enhancement of dengue infection in monocytes related to a previous infection by another serotype or by the transfer of maternal antibodies in infants (Kliks et al. 1989). Also, dengue serotype 2 is most likely to cause DHF, and some strains of this serotype cause DHF more frequently than others (Torres and Castro 2007; Gubler and Clark 1995). Here, I focus on two competing strains of dengue 2 found in tropical America and the Caribbean with different levels of pathogenicity.

Dengue as a Public Health Problem in Tropical America

Epidemic dengue fever was first reported in the late 18th century in tropical Asia, Africa, and America, though the disease is believed to have come from Asia originally (Gubler and Clark 1995; Gubler 2002). Dengue virus has had at least 200 years to evolve in the Americas, and there are endemic American strains for each of the four serotypes. The American strain of dengue 2 does not appear to have the ability to cause DHF (Watts et al. 1999), whereas the Asian strains of dengue 2 often cause DHF in individuals with previous dengue infections, and dengue has therefore been a public health concern in tropical Asia throughout the history of the virus (Gubler 1998; Pinheiro and Corber 1997). Until 1981, dengue in the Americas and the Caribbean was considered a relatively benign disease, but in 1981 Cuba was hit with the first epidemic of DHF in the region, with more than 10,000 cases of DHF and over 150 deaths (Guzman et al. 1999). This epidemic of DHF was later attributed to the spread of dengue 1 from 1977 to 1979, which allowed individuals in the population to build up dengue-1 antibodies, followed by the spread of the Asian strain of dengue 2 during the 1981 epidemic (Kouri et al. 1986; Guzman et al. 1995; Guzman et al

2002). After this epidemic, the Asian strain of dengue 2 has been spreading and evolving throughout the region, causing epidemic DHF, with the new evolving strain often called Asian/American dengue 2 (Torres and Castro 2007; Diaz et al. 2006; Pinheiro and Corber 1997; Siqueira et al. 2005). Here, I investigate the roles that the endemic American strain and Asian/American strain play in the region.

Thesis Questions

With my thesis work, I address questions about the history of dengue 2 in the tropical American and Caribbean region as well as questions about the importance of and interaction between the American and Asian/American strain of dengue 2 in this region. Questions relating to the history of dengue 2 in the region include the following: where has the Asian/American strain infiltrated the dengue 2 population, and at each location it has entered, how long after the 1981 Cuba epidemic did it take to be detected? Are there any correlations between demographic parameters (including human population size, birth rate and death rate) and the ability or length of time it takes for the Asian/American strain to infiltrate the population? Unfortunately, data distinguishing the two strains are scarce, with most case reports in the region reporting at a serotype level at best (PAHO 2009; WHO 2010), and instead I use phylogenetic studies of the region and create my own phylogenetic trees from available sequence data to answer the questions above.

I use mathematical modeling to answer questions about the competition between the two strains of the dengue 2 virus. First, under what circumstances should the Asian/American strain infiltrate and replace the American strain? A study by Anderson and Rico-Hesse in

2006 showed that the Asian/American strain has significantly shorter latency periods in the mosquito than the American strain when the number of viral titers was controlled, which may give it a competitive advantage over the American strain. In 2005, Cologna et al. showed that in vitro, the Asian/American dengue 2 strain had significantly higher viral output than the endemic American strain in both human dendritic cells and *Aedes aegypti* mosquitoes (which likely leads to further shortened latency periods in the host), yet the American strain was able to infect a larger proportion of the human cells. This information suggests that an evolutionary trade-off for the Asian/American strains shorter latency periods in the vector may be a lower transmission rate. Another untested potential trade-off could be a decreased infectious period in the human population, which could be due to hospitalization of people with more severe disease. I use mathematical models to understand under which parameter regimes, given potential trade-offs, the Asian/American strain becomes more competitive than the endemic strain, resulting in strain invasion and/or replacement. If the Asian/American strain will out compete the American strain, how long should it take to do so? I use modeling techniques to explore how the time-scale of invasion changes with varying ecological and epidemiological parameters.

Hypotheses and Expected Results

With the mapping portion of my thesis, I elucidate the spatial spread of the Asian/American strain and American strain of dengue 2 over time. It has been shown that an increased birth rate may result in increased incidence of disease due to the correlation between birth rate and poverty (McClean and Anderson 1988; Bonds and Rohani 2010). I hypothesize that

increasing the birth rate of the population may increase the number of people infected with the endemic strain, which should decrease the rate at which a more competitive strain can invade.

The modeling portion of my thesis explores two main aspects of the competition between the Asian/American and American strain of dengue 2. First, I explore under which conditions and at what time-scale the Asian/American strain of dengue 2 will be able to enter and take over the endemic population. Second, I use the model to help determine which parameters are most important for competition between the two strains. I hypothesize that parameters representing the life cycle of the virus within the mosquito will be most important because mosquitoes have lifespans that are close to the latency period of dengue in the mosquito (Schule 1928; Kyle and Harris 2008). Also, because the latency period is less variable in the mosquito, I expect that shortening the latency period in the mosquito will be more advantageous than lengthening the infectious period in the host.

Finally, using the results of my mapping project and comparing them with the results of my models, I assess which model is the most consistent with the data. I show whether it is appropriate to suspect incomplete immunity between the two strains and whether or not it is necessary to model the latency period in the mosquito as an invariable process. I also investigate which trade-offs for a shortened latency period are consistent with the data, and whether changes in temporal dynamics of the system due to changes in ecological parameters are consistent between the historical data and model results.

Mathematical Modeling of Competing Dengue Strains

Methods

I modeled the competition of the Asian/American dengue 2 strain with the endemic American dengue-2 strain in tropical America and the Caribbean using delayed differential equation Susceptible-Exposed-Infected-Recovered (SEIR) and Susceptible-Exposed-Infected (SEI) models. I calculated the basic reproductive ratio (R_0) for each of the strains to determine which strain is a better competitor given different ecological parameters (human and vector birth/death rate, human and vector population size, vector to human bite rate) and strain-specific parameters (human and vector latency period, human recovery rate, transmission rate, and degree of incomplete immunity). I also explored under what parameter conditions the Asian/American strain will take over the American strain and determined the time scale of these competitive dynamics.

To explore the competitive dynamics, I formulated three temporal models with varying levels of complexity. Each model utilizes the SEIR structure for the human part of the infection cycle and an SEI structure for the mosquito part of the infection cycle, because humans recover from the disease, and mosquitoes generally remain infected for life (Kyle and Harris 2008). I created the models by first making box diagrams for each (Figure 2), and I then used ordinary differential equations and delayed differential equations to model the transition of proportions of the population to and from each class for each of the three models (Equations not shown). Since the *Aedes aegypti* lifespan is generally 2-3 orders of magnitude shorter than the human lifespan, I assume that the mosquito dynamics are at quasi-equilibrium (Keeling and Rohani 2008), and solve for the equilibrium state of the

mosquitoes, only explicitly modeling the human dynamics (Equations 1-24).

The first and simplest model consists of a system of ordinary differential equations (Equations 1-6). It assumes equal birth and death rates in both the human and mosquito populations, as well as complete immunity between the Asian/American strain and American strain of dengue 2, which means that once an individual recovers from one of the strains it cannot become re-infected with either strain. I call this model the complete immunity (CI) model.

The second model is similar to the first, except that it does not consist solely of ordinary differential equations (Equations 7-12). This model includes a time-delayed differential equation to model the transition of mosquitoes from the exposed to infected class, because mosquitoes enter the infected class after a specific amount of time (Schule 1928; Armstrong and Rico-Hesse 2001). The transition to each of the other classes occurs at a constant rate, including the transition of humans from the exposed to infected class (Schule 1928), so all other equations in this model consist of ordinary differential equations. I call this model the delayed complete immunity (DCI) model.

The third model has the same assumptions as the second except it does not assume complete cross-immunity between the two strains (Equations 13-24). If I assume complete cross-immunity, then the more competitive strain will drive the other strain to extinction (Keeling and Rohani 2008). If I do not assume complete cross-immunity, then, under certain conditions, one strain may not completely drive the other strain to extinction, even if it is a better competitor (Vasco et al. 2007; Keeling and Rohani 2008). A study by Russel and McCown in 1976 showed that antibodies made from the Asian strain of dengue 2 were able to successfully neutralize the American strain of dengue 2 virus, and vice

versa. However, I was unable to find any modern studies comparing the American and Asian/American strains of Dengue 2. This model has more compartments than the other two models because there are more disease-class possibilities for the human population (Figure 1c). This model is called the delayed incomplete immunity (DII) model.

For each of the models, I calculate the basic reproductive ratio (R_0) for each of the strains of dengue 2, where R_0 is the expected number of secondary cases resulting from one infected individual in a completely susceptible population, and a good representation of pathogen fitness (Heesterbeek 1996). Since the strain with the highest fitness will replace other strains in deterministic models with complete cross-immunity, a strain will be able to invade a population if

$$\frac{R_{0i}}{R_{0e}} > 1$$

(Keeling and Rohani 2008). The ability of a more competitive strain to invade the human population in a model with incomplete immunity depends both on R_0 and the level of cross-immunity (α):

$$\frac{R_{0i}}{R_{0e}}(1 + \alpha(R_{0e} - 1)) > 1$$

(Vasco et al. 2007). I analytically calculated R_0 for both strains in each of the models using the next generation matrix method, where R_0 is calculated as the spectral radius of the 'next-generation operator', which is a matrix created using the rates at which individuals enter and leave the exposed and infected class (Heesterbeek 2000 and Heffernan et al. 2005). I explore how the value of R_0 changes with varying values of each of the different parameters. To better understand the importance of potential evolutionary trade-offs, I set the vector latency period to 14 days in the American strain and 7 days in the

Asian/American strain and explore the ability of the Asian/American strain to invade as I change various strain-specific and ecological parameters.

In addition to evaluating R_0 for the different strains, I numerically integrate the equations of the models using standard ordinary differential equation and delayed differential equation solvers in Matlab (ODE45 and DDE23) to determine the transient dynamics of the system. Specifically, I determine the length of time it takes for the Asian/American strain to overtake the endemic population. I do this by first solving for the stable equilibrium of the endemic strain for each model (Equations 28-30), setting the initial values equal to these equilibrium values, and then allowing a very small portion of the population to become infected with the Asian/American strain. Next, I observe how changes in ecological and strain-specific parameters as well as differences between the models affects the timing of invasion, and compare these results with the trends observed in the mapping portion of the thesis.

Model Structures

Each of the three models are represented in the box diagrams in Figure 2, where the complete immunity model and the delayed complete immunity model have the same classes: 5 different classes for the mosquitoes and 6 different classes for the humans. The only difference between these two models is in the way the mosquitoes move from the exposed class to the infected class. In the complete immunity model, the transition from one to the other is calculated as the inverse of the average amount of time mosquitoes spend in the latency period (σ_V) multiplied by the proportion of the mosquito population in the exposed

class. In the delayed model, mosquitoes transition from the exposed to infected class after a constant amount of time in the exposed class, corresponding to the average latency period in the vector (τ). The delayed incomplete immunity model has the same five mosquito classes, but it has 12 different human classes instead of six because once a person recovers from one strain of dengue 2, they move into a class that is recovered from that strain but still susceptible to the other strain. This results in a doubling of the number of human classes.

When representing the models as differential equations, each box in the diagram translates to one differential equation, with either proportions of the human or mosquito population moving in and out of each class. However, for the delayed differential equation models, the change in the exposed mosquito class is only dependent on one variable, and can therefore easily be solved and incorporated into the equation keeping track of the change in the infected class. This simplifies the models because it reduces the number of equations by two. After solving for quasi-equilibrium in the mosquitoes, the complete immunity model and the delayed complete immunity model each consist of six differential equations (Equations 1-12), and the delayed incomplete immunity model consists of twelve differential equations (Equations 13-24).

