Dengue Virus–Mosquito Interactions

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Abstract
The mosquito Aedes aegypti is more widely dispersed now than at any time in the past, placing billions of humans at risk of infection with one or more of the four dengue viruses. This review presents and discusses information on mosquito-dengue infection dynamics and describes the prominent role that temperature and rainfall play in controlling dengue viral transmission including discussions of the effect of interannual climate variations and the predicted effect of global warming. Complementary human determinants of dengue epidemiology include viremia titer, variation in viremic period, enhanced viremias, and threshold viremia. Topics covered include epidemiological phenomena such as traveling waves, the generation of genetic diversity of dengue viruses following virgin soil introductions and in hyperendemic settings, and evidence for and against viral virulence as a determinant of the severity of dengue infections. Also described is the crucial role of monotypic and heterotypic herd immunity in shaping dengue epidemic behavior.
**INTRODUCTION**

The mosquito *Aedes aegypti* enjoys greater geographical distribution at present than at any time in the past, and is established in virtually all tropical countries. As a result, approximately 2 billion humans are at risk to infection with one or more of the four dengue viruses (36). These viruses evolved in subhuman primates from a common ancestor and were separately introduced into the urban cycle some 500 years ago (86). The burning issues today do not center on the amazing expansion of *A. aegypti* but rather on the complex causal mechanisms underlying the origin and spread of severe dengue disease (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS). This review focuses on integrative aspects of dengue virus: mosquito interactions and human contributions to dengue epidemic behavior.

*A. aegypti* evolved in Africa, where a sylvatic, ancestral form, *A. formosus*, is enzootic in East and Central Africa (83). The domesticated form spread with the slave trade to the Americas, while in Asia it accompanied commerce and colonization. *A. aegypti* larvae and pupae prefer clean water in many different types of artificial containers. Eggs laid on the walls of containers resist desiccation for months and hatch when submerged in water. Females display a strong anthropophilia and a marked tendency to interrupted and multiple feedings prior to completing a gonotrophic cycle (24, 92). Female mosquitoes are infectious for life (75).

**MOSQUITO DETERMINANTS OF DENGUE EPIDEMIC BEHAVIOR**

**Mosquito Infection Dynamics**

The size and biological status of mosquito vector populations are critical to transmission dynamics of the dengue viruses. The density requirements for transmission, basic reproduction number, and spatial heterogeneity of *A. aegypti* breeding, effect of temperature, and rainfall contribute importantly to infection of mosquitoes by dengue viruses.

**Mosquito density required for transmission.** The minimum density of *A. aegypti* that permits transmission of dengue viruses has long been a topic of fierce debate. For example, a house index of 5 was selected as the target for control of urban yellow fever in Quito, Ecuador (78). In Singapore, where vector density had been held to a house index of less than 1 for many years, dengue infections continued to occur over a long period (10). This does not represent sustained indigenous transmission but undoubtedly is the result of the constant introduction of viremic hosts into Singapore from nearby dengue-endemic countries (60).

**Basic reproduction number.** The basic reproduction number, $R_0$, is the number of secondary infections that results from a single infected individual.
Some formulas are designed to be used early in epidemics and others for endemic settings. The application of these formulas are based on assumptions: (a) that an epidemic is introduced by an infected individual into a virgin soil where all at-risk persons are susceptible, (b) that humans mix homogeneously, (c) that population size is so large that accretion of those that are immune is negligible, and (d) that $R_0$ is measured only during the initial phase of an epidemic. Recent estimations of $R_0$ have been made using one of three methods: (a) final size equation estimated from a serological measurement of an epidemic (48), (b) initial intrinsic growth rate using data from the epidemic curve at the initial stage (50), and (c) age distribution of antibodies (18).

Basic reproduction numbers estimated for dengue range between 1.33 and 11.6. Early estimates by Koopman et al. (48), Newton & Reiter (58), and Marques et al. (50) were relatively low. Using age-stratified dengue-neutralizing antibody data obtained in an area of high dengue endemicity, Ferguson et al. (18) derived dengue type-specific $R_0$ values of 4.3–5.8. Favier et al. (15) analyzed epidemic data from several cities in Brazil and developed values ranging from 3.8 to 5.1, and Massad et al. (52) used data from other Brazilian communities and developed values ranging from 2.7 to 11.6.

