Transmission-clearance trade-offs indicate that dengue virulence evolution depends on epidemiological context

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An extensive body of theory addresses the topic of pathogen virulence evolution, yet few studies have empirically demonstrated the presence of fitness trade-offs that would select for intermediate virulence. Here we show the presence of transmission-clearance trade-offs in dengue virus using viremia measurements. By fitting a within-host model to these data, we further find that the interaction between dengue and the host immune response can account for the observed trade-offs. Finally, we consider dengue virulence evolution when selection acts on the virus’s production rate. By combining within-host model simulations with empirical findings on how host viral load affects human-to-mosquito transmission success, we show that the virus’s transmission potential is maximized at production rates associated with intermediate virulence and that the optimal production rate critically depends on dengue’s epidemiological context. These results indicate that long-term changes in dengue’s global distribution impact the invasion and spread of virulent dengue virus genotypes.
Evolution of virulence theory proposes that parasites will evolve to an intermediate level of virulence when a trade-off exists between parasite transmissibility and virulence, where virulence is most commonly defined as the rate at which hosts experience disease-induced mortality. Although a large body of literature contributes to virulence theory, few studies have empirically demonstrated the existence of a fitness trade-off that would result in evolution towards intermediate virulence. Among human pathogens, HIV provides a rare, well-documented example. Human malaria provides another example, where observed, broad patterns between virulence and transmissibility are consistent with evolution of virulence theory. While a trade-off between transmission rate and disease-induced mortality is the classical trade-off considered in evolution of virulence theory, early work has also considered alternative trade-offs, for example, between the rate of recovery and the rate of disease-induced mortality.

More generally, virulence can be defined as the ability of a pathogen to cause disease. In this case, evolution towards intermediate virulence can also result from trade-offs in many different fitness components, rather than only through the classical trade-off between transmission and disease-induced host mortality. Indeed, recent studies have indicated that the pathogen clearance rate is likely to be an important fitness component to consider in the evolution of virulence, defined as disease severity. These studies further underscore that the interaction between pathogens and the host immune response may be an important factor in the evolution of pathogen virulence. Here, with dengue virus, we document an example of a human pathogen subject to a fitness trade-off involving viral clearance.

In brief, dengue is a vector-borne virus that infects up to 400 million individuals annually. Dengue infections can be asymptomatic, result in symptomatic dengue fever (DF) or result in severe disease, defined as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The virus comprises four antigenically distinct serotypes, each of which is genetically structured into several clades called genotypes, based on levels of nucleotide divergence exceeding 6%. Dengue virus infection induces long-lived immunity against the infecting serotype, but only a transient period of cross-protection against heterologous dengue serotypes. Primary dengue infections can result in severe disease, but they do so rarely, with the majority of these infections being asymptomatic or resulting in DF. In contrast, secondary heterologous infections have an appreciably higher risk of developing into severe disease. Tertiary and quaternary (henceforth, "post-secondary") infections contribute very little to dengue hospital admissions, although they are known to occur. The elevated risk of developing severe disease with a heterologous secondary infection is due at least in part to antibody-dependent enhancement (ADE), a process by which antibodies generated during a primary infection facilitate viral entry into host target cells bearing Fc-γ receptors during a secondary infection. This process, among others, results in an elevated number of cytokine-secreting infected cells, which can initiate a cytokine storm that ultimately leads to the development of vascular leakage, the hallmark of DHF. Severe dengue disease is therefore a result of immunopathology, whereby the host's own immune response, rather than the presence of the pathogen, is the direct cause of damage to host tissue.

Using viremia measurements from symptomatic dengue-infected patients, we first document an empirical trade-off between peak viral load and the viral clearance rate, indicating that high viral loads come at the cost of accelerated viral clearance in both primary and secondary dengue infections. By fitting an existing within-host dengue model to the observed viremia measurements, we show that this observed trade-off can be explained by the interaction of the virus with the host immune response, consistent with recent arguments that the host immune response can be an important factor limiting the evolution of higher transmission rates.

Given that the probability of successfully transmitting to dengue's mosquito vector strongly depends on host viral load, and that high peak viral load is a strong predictor of the probability of triggering severe dengue disease, the empirical trade-off we document between peak viral load and viral clearance rate sets the stage for the possibility that dengue virus strains associated with intermediate virulence maximize the virus's transmission potential. We consider how selection may act on a within-host viral phenotype—the viral production rate—and the implications of this selection on dengue virulence evolution. We show that dengue virus transmission potential is maximized at production rates that are associated with intermediate peak viral loads, and thus an intermediate risk of triggering severe disease. Our analysis further shows that optimal virulence, defined as disease severity, depends on epidemiological context. Specifically, we show that even though secondary infections cause more severe disease than primary infections, they may place selection pressures on the virus to evolve lower virulence in any given infection. These results indicate that the changing global landscape of dengue endemism may in the long-term affect not only infection levels, but, via evolution, disease risk.

