Viral and Epidemiological Determinants of the Invasion Dynamics of Novel Dengue Genotypes

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Abstract

Background: Dengue has become a major concern for international public health. Frequent epidemic outbreaks are believed to be driven by a complex interplay of immunological interactions between its four co-circulating serotypes and large fluctuations in mosquito densities. Viral lineage replacement events, caused for example by different levels of cross-protection or differences in viral fitness, have also been linked to a temporary change in dengue epidemiology. A major replacement event was recently described for South-East Asia where the Asian-1 genotype of dengue serotype 2 replaced the resident Asian/American type. Although this was proposed to be due to increased viral fitness in terms of enhanced human-to-mosquito transmission, no major change in dengue epidemiology could be observed.

Methods/Results: Here we investigate the invasion dynamics of a novel, advantageous dengue genotype within a model system and determine the factors influencing the success and rate of fixation as well as their epidemiological consequences. We find that while viral fitness overall correlates with invasion success and competitive exclusion of the resident genotype, the epidemiological landscape plays a more significant role for successful emergence. Novel genotypes can thus face high risks of stochastic extinction despite their fitness advantage if they get introduced during episodes of high dengue prevalence, especially with respect to that particular serotype.

Conclusion: The rarity of markers for positive selection has often been explained by strong purifying selection whereby the constraints imposed by dengue’s two-host cycle are expected to result in a high rate of deleterious mutations. Our results demonstrate that even highly beneficial mutants are under severe threat of extinction, which would suggest that apart from purifying selection, stochastic effects and genetic drift beyond seasonal bottlenecks are equally important in shaping dengue’s viral ecology and evolution.

Introduction

Dengue virus (DENV) is the most wide-spread arbovirus affecting human populations. During the last decades it has increasingly become a major public health problem with significant economic and social impact [1–3]. It is transmitted between humans in urban and peri-urban settings predominantly by the *Aedes aegypti* and *Aedes albopictus* mosquitoes vector [4]. *Ae. aegypti* is extremely well adapted to urban environments where it efficiently breeds in artificial water containers, such as flower pots, plastic bags or discarded car tires, near human habitations. Both vectors have undergone rapid expansion worldwide in the last couple of decades leading to DENV endemicity in more than 100 countries [5].

There are four closely related and potentially co-circulating serotypes of DENV (DENV1-DENV4) [6,7] and recovery from infection is believed to provide life-long immunity to the infecting serotype but only a brief period of heterologous protection to all other serotypes [8]. Most primary infections are self-limited and clinically silent but can occasionally result in a short-lived febrile illness which is commonly known as dengue fever (DF). In some cases this may progress to more severe and life-threatening illness such as dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) [9]. While several risk factors for developing DHF/DSS have been described, including host genetic background, viral genotype, order of infecting serotype, time between infections or age of infection [1,9], the most widely cited explanation is that of Antibody Dependent Enhancement (ADE) (e.g. [10–13]) whereby subneutralizing antibodies from primary infection can mediate viral entry into host cells leading to increased replication and disease manifestations [14–18].

The temporal epidemiological pattern of dengue is characterized by semi-periodic outbreaks whilst the inter-epidemic cycles in DF/DHF incidence highly correlate with the seasonal variations in vector population size (see e.g. [19]). Furthermore, individual serotype prevalences show cyclical replacements in dominance (Figure 1A) which are believed to be induced by the immune profile of the human population [20,21].

Phylogenetic studies based on complete sequences of structural genes of all 4 serotypes have demonstrated the existence of
Dengue Genotype Replacement

Author Summary

Dengue fever and the more severe dengue haemorrhagic fever and dengue shock syndrome are mosquito borne viral infections that have seen a major increase in terms of global distribution and total case numbers over the last few decades. There are currently four antigenically distinct and potentially co-circulating dengue serotypes and each serotype shows substantial genetic diversity, organised into phylogenetically distinct genotypes or lineages. While there is some evidence for positive selection, the evolutionary dynamics of dengue virus (DENV) is supposed to be mostly dominated by purifying selection due to the constraints imposed by its two-host life-cycle. Motivated by a recent genotype replacement event whereby the resident American/Asian lineage of dengue virus serotype 2 (DENV2) had been displaced by the fitter Asian-1 lineage we investigated some of the epidemiological factors that might determine the success and invasion dynamics of a novel, advantageous dengue genotype. Our results show that although small differences in viral fitness can explain the rapid expansion and fixation of novel genotypes, their fate is ultimately determined by the epidemiological landscape in which they arise.