Fitness Relationships Between Dengue 2 Strains

I used the next generation method to analytically calculate the net reproductive ratio (R_0) for each of the three models (Heesterbeek 2000 and Heffernan et al. 2005); Equations 25-27 describe R_0 for each model. To better understand how individual parameters affect R_0, I graphed how R_0 changes when I vary each parameter separately, within each param-

eters range of uncertainty (Figure 3). For each of the models, R_0 increases linearly with the increase of transmission rate (T_i) and vector bite rate (r), increases non-linearly with the increase of vector population size (N_V), and increases non-linearly with the decrease of vector birth/death rate (μ_V), human population size (N_H), vector latency period (τ_i or ${\sigma_{Vi}}^{-1}$) and human recovery rate (γ_{Hi}).

To understand the competition between the two strains, the individual values of R_0 are less important than their ratio, because in the complete immunity models this value determines whether one strain can outcompete the other. The ratio of the R_0s of the strains (R_{0i}/R_{0e}) for each model is represented in Equations 31-33. Most of the human and mosquito demographic parameters are eliminated, and only human and vector birth/death rate (μ_H and μ_V) and the strain-specific parameters (transmission rate, rate of transition from vector exposed to infected class, latency period, and recovery rate) remain. Realistic changes in the human birth/death rate do not significantly change the ratio of R_0 in any of the models because human demographic processes occur over time scales three orders of magnitude larger than the infection process ($\mu_H << \gamma_H, \sigma_H$). However, each of the other parameters could play a significant role in the competition between the two strains.

To explore potential evolutionary trade-offs for a decreased latency period/increased rate of movement into the infectious class, I plot the appropriate invasion equation (Equation 34 for CI and DCI models and Equation 35 for DII model) against vector birth/death rate, ratios of transmission rate, and ratios of human recovery rate assuming that the vector latency period is 14 days in the endemic strain and 7 days in the Asian/American strain (Figure 4). Here, when equation 34 is true (or $\log(R_{0i}/R_{0e}) > 0$) the Asian/American strain will be able to invade in the complete immunity models. For the complete immu-

nity model, the human recovery rate for the Asian/American strain must be at least $\sim$25% higher and the transmission rate must be at least $\sim$20% lower than the endemic strain to decrease the fitness of the invading strain enough to prevent invasion (Figure 4a,d). For the delayed complete immunity model, the human recovery rate for the Asian/American strain must be at least $\sim$65% higher and the transmission rate must be at least $\sim$25% lower than the endemic strain to decrease its fitness enough to prevent invasion (Figure 4b,e). When equation 35 is true (or $\log(\frac{R_{0i}}{R_{0e}}(1+\alpha(R_{0e}-1))) > 0$), the Asian/American strain can infiltrate with the delayed incomplete immunity model. With the delayed incomplete immunity model, the human recovery rate for the Asian/American strain must be at least twice that of the endemic strain and the transmission rate must be at least $\sim$35% lower than that of the endemic strain to decrease its fitness enough to prevent invasion (Figure 4c,f). In the complete immunity models, decreasing mosquito birth/death rate decreases R_{0i}/R_{0e}, however, no realistic values of mosquito birth/death rate are able to bring the R_{0i}/R_{0e} low enough to prevent invasion (Figure 4g-h). The mosquito birth/death rate decreases the ability for the Asian/American strain to invade until it reaches $\sim$0.045 days^{-1} (for the combination of parameter values used in Figure 4), and after that value the ability to invade increases (Figure 4i). As in the complete immunity models, realistic values of the vector birth/death rate are not sufficient to prevent invasion.

I then looked at the effect of mosquito birth/death rate on the two potential evolutionary trade-offs: human recovery rate and virus transmission rate. For the complete immunity model, the vector birth/death rate has little effect on changing either the human recovery rate or the transmission rate (Figure 5a,d). However, decreasing vector birth/death rate in the delayed complete immunity model serves to decrease the necessary decrease in the

transmission rate ratio (as low as 10% instead of 25%) or increase in human recovery rate ratio (as low as 22% instead of 65%) between the two strains to prevent invasion (Figure 5b,e). For the incomplete immunity model, changing vector birth/death rate from the average value generally increases the necessary difference in human recovery rate ratio or transmission rate ratio to prevent invasion (Figure 5c,f).

Figure 6 shows how adjusting both the relative transmission rates and the relative human recovery rates affects the ability of the Asian/American strain to invade for each model, assuming that the invading strain has a vector latency period that is half the length of the endemic strain. These three strain-specific parameters can interact to determine whether or not the Asian/American strain can infiltrate a population.

In summary, the results from exploring R_0 show that of the three potential evolutionary trade-offs for a decreased vector latency period in each of the models (increased human latency period, decreased transmission rate, and increased human recovery rate), transmission rate is the most important, followed by human recovery rate. The human latency period does not significantly change the fitness of a strain because the human lifespan is so much greater than the time-scale of an epidemic. Also, one ecological parameter proves to be important in predicting which strain is more competitive: vector birth/death rate. Decreasing the vector birth/death rate decreases the importance of differences in the vector latency period in the delayed complete immunity model. These results suggest that under some evolutionary and ecological circumstances, the Asian/American strain of Dengue may be able to invade some regions of the Americas but not others.

Modeling Invasion Timing

To better understand the temporal dynamics of the two competing strains, I numerically solve the models given initial conditions and specific values for each of the parameters. I analytically calculate the disease-free equilibrium value for one strain to get initial conditions for the endemic strain (Equations 28-30). I then observe how each parameter affects the timing of invasion by the Asian/American strain, assuming that the Asian/American strain is more competitive than the endemic American strain, allowing a small proportion of the initial population to be infected with the invading strain, and calculating the time it takes for the invading strain to become more prevalent in the human population than the endemic strain. Most of the parameters only changed the timing of invasion by less than 100 days, which is relatively insignificant with a time range of invasion of around 25 years (Figure 7). For each of the models, none of the ecological parameters were able to delay virus takeover for more than a year when the only difference between the two strains is that the Asian/American strain has a 7-day vector latency period and the American strain has a 14-day vector latency period.

The strain-specific parameters, however, did impact the timing of invasion. As the difference between the vector latency periods decreases, the timing of invasion significantly increases in each of the models, but is most important in the complete immunity models (Figure 8a-c). Decreasing the transmission rate in the invading strain delayed the invasion for up to almost five years in the delayed DCI model, up to four years in the CI model, and up to two years in the DII model (Figure 8d-f). Increasing the human recovery rate in the invading strain delays invasion for up to 6 years in the CI model, up to 5 years in the DCI

model, and up to nearly 2 years in the DII model (Figure 8g-i).

Generally, these results show that parameter values that decrease the difference in fitness (R_0) between the two strains increase the time it takes for the Asian/American strain to enter the population. Parameters that have little effect on the R_0 ratio tend to have little effect on epidemic timing. Also, changing strain-specific parameters generally delayed the invasion the longest in the complete immunity model, then the delayed complete immunity model, and never more than two years in the delayed incomplete immunity model.

Historical Mapping of Dengue 2 in Tropical America and the Caribbean

Methods

To complement the mathematical modeling portion of my thesis, I map the presence of the American strain and Asian/American strain throughout tropical America and the Caribbean over time. I use phylogenetic data obtained from the literature (Lewis et. al 1993; Halstead et. al 2001; Montoya et. al 2003; Carington et. al 2005; Cologna et. al 2005; Bennett et. al 2006; Vasilakis et. al 2007; Diaz et. al 2008; Vasilakis et. al 2008; Oliveira et. al 2010) as well as disease reports from the Pan American Health Organization (PAHO) (PAHO 2009), to create a timeline for each country in the region with available data (number of countries, number of years). The timeline includes strain-specific presence-absence data in each country on a yearly basis, as well as incidence data for all major dengue 2 epidemics in the region. Since the available data from the literature and PAHO is scarce, I used the

dengue virus variation database from NCBI (Resch et al. 2009) to collect all the dengue 2 sequences that are dated and include a country of origin in the NCBI nucleotide database. The sequences available included some complete genomes (N=284) as well as portions of the envelope gene (N=244), capsid gene (N=29), and non-structural gene 1 (NS-1, N=130). I group the complete genomes with each of the genes, and align the three groups using Multiple Alignment using Fast Fourier Transform (MAFFT, Katoh et al. 2002). I then use PHYML to build my trees (Guindon and Gascuel 2003). I use the trees that I built to identify each DNA sequence as Asian, Asian/American, or American strain. I add this information to the timeline for each country, and also use the phylogenetic tree to determine whether the Asian strain entered the region multiple times, or if it entered only once in 1981, and then spread and evolved throughout the region. I visualize the timelines on a map of the region to better illustrate the timing of infiltration of the Asian/American strain in the region.

In addition to information on the history of dengue-2 in the region, I collected other available data for each country including the incidence of epidemics of other dengue serotypes in the region, human population size, and human birth and death rates. These data were collected from publications from PAHO (PAHO 2010). I plot timing of identification of invasion in each country by the Asian/American strain against human population size and human birth and death rates to explore potential relationships between the timing and these parameters. I compare these data to the results of the three models. Finally, I compare the trends in the historical data I collected with the results of the models to determine which model best describes the dynamics of the Asian/American and American strain of dengue 2 in the region.

Results

Table 1 summarizes the history of the evidence for the American strain and Asian/American strain for each country and Figure 1 summarizes the invasion of the Asian/American strain of dengue 2 in the region spatially. The endemic strain of dengue 2 was first identified in the region in 1954 in Trinidad and Tobago, and was last identified in 2000 in Piura, Peru. The Asian strain was first identified in 1981 in Cuba, and has spread throughout the region. The map shows high variability from country to country, but generally the Asian or Asian/American strain first came into the Caribbean in the early 1980s, then spread to tropical South America in the mid 1980s, and finally came to Central America in the late 1990s. A few countries in the region lack any clear evidence of either strains, including some small island countries in the Caribbean and Panama and Belize. Two countries had evidence of the endemic dengue 2 strain, but not the Asian or Asian/American strain: Guatemala and Honduras.

I compare the results of the models with historical data to help determine if the predictions of the model fit the data and to help determine which of the models is most accurate. Unfortunately, historical data are scarce. The Asian/American strain was first detected in the population of different countries in the Caribbean and South and Central America between 1981 and 2005, indicating that there is up to a 25-year delay in some countries (Figure 1, Table 1). In my extensive search to find evidence of each strain of dengue 2, I was unable to find any example of the Asian/American strain being detected in one country, followed by the detection of the American strain. Figure 9 shows the invasion timing data plotted against human birth rate, human death rate, and human population size of each

country. There are no significant trends with human death rate or human population size, but linear regression of the human birth rate data shows a linear increase in date of detection of Asian/American dengue 2 virus with increased birth rate ($R^2 = 0.33$). In addition, I plotted the year of invasion against gross domestic product (GDP), GDP growth rate, and percentage of population in urban areas, none of which showed any trends or correlations (figures not shown).

Discussion

The replacement of endemic American strains of dengue with more virulent strains of Asian origin has caused an important public health problem in the region (Gubler 1995). Though this strain replacement has been occurring for almost 30 years, incidence report data at a strain-specific level are not present in the literature, and as a result there is currently no clear understanding of whether or not the Asian/American strain of dengue 2 has completely taken over the American strain in the region, and when replacement occurred in different parts of the region. Distinguishing between the two strains requires sequencing of the virus RNA and comparison with a phylogeny of the two strains. Many phylogenetic studies have taken dengue 2 virus samples from throughout the region and used them to better understand the evolution of the various strains (Lewis et. al 1993; Halstead et. al 2001; Montoya et. al 2003; Carington et. al 2005; Cologna et. al 2005; Bennett et. al 2006; Vasilakis et. al 2007; Diaz et. al 2008; Vasilakis et. al 2008; Oliveira et. al 2010), and many of the samples have specific dates and countries of origin associated with them; however, there is no summary of these data in the literature. Because of this lack of synthesis,

scientists have a difficult time figuring out which strain is where. For instance, one study stated that the Asian/American strain had not yet entered the population of Peru as of 2006, although three years prior a phylogenetic study showed that the Asian/American virus entered the Peruvian population in 2001 (Montoya et al. 2003; Anderson and Rico-Hesse 2006). I have compiled all of the strain-specific data I could find in the literature that includes a collection date and associated country, and I present a map that summarizes the earliest date the Asian/American strain can be identified in each country of the region (Figure 1).