Many of these measurements suffer from limitations in epidemiological, entomological, or serological data or methodologies. Studies that estimate the total number of infected persons during an epidemic using the hemagglutination inhibition (HI) or the IgG enzyme-linked immunosorbent assay (ELISA) tests are unable to measure type-specific dengue antibodies and may confuse infections with other flaviviruses with past dengue infection. For example, an HI antibody study in Mexico conducted in 1986 putatively measured dengue 1 transmission, a virus that had been introduced into the Western Hemisphere in 1977, but may also have measured previous dengue 4 and dengue 2 infections, these viruses having been introduced into the region in 1981 (48). Additionally, this study may have undermeasured dengue antibody prevalence because the HI test is insufficiently sensitive to detect the low antibody titers generated in response to inapparent infections. Calculations of $R_0$ made by counting reported cases are likely to be biased by underreporting and by an unknown fraction of silent infections. Even dengue 1, a virus that produces a high rate of clinically apparent disease in adults, results in mild or inapparent infections in children. Thus, in Brazil, as much as 40% of the population may not participate in data used to calculate reproduction numbers (15, 50, 52).

**Spatial heterogeneity.** Particularly in North and South America, *A. aegypti* infestations are not uniformly present throughout residential areas. Well understood by entomologists, the problem of spatial heterogeneity was identified by modelers during a virgin soil epidemic on Easter Island. Heterogeneity occurred because not all houses contained *A. aegypti*; their occupants were at low risk of dengue 1 virus infection. When the authors applied corrections for heterogeneity, they improved simulated epidemic curves for the Easter Island outbreak as well as for outbreaks reported in Belém and Brasília, Brazil (16, 17).

**Effect of Temperature and Rainfall**

The epidemic behavior of dengue viruses seemingly correlates closely with fluctuations in temperature and rainfall. This tautology is challenged by frequent outliers. For example, dengue outbreaks on the Indian subcontinent frequently occur during the hot, dry season because *A. aegypti* breeds abundantly in the reservoirs of desert coolers. *Aegypti*-borne viral diseases were widespread in temperate latitudes during the Little Ice Age (1600–1700 AD) because water for human consumption was stored in rain barrels, which supported the populations of mosquitoes needed to transmit viruses that were introduced during summer seasons.

**ELISA:** enzyme-linked immunosorbent assay
Temperature. Experiments to measure survival of adult *A. aegypti* in the field are notoriously noisy, but a consensus value lies somewhere between 0.87 and 0.91 attrition per day in most locations in the dengue-endemic countries of Southeast Asia (22). The integral of the survival time (Sa) provides the average life span of the female; for Sa = 0.89 the average life span is approximately 8.6 days. Because the population declines exponentially with age, and although the majority of females die at an early age, the tail of this distribution contains the rare but older individuals with the potential to transmit dengue viruses. When a female takes an infectious bite on her first day of life and if the length of time required for infection to disseminate is just one day, a substantial portion of the average life span will have passed. Most females are not capable of transmission prior to their deaths. Once it is disseminated to salivary glands, the probability of transmitting virus varies with biting frequency, which is related to the length of the gonotrophic development period. *A. aegypti* females are observed to take multiple potentially infectious bites per replete feed, as high as two or three interrupted feeding attempts with resumption on the same or different host. From an epidemiological perspective, the increased number of interrupted feedings per replete feed as a result of a two- or three-degree increase in temperature is equivalent to a doubling of the density of *A. aegypti*.

Temperature affects the length of the gonotrophic cycle, contributing another factor correlated with seasonality of dengue in tropical Southeast Asia (62). Under circumstances in which a majority of breeding sites are indoors, warm temperature and high moisture contribute to increased adult survival. In addition, warmer temperatures shorten the extrinsic incubation period (EIP). However, the lowest daily temperature, rather than the average temperature, is thought to be the most important determinant of dengue transmission seasonality in Bangkok (92).

Rainfall. *A. aegypti* population changes may correlate with various weather phenomena. Daily, seasonal, and interannual variability in temperature, atmospheric moisture, and rainfall can influence mosquito populations and vectorial competence in a variety of ways. Two examples illustrate contradictory roles of rainfall on *A. aegypti* populations.

In the late 1950s, the World Health Organization, at the request of the government of Thailand, set up an *Aedes* Research Unit to study the ecology and control of *A. aegypti*. The hypothesis tested was that during the warm, rainy season the density of vector mosquitoes increased, which correlated with annual outbreaks of dengue. A series of year-long studies was conducted from 1966 to 1968 in the residential compound of a Buddhist temple. Temple housing was similar to residences outside the compound, with typical types of water-filled containers, primarily large 100- to 200-liter water storage jars, flower pot plates, and ant traps. *A. aegypti* was the only mosquito breeding in most of the containers (62, 74, 82). Throughout the study period the number of water-filled containers and the proportion containing *A. aegypti* were remarkably constant. With the exception of a portion of the ant traps, all containers were manually filled and not influenced by rainfall. It was observed that in the mosquito population under study there were no fluctuations in adult production and densities in response to rainfall. Instead, there was a seasonal increase in adult survival due to temperature and atmospheric moisture. The association of dengue epidemics with rainfall could be explained by increases in adult survival and feeding activity of the vector mosquito.