Methods

Description of the model

The model is an extension of the 4-serotype mathematical framework analysed by Recker et al. [34] and includes a mosquito vector component, temporary cross-immunity after primary infection and seasonal forcing in mosquito biting. In summary, we disregard the effect of maternal antibodies and instead assume that human individuals are born susceptible to all 4 serotypes. After recovery from primary infection they acquire life-long immunity to the infecting serotype and cross-immunity to any other serotype for a short period of time. As temporary immunity wanes, individuals become susceptible to secondary heterologous infection. For simplicity and because of the relative rarity of reported third and fourth infections we assume that after recovery from secondary infections individuals remain fully protected against further challenges [4,35]. The system can then be given by the following set of differential equations describing the rate of infection and seasonal forcing in mosquito biting. In summary, we show that invasion success and total time required for fixation are strongly influenced by inter- and intra-serotype competition at the time of introduction.

\[
\begin{align*}
\frac{dS}{dt} &= \mu N - \left( \sum_i \lambda_i^0 + \mu \right) S \\
\frac{dI_1}{dt} &= \lambda_1^0 S - (\sigma_1 + \mu) I_1 \\
\frac{dI_2}{dt} &= \sigma_1 I_1 - (\sigma_2 + \mu) I_2 \\
\frac{dR_2}{dt} &= \lambda_2^0 X_2 - \left( \sum_i \gamma_i^0 + \mu \right) R_2 \\
\frac{dI_3}{dt} &= \gamma_3^0 R_3 - (\sigma_3 + \mu) I_3 \\
\frac{dI_4}{dt} &= \gamma_4^0 R_4 - (\sigma_4 + \mu) I_4
\end{align*}
\]
\[
\frac{dR}{dt} = \sum_{j \neq i} \sigma_i I_j - \mu R
\]  

(6)

with the force of infection of serotype \( i \) affecting the human population, \( \lambda_i^r \), given as

\[
\lambda_i^r = \eta \beta_i^{v\rightarrow h} \frac{I_i}{N_v}
\]  

(7)

We denote \( \eta \) as the mosquito biting rate and \( \beta_i^{v\rightarrow h} \) as the vector-to-human transmission probability; \( 1/\sigma_i \) and \( 1/\alpha \) are the respective durations of infection and cross-immunity. Given the short period of infection we do not account for the possibility of co-infections by two or more serotypes. We assume a constant human population size \( N_h = S + \sum_i (I_i + X_i + R_i + \sum_j J_{ij}) + R \) and further assume that infection has a negligible effect on the average death rate, \( \mu \). To account for seasonal variation we assume a periodically forced biting rate, that is we set

\[
\eta = \eta_0 (1 + \epsilon \sin (\pi t^k) / k),
\]  

(8)

where \( k \) is a positive integer influencing the ‘seasonality’ where \( k > 1 \) results in shorter and more pronounced seasons.

The dynamics of the mosquito population is given as follows:

\[
\frac{dS}{dt} = \mu^v N^v - \left( \sum_i \beta_i^{h\rightarrow v} \right) S^v
\]  

(9)

\[
\frac{dI_i}{dt} = \lambda_i^v S^v - \mu^v I_i
\]  

(10)
with the force of infection from humans to mosquitoes given as

\[ \lambda_i^h = \eta \frac{p_i^{h+v}}{N^h} \left( I_i + \sum_j \phi_j I_j \right) \]  

(11)

In accordance with our previous model [34] we assume that antibody-dependent enhancement acts to increase both susceptibility to and transmissibility of secondary heterologous infection by factors \( \gamma \) and \( \phi \), respectively, with values described in Table 1.

To investigate the invasion patterns of a novel and fitter dengue genotype we assume that DENV2 is represented by two genotypes which differ in relative fitness but are antigenically equivalent. That is, individuals previously infected by DENV2 are immune to type \( 2 \) and vice versa. We consider four different fitness traits which we can vary independently: (i) transmissibility from human to mosquito, \( \beta_2^{h+v} \); (ii) transmissibility from human to mosquito, \( \beta_2^{h+v} \); (iii) longer life-expectancy of mosquitoes infected with DENV2' to emulate a shorter extrinsic incubation period (EIP), \( \mu_2^e \); (iii) longer infectious period in humans, \( 1/\sigma_2 \); and (iv) an increased level of enhancement of secondary infections, \( \phi_2 \). These can simply be given using:

\[ \beta_2^{h+v} = \beta_2^{h+v}(1 + \rho_p) \]  

(12)

| parameter | definition | value |
|-----------|------------|-------|
| \( \mu \) | host lifespan | 70 years |
| \( \tau \) | temporary heterologous immunity | 5 months |
| \( \sigma \) | infectious period | 3.65 days |
| \( \gamma \) | susceptibility enhancement | 1.33 |
| \( \phi \) | transmissibility enhancement | 1.66 |
| \( \rho_p \) | increase in probability of human-to-mosquito transmission | \( 0 \leq \rho_p \leq 1 \) |
| \( \rho_e \) | increase in infectious period | \( 0 \leq \rho_e \leq 1 \) |
| \( \rho_s \) | increase in enhancement of secondary infections | \( 0 \leq \rho_s \leq 1 \) |
| \( N^h \) | host population size | 9 million |
| \( N^v \) | vector population size | 22.5 million |
| \( \mu^v \) | vector lifespan | 16 days |
| \( c \) | amplitude in seasonality | 0.3 |
| \( \theta_b \) | biting rate | 115 per year |
| \( \beta_2^{h+v} \) | transmission probability human→mosquito | 0.9 |
| \( \beta_2^{h+v} \) | transmission probability mosquito→human | 0.8 |
| \( k \) | speed in seasonality change | 2 |
|  | detection threshold (relative frequency) | 10% |
|  | deterministic fixation threshold (relative frequency) | 99% |
|  | deterministic number of introduced DENV2' (cases) | 1 infected mosquito |
|  | stochastic fixation threshold (cases) | 0 |
|  | stochastic number of introduced DENV2' (cases) | 2 per infectious class |

Table 1. Model Parameters.