The data I collected are patchy, likely because studies that involve collecting and sequencing viral RNA can be time consuming and costly. Many small countries in the Caribbean as well as some larger countries in Central America (Panama and Belize) have no sequence data for dengue 2 at all, while other countries have many data points. Another potential problem with the data is that the viral sequences are most often collected from people who show clinical symptoms of the disease, and since the Asian/American strain is more virulent than the American strain, I expect bias toward that strain.

Despite the inconsistent quality of the invasion data, there are some conclusions that can be drawn from the historical map. The last published sequence of American strain dengue 2 was collected in Peru in 2000 (Montoya et al. 2003), indicating that if clade replacement has occurred throughout the region, it took at least 19 years since first being introduced to Cuba in 1981. Also, the timing of invasion for each country is highly variable, implying that either ecological parameters play a role in invasion timing or there is a spatial or stochastic element to the system. There are two countries that have evidence of the American strain of dengue 2 but no evidence for the Asian/American strain (Guatemala and Honduras);

however, it is uncertain whether this is because the Asian/American strain has not invaded and replaced the American strain or if it has and there are gaps in the data available.

When I compare the phylogenetic invasion data with demographic data for each country, the number of years it takes for the Asian/American strain to be detected in a country correlates with human birth rate (Figure 9a), as hypothesized. The results of the models do not show a significant delay in invasion timing due to birth rate, and it is likely that human birth rate does not directly change the timing of invasion. Higher birth rates in a human population are generally correlated with an increase in poverty (McClean and Anderson 1988). The wealth of a nation may affect a number of parameters, including vector population size and vector birth/death rate, which can significantly alter timing of invasion, according to my models (Figure 8). In addition, more wealthy countries may have a higher DNA sequence sampling rate, resulting in earlier detection of invasion.

I implemented three different models to better understand the competition between the invading Asian/American strain of dengue 2 and the endemic American strain of dengue 2 in the Caribbean and tropical America. The models help me to understand possible evolutionary trade-offs that exist in the system as well as causes for differences in timing of invasion and the ability for the Asian/American strain to take over in different regions. Each model produced similar results with a few key differences.

For each of the models, differences in vector transmission rate, human recovery rate, and vector latency period between the two strains were most important in determining if an invading strain will be able to infiltrate the endemic population (Figure 4). In the delayed complete immunity model and incomplete immunity models, the vector birth/death rate significantly affected the ability for the Asian/American strain to invade the popula-

tion, but no ecological parameters had a significant effect in the complete immunity model (Figure 5). Specifically, in the delayed complete immunity model, as vector birth/death rate decreases, the fitness gain that results from decreasing the latency period in the vector becomes less important and the fitness gains resulting from increasing transmission rate and decreasing the recovery rate become more important. Here, the effect of the vector birth/death rate can be attributed to the death rate of the vector because the birth/death rate parameter that remains in the equation represents the proportion of mosquitoes leaving the exposed class due to death (Equation 32). In the delayed incomplete immunity model, invasion is dependent on the vector birth/death rate due to births and deaths, which act against each other. In this model, when vector birth/death rate is at intermediate values, the fitness gains from a decreased vector latency period become less important, and they become more important at extreme values of vector birth/death rate.

After searching the literature, I came across only one research group studying the differences of these strain-specific parameters between the Asian, Asian/American, and American strain of dengue 2. This research group, led by Rebeca Rico-Hesse, performed a series of studies comparing the ability of the two strains to infect and disseminate in both *Aedes* aegypti and human blood cells (Armstrong and Rico-Hesse 2001; Armstrong and Rico-Hesse 2003; Cologna et al. 2005; Anderson and Rico-Hesse 2006). In *Aedes aegypti*, they showed that the latency period in the mosquito was approximately 7 days for the Asian/American strain and 14 days for the American strain when mosquitoes were infected with blood containing a controlled concentration of virus (Anderson and Rico-Hesse 2006). This was the only direct measurement of one of the strain-specific parameters in the models in any of the studies. However, in one study, human blood cells infected with each

strain showed a significantly higher percentage of human cells that were successfully infected by the American virus than the Asian/American virus12% and 8% in the American strain and Asian/American strain, respectively (Cologna et al. 2005). These differences in the number of human blood cells infected could potentially indicate differences in vector to human transmission rate. None of the studies observed differences in human recovery rate, because the human cell studies were done *in vitro*.

The invasion of the Asian strain into different areas of tropical America occurred over a 25 year period with a high degree of spatial variability in timing (Figure 1). For all of the models, the only parameters that significantly impacted timingdelayed invasion for more than one yearwere the parameters that affect the ability of the Asian/American strain to invade (Figures 7-8). This indicates that parameters that change the comparative fitness between the two strains are most important, including transmission rate, human recovery rate and latency period in all of the models, and vector birth/death rate in the delayed models. For the complete immunity models, if the ratio of R_0s for the two strains is very close to 1 this results in delays of up to six years, yet changes in the ability of the Asian/American strain to invade in the incomplete immunity model result in delays of less than two years. This is because a strain that does not confer complete immunity to another strain is competing less with the other strain. No ecological parameters can significantly change timing in the complete immunity model; therefore, the only model that can account for long delays in invasion timing under different ecological parameters is the delayed complete immunity model, assuming that the spatial spread of the disease was rapid.

The delayed complete immunity model appears to best fit the situation in tropical America and the Caribbean. Empirical studies have shown that the vector latency period is rela-

tively invariable in constant environments due to the time it takes for the virus to replicate in the gut and then migrate to and replicate in the salivary glands (Schule 1928; Armstrong and Rico-Hesse 2001). The results of the complete immunity model are sufficiently different than the results of the delayed complete immunity model to warrant using the DCI model. Specifically, the ability and timing of invasion of the Asian/American strain of dengue 2 in the CI model does not significantly depend on any non-strain-specific data, unlike the DCI model. Also, the historical data suggests that non-strain-specific data are important in invasion timing due to the high variability in timing of invasion throughout the region, though it is possible that this is due to chance. The DII model does not fit the data as well as the DCI model because in the mapping portion of the project, there is no evidence of the persistence of both strains either simultaneously or in an oscillatory pattern. In addition, no strain-specific or ecological parameters could delay invasion for more than two years.

The delayed complete immunity model suggests that an increased vector death rate while in the exposed class increases the fitness gain resulting from the decrease in the vector latency period. In tropical America and the Caribbean, the more pathogenic Asian/American strain of dengue 2 has a shorter vector latency period than the less pathogenic American strain. This likely expands to all of the more pathogenic strains of dengue virus because studies show increased human pathogenicity is correlated with higher viremia in humans (Cologna et al. 2005), and the extrinsic incubation period in *Aedes aegypti* mosquitoes that were given larger concentrations of dengue virus in blood meals were shorter (Watts et al. 1987). The literature suggests that decreasing vector numbers by reducing vector habitat, and therefore decreasing birth rate may be more effective in controlling vector-borne dis-

eases than spraying adult mosquitoes with pesticide and thus increasing death rate (Gubler 1989; Newton and Reiter 1992). In addition, my delayed complete immunity model suggests that decreasing vector number by killing adult mosquitoes results in shortening the vector lifespan, selecting for more virulent virus strains with shorter latency periods. This indicates that killing vector populations with pesticides may actually enhance the spread of more dangerous strains of dengue virus.

My models have a few key limitations that should be studied further. Since none of my models are stochastic, they do not account for probabilistic delays in invasion timing. Also, the models do not include seasonal forcing or cycling of epidemics of different dengue serotypes. The dengue 2 population is not necessarily in the endemic state that I assumed in my model. *Aedes* vector populations are highly dependent on climate conditions including temperature and humidity (Hopp and Foley 2003; Halstead 2008), which vary throughout each year. In addition, the *Aedes* population was eradicated from most of South and Central America by the 1960sbut not the Caribbean or Venezuelaand reintroduced in the1970s (Soper 1963; Gubler 1989). When a population becomes infected with one serotype of dengue, recovered individuals gain temporary immunity from all dengue serotypes (Sabin 1952), resulting in cyclical dynamics of native dengue strains. It is possible that variations in timing of invasion are due to the actual proportion of individuals infected with the endemic strain in different populations being higher or lower than expected at equilibrium.

Here I showed that incorporating a delay in vector latency period can significantly change the results of dengue competition models. The delayed complete and incomplete immunity models can both be used to better understand dengue competition dynamics

throughout the world. Though the delayed incomplete immunity model appears to be inaccurate at describing the problem that I focus on in this study, it could be used to look at the competitive dynamics between different serotypes of dengue as well as other *flaviviridae* viruses. For instance, genotype replacement in West Nile virus has also been associated with differences in vector latency period (Moudy et al. 2007). In addition, the delayed complete immunity model may be used to model dynamics between multiple strains of other dengue serotypes in the Americas region or dynamics between strains of dengue in other regions. A similar situation of dengue 2 clade replacement has occurred in India (Kumar et al. 2010), and further work could use that data to continue to test my model.

Equations

Complete Immunity Model (CIM)

$$\frac{dS_H}{dt} = \mu_H - S_H \left[\left(\frac{N_V}{N_H} \right) \frac{(rT_1)\, 2\frac{\sigma_{V1}}{\sigma_{V1}+\mu_V} I_{H1} + (rT_2)\, 2\frac{\sigma_{V2}}{\sigma_{V2}+\mu_V} I_{H2}}{rT_1 I_{H1} + rT_2 I_{H2} + \mu_V} + \mu_H \right] \tag{1}$$

$$\frac{dE_{H1}}{dt} = S_H I_{H1} \left(\frac{N_V}{N_H} \right) \frac{(rT_1)\, 2\frac{\sigma_{V1}}{\sigma_{V1}+\mu_V}}{rT_1 I_{H1} + rT_2 I_{H2} + \mu_V} - (\sigma_{H1} + \mu_H) E_{H1} \tag{2}$$

$$\frac{dE_{H2}}{dt} = S_H I_{H2} \left(\frac{N_V}{N_H} \right) \frac{(rT_1)\, 2\frac{\sigma_{V2}}{\sigma_{V2}+\mu_V}}{rT_1 I_{H1} + rT_2 I_{H2} + \mu_V} - (\sigma_{H2} + \mu_H) E_{H2} \tag{3}$$

$$\frac{dI_{H1}}{dt} = \sigma_{H1} E_{H1} - (\gamma_{H1} + \mu_H) I_{H1} \tag{4}$$

$$\frac{dI_{H2}}{dt} = \sigma_{H2} E_{H2} - (\gamma_{H2} + \mu_H) I_{H2} \tag{5}$$

$$\frac{dR_H}{dt} = \gamma_{H1} I_{H1} + \gamma_{H2} I_{H2} - \mu_H R_H \tag{6}$$

Delayed Complete Immunity Model (DCIM)

$$\frac{dS_H}{dt} = \mu_H - S_H \left[\left(\frac{N_V}{N_H} \right) \frac{(rT_1)\, 2I_{H1}(t-\tau_1)\, e^{-\mu_V \tau_1} + (rT_2)\, 2I_{H2}(t-\tau_2)\, e^{-\mu_V \tau_2}}{rT_1 I_{H1} + rT_2 I_{H2} + \mu_V} + \mu_H \right] \tag{7}$$

$$\frac{dE_{H1}}{dt} = S_H \left(\frac{N_V}{N_H} \right) \frac{(rT_1)\, 2I_{H1}(t-\tau_1)\, e^{-\mu_V \tau_1}}{rT_1 I_{H1} + rT_2 I_{H2} + \mu_V} - (\sigma_{H1} + \mu_H) E_{H1} \tag{8}$$

$$\frac{dE_{H2}}{dt} = S_H \left(\frac{N_V}{N_H} \right) \frac{(rT_2)\, 2I_{H2}(t-\tau_2)\, e^{-\mu_V \tau_2}}{rT_1 I_{H1} + rT_2 I_{H2} + \mu_V} - (\sigma_{H2} + \mu_H) E_{H2} \tag{9}$$

$$\frac{dI_{H1}}{dt} = \sigma_{H1} E_{H1} - (\gamma_{H1} + \mu_H) I_{H1} \tag{10}$$

$$\frac{dI_{H2}}{dt} = \sigma_{H2} E_{H2} - (\gamma_{H2} + \mu_H) I_{H2} \tag{11}$$

$$\frac{dR_H}{dt} = \gamma_{H1} I_{H1} + \gamma_{H2} I_{H2} - \mu_H R_H \tag{12}$$

