

Why are dengue virus serotypes so distantly related? Enhancement and limiting serotype similarity between dengue virus strains

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Dengue virus, the causative agent of dengue fever, has four major serotypes characterized by large genetic and immunological distances. We propose that the unusually large distances between the serotypes can be explained in the light of a process of antibody-dependent enhancement (ADE) leading to increased mortality. Antibody-dependent enhancement results from a new infection with a particular serotype in an individual with acquired immunity to a different serotype. Classical dengue fever causes negligible mortality, but ADE leads to the risk of developing the significantly more dangerous dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). A mathematical model is presented that describes the epidemiological dynamics of two serotypes of a pathogen where there is the possibility of co-infection and reinfection by a different serotype, along with increased mortality as a result of enhancement. We show that if there is no or slightly increased mortality after reinfection (enhancement), serotypes with a small immunological distance can stably coexist. This suggests that a cloud of serotypes with minor serological differences will constitute the viral population. By contrast, if enhancement is sufficiently great, a substantial immunological distance is necessary for two serotypes to stably coexist in the population. Therefore, high mortality owing to enhancement leads to an evolutionarily stable viral community comprising a set of distantly separated serotypes.

Keywords: dengue virus; dengue haemorrhagic fever; enhancement; genetic distance; coexistence; epidemiology

1. INTRODUCTION

Classical dengue fever is a mosquito-borne disease endemic in South-East Asia, Africa and Central-South America that normally causes a relatively mild illness (Gubler & Kuno 1997). Sufferers recover within one to two weeks after the onset of fever, while mortality during infection is negligibly small (see Gubler & Kuno 1997). However, some individuals develop Dengue haemorrhagic fever (DHF) or Dengue shock syndrome (DSS) (Halstead *et al.* 1970) where the severity of the disease is drastically increased, with an associated mortality rate of as much as 5–15% without clinical care (see Gubler & Kuno 1997). It should be noted that DHF/DSS is caused by dengue strains that normally cause classic dengue fever with its negligible fatality in primary infections and that secondary infection, rather than the differential pathogenicity among strains, is the key risk factor as regards DHF. More specifically, the presence of antibodies to one strain—including those that are maternally derived—leads to a risk of DHF/DSS on subsequent infection with a different strain (Halstead *et al.* 1973; Kliks *et al.* 1988). This effect is thought to be related to the phenomenon observed *in vitro* of antibody-dependent enhancement (ADE) of infection of mononuclear phagocytes observed in dengue and other flaviviruses (Halstead *et al.* 1970; Kliks *et al.* 1988). ADE occurs because the viruses, when complexed with non-neutralizing antibodies, more easily infect cells. As

well as secondary infection, there is some evidence for a number of other risk factors of DHF including variation in serotype virulence. However, even investigators who have examined other risk factors (Vaughn *et al.* 2003) cite reinfection of recovered individuals as the main risk factor in DHF and the hypothesis that DHF is caused by ADE through reinfection is commonly accepted.

The infectious agent of dengue fever, dengue virus (genus *Flavivirus*), has four distinct serotypes, called Den-1, Den-2, Den-3 and Den-4 (Zanotto *et al.* 1996). Since there is a large serological distance between these serotypes (Zanotto *et al.* 1996), it is likely that a host that has recovered and acquired immunity to one of the serotypes can be easily infected by another serotype. The coexistence of the different serotypes of dengue virus is different from the situation in other diseases such as influenza, since dengue shows the coexistence of markedly different types in the same place and at the same time. The antigenic drift of the influenza virus, for example, is a polymorphism in time; different strains (subtypes) occur in different time periods, but the population is nearly monomorphic in terms of subtypes (although there is continuous antigenic diversity within a subtype) (Fitch *et al.* 1997). All four dengue serotypes are considered to have originated in Asian forests and subsequently spread worldwide relatively recently in association with the migration of people, and commerce (Gubler & Kuno 1997). Furthermore, all the serotypes have been documented in Asia, suggesting that they have coexisted in the same geographical region rather than being isolated strains that have mixed through recent human migration.

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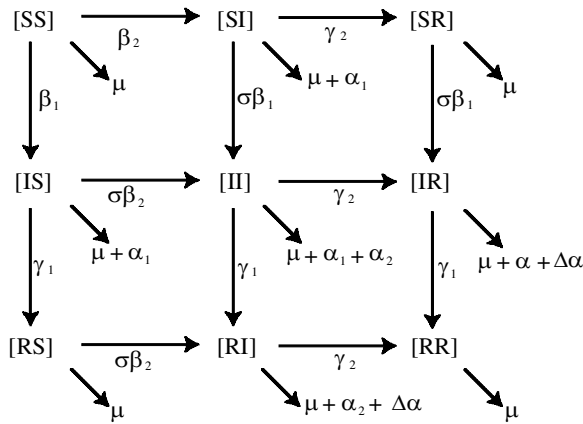


Figure 1. A schematic diagram of the model.