That is, \( \rho_p \) can be considered as the degree of the fitness advantage. In line with the suggestion by Hang et al. [33], most of our analysis is concentrated on the fitness advantage due to increased viral load and thus transmissibility from the infected human individual to the mosquito vector, \( \beta_2^{h+v} \). In fact, we found that the results presented here are invariant to the actual viral trait that is enhanced; results obtained under changes to other viral traits can be found in the supporting material.

**Stochastic simulations**

To address certain aspects of the invasion process of a more probabilistic nature, such as invasion success rates and fixation events, we also implemented the above model as a stochastic framework using a tau-leap Gillespie algorithm [36]. Stochastic simulations were initialized with equilibrium population status derived from the deterministic framework with parameter values the same as given in Table 1 (see Figure S7 and S8 for general model behaviour).

**Results**

We used a simple epidemiological model of dengue to investigate the effect of host population immunity structures and transmission settings on the invasion pattern of a novel DENV2 genotype, hereby denoted as DENV2'. The model is based on a previously introduced deterministic, multi-serotype framework (e.g. [34,37,38]) but extended to include the mosquito vector population, with seasonal fluctuations in biting frequencies, and a period of temporary cross-immunity; full model details are given in the Methods section. We verified our model predictions within a stochastic framework which allowed us more adequately address and further explore certain aspects of the invasion and replacement dynamics and their determinants [39].

The general dynamics generated by our model under parameter values given in Table 1 and prior to the introduction of a novel DENV2 genotype are characterised by semi-regular epidemic outbreaks and asynchronous cyclical behaviour in serotype prevalence (Figure 2). In accordance with previous studies (e.g. [34,37,40]) a wide range of incidence and serotype dynamics with different inter-epidemic periods can also be found under changes to key parameters values, especially those relating to the degree of enhancement of secondary infection or the period of temporary cross-immunity (Figures S1 and S2). For the remainder of this work, however, we kept most parameter values constant to allow for better comparisons between invasion patterns and their epidemiological determinants.

**Genotype invasion and replacement**

We examined the dynamics of a novel genotype introduced into a dengue endemic population by either an infected human individual or via an infected mosquito. The novel genotype is here denoted as DENV2', to represent the Asian-1 genotype of serotype 2, whereas the resident type is denoted as DENV2 to represent the Asian/
American type. Figure 3 shows the result of an invasion scenario where the invading genotype has a small fitness advantage over the resident type ($\rho_b = 0.045$, corresponding to a fitness advantage of 4.5%). In this case, higher viral fitness was realised through enhanced transmissibility from infected human individuals to the mosquito vectors, i.e. $\beta_{2w}^{b,x} > \beta_{2w}^{b,-x}$. In agreement with the data, two important features of the invasion dynamics can be observed and are highlighted in Figure 3B. Despite the eventual fast rate at which the advantageous genotype replaces the resident type, there is a significant lag between the point of introduction and the time when DENV2’ genotype would reach a detectable level of prevalence within the population; we refer to this level of prevalence as detection threshold. Furthermore, despite the expected temporary rise in dengue incidence, compared to the situation without invasion, the overall dynamics in both disease incidence and serotype prevalence remain largely invariant (Figure 3A). This suggests that both the time lag between introduction and first detection and also the rapid exclusion of the resident genotype, such as reported by Hang et al. [33], can be explained by a relatively small fitness advantage of the invading genotype.

The same qualitative behaviour can be also found when changing other viral traits which could determine the fitness advantage. That is, shortening the extrinsic incubation period, $\rho_d$, increasing the duration of infection, $\rho_s$, or the level of enhancement of secondary infection, $\rho_d$, have the same effect as increasing the transmission probability from infected humans to mosquitoes, $\rho_B$. Notably, though, when considering low advantages, smaller differences in terms of viral fitness are required to achieve the same rate of fixation if the fitness advantage manifests itself in longer infectious periods compared to an increase in transmissibility (Figure S3). Interestingly, while similar levels of fitness advantages in either EIP or transmissibility result in the same fixation times (Figure S4), the disturbance on the epidemiological pattern of dengue is less severe when the fitness advantage is expressed in the mosquito (Figure S5). From now on, we concentrate only on a fitness advantage through the proposed increase in human-to-vector transmission.

**The effect of viral fitness and time of introduction**

As shown in Figure 3, a small increase in transmissibility from human to mosquito seems sufficient for a novel genotype to displace a resident type within a short period of time. The actual rate of competitive exclusion and overall time from introduction of the advantageous genotype to its fixation in the population is likely to depend on various factors including fitness advantage, rate of transmission and immune profile within the human population. As shown in Figure 4A, increasing viral fitness accelerates the rate at which the invading genotype drives the resident type, DENV2, to extinction, resulting in a shorter period between introduction and fixation. For example, increasing the fitness advantage from 8% to 28% reduces the time to fixation from $\approx 8$ years down to $\approx 2$ years. However, this increase in viral fitness has a major effect on dengue incidence patterns and the dynamics of the other serotypes. In this case it leads to a significantly bigger epidemic outbreak at the time of replacement followed by a long period of low transmission and low prevalence of serotype 2 which could endanger its continuous persistence; this is highlighted in Figure 4B (compare to Figure 3A).