Delayed Incomplete Immunity Model (DIIM)

$$\begin{aligned}
\frac{dSS}{dt} &= \mu_H - SS\left(\frac{N_V}{N_H}\right)\frac{(rT_1)2e^{-\mu_V\tau_1}\left(IS(t-\tau_1)+IR(t-\tau_1)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V} \\
&\quad +SS\frac{(rT_2)2e^{-\mu_V\tau_2}\left(SI(t-\tau_2)+RI(t-\tau_2)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}+SS\mu_H \qquad (13)\\
\frac{dES}{dt} &= SS\left(\frac{N_V}{N_H}\right)\frac{(rT_1)2e^{-\mu_V\tau_1}\left(IS(t-\tau_1)+IR(t-\tau_1)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}-(\mu_H+\sigma_{H1})ES \qquad (14)\\
\frac{dSE}{dt} &= SS\left(\frac{N_V}{N_H}\right)\frac{(rT_2)2e^{-\mu_V\tau_2}\left(SI(t-\tau_2)+RI(t-\tau_2)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}-(\mu_H+\sigma_{H2})SE \qquad (15)\\
\frac{dIS}{dt} &= \sigma_{H1}ES-(\gamma_{H1}+\mu_H)IS \qquad (16)\\
\frac{dSI}{dt} &= \sigma_{H2}SE-(\gamma_{H2}+\mu_H)SI \qquad (17)\\
\frac{dRS}{dt} &= \gamma_{H1}IS-RS\left[\left(\frac{N_V}{N_H}\right)\frac{\alpha_2(rT_2)2e^{-\mu_V\tau_2}\left(SI(t-\tau_2)+RI(t-\tau_2)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}+\mu_H\right] \qquad (18)\\
\frac{dSR}{dt} &= \gamma_{H2}SI-SR\left[\left(\frac{N_V}{N_H}\right)\frac{\alpha_1(rT_1)2e^{-\mu_V\tau_1}\left(IS(t-\tau_1)+IR(t-\tau_1)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}+\mu_H\right] \qquad (19)\\
\frac{dRE}{dt} &= RS\left(\frac{N_V}{N_H}\right)\frac{\alpha_2(rT_2)2e^{-\mu_V\tau_2}\left(SI(t-\tau_2)+RI(t-\tau_2)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}-(\sigma_{H2}+\mu_H)RE \qquad (20)\\
\frac{dER}{dt} &= RS\left(\frac{N_V}{N_H}\right)\frac{\alpha_1(rT_1)2e^{-\mu_V\tau_1}\left(IS(t-\tau_1)+IR(t-\tau_1)\right)}{rT_1\left(IS+IR\right)+rT_2\left(SI+RI\right)+\mu_V}-(\sigma_{H1}+\mu_H)ER \qquad (21)\\
\frac{dRI}{dt} &= \sigma_{H2}RE-(\gamma_{H2}+\mu_H)RI \qquad (22)\\
\frac{dIR}{dt} &= \sigma_{H1}ER-(\gamma_{H1}+\mu_H)IR \qquad (23)\\
\frac{dRR}{dt} &= \gamma_{H1}IR+\gamma_{H2}RI-\mu_hRR \qquad (24)
\end{aligned}$$

R_0 for Strain i

$$\begin{aligned}
R_{0i(CIM)} &= \sqrt{\frac{N_V\nu_V\nu_H(rT_i)2\frac{\sigma_{Vi}}{\sigma_{Vi}+\mu_V}\sigma_{Hi}}{\mu_V^2\mu_HN_H(\sigma_{Hi}+\mu_H)(\gamma_{Hi}+\mu_H)}} \qquad (25)\\
R_{0i(DCIM)} &= \sqrt{\frac{N_V\nu_V\nu_H(rT_i)^2e^{-\mu_V\tau_i}\sigma_{Hi}}{\mu_V^2\mu_HN_H(\sigma_{Hi}+\mu_H)(\gamma_{Hi}+\mu_H)}} \qquad (26)\\
R_{0i(DIIM)} &= \sqrt{\frac{N_V\nu_V\nu_H(rT_i)^2e^{-\mu_V\tau_i}\sigma_{Hi}}{\mu_V^2\mu_HN_H(\sigma_{Hi}+\mu_H)(\gamma_{Hi}+\mu_H)}} \qquad (27)
\end{aligned}$$

Equilibrium Proportion of Population in Infected Class

$$I_{H1(CIM)} = \frac{\mu_h \sigma_{H1}(rT_1)2\frac{\sigma_{V1}}{\sigma_{V1}+\mu_V}N_V - \mu_H\mu_V N_H(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)}{(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)rT_1\left(N_V rT_1\frac{\sigma_{V1}}{\sigma_{V1}+\mu_V}+\mu_H N_H\right)} \quad (28)$$

$$I_{H1(DCIM)} = \frac{\mu_h \sigma_{H1}(rT_1)2e^{-\mu_V\tau_1}N_V - \mu_H\mu_V N_H(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)}{(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)rT_1\left(N_V rT_1 e^{-\mu_V\tau_1}+\mu_H N_H\right)} \quad (29)$$

$$I_{H1(DIIM)} = \frac{\mu_h \sigma_{H1}(rT_1)2e^{-\mu_V\tau_1}N_V - \mu_H\mu_V N_H(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)}{(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)rT_1\left(N_V rT_1 e^{-\mu_V\tau_1}+\mu_H N_H\right)} \quad (30)$$

Ratio of R_0 for the Invading strain (i) and the Endemic strain (e)

$$\frac{R_{0i}}{R_{0e\,(CIM)}} = \sqrt{\frac{T_2^2\sigma_{V2}(\sigma_{V2}+\mu_V)\sigma_{H2}(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)}{T_1^2\sigma_{V1}(\sigma_{V1}+\mu_V)\sigma_{H1}(\sigma_{H2}+\mu_H)(\gamma_{H2}+\mu_H)}} \quad (31)$$

$$\frac{R_{0i}}{R_{0e\,(DCIM)}} = \sqrt{\frac{T_2^2 e^{-\mu_V\tau_2}\sigma_{H2}(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)}{T_1^2 e^{-\mu_V\tau_2}\sigma_{H1}(\sigma_{H2}+\mu_H)(\gamma_{H2}+\mu_H)}} \quad (32)$$

$$\frac{R_{0i}}{R_{0e\,(DIIM)}} = \sqrt{\frac{T_2^2 e^{-\mu_V\tau_2}\sigma_{H2}(\sigma_{H1}+\mu_H)(\gamma_{H1}+\mu_H)}{T_1^2 e^{-\mu_V\tau_2}\sigma_{H1}(\sigma_{H2}+\mu_H)(\gamma_{H2}+\mu_H)}} \quad (33)$$

Conditions Necessary for Successful Invasion into a Population

$$\frac{R_{0i}}{R_{0e}} > 1 \quad (34)$$

$$\frac{R_{0i}}{R_{0e}}(1+\alpha(R_{0e}-1)) > 1 \quad (35)$$

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Appendix: Tables and Figures

Country	1st American	Last American	1st Asian/Am.	Last Asian/American
Aruba	-	-	1996	1996
Bahamas	-	-	1989	1989
Barbados	-	-	1987	1998
Bolivia	-	-	1997	1997
Brazil	-	-	1990	2008
Colombia	1971	1986	1992	2005
Costa Rica	1994	1994	2000	2000
Cuba	-	-	1981	1997
Dominica	-	-	1995	1995
Dominican Republic	-	-	1984	2001
Ecuador	-	-	2000	2000
El Salvador	-	-	1999	1999
French Guiana	1970	1970	2005	2005
Guatemala	1979	1979	-	-
Guyana	-	-	2000	2000
Honduras	1991	1991	-	-
Jamaica	1969	1969	1981	1984
Martinique	-	-	1992	1998
Mexico	1981	1995	2000	2006
Nicaragua	-	-	1999	2008
Paraguay	-	-	2001	2005
Peru	1995	2000	2001	2001
Puerto Rico	1969	1977	1986	2007
Saint Lucia	-	-	1999	1999
St. Vincent and the Grenadines	-	-	1998	1998
Suriname	-	-	1986	1999
Trinidad and Tobago	1953	1978	1986	2000
Venezuela	1987	1987	1990	2007

Table 1: The first and last known presence of the American and Asian/American strain of dengue 2, as determined by literature study and phylogenetic analysis.

Parameter	Symbol	Value	Value Range
Vector population size	N_V	50,000	10-1,000 (x1000)
Human population size	N_H	100,000	10-1,000 (x1000)
Vector birth/death rate	μ_V	1/14 day^{-1}	1/5 – 1/30 $days^{-1}$
Human birth/death rate	μ_H	1/60 yr^{-1}	1/50 – 1/70 yr^{-1}
Vector bite rate	r	0.5 day^{-1}	0.33 - 0.5 day^{-1}
Transmission probability	β	0.45	0.33 - 1
Human rate of transition from the exposed to infected class	σ_H	1/5 $days^{-1}$	1/4 – 1/7 $days^{-1}$
Vector rate of transition from the exposed to infected class	σ_V	-	1/20 – 1/5 $days^{-1}$
American strain	-	1/14 $days^{-1}$	-
Asian/American strain	-	1/7 $days^{-1}$	-
Vector latency period	τ	-	5-20 days
American strain	-	14 days	-
Asian/American strain	-	7 days	-
Human recovery rate	γ_H	1/6 $days^{-1}$	1/4 – 1/12 $days^{-1}$
Degree of incomplete immunity	α	0.1	0 – 0.5

Table 2: The parameters used in the models are described and given values, which were used in all figures unless otherwise noted. The value range represents the range used when each parameter was varied.

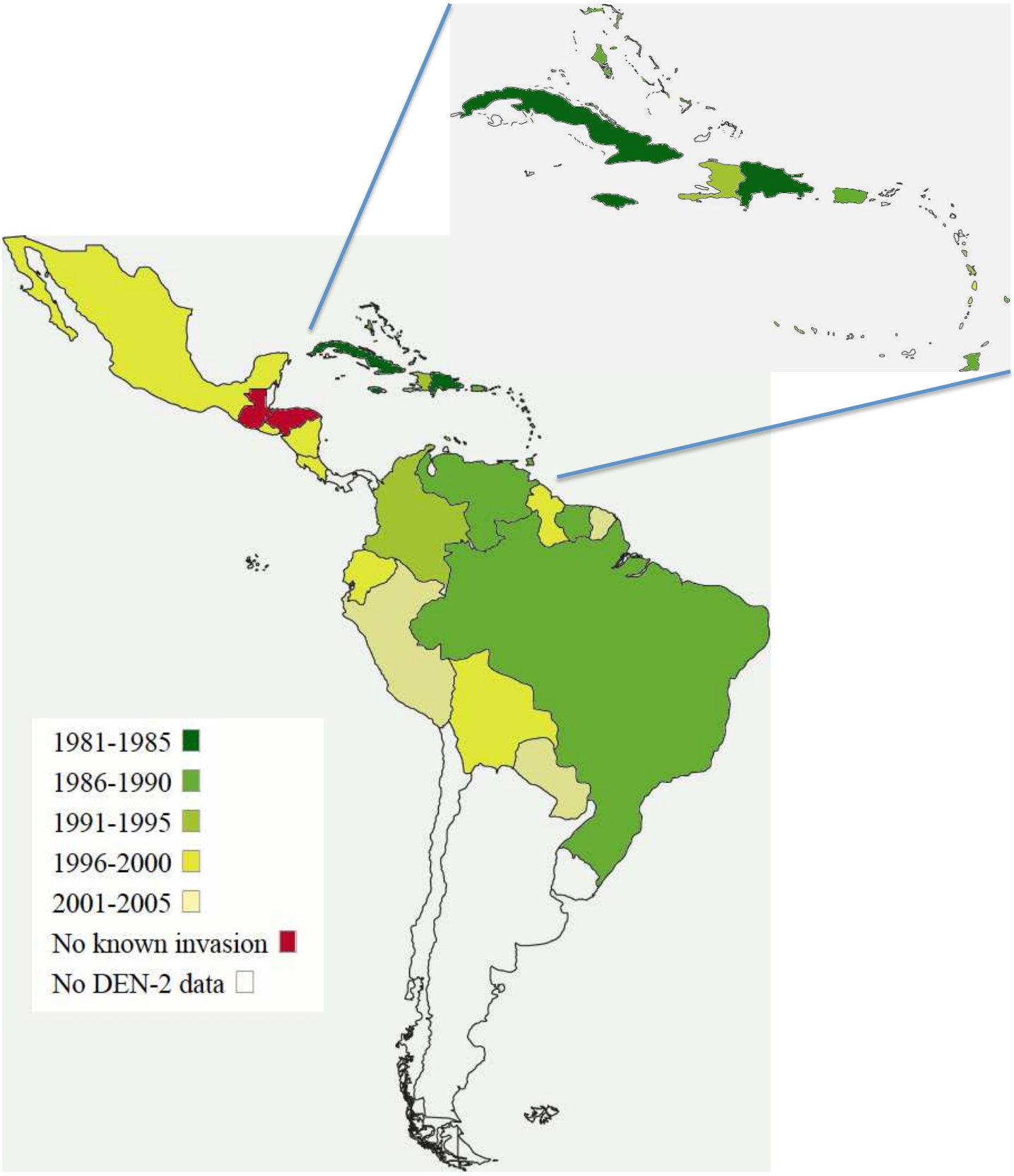


Figure 1: Map of Asian/American strain invasion into American Population, with a close-up of the Caribbean islands. Dates correspond with the first identification of Asian/American strain in each country. White countries have no DNA evidence of dengue 2, and red countries have DNA evidence of American strain dengue 2 but no evidence of Asian/American strain