In a recent model, Ferguson *et al.* (1999) examined ADE in viral diseases, focusing on ADE leading to a form of ‘negative cross immunity’ where there is an increased risk of infection in secondary disease. Although they mention the role of ADE as a risk factor in DHF, they do not examine this effect in their model, concentrating on increased transmission in secondary infections. They analysed a two-strain model with constant host population size, where the chance of reinfection is greater than that of primary infection, and found that there is an increased chance of coexistence and cyclic behaviour. The ADE hypothesis that the increased infection rate of cells leads to DHF/DSS in secondary infections is, however, well established (Gubler & Kuno 1997) and for clarity we will concentrate only on this phenomenon in the present paper.

We analyse a simple epidemiological model with susceptible, infected and recovered individuals, where we allow double infection and reinfection by different viral serotypes. The model also takes into account ADE, leading to additional mortality when there is a secondary infection by a different serotype. We then ask how the exclusion and coexistence of serotypes are influenced by the interplay of cross-immunity between the serotypes, and enhancement. We show that mortality enhancement acts in an opposite way to that of transmission enhancement in terms of its effect on the coexistence of strains. In Ferguson *et al.* (1999) the (transmission) ‘enhancement acts to permit the coexistence of all strains where in its absence only one or a subset would persist’, whereas, as we show below, mortality enhancement drives closely related strains to extinction, permitting only the coexistence of distantly related serotypes. The coexistence of distantly related serotypes characterizes dengue virus and our theoretical work therefore supports the hypothesis of ADE leading to DHF and DSS.

2. MODEL AND ANALYSIS

The model is based on the standard ‘SIR’ epidemiological model in which host status is classified as susceptible (S), infected/infectious (I) and recovered (R). We extend the SIR model to include two strains of the pathogen with different serotypes, and include the possibility of double-infection and reinfection by a different strain. Based on the immunological status of the two serotypes, a host may have one of nine states, illustrated in figure 1, where the

left and right letters of a state represent the host status to serotypes 1 and 2, respectively. For example, [SS] denotes a host susceptible to both serotypes, while [IS] is a host infected by serotype 1 and susceptible to serotype 2. Let β_i be the transmission rate of strain i , γ_i the recovery rate from strain i , and α_i the additional mortality caused by a single infection of strain i . Natural mortality of the host is denoted by μ , and new susceptible individuals (to both strains) are produced at an intrinsic growth rate r . K is the maximum population size (carrying capacity).

A host currently infected or recovered from one strain may experience a double-infection or reinfection by a different strain, but at a rate reduced by cross-immunity. Thus the transmission rate of strain i to a host infected by or immune to the other strain is discounted as $\beta_i\sigma$, where the discounting factor σ ($0 < \sigma < 1$) is a measure of the dissimilarity of two serotypes. If σ approaches zero the serotypes are immunologically indistinguishable and cross-immunity is perfect (the strain cannot co-infect or re-infect a host), whereas if σ approaches 1 the serotypes are completely different and no cross-immunity occurs. The other important process is enhancement. This is the additional mortality ($\Delta\alpha$) of a host that has recovered from one strain but is reinfected by another strain.

The density of hosts belonging to each immunological status changes with time:

$$\begin{aligned}
 d[SS]/dt &= r(1 - N/K)N - \beta_1[SS][I\cdot] - \beta_2[SS][\cdot I] - \mu[SS], \\
 d[SI]/dt &= \beta_2[SS][\cdot I] - \beta_1\sigma[SI][I\cdot] - (\mu + \alpha_2 + \gamma_2)[SI], \\
 d[SR]/dt &= \gamma_2[SI] - \beta_1\sigma[SR][I\cdot] - \mu[SR], \\
 d[IS]/dt &= \beta_1[SS][I\cdot] - \beta_2\sigma[IS][\cdot I] - (\mu + \alpha_1 + \gamma_1)[IS], \\
 d[II]/dt &= \beta_1\sigma[SI][I\cdot] + \beta_2\sigma[IS][\cdot I] - (\mu + \alpha_1 + \alpha_2 + \gamma_1 + \gamma_2)[II], \\
 d[IR]/dt &= \gamma_2[II] + \beta_1\sigma[SR][I\cdot] - (\mu + \alpha_1 + \gamma_1 + \Delta\alpha)[IR], \\
 d[RS]/dt &= \gamma_1[IS] - \beta_2\sigma[RS][\cdot I] - \mu[RS], \\
 d[RI]/dt &= \gamma_1[II] + \beta_2\sigma[RS][I\cdot] - (\mu + \alpha_2 + \gamma_2 + \Delta\alpha)[RI], \\
 d[RR]/dt &= \gamma_1[IR] + \gamma_2[RI] - \mu[RR],
 \end{aligned}
 \tag{2.1}$$