Results

Trade-off between peak viral load and viral clearance rate. In the context of dengue, virulence can be defined as the probability that infection results in severe disease, defined classically by the World Health Organization as DHF/DSS. This probability is known to depend on several factors, including host genetics, host immune status, and infecting virus genotype and serotype. Here, we operationally define virulence as peak viral load because high viral load has been associated with high hemocencentration, thrombocytopenia, and a higher pleural infusion index, all criteria used to define DHF. Peak viral load has been shown to differ between primary and secondary dengue infections, most likely due to differences in the host's adaptive immune response between these infections. Tertiary and quaternary dengue infections, most likely due to differences in the host's adaptive immune response between these infections, may place selection pressures on the virus to evolve lower virulence in any given infection.

Because the probability that severe dengue disease results in death is low, estimated at less than one percent, disease-induced mortality is unlikely to play a role in limiting the evolution of virulence in dengue. We therefore aim to determine whether high viral loads during dengue infections are associated with shortened durations of infection. A negative association between viral load and the duration of infection could lead to a trade-off that would result in evolution towards intermediate virulence. Because dengue is an acute infection, viral load changes dramatically over time and the importance of the duration of infection is modulated by the degree to which an individual is infectious at different timepoints over this period. To determine whether a trade-off exists between dengue viral load and the duration of infection, we thus sought to examine the empirical relationship between peak viral load and the viral clearance rate. To this end, we calculated peak viral loads and viral clearance rates from viremia measurements of 239 symptomatic dengue-infected individuals (Methods). We found a positive relationship between individuals with detectable peak viral load and the viral clearance rate in both primary and secondary dengue infections.
(Fig. 1). These findings are consistent with previous analyses showing that severe dengue infections are associated with both higher viral load peaks and higher viral clearance rates than non-severe dengue infections13,24.

Host immune response dynamics reproduce empirical trade-off. To address the mechanism underlying this empirical trade-off, we fit an existing within-host model for dengue34,35 to the longitudinal viremia measurements from all 239 dengue-infected individuals (Methods; Fig. 2a, b). The within-host model we used was chosen based on its ability to reproduce characteristic features of dengue infections and its consistency with findings from immunological studies in mouse models36,37. The statistical model fit incorporates inter-individual heterogeneity in the viral infectivity rate, which we included to capture variation in viral dynamics arising from host factors specifically, such as host immune status and host genetics (Methods). The statistically parameterized model reproduces the positive relationship between peak viremia and viral clearance rate observed in the data (Fig. 2c, d). It ascribes this positive relationship to the feedback between viral dynamics and the activation of the host immune response, with high viremia levels eliciting a strong innate and/or adaptive immune response, which in turn drives a high viral clearance rate.

It is well known that the overwhelming majority of dengue infections are asymptomatic infections11. Because the viral load data set consists exclusively of symptomatic dengue cases, which are known to have higher viral loads38, a model fit to these data will overestimate peak viral load for average dengue infections. In order to consider asymptomatic infections, we re-fit the model to a subset of the data consisting of patients with lower viral loads (Methods). Simulations of this re-parameterized model reproduced viral load dynamics more consistent with both non-severe and asymptomatic dengue infections (Supplementary Fig. 1), while still reproducing the observed empirical trade-offs (Fig. 2c, d). The ability of these within-host models to quantitatively reproduce the trade-offs shown in Fig. 1 supports the hypothesis that the interaction between dengue virus and the host immune response is responsible for generating the empirical trade-offs observed in both primary and secondary dengue infections.

Dengue virulence evolution. To consider the potential for dengue virulence evolution, we next use our parameterized model to ask how selection may act on viral phenotypes that impact within-host dynamics. We specifically consider the viral production rate, which quantifies the rate at which viral progeny are produced from infected host cells, as an evolvable trait. We consider this viral phenotype because dengue strains are known to exhibit natural variation in this trait39,40 and because this phenotype has in many cases been shown to have a virus genetic basis39,41. Further, this phenotype is known to impact the probability of developing severe disease, with viral strains with higher viral production rates being associated with higher virulence13,40.

In our analyses below, we use the within-host model parameterization fit to the data subset, since this model parameterization we believe more appropriately reflects the dynamics of both asymptomatic and symptomatic infections, rather than only symptomatic infections.