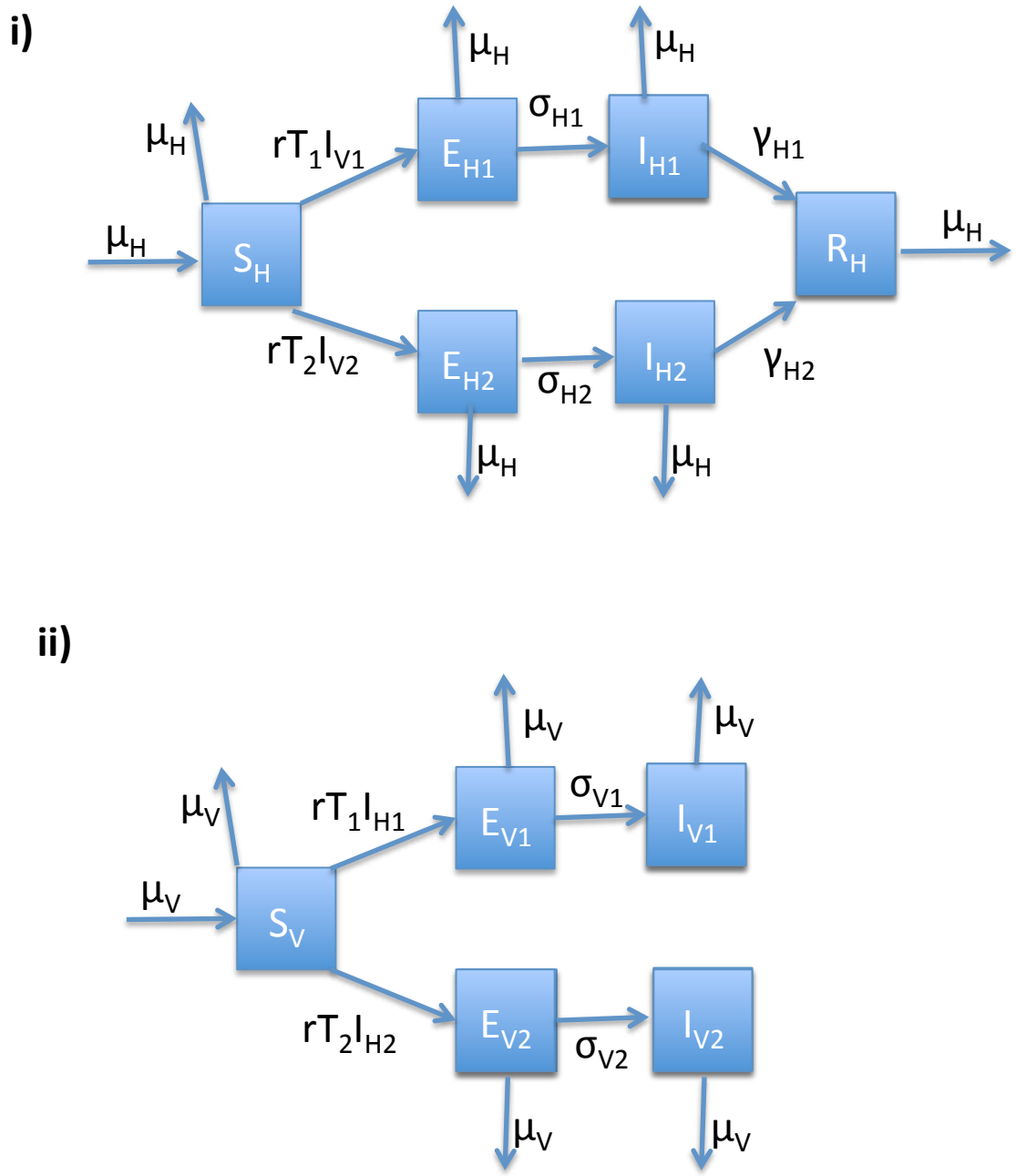


Figure 2a: Box diagram for complete immunity (CI) model where i) represents human (H) dynamics and ii) Represents vector (V) dynamics for strains 1 and 2. S,E,I, and R represent the proportion of the population who are susceptible, exposed, infected, and recovered from the virus. Proportions of the population leave and enter each class according to different rates: birth/death rate (μ), mosquito bite rate (r), transmission rate (T), rate of transition from exposed to infected class (σ), and recovery rate (γ).

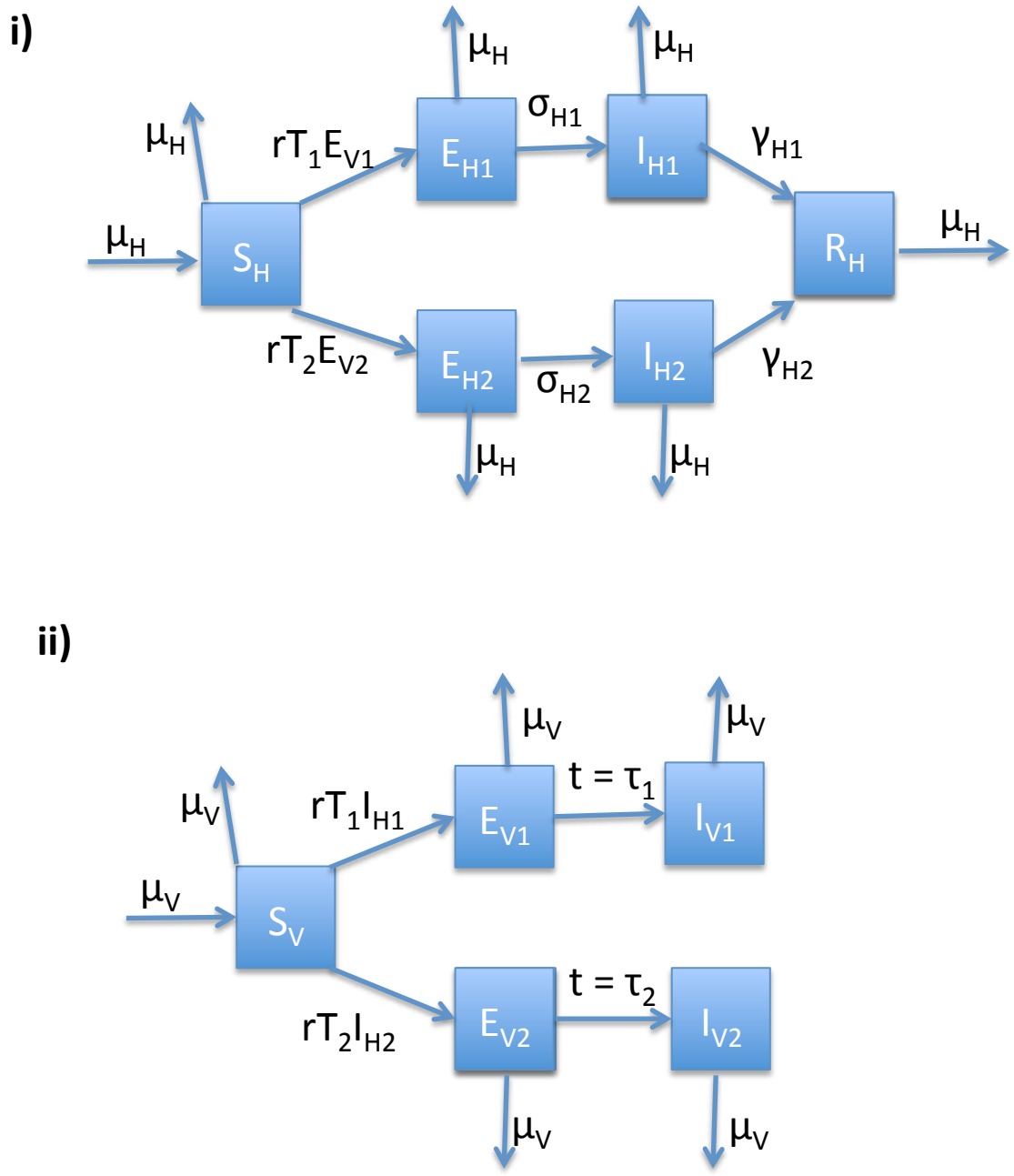


Figure 2b: Box diagram for delayed complete immunity (DCI) model where i) represents human (H) dynamics and ii) Represents vector (V) dynamics for strains 1 and 2. S,E,I, and R represent the proportion of the population who are susceptible, exposed, infected, and recovered from the virus. Proportions of the population leave and enter each class according to different rates: birth/death rate (μ), mosquito bite rate (r), transmission rate (T), rate of transition from exposed to infected class (σ), latency period (τ), and recovery rate (γ).