where $N = [SS] + [IS] + [RS] + [SI] + [II] + [RI] + [SR] + [IR] + [RR]$ is the total density of hosts, $[I\cdot] = [IS] + [II] + [IR]$ is the density of hosts infected by strain 1, and $[\cdot I] = [SI] + [II] + [RI]$ is that infected by strain 2. Throughout the paper, we assume that the basic reproductive rate of strain 2 is greater than that of strain 1, and that both the following ratios are larger than 1:

$$\frac{\beta_2 K_0}{\mu + \alpha_2 + \gamma_2} > \frac{\beta_1 K_0}{\mu + \alpha_1 + \gamma_1} > 1,
 \tag{2.2}$$

where $K_0 = K(r - \mu)/\mu$, and therefore either of the strains can invade the disease-free host population.

The basic reproductive rate is the expected number of secondary infections from an infected host before it dies or recovers (Anderson & May 1991). Conventional wisdom therefore states that the pathogen strain with the greater basic reproductive rate will invade and replace the strain with a smaller basic reproductive rate (Anderson & May 1991). This principle remains true in our model if $\sigma = 0$ (i.e. if no co-infection or reinfection by another strain occurs because of perfect cross-immunity). However, if co-infection and reinfection are allowed ($\sigma > 0$), coexistence of strains is the outcome. There are important consequences to the evolutionary dynamics once we add mortality enhancement in reinfected hosts. When reinfection is caused by a different strain, and the mortality of reinfected hosts is large, an established viral strain tends

to enjoy an advantage over newly introduced strains. Our objective in this paper is to clarify the interplay between cross-immunity and mortality enhancement in regard to the coexistence of strains. We will study the model by local stability analysis of the marginal equilibria (the equilibria in which only one of the strains exists) and the internal equilibria (the equilibria where both strains coexist), along with extensive numerical simulation of the dynamics. We first analyse the local stability of the marginal equilibria. Next we analyse the local stability of the internal or coexisting equilibrium, the position of which is obtained by numerical root finding. Finally we synthesize the results of local stability analysis to clarify how dynamic behaviour and evolutionary outcome depend on the immunological distance between strains and the degree of mortality enhancement.

(a) *Stability of the marginal equilibria*

There are three marginal equilibria in the system: the disease-free equilibrium and the two equilibria in which only one of the strains exists. As is obvious from assumption (2.2), the disease-free equilibrium is unstable and the population allows the invasion of the disease. The analysis here is therefore focused on the local stability of the two single-strain-endemic equilibria. We examine whether one strain of the pathogen can invade a population that is in endemic equilibrium with the other strain.

Suppose that initially only strain 2 is present and therefore we can set [IS] = [IR] = [II] = [RI] = [RS] = [RR] = 0 in equation (2.1). At equilibrium with only strain 2 we have

$$\begin{aligned} d[SS]/dt &= r(1 - N/K)N - \beta_2[SS][SI] - \mu[SS] = 0, \\ d[SI]/dt &= \beta_2[SS][SI] - (\mu + \alpha_2 + \gamma_2)[SI] = 0, \\ d[SR]/dt &= \gamma_2[SI] - \mu[SR] = 0. \end{aligned} \tag{2.3}$$

The endemic equilibrium of the above is denoted by ([SS], [SI], [SR]) = ($\hat{x}_2, \hat{y}_2, \hat{z}_2$) with

$$\begin{aligned} \hat{x}_2 &= K_0/R_0(2), \\ \hat{y}_2 &= K_0\theta_2 \left[\frac{1}{2} \left(1 - \theta_2\zeta_2 + \sqrt{(1 - \theta_2\zeta_2)^2 + 4\theta_2\zeta_2/R_0(2)} \right) - 1/R_0(2) \right], \\ \hat{z}_2 &= K_0(1 - \theta_2) \left[\frac{1}{2} \left(1 - \theta_2\zeta_2 + \sqrt{(1 - \theta_2\zeta_2)^2 + 4\theta_2\zeta_2/R_0(2)} \right) - 1/R_0(2) \right], \end{aligned} \tag{2.4}$$

where $K_0 = K(r - \mu)/\mu$ is the equilibrium density in the disease-free population, $R_0(2) = \beta_2 K_0 / (\mu + \alpha_2 + \gamma_2)$ is the basic reproductive rate of strain 2, $\zeta_2 = \alpha_2 / (r - \mu)$ is the ratio of pathogenicity of strain 2 to the net growth rate of the host, and $\theta_2 = \mu / (\mu + \gamma_2)$. When a small number of strain 1 are introduced to a population at strain 2-endemic equilibrium, the density of hosts infected by or recovered from strain 1 changes, while they remain rare:

$$\begin{aligned} d[IS]/dt &= \beta_1\hat{x}_2[I] - \beta_2\sigma\hat{y}_2[IS] - (\mu + \alpha_1 + \gamma_1)[IS], \\ d[IR]/dt &= \gamma_2[II] + \beta_1\sigma\hat{z}_2[I] - (\mu + \alpha_2 + \gamma_2 + \Delta\alpha)[IR], \\ d[II]/dt &= \beta_1\sigma\hat{y}_2[I] + \beta_2\sigma\hat{y}_2[IS] - (\mu + \alpha_1 + \alpha_2 + \gamma_1 + \gamma_2)[II], \\ d[RS]/dt &= \gamma_1[IS] - \beta_2\sigma\hat{y}_2[RS] - \mu[RS], \\ d[RI]/dt &= \gamma_1[II] + \beta_2\sigma\hat{y}_2[RS] - (\mu + \alpha_1 + \gamma_1 + \Delta\alpha)[RI], \\ d[RR]/dt &= \gamma_1[IR] + \gamma_2[RI] - \mu[RR]. \end{aligned} \tag{2.5}$$

These linearized dynamics can be decoupled into the equations for the strain 1-infected host densities, [IS], [II] and [IR], and the equations including host densities immune to strain 1. The former is given by

$$\frac{d}{dt} \begin{pmatrix} [IS] \\ [II] \\ [IR] \end{pmatrix} = \begin{pmatrix} \beta_1\hat{x}_2 - \beta_2\sigma\hat{y}_2 - \nu_1 & \beta_1\hat{x}_2 & \beta_1\hat{x}_2 \\ (\beta_1 + \beta_2)\sigma\hat{y}_2 & \beta_1\sigma\hat{y}_2 - \mu_{II} & \beta_1\sigma\hat{y}_2 \\ \beta_1\sigma\hat{z}_2 & \beta_1\sigma\hat{z}_2 + \gamma_2 & \beta_1\sigma\hat{z}_2 - \nu_2 - \Delta\alpha \end{pmatrix} \begin{pmatrix} [IS] \\ [II] \\ [IR] \end{pmatrix}, \tag{2.6}$$

where $\nu_i = \mu + \alpha_i + \gamma_i$ ($i = 1, 2$) and $\mu_{II} = \mu + \alpha_1 + \gamma_1 + \alpha_2 + \gamma_2$. If the equilibrium ([IS], [IR], [II]) = (0, 0, 0) is locally stable, the strain-1-infected densities converge to zero, and then the density of hosts immune to strain 1 also goes to zero. Therefore it is sufficient to examine the local stability of the zero solution of equation (2.6) to determine the invasibility of strain 1. By exchanging the role of strains, we can also obtain the condition for the invasibility of strain 2 to the strain-1-endemic population.

The results of local stability analysis using Routh-Hurwitz conditions (Murray 1989) for the Jacobian matrix \mathcal{J} (the matrix on the right-hand side of equation (2.6)) are summarized in figure 2. The region for local stability of the marginal equilibria is shown in the parameter space of the two most important parameters in the present context, cross-immunity σ (from $\sigma = 0$ for perfect cross-immunity to $\sigma = 1$ for no cross-immunity), and increased mortality owing to enhancement, $\Delta\alpha$. Strain 2, with its greater basic reproductive rate, can always invade a population with strain 1 alone (figure 2). Strain 1 (the strain with a smaller basic reproductive rate), by contrast, can invade an established population of strain 2 only when the degree of enhancement is less than a threshold that depends on σ . The condition for strain 1 to invade a strain-2-endemic population is given by $\det(\mathcal{J}) < 0$ or $\Delta\alpha < (\Delta\alpha)_c$, where the threshold enhancement $(\Delta\alpha)_c$ as a function of σ is illustrated in figure 2. If we assume that the host natural mortality μ and pathogenicity α_1 and α_2 for single strain infection are small relative to the recovery rates, $\gamma_2, \mu/\gamma_i = O(\epsilon), \alpha_i/\gamma_i = O(\epsilon)$ (where $O(\epsilon)$ means ‘sufficiently small’, as found in dengue), the invasibility condition is greatly simplified:

$$R_0(1) > \frac{R_0(2)(\Delta\alpha + \gamma_1)}{\Delta\alpha + (1 - \sigma + \sigma R_0(2))\gamma_1} + O(\epsilon), \tag{2.7}$$