In contrast, longitudinal studies in Puerto Rico demonstrated a positive correlation between rainfall and vector abundance, strongest in the dry, south coastal portions of the island (55). Adult abundance varied not with temperature but with the availability and productivity of water-holding containers. In contrast with household breeding in Bangkok, most breeding in Puerto Rico occurred...
outdoors and in rain-filled containers, primarily animal watering dishes and discarded tires. Container productivity was limited not by temperature or oviposition but by larval survival, ultimately driven by the amount of food present or formed photosynthetically within the container (55).

Data demonstrating an increase in mosquito populations with the onset of the rainy season were obtained from studies conducted in 1962 in several Bangkok locales by workers at the Southeast Asia Treaty Organization (SEATO) Medical Research Laboratory. Five areas in Bangkok were monitored for mosquito populations with human-baited traps. Collectors were present in each of these areas from 0400 to 1300 and from 1500 to 2300 5 days a week for the entire year. A total of 3674 A. aegypti were captured as they attempted to feed on collectors (J.E. Scanlon; personal communication, Annual Progress Report, SEATO Medical Research Laboratory, Bangkok, Thailand).

These yielded 21 strains of dengue virus and 7 strains of chikungunya virus, an alphavirus transmitted by A. aegypti that was endemic in Bangkok at that time. The population of A. aegypti females collected in human-baited traps began with the onset of the rainy season and anticipated the DHF hospitalization curve by two months (Figure 1).

The Influence of Interannual Climate Variation

The El Niño Southern Oscillation (ENSO) is an atmosphere-ocean coupled system that produces quasi-periodic short-term climate and sea surface temperature changes over the Pacific region with impacts on weather worldwide, including many countries in the Americas, Africa, and Asia. This system oscillates between two extremes known as El Niño and La Niña that are associated with approximately opposite disturbances to climate worldwide. A chief phenomenon of an El Niño phase is an eastward extension of warm surface waters situated off northwestern Australia toward the west coast of equatorial South America. During the cool phase, La Niña, equatorial westerlies result in an upwelling of cold abyssal water that is transported to the west, creating a tongue of abnormally cool surface waters.

Figure 1

A. aegypti collections and DHF cases, Bangkok 1962 (J.E. Scanlon, personal communication 1963; Annual Progress Report, SEATO Medical Research Laboratory, Bangkok, Thailand).
extending toward Indonesia. Because convection rainfall in this region is limited to sea surface temperatures greater than approximately 26°C–27°C, the spatial distribution of rainfall is associated with equatorial sea surface temperature anomalies associated with the ENSO state. The “Southern Oscillation” refers to the oscillation of atmospheric pressures between the eastern and western Pacific. One indicator statistic of the ENSO state is the southern oscillation index (SOI), the normalized difference in pressure between Darwin and Tahiti. El Niño and La Niña are associated with negative and positive values of the SOI, respectively. Much of the interannual variability in climate in the central Pacific has been attributed to the state and intensity of ENSO.

Given that dengue incidence is a function of the interaction of many factors, it is not surprising that dengue activity has been correlated with the ENSO state or one of its statistics, SOI in regions (most clearly in the South Pacific region) where ENSO or SOI is correlated with temperature and/or rainfall anomalies (34). The ENSO has not been shown to affect periodicity of dengue activity in Southeast Asia independent of factors such as herd immunity and infection enhancement (89).

HUMAN DETERMINANTS OF DENGUE EPIDEMIC BEHAVIOR

Viremia Titer, Variation in Viremic Periods, and Enhanced Viremias

The size of the virus inoculum, the product of viral titer and quantity of blood meal taken by the insect, influences the probability that the vector will subsequently disseminate infection to the salivary glands (25, 70). The duration of the dissemination period can vary with viremia titer. Watts et al. (87) reported that the EIP for dengue in A. aegypti at 30°C was 12 and 25 days for mosquitoes infected with high and low doses, respectively. The classic experiments on human volunteers by Siler (75) and Simmons (76) established that the intrinsic incubation period averaged between 4.5 and 7 days, with a small number of cases exceeding 10 days, and that viremia may ensue 6 to 18 h before onset of fever. The symptomatic viremia is 4 to 5 days but may be as long as 12 days (75, 76). The duration of viremia may be a function of viral dose delivered by infected mosquitoes (59).