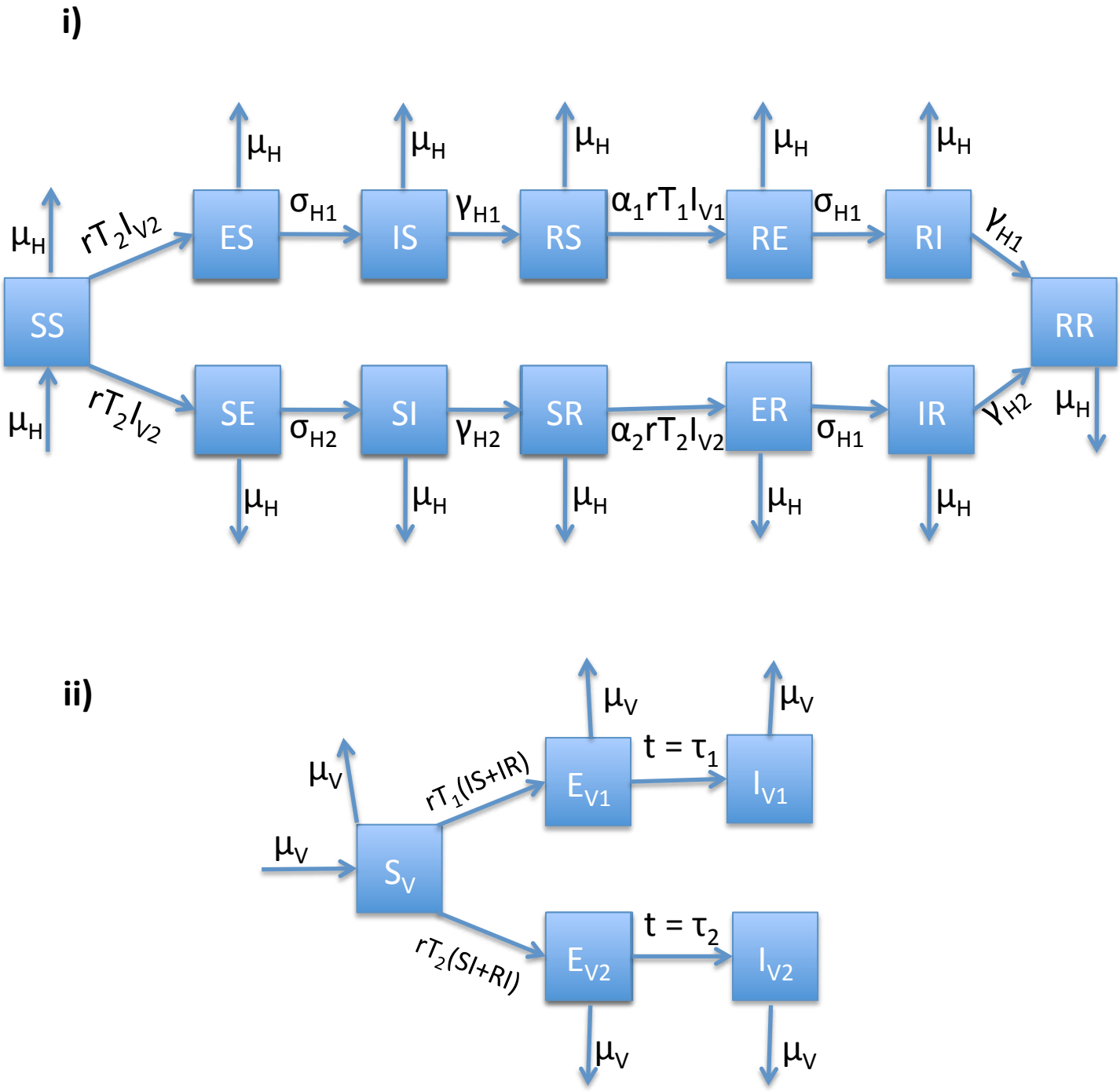


Figure 2b: Box diagram for delayed complete immunity (DCI) model where i) represents human (H) dynamics and ii) Represents vector (V) dynamics for strains 1 and 2. S,E,I, and R represent the proportion of the population who are susceptible, exposed, infected, and recovered from the virus. Proportions of the population leave and enter each class according to different rates: birth/death rate (μ), mosquito bite rate (r), transmission rate (T), rate of transition from exposed to infected class (σ), latency period (τ), degree of incomplete immunity (α) and recovery rate (γ).

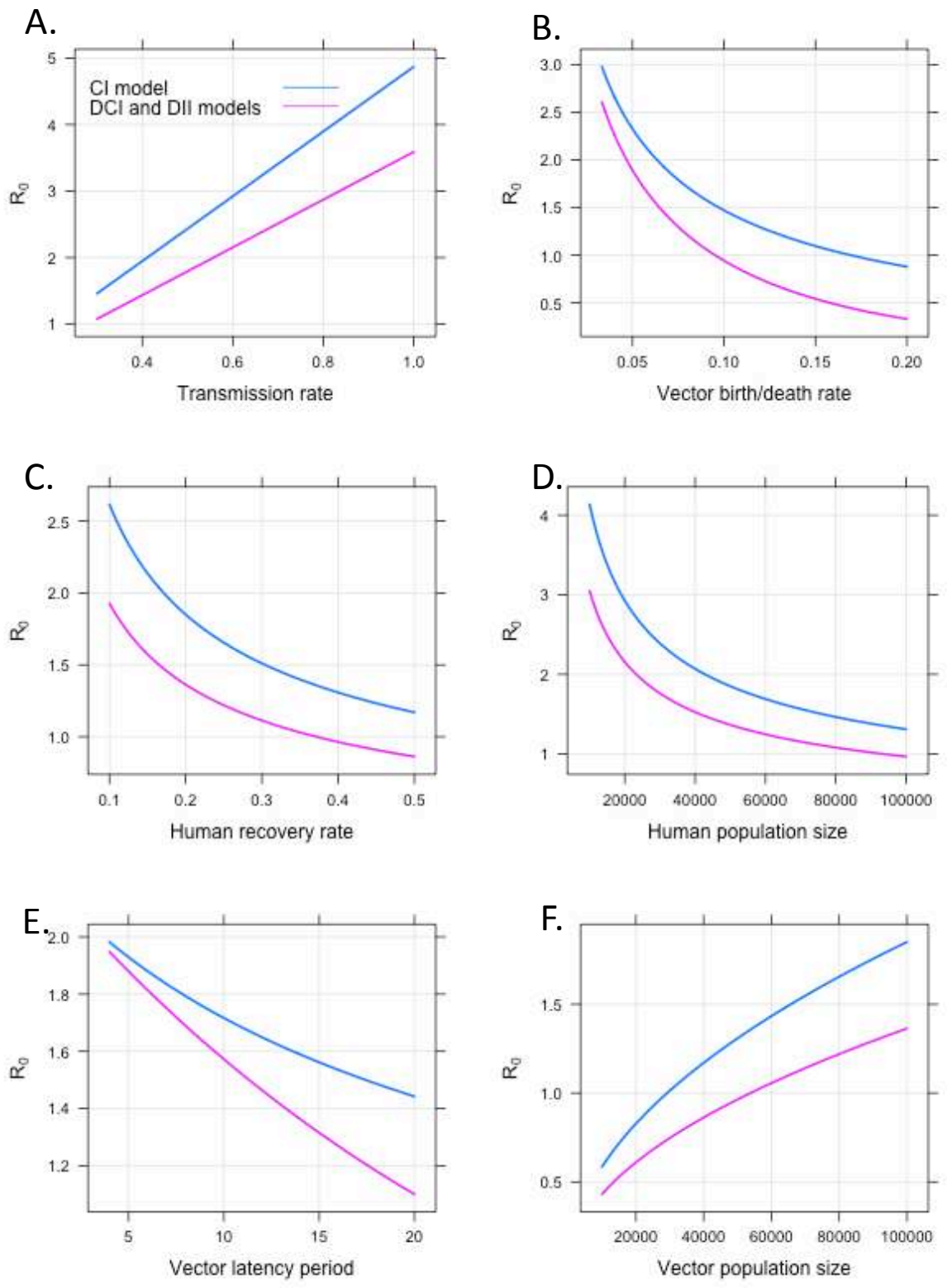
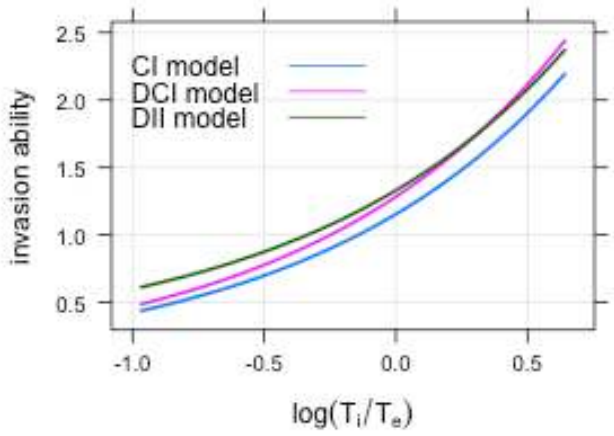
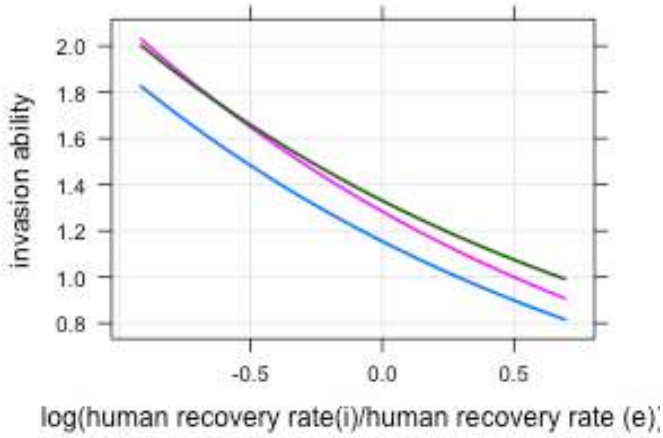


Figure 3: Change in R_0 with change in model parameters: transmission rate (a), vector birth/ death rate (b), human recovery rate (c), human population size (d), vector latency period (e), and vector population size (f).





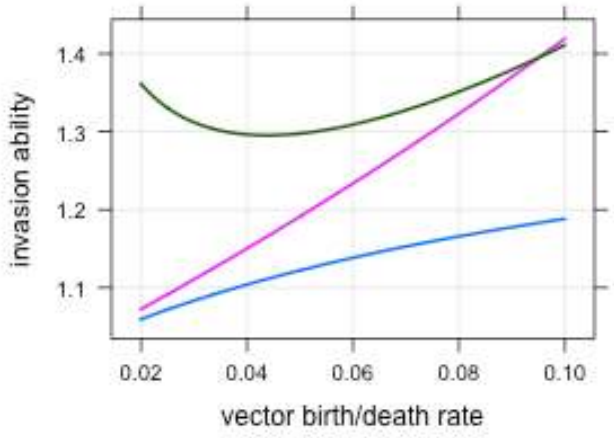


Figure 4: Ability for Asian/American strain to invade with change in parameters: ratio of transmission rates (a), ratio of human recovery rates (b), and mosquito birth/death rate (c). Ability to invade calculated with equation 34 for the CI and DCI models and equation 35 for the DII model, where the Asian/American strain can invade the endemic population if invasion ability > 1.

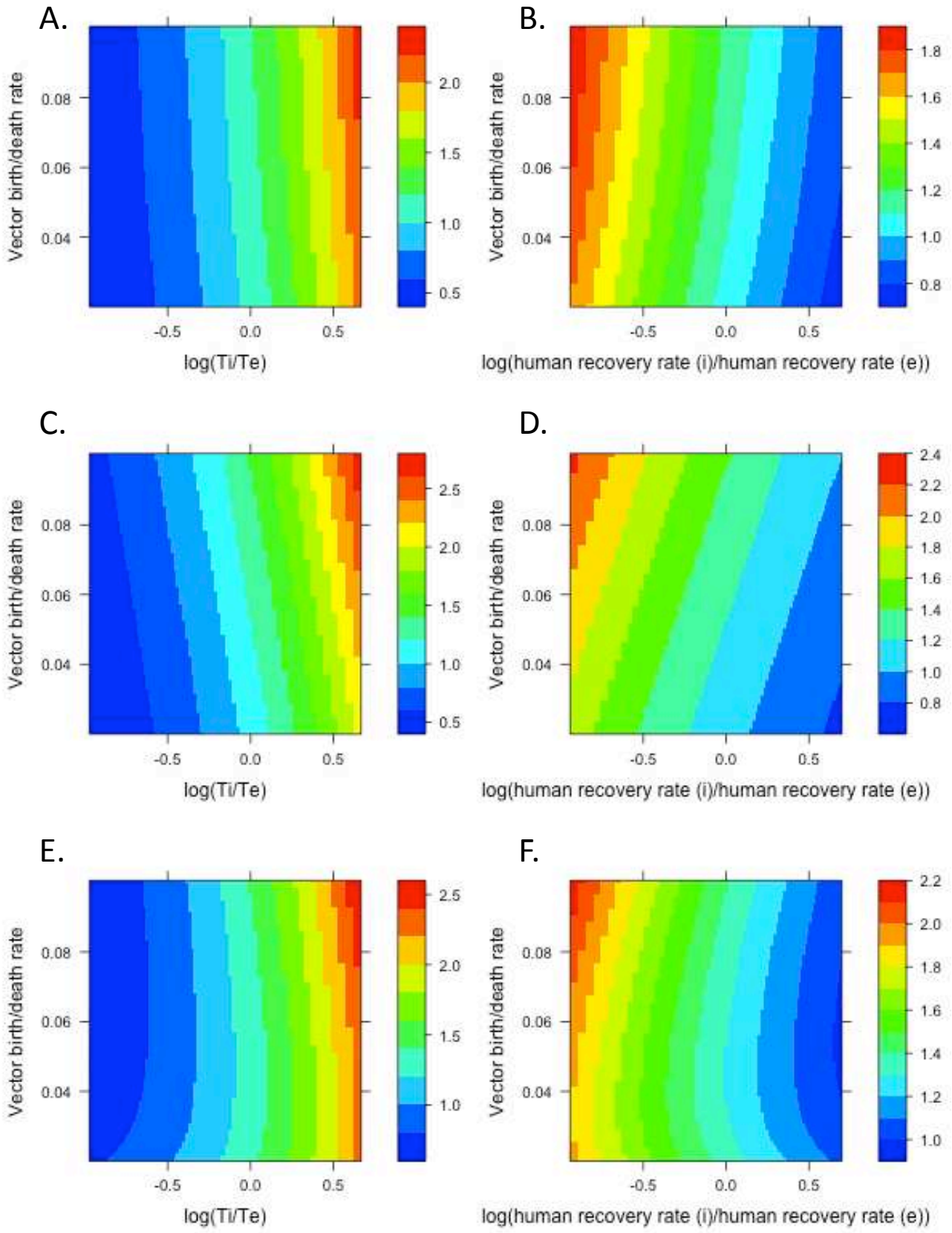


Figure 5: Ability for Asian/American strain to invade when ratio of transmission rates (a,c,e) and ratio of human recovery rates (b,d,f) vary with and mosquito birth/death rate. Ability to invade calculated with equation 34 for the CI (a,b) and DCI (c,d) models and equation 35 for the DII model (e,f), where the Asian/American strain can invade the endemic population if invasion ability > 1.