We next addressed the effect of the time of introduction on the invasion dynamics. This was simply motivated by the fact that serotype competition is not constant over time but is strongly affected by the level of transmission which itself is dependent on host immunity level and seasonal variation in mosquito densities. Not surprisingly, we found that the time of introduction can significantly alter the time taken for a novel genotype to reach fixation. Figure 5A shows the decrease in the frequency of DENV2, relative to the fitter genotype DENV2’, for two different time points of introduction. However, while the overall duration from invasion to fixation is dependent on the time when DENV2’ gets introduced, the actual rate of replacement remains constant. In other words, the time taken from DENV2’ passing a detection threshold, relative to DENV2, to reaching fixation is independent of the time of introduction (Figure 5B) and therefore independent of the overall epidemiological dynamics. This, on the other hand, suggests that the time lag between introduction and the point when it has spread sufficiently for detection, or waiting time, is strongly influenced by the epidemiological profile at that time.

To investigate further the determinants for fixation time we simulated a number of invasion events at various time points over a four year period and recorded the total time to fixation for each event with respect to (i) the number of naive individuals, (ii) serotype 2 susceptible individuals, (iii) disease prevalence and (iv) mosquito biting frequency. While we could not find a clear correlation between any of these population profiles and fixation...
time, we observed a trend for longer fixation times during the time window where the relative prevalence of serotype 2 was increasing (Figure S6).

The effect of serotype competition on emergence time and invasion success

The results from our deterministic model suggest that novel genotypes can face long periods at very low prevalence before breaching a detection threshold and going to fixation. Within a more realistic setting these periods signify an enhanced risk of stochastic extinction of the novel type despite its fitness advantage over the resident type. To better address the invasion success of DENV2 we used a stochastic formulation of our model (see Methods) and simulated a number of invasion events over a period of four years and recorded the success rate of invasion, here defined as the successful introduction into a population followed by competitive exclusion of the resident type. As demonstrated in Figure 6A we observed that invasion success shows an oscillatory behaviour whose phase seems negatively correlated to total dengue prevalence at time of introduction. This suggests that the invasion of a newly advantageous genotype can be hampered by serotype competition during epidemics and favoured during off-season periods. Moreover, the amplitude of oscillation, i.e. the maximum success rate, is dependent on and again negatively correlated to serotype 2 prevalence. Figure 6B shows the increase in relative prevalence of DENV2 over the 4-year period which clearly correlates with a decline in the success rate of DENV2'.

Since the time taken from passing a detection threshold to reaching fixation was shown to be independent of the time of introduction (Figure 5B), we focused on the relationship between serotype 2 prevalence and the time to emergence, i.e. the period between introduction and reaching a 10% prevalence threshold. Figure 7 clearly illustrates that a novel and advantageous genotype entering the population during periods of high DENV2 prevalence will face significantly longer emergence times than those introduced during periods of low prevalence. Together our results indicate that

Figure 3. Dynamics of an invading genotype. (A) Plotting the frequency of DENV2’ relative to DENV2 highlights two phases of the invasion process: a period of very low frequency and a subsequent rapid shift in dominance and competitive exclusion. The fitness advantage in both plots is due to increased human-to-vector transmission rate ($\beta_v = 0.045$) over the resident type. (B) The cyclical serotype behaviour remains invariant to the introduction of a fitter genotype of serotype 2, DENV2' (cyan line), which enters the population at time $t = 1239.5$ (pink arrow) and drives the resident type, DENV2 (blue line), to extinction after ~13 years. Comparing the equivalent time series in Figure 2, no major changes in disease levels or inter-epidemic period can be observed. Other parameters as in Table 1.

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the fate of a novel genotype is strongly determined by both inter-
and intra-serotype competition at the time of introduction.

Discussion

We analysed the invasion pattern of a novel dengue genotype
into an endemic population with 4 co-circulating serotypes. Within
our framework we assumed that the invading genotype, represent-
ing the Asian-1 genotype of dengue virus serotype 2, possesses
a fitness advantage over the resident type, the Asian/American
genotype, through enhanced transmissibility from infected human
individuals to the mosquito vectors. This assumption was based on
the findings by Hang et al. [33] which showed increased plasma
viraemia levels in patients infected by Asian-1 DENV2 viruses. In
contrast to other studies [30,41], Hang and colleagues did not find
increased infectivity of Asian-1 viruses to Ae. aegypti mosquitoes per
sec; however, it is easy to envisage how higher viral titers could
enhance the ‘per bite’ probability of human-to-vector transmis-
sion. By thus focusing on the hypothesis of a small increase in
transmissibility during primary and secondary infections, and in
agreement with the data, we observed that the total time for
genotype replacement is composed of a period during which the
invading type can circulate at very low prevalence levels for several
years, followed by a rapid shift in dominance and competitive
exclusion after the invading genotype had emerged; here we
defined ‘emergence’ as a threshold level of prevalence where
widespread detection would be highly likely.