Cummings et al. (13) examined the epidemiological impact of enhanced viremia on the prevalence and persistence of viral serotypes. Using a dynamical system model of n cocirculating dengue serotypes, the authors observed that antibody-dependent enhancement (ADE) provides a competitive advantage to those serotypes that undergo enhancement compared with those that do not, and

Global Warming

It is frequently predicted that increases in mean temperatures will increase dengue transmission in temperate countries. This prediction is premised upon a hypothesized temperature restriction to the geographic distribution of A. aegypti (63). This hypothesis is simply false. The history of dengue and yellow fever epidemics in the United States provides the strongest possible evidence of a lack of geographical limit to its distribution. During the eighteenth and nineteenth centuries, A. aegypti–borne viral diseases, yellow fever and dengue, were widespread in U.S. cities along the Atlantic and Gulf seaboards and throughout the Mississippi River basin (65). A. aegypti merely required the introduction of viremic travelers during the summer season to produce outbreaks of dengue and yellow fever (64). Initially, both diseases were controlled in the United States using anti-Aedes measures such as oiling standing water and septic tanks or sealing mosquito-breeding rain barrels. These efforts were succeeded enduringly by the ubiquity of modern air-conditioned houses serviced by reliable piped water so that American cities no longer provide ample A. aegypti breeding sites due to phenomena completely independent of temperature (66).
that this advantage increases with increasing numbers of cocirculating serotypes. Paradoxically, there are limits to the selective advantage provided by increasing levels of ADE, as greater levels of enhancement induce large-amplitude oscillations in incidence of all dengue virus infections, threatening the persistence of both enhanced and nonenhanced serotypes. Their results suggest that enhancement is most advantageous in settings where multiple serotypes circulate, and where a large host population is available to support pathogen persistence during the deep troughs following ADE-induced large-amplitude oscillations.

In all dengue infections, viremia peaks shortly after the onset of fever. For those individuals who develop severe disease and are at risk of death, peak viremia has occurred before the onset of incapacitating symptoms and signs when they are ambulatory and fully exposed to mosquitoes. An assumption often made in mathematical models of dengue infection is that fatal cases (individuals with enhanced viremia) are removed from the population (by death) and thus not accessible to vector mosquitoes, resulting in the failure of dengue viruses to survive (89). For the reasons stated above, this assumption is false. Based on the assumption that enhanced viremia occurs only in individuals who are symptomatic during secondary dengue infections, the fraction of all dengue infections with enhanced viremia is small, possibly only 10% of second dengue infections (38).

**Threshold Viremia Required to Infect Mosquitoes**

The threshold of viremia in humans required to infect mosquitoes has not been measured accurately. In natural infections, virus titers in humans rise as high as 10^8 Mosquito Infectious Doses_50_ (MID$_{50}$) per ml. In monkeys, dengue viremia was as high as 10^6 MID$_{50}$, yet 10%–15% of *Aedes albopictus* mosquitoes allowed to feed on these monkeys were infected. As for the impact of dengue strain variation, a considerable degree of variation in the EIP has been observed. For example, with mosquitoes feeding on humans infected with an unadapted strain of dengue 1, the EIP was 14 days, whereas with mosquitoes feeding on humans infected with the strains at low mouse passage levels, the EIP was 22 days (71). Long EIPs have been observed with dengue virus strains with attenuation characteristics. Often, although infection is established, mosquitoes fail to transmit virus (4, 53).

**Household Transmission**

Once an infective mosquito enters a house or a member of a household becomes infected, the probability of multiple infections in the household increases and may result in clusters of dengue infections (57). In a Chinese household, 29 of 30 members were infected (93). Multiple DHF patients per household have been documented. In Thailand, among 271 families in which at least one member contracted DHF, 35 (12.9%) had two or more DHF patients per household (41).

**Dispersal of Virus**

Dispersal of dengue viruses is by human agency. Over the years public health authorities have directed attention to the spread of dengue viruses in infected mosquitoes by implementing mosquito abatement programs at international airports and spraying adulticides into passenger cabins of arriving aircraft. These efforts to prevent the introduction of dengue-infected mosquitoes are not supported by evidence. Viremic humans are the most likely source of the importation of dengue viruses throughout the world.