where $R_0(i)$ is the basic reproductive rate of strain i defined before. If there is no or slight mortality enhancement ($\Delta \rightarrow 0$), strain 1 can invade the population with strain 2 when

$$R_0(1)/R_0(2) > 1/[1 + \sigma(R_0(2) - 1)]. \tag{2.8}$$

Thus, strain 1, which is characterized by a lower basic reproductive rate than strain 2, is able to invade the population when $\sigma > \sigma_c \equiv (R_0(2)/R_0(1) - 1)/(R_0(2) - 1) > 0$, i.e. when the immunity caused by previous infection by strain 2 is not perfect against the reinfection of strain 1. Interestingly, this effect of promoting coexistence of a competitively inferior strain (having a fixed ratio f lower basic reproductive rate than the resident, $R_0(1)/R_0(2) = f < 1$) is stronger when the pre-existing strain has a larger basic reproductive ratio (i.e. the right-hand side of equation (2.8) decreases with $R_0(2)$). At the limit of perfect cross-immunity ($\sigma \rightarrow 0$), the invasibility condition is reduced to $R_0(1) > R_0(2)$, i.e. only a strain with a greater basic reproductive rate than the resident strain can invade. By contrast, at the limit of no cross-immunity ($\sigma \rightarrow 1$), the invasibility condition for strain 1 becomes $R_0(1) > 1$, i.e.

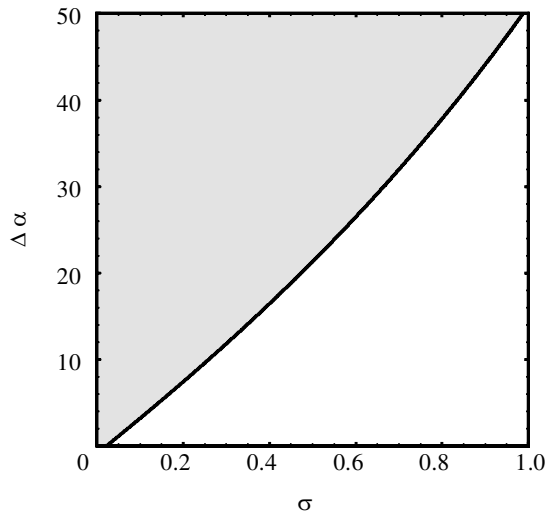


Figure 2. The local stability of the marginal endemic equilibria with only one strain, plotted against the invasion of the other strain. The parameter space of immunological distance σ between the serotypes (horizontal axis) and the degree of mortality enhancement $\Delta\alpha$ in reinfection (vertical axis) is divided into two regions, according to the local stability of two marginal equilibria. The dividing curve indicates the threshold mortality enhancement (equation (2.7)) for the invisibility of strain 1, with less basic reproductive rate R_0 . Strain 2, with a larger R_0 , can always invade the population with only strain 1. In the grey region strain 2 can repel invasion by strain 1; in the white region both strains allow the invasion of the other. Parameters are $\alpha_1 = 0.01$, $\alpha_2 = 0.02$, $\beta_1 = 0.025$, $\beta_2 = 0.05$, $\gamma_1 = 0.9$, $\gamma_2 = 0.7$, $\mu = 0.01$, $r = 1$, $K = 1000$.

any strain that can invade the disease-free population can also invade a population with the other strain. This effect of imperfect cross-immunity in promoting serotypic diversity is greatly diminished by the presence of mortality enhancement, as shown in equation (2.7). At the limit of strong enhancement, $\Delta/\gamma_1 \gg 1$, the invisibility condition (2.7) for strain 1 approaches $R_0(1) > R_0(2)$, i.e. only a strain with a greater basic reproductive rate than that of the resident can invade the population.

Equation (2.7) characterizes the way that cross-immunity and mortality enhancement affect the coexistence of strains. To summarize: (i) imperfect cross-immunity ($\sigma > 0$) greatly promotes the invasion of competitively inferior strains (those with smaller R_0 than the resident strain); (ii) this effect is stronger when the resident strain has a greater basic reproductive ratio; (iii) mortality enhancement, by contrast, reduces the chance of invasion by a new strain—high enhancement virtually restricts invasion to only those strains that have a greater basic reproductive rate than that of the resident. Note again that equation (2.7) is only valid for small natural (μ) and first-infection-induced (α) mortalities and the exact invisibility condition is given by $\det(\mathcal{J}) < 0$ (figure 2).