We first consider the effects of the viral production rate on dengue virulence (as ascertained by peak viral load) and on transmission fitness in the context of primary dengue infections. Figure 3a shows how peak viral load depends on the viral production rate, as predicted by the within-host model. The predicted positive relationship between peak viral load and the viral production rate is consistent with empirical findings that higher viral production rates result in higher probabilities of triggering severe disease39. Using an empirically estimated relationship between host viral load and transmission probability to dengue’s mosquito vector22, we next predicted the relationship between the viral production rate and dengue’s transmission potential (Methods), which is proportional to the basic reproduction number $R_0$. Figure 3b shows this relationship, with transmission potential being maximized at intermediate viral production rates, and therewith at intermediate peak viral loads. Figure 3c combines these results to more clearly demonstrate that dengue’s transmission potential from individuals experiencing a primary infection is maximized at intermediate peak viral loads, reflecting intermediate virulence. Maximization of dengue’s transmission potential at an intermediate viral production rate results from a trade-off between peak viral load and the viral clearance rate in primary infections (Supplementary Fig. 2), analogous to the trade-off that was reproduced by the within-host model incorporating inter-individual variation in dengue’s viral infectivity rate (Fig. 2c). This within-host trade-off scales up to a transmission fitness trade-off because viral transmission success to dengue’s mosquito vector saturates at high host viremia, while fitness costs associated with accelerated viral clearance continue to rise at higher viral production rates. Note that the results shown in Fig. 3 assume that the viral production rate only

Fig. 1 Empirical relationship between peak viral loads and viral clearance rates in dengue. a Peak viral loads plotted alongside maximum daily viral clearance rates for each of the individuals in the Ho Chi Minh City (HCMC) data set experiencing a primary dengue infection with a detectable viral peak ($n = 15$). The relationship is positive with a slope of $m = 0.33$, trending towards significance ($p = 0.14$, t-test). b Peak viral loads plotted alongside maximum daily viral clearance rates for each of the individuals in the HCMC data set experiencing a secondary dengue infection with a detectable viral peak ($n = 51$). The relationship is positive with a slope of $m = 0.32$ ($p < 0.01$, t-test)
impacts viral load dynamics in the human host, and therewith, the transmission success of dengue virus from humans to its mosquito vector. Our shown results implicitly assume that the viral production rate does not impact the probability that an infected mosquito transmits the virus to a susceptible host, an assumption we return to in the “Discussion” section.

Similar to our findings for primary infections, peak viral loads in secondary infections are also higher at viral production rates (Fig. 3a). Dengue’s transmission potential is again maximized at an intermediate viral production rate (Fig. 3b) due to a trade-off between peak viral load and the viral clearance rate. However, the viral production rate that maximizes dengue’s transmission potential is substantially lower in a secondary dengue infection than in a primary dengue infection because the adaptive immune response plays an important role in clearing secondary dengue virus infections. If we instead assume, as for primary infections, that it is the innate immune response that clears secondary infections (Supplementary Fig. 3), transmission potential is still maximized at lower viral production rates than in primary infections (Supplementary Fig. 4), but in this case, transmission potential is maximized at viral production rates that yield a viral peak of approximately 7 log genome copies/ml (rather than 6 log genome copies/ml, as seen in Fig. 3c). Our finding that secondary dengue infections select for viral production rates associated with lower peak viral loads than primary dengue infections is also robust to alternative adaptive immune response formulations, such as one that has T-cell activation rates saturate at high antigen levels (see Supplementary Note 1 and Supplementary Fig. 5). This finding also holds for the model parameterization using the full data set (Supplementary Fig. 6).

Given our finding that dengue’s fitness trade-offs depends on host immune status, we here synthesize our results to consider the role of epidemiological context in shaping dengue virulence evolution. We examine virulence evolution in two distinct epidemiological contexts: in a population with only a single dengue serotype endemically circulating and in a population with
two serotypes circulating. To determine the viral production rate at which viral fitness is maximized in each of these epidemiological contexts, we use an adaptive dynamics framework \(^42\) (Methods).

When only one serotype is circulating, all infections are primary infections, and the evolutionary stable viral production rate is one that maximizes the transmission potential of a primary dengue infection (Fig. 4a). This optimal viral production rate is not impacted by the transmission intensity of the virus (Fig. 4b), which may differ because of geographic differences in mosquito densities or in the mosquito biting rate. This is because, in a situation with only one serotype circulating, all infections are primary infections, regardless of the extent of dengue endemism. In a population with two circulating dengue serotypes, the evolutionary stable viral production rate is always lower than in the context of only one circulating serotype (Fig. 4b). This is because secondary infections select for lower viral production rates (Fig. 3b). As such, at evolutionary equilibrium, primary infections are expected to result in a lower viral peak (and therefore a lower probability of triggering severe dengue disease) in a population with two circulating serotypes than in a population with only one serotype circulating (Fig. 4c). In a population with two circulating serotypes, however, the transmission intensity of the virus does impact the optimal viral production rate (Fig. 4b), since this transmission intensity impacts the proportion of dengue infections that are primary versus secondary infections. At higher transmission intensities, and under the assumption of no heterologous cross-immunity between the serotypes, the proportion of dengue infections that are secondary infections relative to primary infections increases, saturating at 50%. Under this assumption, the optimal viral production rate therefore decreases with higher transmission intensities. Optimal viral production rates are to some extent impacted when a period of heterologous cross-immunity is assumed between the circulating serotypes (Fig. 4b). When cross-immunity acts to temporarily shield an individual from infection, lower viral production rates are selected for at higher transmission intensities, similar to the case with no heterologous cross-protection. However, under the assumption that cross-immunity shields not from infection, but only from symptomatic disease ("clinical cross-protection" \(^43\)), optimal viral production rates change non-monotonically with transmission intensity (Fig. 4b). In all cases, however, populations with two circulating serotypes are expected to select for lower viral production rates than populations with only a single circulating serotype. The within-host model parameterized using the full data set yields qualitatively similar results (results not shown).