Of particular interest is the time lag between introduction and
emergence, or waiting time, when the detection of the new dengue
genotype might be difficult by surveillance systems based on low
viral sampling numbers and/or infrequent genotyping. Not
surprisingly, we found that this period is strongly and positively
affected by the difference in viral fitness between the resident and
novel genotype. In the case of small fitness advantages several
years could pass before the invading type has spread sufficiently to
outcompete the resident type on a population-wide level.
Furthermore, as the epidemiological pattern would remain largely
invariant, passive surveillance systems based simply on case
numbers could also easily fail to detect this intra-serotype
replacement event. These results therefore support the findings
of Hang et al. [33] who hypothesised that a small enhancement of
human-to-mosquito transmission through increased viral load is
sufficient to explain the observed invasion pattern in Southern Viet Nam where Asian-1 was first detected in 2003 despite the phylogenetic analyses dating the introductory event sometime during the late 1990’s.

Apart from increased transmission from infected humans to the mosquito vectors we also considered other viral traits that could be enhanced in the Asian-1 genotype, such as longer infectious periods or shorter extrinsic incubation periods (EIP). The latter is of particular interest as it can potentially lead to a significantly increase in vectorial capacity [31]. While the actual viral trait which is enhanced does not alter the overall invasion pattern or results presented in this work (Figures S3, S4, S5, S9, S10, and S11), we found that viral fitness traits have an additive effect (Figure S4). This means that even smaller individual enhancements are sufficient to explain the observed invasion dynamics of the Asian-1 genotype, especially under the assumption that this replacement event did not have a major effect on the sero-epidemiological pattern of dengue.

Interestingly, though, our results suggest that dengue incidence and serotype dynamics are less disturbed when the fitness advantage is manifested through shorter EIP than increased infectivity or transmissibility (Figure S5).

In addition to viral fitness, the time point at which a novel genotype enters a population is crucially important in determining its invasion dynamics and ultimately success. Whereas the relative fitness advantage affects the overall time between introduction and fixation, the epidemiological profile more strongly determines the period of low level prevalence before the advantageous genotype emerges. We tested various epidemiological factors for their influence on the waiting time but to our surprise only found the relative prevalence of DENV2 to have a strong effect. That is, whereas population susceptibility to either dengue in general or serotype 2 in particular had no immediate influence on the time between introduction and wide-spread detection, we found that the relative prevalence of DENV2 at the time of introduction positively correlates with extended periods during which the novel genotype circulates below a detection threshold. Therefore, while transmission intensities strongly affect the success of an invasion event, the dominance level of serotype 2 within the population

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**Figure 5. The effect of the time of introduction on the rate of fixation.** (A) The graph shows the increase in the frequency of DENV2*, relative to DENV2, for two different time points of introduction (TPI). Despite a discernible difference in the total time for DENV2* to reach fixation and competitively exclude the resident type, the actual rate of displacement (highlighted as dashed lines) remains the same. That is, the differences in fixation times in both cases are solely due to the differences in the initial expansion period of the invading genotype before it reaches wide-spread detection level (here arbitrarily set at 10% relative prevalence). (B) Whereas the relative fitness advantage of the invading genotype has a significant effect on the rate of replacement, it remains invariant to the time at which it is introduced into the population. All parameters as in Table 1 and $p_{a}=0.045$ for (A).
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determines both the invasion success rate and, independently, the period before the invading genotype would reach a sufficient level of prevalence to be widely detectable. Our results thus confirm that serotype interactions and the resulting epidemiological landscape can have a big influence on intra-serotype dynamics and thus viral evolution, as previously noted by Zhang and colleagues [23].

There is considerable interest in determining the evolutionary processes that underlie the observed structures and genetic variation of dengue virus populations (both inter- and intra-serotypic). Overall, low estimates of selection pressure, in terms of average $dN/dS$ values, and the fact that dengue has a two-host life-cycle are commonly used to place purifying selection as the strongest selective force acting on dengue evolution [23,26,42]. Various phylogenetic studies have identified frequent DENV lineage turnover events which have resulted in the characteristic, ladder-like tree (e.g. [24,42]) and which are commonly ascribed to positive selection [24,32,43]. In addition, genetic drift has also been proposed to play a major part in dengue evolution such that the replacement of viral lineages or clades could be explained through stochastic processes alone. For example, repeated bottlenecks due to large seasonal fluctuations in mosquito densities imply that the emergence of novel and possibly advantageous genotypes could be a recurrent phenomenon followed by a strong probability for extinction in the subsequent circulating seasons which could explain the weak signature for positive selection in the data (compared to purifying selection). This in turn would also suggest that the success of a genotype does not always reflect its viral fitness [7]. In fact, we have shown that novel genotypes, especially those that arise during large epidemic outbreaks, can face high risks of extinction despite possessing a fitness advantage. Furthermore, even successful genotypes, i.e. those that eventually reach fixation, potentially undergo prolonged periods of low frequency which can span for

Figure 6. The effect of transmission and serotype competition on invasion success. The success rate of the invading genotype, DENV2', strongly varies depending on the number of total infected individuals and the relative prevalence of serotype 2 in the population at the time point of introduction (TPI). (A) The invasion success (orange line) oscillates out of phase with total dengue incidence (grey line) and is minimized when disease prevalence peaks, demonstrating how the current level of transmission can influence the invasion success of new advantageous genotypes. (B) The highest rates of successful invasions can be observed during periods of low relative prevalence of serotype 2 (blue line). In contrast, the probability of an invading advantageous genotype to get established and reach fixation is significantly reduced as serotype 2 gains wide-spread dominant within the population. Parameters as in Table 1 and $\rho_0 = 0.045$.