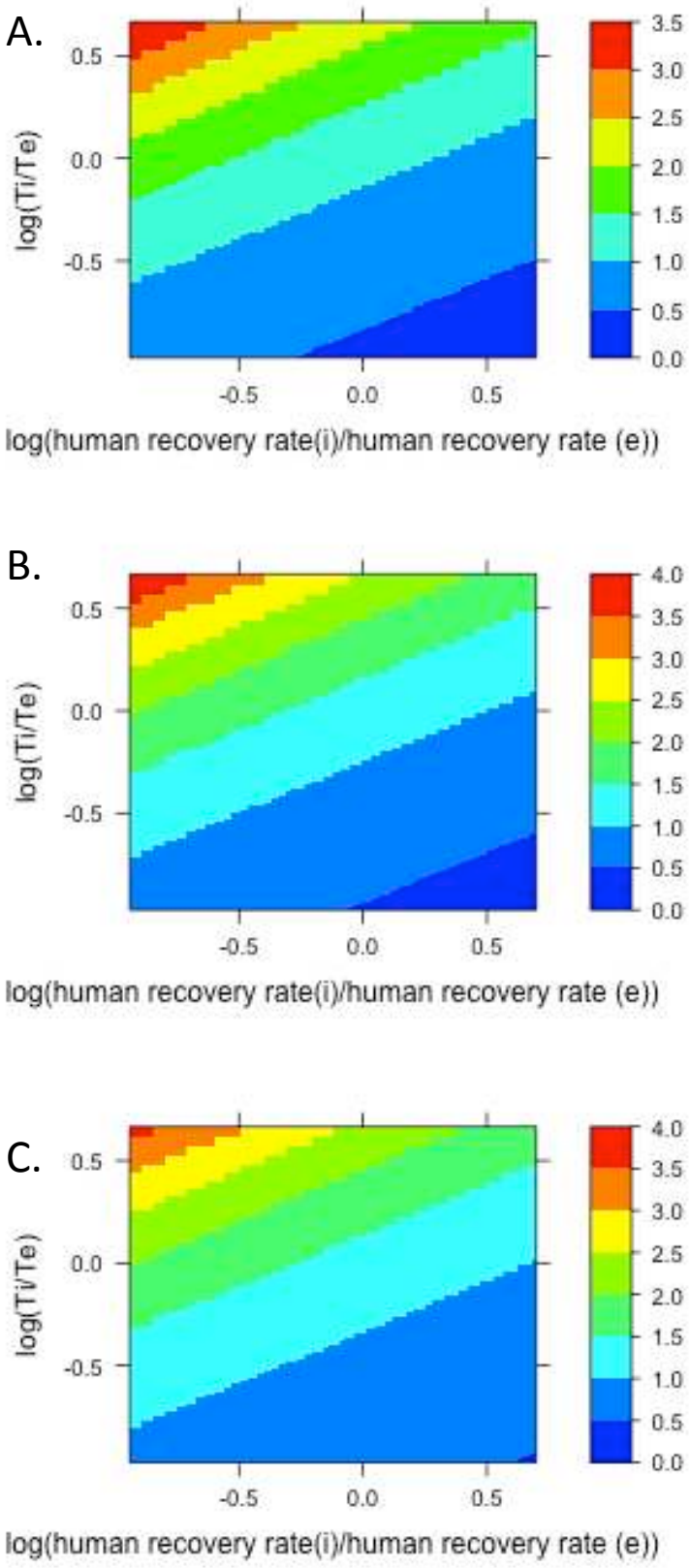


Figure 6: Ability for Asian/American strain to invade when ratio of transmission rates varies with ratio of human recovery rates. Ability to invade calculated with equation 34 for the CI (a) and DCI (b) models and equation 35 for the DII model (c), where the Asian/American strain can invade the endemic population if invasion ability > 1.

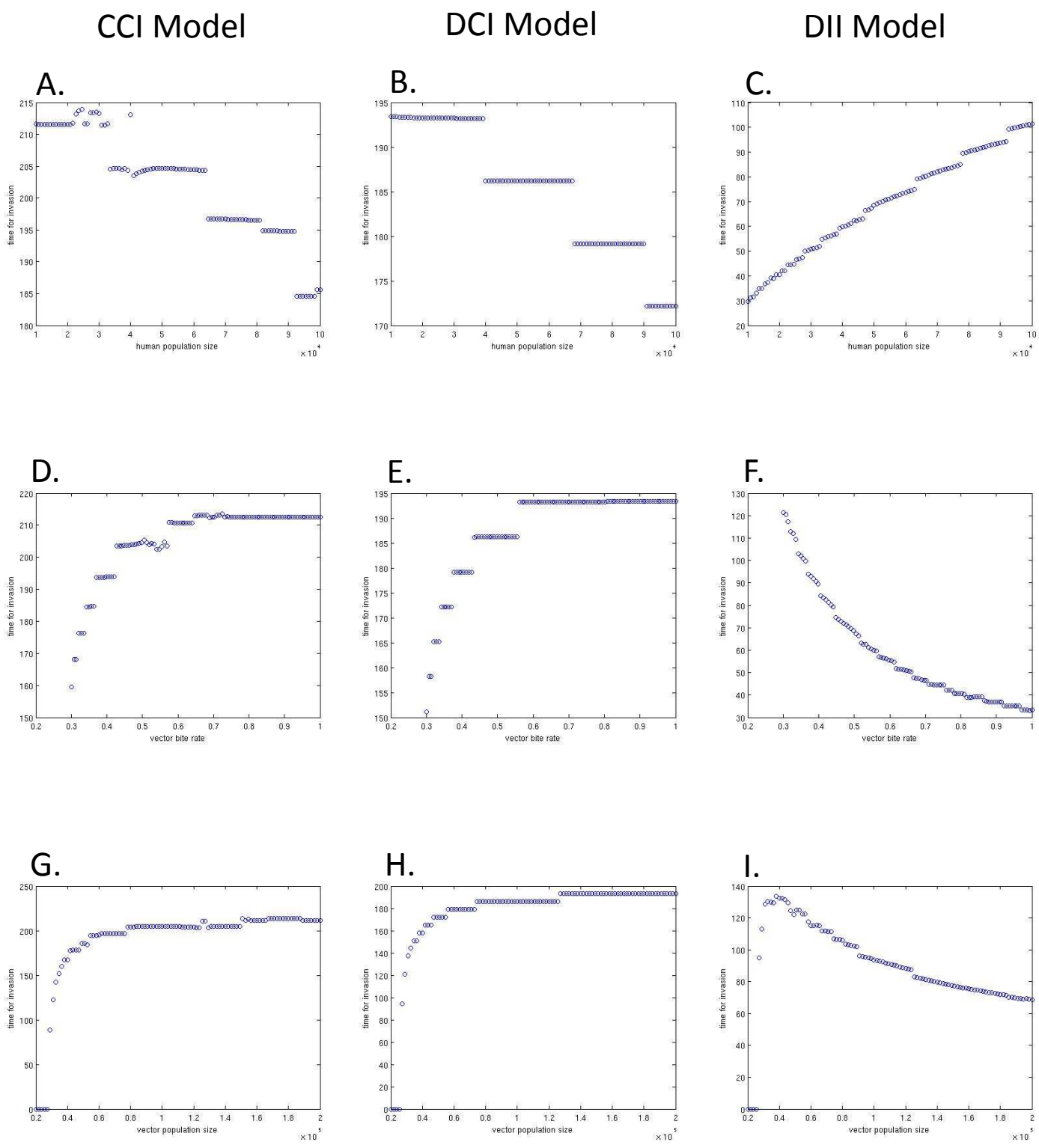


Figure 7a-i: Timing of invasion when ecological parameters vary: human population size (a-c), vector bite rate (d-f), and vector population size (g-i). The plots do not appear smooth because of numerical artifacts and step sizes in the numerical solvers.

CCI Model DCI Model DII Model

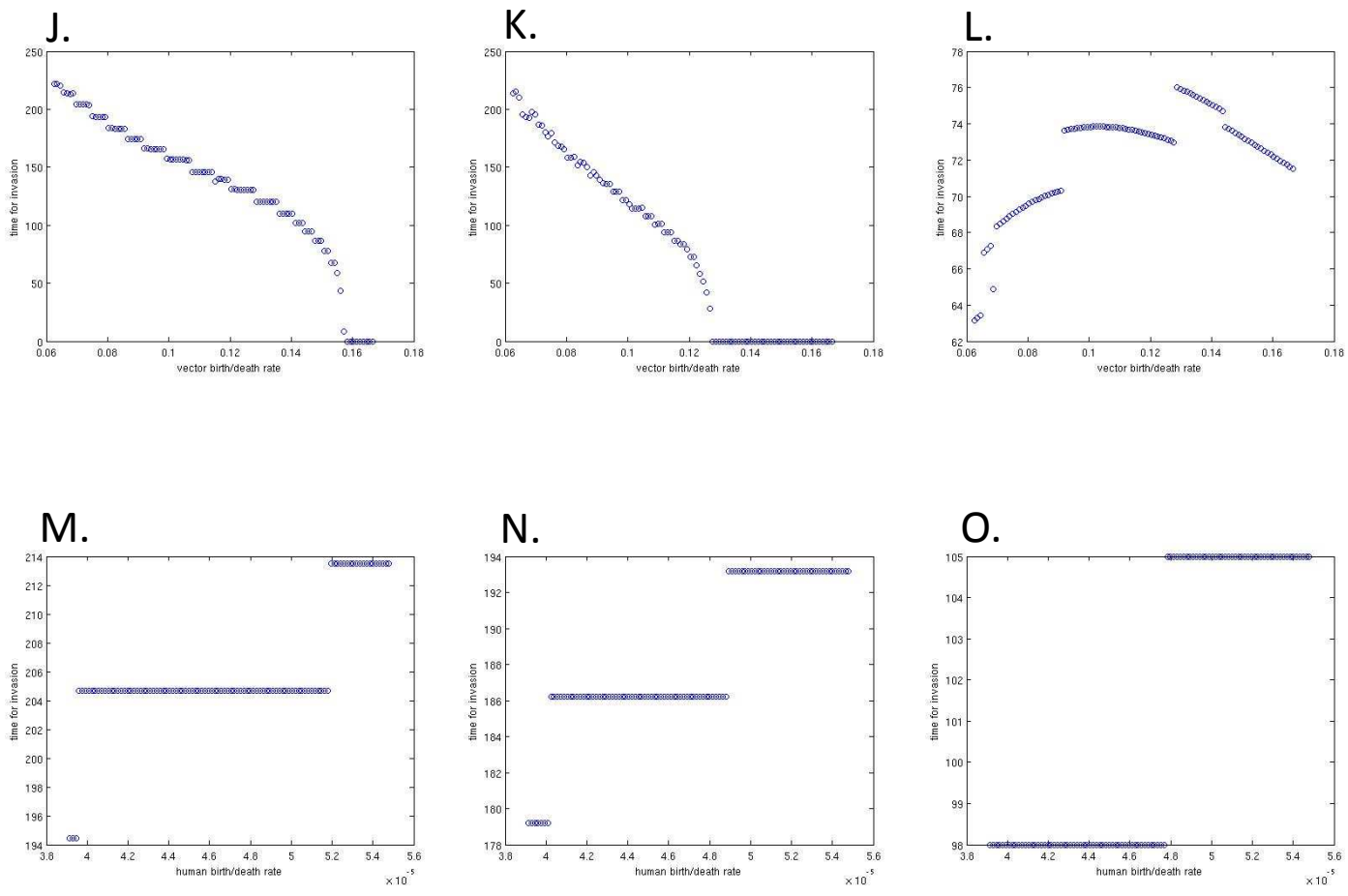


Figure 7j-o: Timing of invasion when ecological parameters vary: vector birth/death rate (j-l), human birth/death rate (m-o). The plots do not appear smooth because of numerical artifacts and step sizes in the numerical solvers.