**Discussion**

Here we have shown empirical support for a trade-off between peak viral load and the viral clearance rate in dengue, the most prevalent vector-borne viral disease of humans. Using a within-host model fit to viremia measurements from symptomatic dengue-infected individuals, we found that this trade-off can be explained by the interaction of the virus with the host immune response, providing empirical support for this hypothesis from theoretical studies \(^10,21\). Our findings are further consistent with an analysis of the within-host dynamics of arboviruses (including dengue) in experimentally challenged vertebrate hosts \(^44\). That analysis showed that arboviruses appear to exhibit a trade-off between peak viremia and the duration of infection, a trade-off that was made apparent when considering various doses of virus administered \(^44\). This study further invoked immune clearance as the likely mechanism for the observed trade-off, given that target cell limitation is unlikely, especially for dengue.

We next considered whether a transmission-clearance trade-off analogous to the trade-off we observed empirically could result in selection pressures on dengue virus to evolve to intermediate virulence. We specifically considered the possibility for dengue virus to evolve its viral production rate. By combining within-host model simulations with empirical estimates of transmission success to dengue’s mosquito vector, we found that viral fitness, as measured by transmission potential, is maximized at intermediate dengue virus production rates. These intermediate production rates are associated with intermediate peak viral loads and thus an intermediate risk for triggering severe disease (i.e., intermediate virulence). We further found that the viral production rate at which transmission potential was maximized differed by immune status, with secondary infections selecting for lower viral production rates than primary infections. Because lower viral production rates result in lower peak viral loads, this means that secondary infections select for genetically less virulent viruses.
Furthermore, previous work has shown that epidemiological context impacts the viral production rate that is evolutionary stable, with single-serotype epidemiological settings selecting for higher within-host viral production rates than epidemiological settings with two circulating serotypes. Our conclusions are robust to two different within-host model parameterizations, one fit to the full data set and one fit to a data subset of lower viral loads that attempts to capture asymptomatic infections.

Finally, as a consequence of these immune-status effects, we have shown that epidemiological context impacts the viral production rate that is evolutionary stable, with single-serotype epidemiological settings selecting for higher within-host viral production rates than epidemiological settings with two circulating serotypes. Our conclusions are robust to two different within-host model parameterizations, one fit to the full data set and one fit to a data subset of lower viral loads that attempts to capture asymptomatic infections.

Our finding that dengue virus should evolve towards intermediate virulence depends on the formulation of our within-host model, specifically on our assumptions of how the host’s immune system responds to viral infection. The within-host model we fit is based on an existing within-host model for dengue virus that has been shown to successfully reproduce characteristic features of dengue infections, including the timing and magnitude of peak viremia, and the viral clearance rate, as well as immunological features of dengue infections. Furthermore, previous work has shown that this model can recover findings from virological and immunological studies of dengue infection when statistically fit to viral load data. As such, although the fitness trade-offs derived here depend on a within-host model formulation, the model formulation we have used is one that has considerable empirical support. With the use of this model, a transmission fitness trade-off occurs because both the innate and adaptive immune responses are activated in an antigen-dependent manner. High levels of viremia therefore result in strong activation of the immune response and a shorter duration of infection (consistent with the patterns shown in Fig. 1). A model in which the immune response is generated in an antigen-independent manner would not generate a transmission-virulence trade-off because no costs would be incurred by a virus for an increase in its viral production rate. However, a completely antigen-independent stimulation of the immune response is unlikely, given the observed within-host trade-offs (Fig. 1), as well as existing empirical findings that show a relationship between the strength of innate and adaptive immune responses and viral load and/or the severity of dengue disease.