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several transmission seasons independently of the epidemics therein. Therefore, low measures of adaptive selection in this case would not necessarily imply strong purifying selection but could equally be explained by other epidemiological factors. This, however, needs to be confirmed within a more rigorous framework.

Dengue’s two-host life-cycle implies a significant evolutionary constraint whereby the majority of newly arising variants are likely to be deleterious and selectively removed from the population. We have shown that even novel and advantageous DENV genotypes can undergo periods of several years prior reaching sufficiently large population sizes to escape the risk of extinction. Our results thus indicate that in addition to purifying selection, the epidemiological landscape and stochastic effects might be equally important determinants in shaping the viral evolutionary ecology.

Supporting Information

Figure S1 Model behaviour under different levels of enhancement. Under a wide range of parameter values, the model reproduces the observed epidemiological pattern of dengue. In agreement with previous models, the level of ADE, either in terms of transmission or susceptibility enhancement ($\varphi$ and $\gamma$, respectively), has a significant effect on the qualitative dynamics, with greater degrees of ADE generally leading to more pronounced epidemic outbreaks and chaotic serotype oscillations. These simulated time series show the cyclical behaviour in serotype prevalence (coloured lines) and regular epidemic outbreaks (grey) for (A) $\varphi = \gamma = 1.0$ (B) $\varphi = \gamma = 1.3$ (C) $\varphi = 1.9 \gamma = 1.3$ (D) $\varphi = 1.3 \gamma = 1.9$. Other parameter values as in Table 1 (main text).

Found at: doi:10.1371/journal.pntd.0000894.s001 (1.57 MB TIF)

Figure S2 Model behaviour under different levels of temporary heterologous immunity. Under various periods of temporary heterologous immunity ($\alpha$), the model reproduces the observed epidemiological pattern of dengue. Increasing the value of $\alpha$ - (A) 3.5, (B) 4.5, (C) 5.5, (D) 6.5 - leads to higher interepidemic periods as epidemics caused by one serotype build temporary immunity and prevent DENV from exploring the human population until immunity wanes.

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Figure S3 The effect of viral fitness assuming changes in infectious period and secondary infections. The graph demonstrates the increased rate in competitive exclusion of the resident genotype DENV2 for increasing levels of viral fitness of DENV2* expressed as (A) infectious period ($\rho_\text{a}$) and (B) increased infectivity in secondary infections ($\rho_\text{b}$). (A) Similar fitness differences are required for displacement to take place in the same time window as in Figure 4, main text. (B) Higher fitness differences are required for displacement to take place in the same time window as in Figure 4, main text. Other parameter values as in Table 1 (main text).

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Figure S4 The synergistic effect of viral fitness assuming changes in the extrinsic incubation period and human-to-vector transmission. The graph demonstrates the increased rate in competitive exclusion of the resident genotype DENV2 for increasing levels of viral fitness of DENV2* expressed as a shorter extrinsic incubation period ($\rho_\text{c}$) and increased human-to-vector transmission ($\rho_\text{b}$) (see Methods in main text). (A,B) Equal fitness differences either expressed as shorter extrinsic incubation period or increased human-to-vector transmission lead to similar emergence and fixation times. (C) The effect of $\rho_\text{a}$ and $\rho_\text{b}$ on the invasion dynamics is additive. Other parameter values as in Table 1 (main text).

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Figure S5 The effect of viral fitness assuming changes extrinsic incubation period. The graph demonstrates the increased rate in competitive exclusion of the resident genotype DENV2 for increasing levels of viral fitness of DENV2* expressed as a shorter extrinsic incubation period ($\rho_\text{c}$) (see Methods). (A) Higher fitness differences lead to shorter waiting and fixation times. (B) Interestingly, even significant advantages, here $\rho_\text{c} = 0.2$, i.e. a 20% fitter genotype, does not result in severe disruption of...
the incidence patterns of dengue. Other parameter values as in Table 1 (main text).

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Figure S6 Effects of other population status on total time of fixation. The graphs show the time taken for a novel serotype 2 genotype to reach fixation given (A) the number of susceptible (naïve) individuals, (B) dengue disease prevalence, (C) number of susceptible individuals to serotype 2 and (D) seasonality, at the time point of introduction of the invading genotype (black curves). Points represent an introduction event, given a certain population status, and are coloured according to the total time for fixation. A clear increase in total time is observed in all 4 plots along the chosen time window with no correlation between any of the variables in A, B, C or D. \( p_r = 0.045 \) all other parameter values as in Table 1 (main text).

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Figure S7 Stochastic model behaviour. Initialized with the population state and parameters of the deterministic model at \( t = 1250 \), the stochastic model exhibits a similar time series as presented in Figure 2 (main text) with persistence of all serotypes. This simulated time series shows the cyclical behaviour in serotype prevalence (coloured lines) and regular epidemic outbreaks (grey). Parameter values as in Table 1 (main text).