**Traveling waves in endemic countries.**

Since the early vector control campaigns against urban yellow fever in the Americas, it was recognized that viruses were maintained in and spread from urban centers. Studies by Cummings et al. (12) demonstrated that
Dengue viruses reside in and spread out of large urban centers in the highly endemic countries of Southeast Asia. When hospital statistics from each of the 72 provinces of Thailand from 1983 to 1997 were analyzed, Bangkok, a metropolitan area of over 9 million people, was found to be the country’s endemic center. Longitudinal studies on dengue patients hospitalized in Bangkok Children’s Hospital from 1973 through 1999 demonstrated a roughly three-year periodicity between large outbreaks as well as a pattern of successive predominance of different dengue viruses: dengue virus type 1 (DENV-1) in 1990–1992, DENV-2 in 1973–1986 and 1988–1989, DENV-3 in 1987 and 1995–1999, and DENV-4 in 1993–1994. Following these periodic increases and decreases in hospitalization rates and successive predominating dengue viruses, waves of severe cases progressed from the capital in all directions, moving at a speed of 148 km per month.

**Importation and spread of dengue viruses.**
A significant part of the history of dengue viruses in the mid-twentieth century involves the reintroduction of *A. aegypti* into areas previously under vector control and the subsequent importation of dengue viruses. The most important importation route has been from Southeast Asia to North and South America. During the first half of the twentieth century the only dengue virus known to circulate in North and South America was the DENV-2 American genotype. In 1963, a dengue 3 virus of Asian origin was imported into Puerto Rico, producing an epidemic (57). After traversing the Caribbean Islands and possibly producing outbreaks in Venezuela and Colombia, this virus apparently became extinct. In 1977, a dengue 1 of Southeast Asian origin was imported into the Americas. In 1981, the region’s first epidemic of DHF was reported, caused by an imported Asian strain of DENV-2 distinct from the American subtype circulating previously (28, 49). In addition, in 1981, DENV-4 subtype II of Asian origin was recovered in the Americas and caused epidemics of dengue fever (DF) throughout the region. Finally, in 1994, genotype II DENV-3 was recognized first in Nicaragua and subsequently circulated widely throughout the Caribbean and throughout South America.

Despite the importance of these epidemics, little is known about the rates or determinants of viral spread among island and mainland populations or their directions of movement. A Bayesian method of a coalescent approach was used to investigate transmission histories and a parsimony method to assess patterns of strain migration of DENV-2 and DENV-4 after their introduction in 1981. Using isolates from 1981 to 2004, Carrington et al. (9) studied the dispersal of these two viruses. For both types of viruses there was an initial invasion phase characterized by an exponential increase in the number of DENV lineages, after which levels of genetic diversity remained constant despite reported fluctuations in DENV-2 and DENV-4 activity. Strikingly, viral lineage numbers increased 16-fold more rapidly in DENV-4 than in DENV-2, indicating a more rapid growth rate or a higher rate of geographic dispersal of DENV-4. The most obvious explanation for the more rapid spread of DENV-4 is that the American population was fully susceptible to DENV-4 but partially immune to DENV-2 from long exposure to DENV-2 American genotype. It is also possible that immunity to either/or DENV-1 or DENV-3 raised antibodies capable of protecting against DENV-2 infections or enhancing DENV-4 viremias, thus accelerating epidemic spread.

Each of the four dengue viruses of Asian origin in the American region has evolved after the introduction of single strains in the late 1970s and early 1980s. The transmission dynamics of the remaining two serotypes of dengue virus in the Americas might also reflect the immunological landscape. Epidemiological evidence suggests that when DENV-1 first appeared in the Americas its pattern of spread was similar to that of DENV-4. This serotype was first reported in Jamaica in

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1977 (61), and within only one year it spread throughout the region with at least 30 countries reporting activity (8). Preliminary data on DENV-3 isolates from the Americas suggest that this virus, which was absent from the region for 17 years before it was reintroduced in 1994, has expanded at a faster rate than DENV-2 but not as rapidly as DENV-4 (9). Hence, some protective immunity against DENV-3 may remain, although this conclusion is tentative because the time frame and overall host population size differ.