Combining these two results for the local stability of the marginal equilibria, there are two different outcomes when a new strain is introduced to a population (figure 2). If the mortality increment $\Delta\alpha$ by enhancement is greater than the critical enhancement $(\Delta\alpha)_c$, only the strain with the greater basic reproductive rate can repel invasion of

the other strain. If enhancement is less pronounced ($\Delta\alpha < (\Delta\alpha)_c$), both marginal equilibria are locally unstable, allowing invasion of the other strain. This implies that we should find two strains coexisting in the population and that both strains are protected from extinction as long as $\Delta\alpha < (\Delta\alpha)_c$.

(b) Stability of the two-strain equilibrium

The above analysis is based only on the local stability of the marginal equilibria, and it is assumed that the invading strain has a very low density. However, even when the invasion of a second strain with low density cannot occur, a population may stably maintain both strains, and may also allow a mass invasion of a large number of the new strain introduced to the population by processes such as colonization. In our model there is a region in which one of the marginal equilibria and the internal equilibrium for co-circulation are simultaneously stable. To demonstrate this we need to examine the local stability of the internal equilibrium, and we therefore numerically analyse the bifurcation diagram of the system. We have chosen σ as the bifurcation parameter, with the stable and unstable steady states and the maximum and minimum of limit cycles plotted for the density of [SI] when σ is continuously varied (figure 3).

Figure 3*a* illustrates a typical bifurcation diagram for a relatively small degree of mortality enhancement ($\Delta\alpha = 7.5$). Four different phases arise when we increase σ from $\sigma = 0$ (perfect cross-immunity) to $\sigma = 1$ (no cross-immunity). (i) When σ is close to 0, strain 1 (having smaller R_0) goes to extinction and the population will reach a stable endemic equilibrium with only strain 2. (ii) When σ is just above the first threshold, which is the threshold (2.7) calculated from the analysis of marginal equilibria (figure 2), strain 1 is now able to invade. The population then maintains both strains in a stable endemic equilibrium. (iii) When σ exceeds the second threshold this internal equilibrium loses stability and a Hopf bifurcation takes place. The population then shows stable limit cycles with a nearly synchronized burst of both strains. (iv) When σ is further increased, the limit cycles coalesce into a stable equilibrium. The population again maintains both of the strains at steady densities of infected individuals.

If the degree of enhancement is sufficiently large ($\Delta\alpha = 20$, figure 3*b*; $\Delta\alpha = 40$, figure 3*c*), the bifurcation diagram is qualitatively different from that illustrated in figure 3*a*. The dynamic behaviour of the two ends of the σ axis are the same as before: strain 1 goes to extinction if σ is sufficiently small, and both strains are stably maintained at steady densities if σ is sufficiently large. However, the region of intermediate σ has a different pattern. The region for the limit cycles disappears, and instead a region of bi-stability arises. In the bi-stable region the population converges to either internal equilibrium with both strains or to marginal equilibrium with only strain 2, depending on initial conditions. This phase diagram is summarized in figure 4.

When bi-stability arises at intermediate σ (figure 3*b,c*), the infected density of strain 2 is larger in the population converged to a co-circulating equilibrium than in the population converged to the strain-2-only equilibrium.