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Figure S8 Effect of fitness advantage on invasion success. Considering a fixed time point for introduction, increasing values of \( p_r \) result in higher invasion success rates of DENV2’ and lowers fixation time. Time of introduction 1259.3, parameter values as in Table 1 (main text).

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Figure S9 The effect of transmission and serotype competition on invasion success and emergence time of successful fixation events, assuming changes in the EIP. The success rate of the invading genotype, DENV2’, strongly varies depending on the number of total infected individuals and the relative prevalence of serotype 2 in the population at the time point of introduction (TPI). The total time required for a novel (and eventually successful) genotype DENV2’ to reach detection level is highly dependent on the relative prevalence of serotype 2 at the time it enters the population. (A) The invasion success (orange line) oscillates out of phase with total dengue incidence (grey line) and is minimized when disease prevalence peaks, demonstrating how the current level of transmission can influence the invasion success of new advantageous genotypes. (B) The highest rates of successful invasions can be observed during periods of low relative prevalence of serotype 2 (blue line). In contrast, the probability of an invading advantageous genotype to get established and reach fixation is significantly reduced as serotype 2 gains wide-spread dominant within the population. (C) The red points show how the average emergence times, i.e. the period between introduction and reaching a 10% detection threshold, of successful invasion events increases with the relative prevalence of DENV2 at the time of introduction (blue line). Standard deviations, based on 10 simulated successful invasion events, are shown as light-blue bars. Parameters as in Table 1 and \( p_s = 0.045 \).

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Figure S10 The effect of transmission and serotype competition on invasion success and emergence time of successful fixation events, assuming changes in human infectious period. The success rate of the invading genotype, DENV2’, strongly varies depending on the number of total infected individuals and the relative prevalence of serotype 2 in the population at the time point of introduction (TPI). The total time required for a novel (and eventually successful) genotype DENV2’ to reach detection level is highly dependent on the relative prevalence of serotype 2 at the time it enters the population. (A) The invasion success (orange line) oscillates out of phase with total dengue incidence (grey line) and is minimized when disease prevalence peaks, demonstrating how the current level of transmission can influence the invasion success of new advantageous genotypes. (B) The highest rates of successful invasions can be observed during periods of low relative prevalence of serotype 2 (blue line). In contrast, the probability of an invading advantageous genotype to get established and reach fixation is significantly reduced as serotype 2 gains wide-spread dominant within the population. (C) The red points show how the average emergence times, i.e. the period between introduction and reaching a 10% detection threshold, of successful invasion events increases with the relative prevalence of DENV2 at the time of introduction (blue line). Standard deviations, based on 10 simulated successful invasion events, are shown as light-blue bars. Parameters as in Table 1 and \( p_s = 0.045 \).

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Figure S11 The effect of transmission and serotype competition on invasion success and emergence time of successful fixation events, assuming changes in transmissibility of secondary infections. The success rate of the invading genotype, DENV2’, strongly varies depending on the number of total infected individuals and the relative prevalence of serotype 2 in the population at the time point of introduction (TPI). The total time required for a novel (and eventually successful) genotype DENV2’ to reach detection level is highly dependent on the relative prevalence of serotype 2 at the time it enters the population. (A) The invasion success (orange line) oscillates out of phase with total dengue incidence (grey line) and is minimized when disease prevalence peaks, demonstrating how the current level of transmission can influence the invasion success of new advantageous genotypes. (B) The highest rates of successful invasions can be observed during periods of low relative prevalence of serotype 2 (blue line). In contrast, the probability of an invading advantageous genotype to get established and reach fixation is significantly reduced as serotype 2 gains wide-spread dominant within the population. (C) The red points show how the average emergence times, i.e. the period between introduction and reaching a 10% detection threshold, of successful invasion events increases with the relative prevalence of DENV2 at the time of introduction (blue line). Standard deviations, based on 10 simulated successful invasion events, are shown as light-blue bars. Parameters as in Table 1 and \( p_s = 0.075 \).

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Author Contributions
Conceived and designed the experiments: JL MR. Performed the experiments: JL. Analyzed the data: JL. Wrote the paper: JL MR.

References
1. Kyle JL, Harris E (2008) Global spread and persistence of dengue. Annual Review of Microbiology 62: 71–92.
2. San Martin J, Brathwaite O, Zambrano B, Solozzano J, Bouckenooghe A, et al. (2010) The epidemiology of dengue in the americas over the last three decades: a
Dengue Genotype Replacement

1. World Health Organization (2000) Strengthening implementation of the global strategy for dengue prevention and control. Presented at Report of the Informal Consultation, Geneva, Switzerland.

2. Nisalak A, Endy TP, Nimmannitya S, Kalayanarooj S, Thisayakorn U, et al. (1997) Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. American Journal of Epidemiology 120: 653–669.

3. Hattersley EJ, O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. Infection, Genetics and Evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases. pp 523–540.

4. Thiem S, Aung MM, Shwe TN, Zaw A, et al. (1997) Risk factors in dengue shock syndrome. The American Journal of Tropical Medicine and Hygiene 56: 566–572.