Repeated introductions. In islands in the Pacific, a more complex pattern of viral strain introduction has been found. Outbreaks of dengue due to DENV-1 occurred almost simultaneously in 2001 in Myanmar and at multiple sites almost 10,000 km away in the Pacific (1). Phylogenetic analyses of the E protein genes of DENV-1 strains recovered from Asia and the Pacific revealed three major viral genotypes (I, II, and III), each having distinct clades. The majority of strains from the Pacific and Myanmar, and a number of other Asian strains, fell into genotype I. Genotype II comprised a smaller set of Asian and Pacific strains, and genotype III contained viruses from diverse geographical localities. Analyses suggested that outbreak of DENV-1 in the period 2000–2003 in the Pacific was due to multiple introductions of DENV-1 from several different Asian localities (Palau, Western Samoa—Philippines; New Caledonia, Fiji—Myanmar/Thailand; New Caledonia, Tahiti—Malaysia; and New Caledonia—Cambodia). For example, it was learned that approximately 2500 individuals from Oceania visit Thailand each year, providing a ready source of human hosts to transport dengue viruses back to their country of origin. It is remarkable that only DENV-1 viruses resulted in overt disease in Oceania despite the evidence that all four dengue viruses were circulating in Southeast Asian countries and thus should have resulted in the importation of viruses other than DENV-1. It is likely that other dengue serotypes were introduced to the Pacific that did not result in overt disease or did not result in sustained cycles of transmission possibly because protective immunity might have prevented sustained transmission (46).

Pre-infection Factors and Early Infection Events

Various preinfection factors that may influence the outcome of dengue infections include infection parity, protective immunity, herd immunity, enhancing antibodies, age, sex, race, and nutritional status. These have been discussed in detail elsewhere (37, 38). Factors of interest to this review are described.

Infection parity. The most fundamental epidemiological observation relating to DHF and DSS is that they occur regularly in locales where two or more dengue viruses are simultaneously or sequentially epidemic. In these locales, DHF and DSS occur in two immunological settings: (a) in individuals age one year old and older who have been infected with two or more different dengue viruses at intervals of at least one to more than 20 years, and (b) in dengue hyperendemic areas in individuals under one year of age who circulate passively acquired dengue antibodies and who are infected with a dengue virus for the first time.

Early evidence that two infections are required to produce DHF/DSS was embedded in the shape of the curve describing age distribution of hospitalized patients in the highly dengue-endemic city of Bangkok. In 1970, Fischer & Halstead (21) explored in a mathematical model the age distributions resulting from first, second, third, and fourth dengue infections. The best fit was a two-infection model, which when applied to actual data for the years 1958–1964 reconstructed the 1964 age distribution of patients in Bangkok Children’s Hospital almost exactly. When this study was undertaken, the intrinsic age susceptibility to dengue vascular permeability syndromes was unknown.
Subsequently, it was observed that the youngest children are more susceptible than older children and adults to vascular permeability during a second dengue infection (30). This innate susceptibility erases a mathematical requirement that severe disease accompanies infections spaced apart by less than five years (21). DHF and DSS have occurred when the interval between first and second infections was nearly 30 years (2).

Finally, second infections have been associated with large, classical outbreaks of DHF/DSS. This is best illustrated in Cuba, where two different dengue viruses were introduced sequentially into a largely susceptible population. In 1977–1979, dengue 1 circulated throughout the country in a population that had not been exposed to any dengue infections since just prior to World War II (49). Neutralizing antibody studies showed the earlier dengue infection to be DENV-2, in all likelihood American genotype dengue 2. As many as 50% of individuals who were 40 years of age and older were immune to dengue 2, and in 1997 half of these individuals were infected by dengue 1. None of these sequential dengue 2–dengue 1 infections produced severe disease. In 1981, a dengue 2 virus of Southeast Asian origin introduced into Cuba produced DHF/DSS in persons immune to DENV-1 and then infected with DENV-2. Older individuals infected with dengue 2 prior to World War II were protected against DHF/DSS. In 1997, 20 years after the introduction of dengue 1, an Asian genotype dengue 2 again circulated in the city of Santiago de Cuba and environs (32). Individuals immune to dengue 1 but infected with dengue 2 developed overt disease, including DHF/DSS. Although the viruses causing the 1981 and 1997 epidemics were genetically similar, when comparing attack rates and case fatality rates in the same age groups, the 1997 epidemic was more severe than that in 1981 (28, 33, 69).

Viremic tertiary dengue infections have been reported in rhesus monkeys (39, 40). In prospective cohort studies, a few DHF/DSS cases have been documented to accompany infection with a third dengue infection (M.P. Mammen, personal communication). The fraction of total DHF/DSS contributed by tertiary dengue infections is unknown. Studies on rhesus monkeys have shown that animals immune to three different dengue viruses were susceptible to infection with the fourth virus (40). What fraction of humans previously infected with three dengue viruses may be susceptible to a fourth infection and whether clinical disease accompanies a fourth dengue infection are not known.