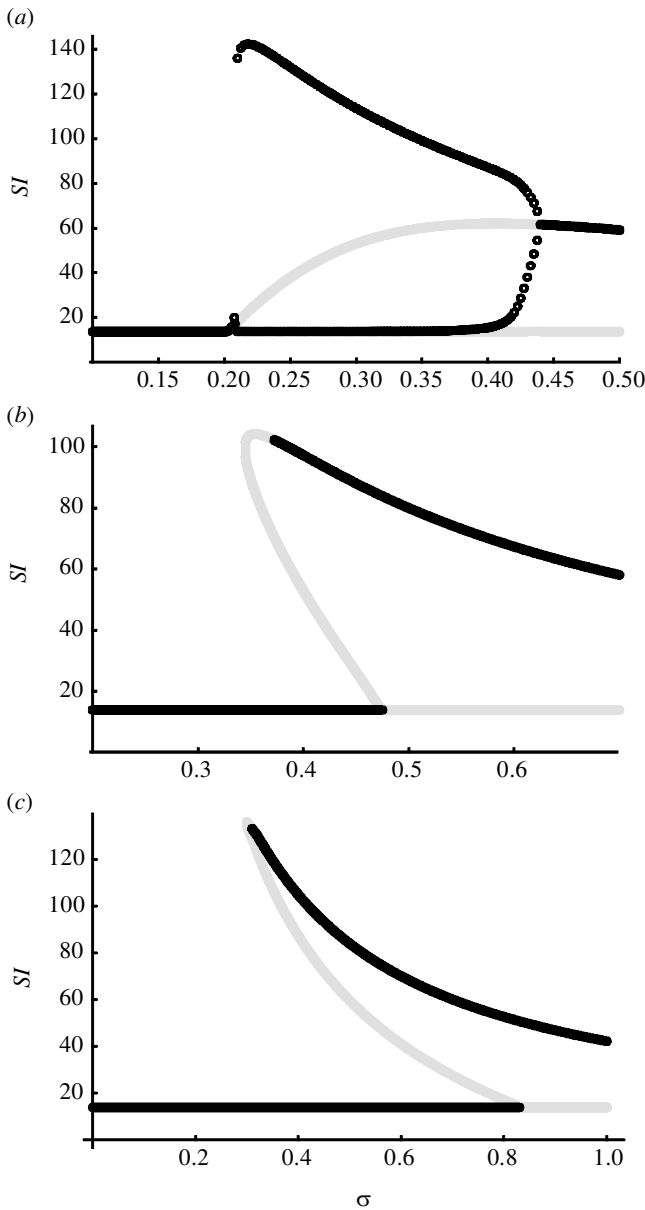


Figure 3. A bifurcation diagram illustrating the stable (solid line) and unstable (grey line) steady state, and the maximum and minimum of limit cycles (open circles) for the density of [SI] (host infected by strain 2 but susceptible to strain 1). The cross-immunity parameter σ is chosen as a bifurcation parameter. The three panels are the bifurcation diagrams for different degrees of mortality enhancement $\Delta\alpha$ when σ is continuously varied from $\sigma = 0$ (perfect cross-immunity) to $\sigma = 1$ (no cross-immunity): (a) $\Delta\alpha = 7.5$, (b) $\Delta\alpha = 20$; (c) $\Delta\alpha = 40$. The parameters other than σ and $\Delta\alpha$ are the same as in figure 2.

Thus the presence of the competing strain (strain 1) increases the density of strain 2. This apparent 'cooperative' behaviour (which might be wrongly interpreted as an indication of transmission enhancement at the population level) is caused by a reduction in the recovered density in the co-circulating population by mortality enhancement. As reinfected hosts have a far greater mortality rate, the recruitment rate of susceptible hosts is greater in the co-circulating population than in the population with only strain 2.

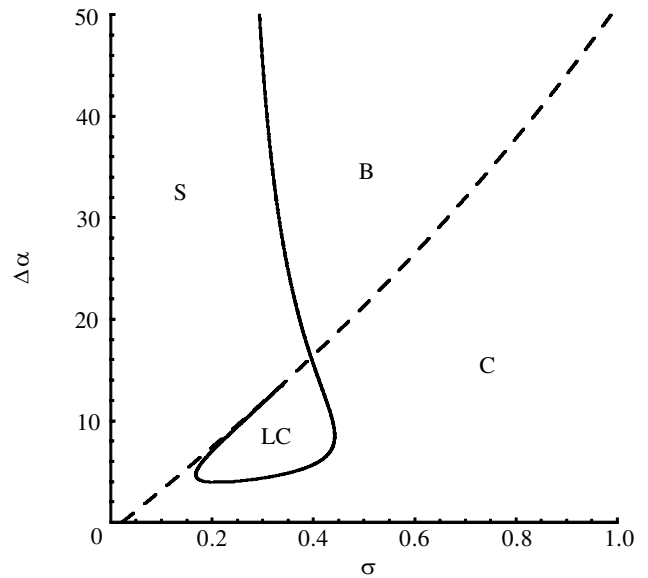


Figure 4. Phase diagram drawn from the stability analysis of the two marginal equilibria with single strain populations, and that of the internal equilibrium with both strains, in the parameter space of σ and $\Delta\alpha$. In region S, only the marginal equilibrium with strain 2 is stable (strain 2 only). In region C, only the internal equilibrium is stable (steady co-circulation). In region B, both the internal equilibrium and the marginal equilibrium with only strain 2 are simultaneously stable. The population then converges to one of the two equilibria depending on the initial conditions (bi-stable region). In region LC, the population converges to stable limit cycles of both strains (co-circulation with limit cycles). The solid curve dividing the strain-2-only region from the bi-stable and steady co-circulation regions is the same as depicted in figure 2. The broken curve is drawn from numerically obtained bifurcation points in the parameter space of internal equilibrium—the stable and unstable branches of internal equilibrium are obtained by continuation through numerical root finding. Local stability is determined by evaluating the eigenvalues at internal equilibrium. The parameters are the same as in figures 2 and 3.

3. DISCUSSION

By developing a mathematical model we have examined in detail the interplay between immunological distance as a correlate of the degree of partial cross-immunity and antibody-dependent enhancement leading to increased mortality. With little or no enhancement the coexistence of closely related strains is found, but as enhancement increases, coexistence is only possible between distantly related strains. We have also demonstrated several interesting behaviours including the possibility of cyclic behaviour leading to bursts of coexisting strains as well as bi-stability between coexistence and the existence of a single strain.