Our finding of a transmission fitness trade-off in dengue also critically depends on quantitative estimates of how within-host viral load impacts the probability that a susceptible mosquito becomes infected following a bite from an infected host. While our model has incorporated this demonstrated relationship between host viral load and transmission success to the mosquito, our model implicitly made several other simplifying assumptions. First, we assumed that host viral load only impacted transmission success to the mosquito, but not viral dynamics within the mosquito, which may in turn affect transmission success from mosquito to host. When we considered selection on the dengue virus production rate, we further assumed that this viral trait only affected viral dynamics in human hosts. It could be the case that the viral production rate also impacts viral dynamics in the mosquito vector and, therewith potentially onward transmission success to susceptible hosts. Indeed, several studies have indicated that more virulent dengue virus genotypes may have a fitness advantage in mosquitoes. Specifically, Hanley and colleagues showed that a more virulent genotype of DENV-3 reached higher titers in the midgut of the primary mosquito vector of dengue virus, *Aedes aegypti*, relative to a less virulent DENV-3 genotype. They further showed that the more virulent genotype more effectively disseminated throughout the body of the mosquito vector, indicating that the probability that an infected mosquito successfully transmits the virus to a susceptible human host is likely higher for the more virulent genotype compared to the less virulent genotype. Cologna and colleagues also found that more virulent dengue genotypes replicated more efficiently in
mosquito vectors. Finally, OhAinle and colleagues found that a dengue clade associated with higher viremia in a hospital study had a fitness advantage during viral competition assays in a mosquito cell line. The findings of these studies can be incorporated into our virulence evolution analysis by assuming that higher viral production rates increase the probability of dengue transmission success from infected mosquito to susceptible host (Supplementary Note 3). Incorporation of this fitness benefit in mosquitoes would lead to higher optimal viral production rates in both primary and secondary infections (Supplementary Fig. 8). Finally, our model did not consider the effect that within-host effects on transmissibility, which can differ between individuals because of host genetics or other reasons. Consistent with theory, incorporating this within-host heterogeneity does not affect our general conclusion that viral transmission fitness is maximized at intermediate virulence.

Our analyses show that, for dengue, understanding virulence evolution is incomplete without explicitly considering an epidemiological context. Specifically, we found that regions with only a single serotype present will select for a more virulent within-host phenotype than regions with two serotypes circulating. These findings may be consistent with the emergence of virulent DENV-2 genotypes in many countries in Latin America in the late 20th century where one serotype typically predominated at a time. Further characterization of post-secondary infections are needed to consider hyperendemic scenarios with four circulating genotypes. Our analyses show that, for dengue, understanding virulence evolution is incomplete without explicitly considering an epidemiological context. Specifically, we found that regions with only a single serotype present will select for a more virulent within-host phenotype than regions with two serotypes circulating. These findings may be consistent with the emergence of virulent DENV-2 genotypes in many countries in Latin America in the late 20th century where one serotype typically predominated at a time. Further characterization of post-secondary infections are needed to consider hyperendemic scenarios with four circulating genotypes. A hyperendemic scenario may select for higher or lower viremia relative to a two-circulating serotype scenario depending on whether optimal viral production rates of post-secondary infections are more similar to primary or secondary infections. The epidemiological context on which we have focused are also all endemic contexts, where viruses maximize fitness by maximizing their basic reproduction numbers. However, transient dengue epidemics may select for within-host viral phenotypes that instead maximize the intrinsic population growth rate of the virus. This suggests that viral virulence may evolve to higher levels in both primary and secondary infections than predicted by our endemic analyses. We would still expect, however, that primary dengue infections select for more virulent phenotypes than secondary dengue infections in these epidemic contexts.

Our results indicate that geographic regions will likely differ in the evolutionary selection pressures they place on dengue virus, and future work should focus on identifying the regions that will most likely be the sources of more virulent dengue strains. Empirically characterizing a region’s epidemiological context is complicated by the difficulty in detecting asymptomatic infections and distinguishing between secondary and post-secondary infections. However, cohort studies that detect asymptomatic infections such as in Vietnam and the long-running longitudinal pediatric dengue cohort study in Nicaragua may be useful in characterizing epidemiological context more accurately. Latin America is an especially good area for studying the links between viral load kinetics and onward transmission potential because in many Latin American countries, only one serotype predominates over time, possibly decreasing the complexity of epidemiological context.

In the 21st century we have seen a shift to hyperendemicity in many countries around the world, as well as the emergence of dengue in new regions. While many epidemiological studies focus on case severity, our results here show that regions with low case severity may play a critical role in incubating highly virulent dengue strains. As the global distribution of dengue shifts, understanding the changing patterns of infection histories is crucial for predicting sources of virulent dengue strains. More broadly, these results provide data-driven support for how
complex patterns of immunity can have large effects in shaping pathogen virulence evolution.