### Herd immunity
Values of $R_0$ can be used to estimate herd immunity. The higher the value of $R_0$, the bigger the fraction of the population that must be immune to stop transmission. As illustrated by Ferguson et al. (18), approximate levels for herd immunity (or the desired level of immunization of the population using vaccines), $p$, may be derived given the relationship $p > 1 - 1/R_0$. Where many dengue viruses are endemic, vaccine consists of a cocktail of antigens, and the magnitude of $p$ will be set by the virus type with the highest $R_0$ value. For example, for a $R_0$ value of 5.6, the magnitude of $p$ is 0.82. In other words, to protect against continuing circulation of dengue viruses, roughly 85% of the population would have to be immunized.

The value of the reproduction number and herd immunity are also affected by spatial heterogeneity of the distribution of vector mosquitoes (see above). For example, in Cuba, in 1977, dengue 1 was introduced into a population whose only previous dengue experience was with type 2 before World War II. This resulted in a mild island-wide epidemic that failed to stimulate massive vector control. The epidemic continued until it stopped spontaneously in 1979. An island-wide serological survey detected HI antibodies in 44.5% of the population (51). This prevalence of dengue antibody does not mean that dengue 1 transmission stopped because 44.5% of the
population was immune. Clearly, a significant fraction of Cuban households was not infested with A. aegypti.

The protective effect of heterotypic dengue immunity. Sabin was the first to demonstrate in human volunteers that heterotypic immunity can prevent disease by a different dengue virus. This was observed when DENV-2 was given at an interval of less than 3 months after DENV-1 (71). In dengue-endemic areas of Asia where premises indices approach 100% and there is ongoing circulation of multiple serotypes, heterotypic antibodies must profoundly affect herd immunity. Unfortunately, this phenomenon, although real, has attracted little scientific interest. The clearest instance of cross-reactive heterotypic immunity was the cross-reactive neutralization of American genotype DENV-2 viruses by human sera containing monoclonal DENV-1 antibodies. In this situation, DENV-1 immunity did not appear to prevent DENV-2 infections, but partial immunity may have downregulated infections, permitting only mild disease during secondary dengue infections (47).

Dengue Virus Virulence

Different strains of the same virus appear to vary in their ability to cause overt disease or inapparent infections (27). When infection with an organism is associated with disease the microorganism is said to be pathogenic. Differences in pathogenicity may be illustrated by responses to DENV-2 infections in susceptible adults. Sabin and Schlesinger (71, 72) observed overt DF in susceptible volunteers infected with DENV-2 New Guinea C virus, as did physicians in Singapore during an outbreak of a DENV-2 cosmopolitan genotype. In contrast, no disease accompanied primary infections with DENV-2 genotype III strains in the 1997 Cuban outbreak (29). This latter strain might be referred to as nonpathogenic during a primary infection.

An explanation frequently given for the occurrence of DHF/DSS is that the infecting virus is virulent, whereas viruses that cause only DF are nonvirulent. Virulence is best understood as a ratio of severe or mild outcomes to the total number of individuals infected with an organism. For example, rabies is a classical virulent virus, as nearly all infections result in death. The closer the ratio is to 1, the more virulent the virus. The American genotype DENV-2 is said by some workers to be nonvirulent on the basis of laboratory observations that the ability of this virus to grow in cell cultures and mosquitoes is reduced compared with an Asian genotype DENV-2 (11, 67). From these observations it has been predicted that in nature this virus will be poorly transmitted by mosquitoes and that disease in humans will be mild (11). However, these predictions are not consistent with field observations. In 1995, a large epidemic of American genotype DENV-2 occurred in the Amazonian city of Iquitos, Peru (88). This virus must have been transported up the Amazon River to Peru, a process undoubtedly accompanied by silent infections. The arrival of dengue virus in Iquitos implies its prior efficient transmission from humans to mosquitoes to humans by bites of geographically dispersed and genetically distinct populations of A. aegypti.

Because severe dengue disease occurs consistently with secondary dengue infections, virulence must be defined in a two-infection context. A prior dengue infection somehow alters human susceptibility to permit a virulent infection. If virological factors are involved, they likely reside in antigenic features in common between the first and second infecting viruses. An excellent example is the neutralization of American genotype DENV-2 by human antibodies to DENV-1. These results suggest that the lower virulence of American genotype DENV-2 results from a DENV-1-like surface epitope on this virus that permits partial neutralization (and downregulation of disease) by DENV-1 antibodies (47).
INTEGRATIVE DETERMINANTS OF DENGUE EPIDEMIC BEHAVIOR

Mosquito Passage and Viral Genetics

The availability of huge numbers of partially or completely sequenced dengue viruses has led to the growth of major analyses of the genetic changes observed in populations of viruses belonging to a single dengue type as well as hints about the survival of one dengue virus population with respect to another. By necessity, almost all dengue viruses have been obtained from diseased humans. A continuing effort has been made to find consistent differences in viruses recovered from severe versus mild human disease. A recent trend has been to study the transmission of dengue viruses in foci—family members of index cases. The recovery of viruses from silently infected humans should provide an even stern test of the hypothesis that different viruses are associated with disease compared with inapparent infections.