Our key result is that increased enhancement leads to the need for a greater immunological distance for strains to coexist. Without enhancement our model would predict that a cloud of closely related serotypes would coexist. This is a fairly common situation in nature and can be seen within the tick-borne encephalitis complex, which also belongs to flaviviruses, as does the dengue virus (Zanotto *et al.* 1996). In contrast, the dengue viruses show

four distinct serotypes. The mortality enhancement described in dengue is an unusual phenomenon and we are unaware of other diseases where it has been proposed (though ADE has been observed *in vitro* in other flaviviruses and HIV) (Takeda *et al.* 1988). Our model demonstrates that mortality enhancement can explain the observed serological distance, and the fact that this highly unusual phenomenon is associated with the highly unusual evolutionary pattern of the dengue viruses makes us confident that enhancement explains the pattern of serotypes seen in dengue.

Enhancement produces a selective force favouring an established or a commoner serotype. If a serotype exists in a local population, a large fraction of the population is immune to it. In particular, in pathogens such as dengue whose severity in primary infection is low and the recovery rate high (the infectious period is typically one to two weeks in dengue fever), the fraction of recovered individuals is high while the proportion of susceptible individuals is low. A new serotype introduced to the population must therefore mainly spread through hosts that have already been infected by the wild-type. Thus, to establish itself a new serotype must override a 'wall of enhancement'—a large additional mortality that the wild-type did not suffer when it established itself in the disease-free population. The consequence of this is that a new mutant can invade a population that is already endemic with another strain only if it is immunologically distant from the wild-type. A new strain then gains the advantage of exploiting hosts that have recovered from the wild-type but who are still partly susceptible to the new strain, and it thereby overcomes the disadvantage of enhancement. In other words, those serotypes closely related to one of the pre-existing serotypes will be culled out when they are introduced by either mutation or migration. It follows therefore that only those serotypes that are sufficiently distant from each other can be stably maintained in the host population. This is the putative reason that we propose, on the basis of the dynamic model, why dengue virus serotypes are so distantly related.

The results of theoretical studies suggest that cross-immunity strongly affects the patterns of outbreaks when more than two serotypes are stably or transiently co-circulating (Andreasen *et al.* 1997; Feng & VelascoHernandez 1997; Gomes *et al.* 2002) and when new serotypes are continuously introduced by mutation (Pease 1987; Adler 1991; Haraguchi & Sasaki 1997; Gog & Grenfell 2002). A genetic model with a number of pathogen strains has shown that cross-immunity can also promote the coexistence of distantly related genotypes (Gupta *et al.* 1998). When a continuum of antigenic strains compete, sufficiently strong cross-immunity may destabilize the evolutionary and epidemiological dynamics, causing outbreaks to occur discontinuously in both time and genotype space (Haraguchi & Sasaki 1997). This is an example of the discreteness principle arising in a variety of ecological/evolutionary phenomena (Sasaki & Ellner 1995, 1997; Ellner & Sasaki 1996; Sasaki 1997; Sasaki & Godfray 1999)—the evolutionarily stable population/community consists of a set of distinctly distant genotypes/species, as a consequence of graded competition between similar phenotypes in a continuous trait space. Mortality enhancement, however, greatly exaggerates this tendency.

The dynamic behaviour of the coexistence equilibrium is interesting. As the degree of cross-immunity increases we may move from stable coexistence to a period of limit cycles, returning to stability again at even higher cross-immunity. It is important to note the possibility of limit cycles as this predicts the episodic outbreak of multiple strains simultaneously. This would result not only in bursts of dengue fever, but also, more importantly, bursts of DHF. It may be that this intrinsic epidemiological dynamic is driving the noted outbreak dynamics of DHF (Gubler & Kuno 1997). At higher levels of enhancement we see a different pattern. Now instead of limit cycles we find bi-stability between the coexistence of the strains and one strain alone. Since the outcome depends on initial conditions, in the more stochastic natural world we might expect to find flips between co-circulation of strains and periods where only one strain exists. We would in this case predict that there would be periods of classical dengue fever followed by periods of DHF/DSS.

To conclude, our model provides support for the hypothesis that reinfection in recovered individuals leads to DHF/DSS. This mortality enhancement can explain the unusual phylogeny that we see in dengue. It should be noted that enhancement makes the control of dengue viruses difficult. Antibody production against dengue in a vaccinated host may increase their risk of developing DHF/DSS if they are reinfected. In addition, vaccination itself may cause DHF/DSS in a host previously infected by a different wild dengue virus. A theoretical understanding of the role of enhancement in the epidemiology and evolution of dengue, in addition to immunological and pathological studies, is therefore of great practical importance in the control of dengue.

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