Methods

Data. The empirical data set of viremia measurements from 239 symptomatic dengue-infected individuals originates from a clinical trial of adult dengue patients at the Hospital for Tropical Diseases in Ho Chi Minh City (HCMC), Vietnam.

The trial studied the effects of the antiviral drug chloroquine on dengue, but the drug had no measurable effect on viral load dynamics. As in previous analyses of this data set, we therefore do not distinguish between chloroquine-treated patients and control patients. Patients were recruited into the study if they met the following criteria: if their first viral load measurement was within 72 h of the onset of symptoms. Viral load was then measured twice per day using RT-PCR, with the assays having limits of detection of either 1500 genome copies/ml or 15,000 genome copies/ml. The data set consists of individuals infected with one of dengue’s four serotypes, where infected individuals were further stratified by symptom status (primary infection or secondary heterologous infection), and clinical manifestation (DF or DHF), as described previously.

Note that classification of immune status did not include post-secondary infection so some individuals classified with a secondary infection may in fact have been experiencing a post-secondary infection.

For each individual, the maximum daily viral clearance rate was calculated by doubling the maximum decrease in viral load (on the log_{10} scale) between two consecutive viremia measurements (taken 12 h apart). We then calculated the maximum viral load for each individual. Because the onset of dengue symptoms often occurs at or following peak viral load, many of the dengue-infected individuals had viremia measured only during viral decline, with no detectable viral peak. We categorized dengue-infected individuals as either having or not having a detectable viral peak, based on whether viral load was observed to increase following the first viremia measurement. We fit the relationships between maximum viral load and viral clearance rate shown in Fig. 1 using only the subset of individuals with detectable viral peaks. Note that some of these individuals in this subset may have inappropriately been categorized as having a detectable viral peak due to measurement error or to intrinsic fluctuations in viral dynamics. Following an initial regression, we computed Cook’s distance to identify outliers in this data set. Datapoints from two individuals in the primary dengue infection data set and datapoints from five individuals in the secondary dengue infection data set were above the threshold value of three times the mean Cook’s distance so we refit the relationships between maximum viral load and the viral clearance rate once these datapoints were excluded.

We further examined the correlation between peak viral load and the viral clearance rate slope in the subset of individuals having detected viral peaks. In this case, we fit the viral clearance rate slopes using the likelihood function described previously in ref. 34. This alternative analysis again yielded a positive relationship between peak viral load and viral clearance rate in secondary dengue infections, with slope = 0.12 (p = 0.01, t-test). This approach, however, did not yield a significantly positive relationship between peak viral load and viral clearance rate in primary infections.

Within-host model. The within-host model we parameterize using the viremia measurements is given by:

\[
\begin{align*}
\frac{dN}{dt} &= -\beta V N \\
\frac{dV}{dt} &= \beta V N - a N Y - \delta_1 Y T \\
\frac{dY}{dt} &= a Y - k V \\
\frac{dN}{dt} &= q Y - d_N N \\
\frac{dT}{dt} &= q_Y Y T
\end{align*}
\]

where the variables are uninfected target cells X, infected target cells Y, free virus V, natural killer (NK) cells N, and T cells T. NK cells capture the role of the innate immune response. T cells capture the role of the cellular immune response. In the model, uninfected cells become infected with free virus at an overall rate of βV N. Infected cells are cleared by NK cells at an overall rate of α N Y and by T cells at an overall rate of δ_Y T. Virus is produced by infected cells at an overall rate of α_Y V and cleared at rate k V. NK cells are activated at an overall rate q Y, and decay at rate d_N N. T cells are activated at an overall rate of q_Y Y T. The parameter β is known as the viral infectivity rate. The parameter α is known as the viral production rate. The parameter δ_Y is known as the viral clearance rate.

SI) will be higher than that in primary infection model reduces to a model with only the cellular adaptive immune response responsible for clearing infection and we do not include NK cells in the within-host model uninfected cells become infected with free virus at an overall rate of \( \alpha V N \) and by T-cells at an overall rate of \( q\beta \sum_{i=1}^{n} \alpha_i V N \), where the parameter \( \alpha \) is known as the viral production rate. The parameter \( q\beta \) is known as the viral clearance rate.
As expected from the effects of ADE,14,75 \( \beta_{ns} \) was estimated to be higher than \( \beta_{ps} \) (27% higher). A higher viral infectivity rate and a lower innate immune response activation rate \( (q_\alpha = 0) \) results in secondary dengue infections having higher peak viral loads than primary dengue infections (on average, peak viral loads of 10.2 log genome copies/ml compared to 9.7 log genome copies/ml, in the absence of measurement noise). Given our operational definition of virulence, these results indicate that secondary infections should be at higher risk for triggering severe disease than primary infections, consistent with epidemiological studies.23,25,26 Further, viral clearance rates in secondary infections exceed those in primary infections (1.9 log genome copies/ml/day compared to 1.4 log genome copies/ml/day, in the absence of measurement noise), consistent with virological studies.23,25