5. Boonnak K, Slike BM, Burgess TH, Mason RM, Wu SJ, et al. (2008) Role of dendritic cells in antibody-dependent enhancement of dengue virus infection. Journal of Experimental Medicine 144: 3183–3186.

6. Recker M, Blyuss KB, Simmons CP, Hien TT, Wills B, et al. (2009) Immunological serotype interactions and their effect on the epidemiological pattern of dengue. Proceedings Biological sciences/The Royal Society 276: 2541–8.

7. Kungthong C, Zhang C, Mammen MP, Ubol S, Holmes EC (2002) Phylogenetic evidence for adaptive evolution of dengue viruses in nature. Journal of General Virology 83: 1679–1689.

8. Bennett S, Holmes E, Chirivella M, Rodriguez D, Beltran M, et al. (2003) Selection-driven evolution of emergent dengue virus. Molecular Biology and Evolution 19: 1630–8.

9. Bennett S, Holmes E, Chirivella M, Rodriguez D, Beltran M, et al. (2003) Cross-reacting antibodies enhance dengue virus infection in aedes aegypti. Journal of Virology 77: 11296–11299.

10. Bennett S, Holmes E, Chirivella M, Rodriguez D, Beltran M, et al. (2006) Molecular evolution of dengue 2 virus in puerto rico: positive selection in the viral envelope accompanies clade reintroduction. The Journal of General Virology 87: 885–93.

11. Boonnak K, Slike BM, Burgess TH, Mason RM, Wu SJ, et al. (2008) Role of dendritic cells in antibody-dependent enhancement of dengue virus infection. Journal of Experimental Medicine 144: 3183–3186.

12. Recker M, Blyuss KB, Simmons CP, Hien TT, Wills B, et al. (2009) Immunological serotype interactions and their effect on the epidemiological pattern of dengue. Proceedings Biological sciences/The Royal Society 276: 2541–8.

13. Gibbsen RV, Kamaraoj S, Jurman RG, Nisalak A, Vaughn DW, et al. (2007) Analysis of repeat hospital admissions for dengue to estimate the frequency of third or fourth dengue infections resulting in admissions and dengue hemorrhagic fever, and serotype sequences. The American Journal of Tropical Medicine and Hygiene 77: 910–3.

14. Hattersley EJ, O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. Journal of Experimental Medicine 146: 201–217.

15. Dejiarutivai W, Jumnaaisong A, Ousirasal N, Fitos P, Vasanaivathana S, et al. (2010) Cross-reacting antibodies enhance dengue virus infection in humans. Science 326: 743–748.

16. Johansson MA, Dominici F, Gläss GE (2009) Local and global effects of climate on transmission in Puerto Rico. PLoS Neglected Tropical Diseases 3: e382.

17. Adams B, Holmes EC, Zhang C, Mammen MP, Nimmannitya S, et al. (2006) Cross-protective immunity can account for the alternating epidemic pattern of dengue virus serotypes circulating in bangkok. Proceedings of the National Academy of Sciences of the United States of America 103: 14234–9.

18. Hu MH, Lowsky K, Jiang L, Hsiaing T, Holmes E, et al. (2005) Lineage extinction and replacement in dengue type 1 virus populations are due to stochastic events rather than to natural selection. Virology 336: 163–72.

19. Zhang C, Mammen M, Chinnaiooripinan P, Kungthong C, Rodpradit P, et al. (2005) Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. Journal of Virology 79: 13183–90.

20. Halstead SB (2003) Patterns of intra- and interhost nonsynonymous variation reveal strong purifying selection in dengue virus. Journal of Virology 77: 11296–11299.

21. Halstead SB (1970) Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion. Yale Journal of Biology and Medicine 42: 330–362.

22. Gubler DJ (2002) Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends in Microbiology 10: 100–3.

23. Chattopadhyay A, Srinivasan P, Zaki SR, Gubler DJ, et al. (2007) Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. Infection, Genetics and Evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases. pp 329–341.

24. Bennett S, Holmes E, Chirivella M, Rodriguez D, Beltran M, et al. (2003) Selection-driven evolution of emergent dengue virus. Molecular Biology and Evolution 19: 1630–8.

25. Witke V, Robb T, Thu H, Nisalak A, Nimmannitya S, et al. (2002) Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. Virology 301: 148–56.

26. Bennett S, Chirivella M, Rodriguez D, Beltran M, et al. (2003) Cross-reacting antibodies enhance dengue virus infection in aedes aegypti. Journal of Virology 77: 11296–11299.

27. Bennett S, Holmes E, Chirivella M, Rodriguez D, Beltran M, et al. (2006) Molecular evolution of dengue 2 virus in puerto rico: positive selection in the viral envelope accompanies clade reintroduction. The Journal of General Virology 87: 885–93.

28. Hattersley EJ, O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. Journal of Experimental Medicine 146: 201–217.

29. Dejiarutivai W, Jumnaaisong A, Ousirasal N, Fitos P, Vasanaivathana S, et al. (2010) Cross-reacting antibodies enhance dengue virus infection in humans. Science 326: 743–748.

30. Johansson MA, Dominici F, Gläss GE (2009) Local and global effects of climate on transmission in Puerto Rico. PLoS Neglected Tropical Diseases 3: e382.