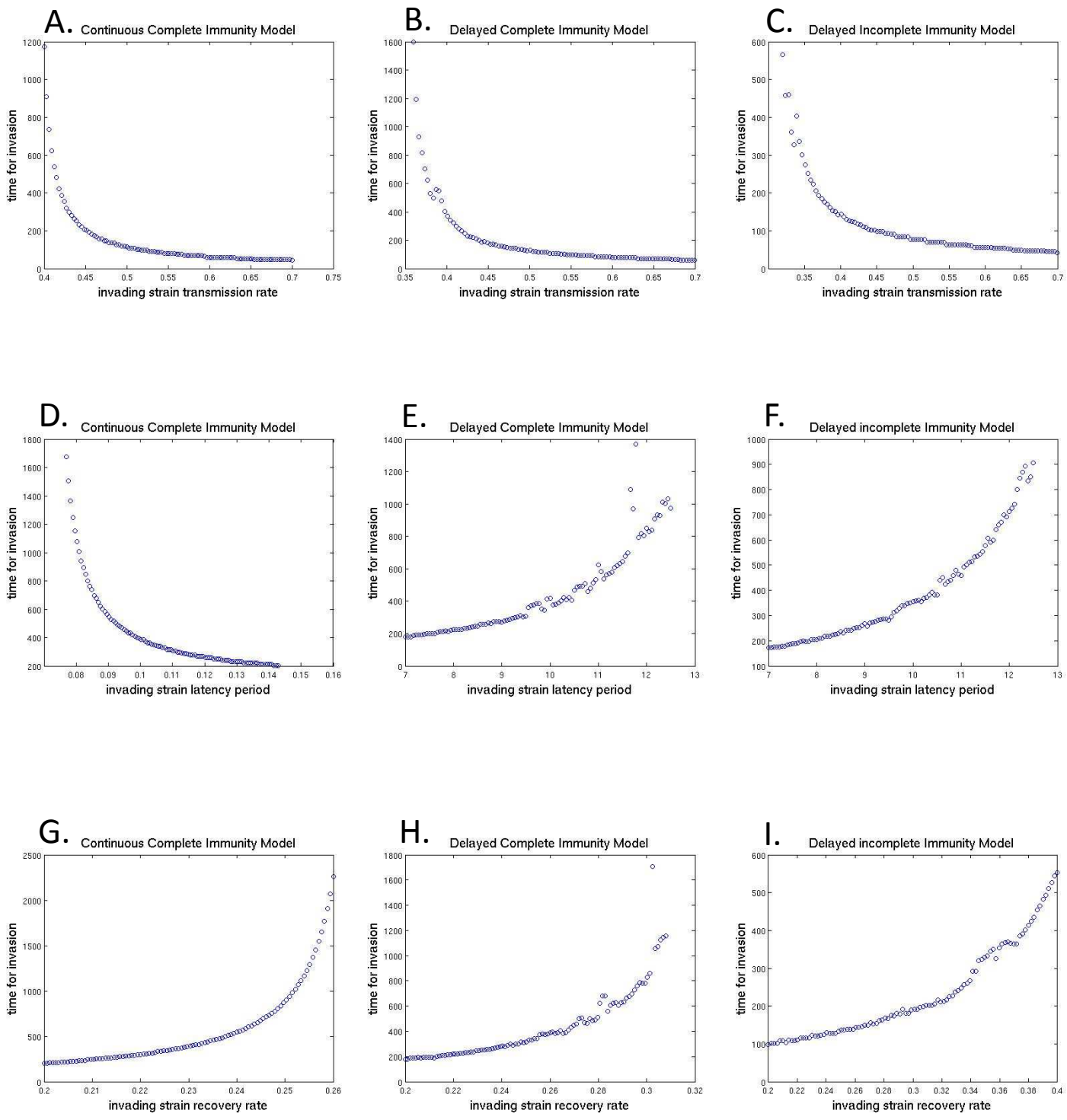
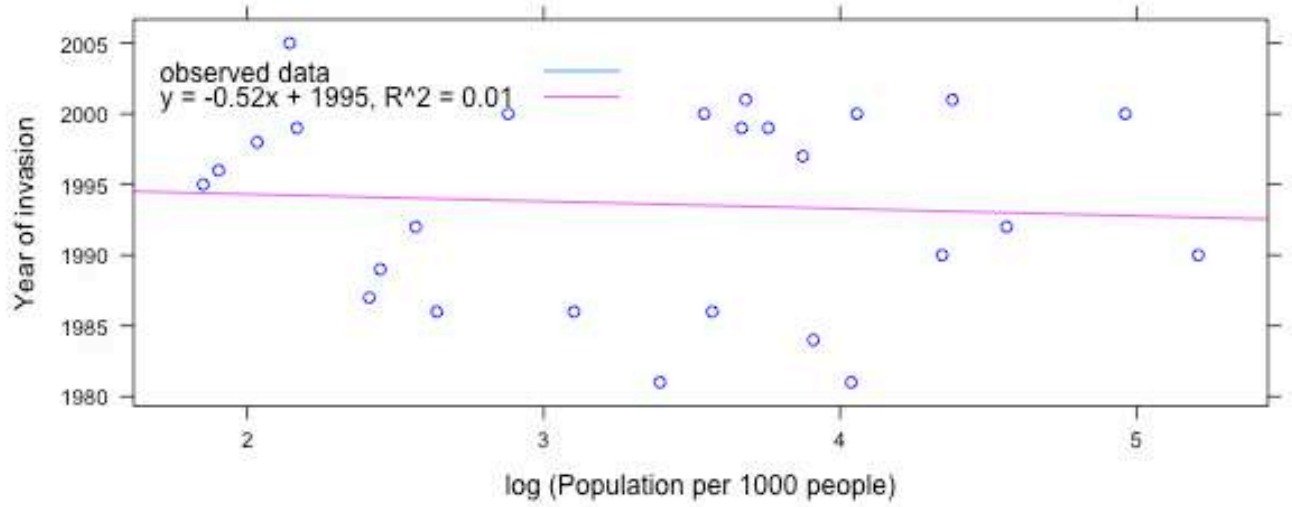
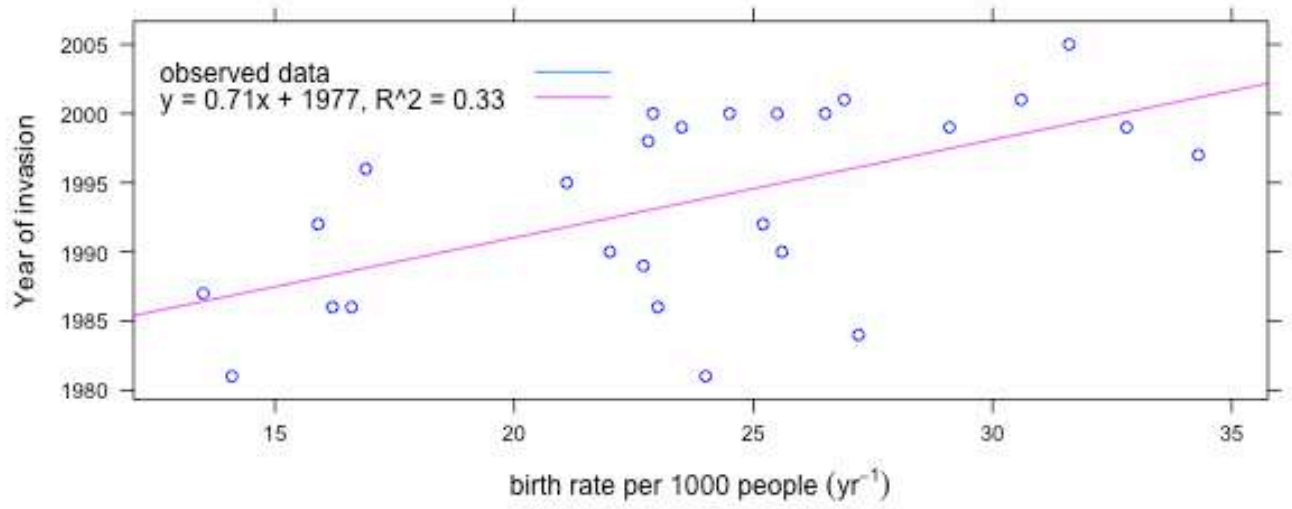


Figure 8: Timing of invasion when strain-specific parameters vary: transmission rate (a-c), latency period (d-f), and human recovery rate (g-i). The plots do not appear smooth because of numerical artifacts and step sizes in the numerical solvers.

A.



B.



C.

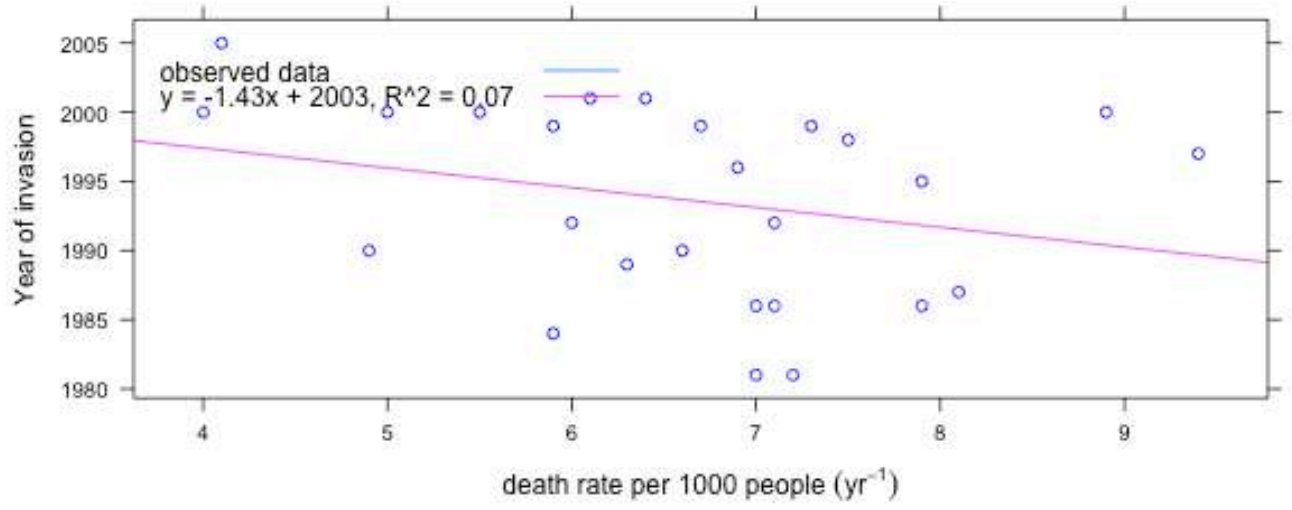


Figure 9: Timing of invasion collected from phylogenies correlated with human demographic data: population size (a), birth rate (b), and death rate (c).