**Within-host parameter modelization using HCMC data subset.** Longitudinal cohort studies indicate that only 6–40% of infections are symptomatic, with the remaining being asymptomatic.6,7,26 Viral load measurements from asymptomatic infections are generally not available, although a recent study has shown that asymptomatic infections exhibit lower viremia than symptomatic infections.38 In an attempt to consider viral load measurements representative of both asymptomatic and symptomatic infections, we re-fit the within-host model given by Eq. (1) to a subset of the HCMC data set. This subset consists of individuals with lower viral loads, specifically, individuals experiencing a primary infection whose maximum viral load was \( \leq 5.8 \) log genome copies/ml and individuals experiencing a secondary infection whose maximum viral load was \( \leq 4.5 \) log genome copies/ml. These viral load levels correspond to the middle range of peak viremia values typically seen in symptomatic dengue infections.23–26,27 A difference of 0.5 log genome copies/ml between peak viral loads of primary versus secondary infections was chosen for consistency with findings in ref. 24. This subset of the viral load data set includes 11 primary infections (37% of all primary infections) and 90 secondary infections (43% of all secondary infections), comprising a total of 1020 viremia measurements.

We expect that the lower viral loads observed in this subset of individuals are due to lower viral infectivity rates \( \beta \), and a stronger immune response (higher \( q_\alpha \) and/or lower \( d_\alpha \) in primary infections, and higher \( q_\tau \) in secondary infections) in these individuals compared to individuals in the full data set. Due to the small number of individuals in the data subset, and because we have no reason to expect that the coefficient of variation \( c \), or the ratio of \( \beta_{ps}/\beta_{ns} \), to differ between the full data set and the data subset, we fit only four parameters to the data subset: \( \beta_{ps}, q_\alpha, q_\tau, \) and \( d_\tau \). We estimated a small value of \( d_\tau = 1 \times 10^{-7} \) day, and therefore performed a likelihood ratio test to determine whether a simpler model with \( d_\tau = 0 \) could be rejected. We were unable to reject this simpler model and therefore set \( d_\tau \) to 0. The final parameter estimates are provided in Supplementary Table 1.

**Quantifying population-level viral transmission potential.** In endemic situations, viral fitness is given by the basic reproduction number \( R_0 \), which for a vector-borne disease depends on the vector biting rate \( b \), the ratio \( m \) of female mosquitoes to humans, the lifespan of mosquitoes \( 1/\mu \), and the probability of an infected human (mosquito) transmitting the virus to a susceptible mosquito (human). The most general formulation of \( R_0 \) for a vector-borne disease is given in Equation (1) of ref. 27. In this work, the authors assumed that the probability of an infected mosquito transmitting dengue to a susceptible human \( p_{m|v} (V(t)) \) is proportional to \( q_{m|v} V(t) \), the proportion of mosquitoes that have detectable virus in their saliva \( \tau \) units of time after having taken a blood meal from an infected human with viral load \( V \). In turn, these authors did not find that \( q_{m|v} (V(t)) \) depended on viral load \( V \). As such, \( R_0 = \int_0^{\tau} p_{m|v} (V(t)) e^{-\mu t} d t \) evaluates to a constant, which we call \( P_{m|v} \).

This leads to the following expression for \( R_0 \):

\[
R_0 = mb\mu p_{m|v} \int_0^\tau p(V(t)) e^{-\mu t} d t
\]

(2)

where \( p(V) \) is the probability that an infected human with viral load \( V \) transmits the virus to a susceptible mosquito upon being bitten and \( p_{m|v} (V) \) is the probability that a human who was infected with dengue \( \tau \) units of time ago has viral load \( V \). Since we simulate our within-host model deterministically under any given parameterization, we can equivalently write this equation as:

\[
R_0 = mb\mu p_{m|v} \int_0^\infty p(V(t)) d t
\]

(3)

where \( V(t) \) is the viral load of an infected human who was infected \( \tau \) units of time ago. We call the integral inside this expression the transmission potential of the strain.

To calculate the transmission potential, we rely on a recent experimental study analyzing factors that influence transmission success of dengue virus to susceptible mosquitoes.22 In this study, 407 susceptible mosquitoes were fed on 208 hospitalized dengue patients. An analysis of whether these blood-fed mosquitoes became infected with dengue (as measured by presence of the virus in their abdomens) showed that host viremia was the strongest covariate explaining survival to mosquito infection.23 Based on this finding, the authors quantified the relationship between patient viremia and the probability of human-to-mosquito transmission using marginal logistic regression for each of dengue's four serotypes. We used this determined relationship to couple the viral load dynamics derived from our within-host model \( (V(t)) \) to the probability of viral transmission from infected human to susceptible mosquito \( p(V(t)) \). We refer the reader to ref. 22 for the experimental data and the parameterization of the logistic regression curves. We used the DENV-1 parameterization from ref. 23 because the majority of dengue infections in the HCMC data set are dengue serotype 1. Our results suggest that the probability to transmit at different viral loads is different for the other three dengue serotypes (Supplementary Note 2, Supplementary Fig. 7).

**Peak viral load and transmission potential.** In Fig. 3a, we plot the relationship between peak viral load and the viral production rate \( \omega \). In Fig. 3b, we plot the relationship between transmission potential \( ( ) \) and the black solid lines in Fig. 3a, b show the peak viral loads and transmission potentials evaluated at mean viral infectivity rates, which depend on whether the infection is a primary or secondary infection. At each value of \( \omega \) considered, we further sample 100 viral infectivity rates from the distribution with mean \( \beta \) and standard deviation \( \sigma \), where \( \beta \) is the coefficient of variation estimate. For each of these sampled viral infectivity rates, we simulate the within-host model, calculate the peak viral load, and evaluate the transmission potential. In practice, based on the results in ref. 22, we assume that when viral load levels are below 1500 genome copies/ml transmission to the mosquito will not occur. The confidence intervals in Fig. 3a, b show the 95% range of values obtained from these 100 samples at each \( \omega \) value. In Fig. 3c, we use the combined set of simulations across \( \omega \) values. Specifically, using a sliding window of peak viral load levels, we find the simulations having peak viral loads in a given window and plot the 95% range in transmission potential values across this set of simulations.

**Virulence evolution in different epidemiological contexts.** We use an adaptive dynamics framework to consider dengue evolution of virulence in different epidemiological contexts. We specifically consider two different endemic contexts: one with a single circulating serotype and one with two circulating serotypes. The transmission dynamics under each of these endemic contexts are based on an existing epidemiological model for dengue. Specifically, the models we use are deterministic, age-structured epidemiological models with age-dependent mortality rates, as described in ref. 22 (the ‘Duke model’). All of the models we consider assume life-long immunity to reinfection with a homologous dengue serotype. The basic parameter in the type and duration of transient cross-protection is assumed between heterologous serotypes. We consider three models: one with no heterologous cross-protection, one with a 2 year period of “classical” cross-protection (where cross-protection temporarily prevents infection with the heterologous serotype), and one with a 2 year period of “clinical” cross-protection (where cross-protection prevents symptom onset and onward transmission, but does not prevent infection or, critically, seroconversion). We assume a 2 year period of cross-protection in the latter two models based on previous findings.6 We do not include seasonality in the model.

For the single circulating serotype endemic context, the epidemiological model collapses to a basic age-structured susceptible-infected-recovered (SIR) model. In this case, the viral production rate that maximizes the transmission potential of primary infections is the evolutionary stable viral phenotypic. To confirm this, we simulated this model with a “resident” dengue strain having an \( R_0 \) that is calculated from its viral production rate. Specifically, we calculate a strain’s \( R_0 \) by multiplying \( Z \) (its calculated transmission potential) which depends on \( \beta \), the basic age-structured transmission intensity factor of \( \beta = mb\mu p_{m|v} \omega \). Once simulations of this model have reached their epidemiological equilibrium, we fix the number of susceptible and recovered hosts at their equilibrium values and determine numerically whether an “invading” strain of the same serotype (which has its own viral production rate, and corresponding \( R_0 \)) can increase when rare. If the invading strain can increase when rare, its reproductive rate \( R_0 \) exceeds 1 in the endemic context of the resident strain and (given full cross-immunity) it would replace the resident strain. The pairwise invasibility plot shown in Fig. 4a shows the results of this one-serotype analysis for combinations of “resident” and “invading” strains when \( f \) is set to 2. The pairwise invasibility plot shows that, however, does not change with a change in \( f \) in Fig. 4b, given that all infections (regardless of their number) are primary infections.

For the two-circulating serotypes endemic context, the epidemiological models remain more complicated, but we adopt a conceptually similar approach to the one used for the single-serotype context to determine the cost of different transmission to the mosquito (as well as the mosquito densities \( \mu \) or biting rates \( b \)).

**Code availability.** All code is available on GitHub at https://github.com/ rbensharac/dengue_virulence_evolution.
Data availability. The viral load data were downloaded from ref. 84.

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Author contributions
R.B.-S. and K.K. jointly conceived the study. R.B.-S. performed within-host model and transmission potential analyses while K.K. performed the pairwise invasibility analyses. Both R.B.-S. and K.K. contributed to analyses and wrote the manuscript.

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