Founder or stochastic effect. Dengue viruses may be lost during the cool season in northern portions of Northern Hemisphere tropical areas. This loss may be due to any or a combination of the following: temperature, low mosquito populations, and herd immunity. In these settings, dengue viruses may be annually reintroduced from more southerly locations each year (56). This reintroduction maintains the mix of serotypes and genotypes within a region. When a genotype does disappear, it may be reintroduced from a single virus or limited genetic pool (bottleneck). A similar stochastic effect is reported for relatively isolated islands of the Pacific that are too small to maintain dengue viral endemicity (1). When virus does arrive, the introduced strain may have been active earlier on larger islands or imported from mainland dengue-endemic countries.

Single introductions: generation of genetic diversity. Genetic changes in DENV-4 viruses, which circulated at irregular intervals, causing disease in Puerto Rico, illustrate how dengue viruses evolve over short periods (6). The genetic structures of 82 viruses from the years 1982, 1986–1987, 1994, 1996, and 1998 were sequenced and analyzed. No significant changes were observed in structural (E) genes, meaning that host immunity played no role in the evolutionary shifts observed. This is in contrast to hemagglutinin (HA) gene of influenza A, which appears to be under strong immunologic selection (7). In dengue virus, constraints on the E gene may be imposed by the need to preserve functions that condition survival in a two-host life cycle (5, 80).

Rates of nucleotide substitution in arboviruses are lower than those seen in many other RNA viruses (45, 90). Against this conservative background genomes steadily accumulated changes, often nonsynonymous changes in the nonstructural gene NS2A. As an example of this process, a dominant Puerto Rican lineage twice descended from earlier rare genotypes, a pattern that suggests that much of the lineage turnover is driven by selection on viral genotype.

The changes described above for DENV-1 and DENV-4 viruses provide evidence for rapid selective and adaptive evolution of dengue viruses from a single introduction. This adaptive evolution is driven by the stochastic nature of the dengue virus life cycle, which favors survival of common variants. Genetic bottlenecks occur at every mosquito feeding event, during seasonal reductions in vector populations, and at variations in the abundance of susceptible human hosts (26).

An interesting example of the evolution of genetic changes was documented during the DENV-2 epidemic in 1997 in Santiago de Cuba. During this outbreak, case fatality rates had increased month by month, a phenomenon that also had been documented during the 1981 DENV-2 Cuba-wide epidemic (31). It had been hypothesized that an observed month-by-month increase in disease severity might result from serial infection of those immune to DENV-1 with Asian
genotype DENV-2 viruses, each successive generation having been selected to escape cross-reactive neutralization from DENV-1 antibodies and thus is more susceptible to the enhancing effects of these same antibodies in the next infected host (31). Available isolates from 1997 were sequenced to look for changes in envelope protein that might signify emergence of neutralization escape mutants. These changes were not found (69). However, significant changes observed in NS5 were indicative of a clear pattern of virus evolution during the epidemic (68). The origin and meaning of these changes require further study.

**Endemic dengue: generation of genetic diversity.** When sampled in single locales in endemic countries, dengue viruses have shown significant and rapid changes in the genetic structure typified by lineage replacement on phylogenetic trees (1, 6, 77, 91). The absence of a proofreading capacity in RNA-dependent RNA polymerases gives rise to approximately one nucleotide change in a dengue virus genome during each cycle of replication (79). Although the majority of mutations that arise within each host are likely to be deleterious, such variation may give rise to diverse viruses able to occupy new ecological niches or to adapt to selective pressures (44). Analyses of the ratio of nonsynonymous to synonymous nucleotide changes per site (dN/dS) within and between DENV populations suggest they are subject to strong purifying selection (6, 84), with relatively little evidence for adaptive evolution (43, 44, 91).

A lack of longitudinal studies of the evolution of DENV at single locations has hampered an understanding of how frequently extinctions of DENV genotypes might have occurred or what evolutionary processes are responsible. The extinction of a lineage of DENV-1 in Myanmar has been documented recently (81). Phylogenetic analyses of the sequences of DENV-1 genomes confirmed that three distinct clades (from two different genotypes) circulated in Myanmar since the early 1970s, when DHF was first recognized in that country, (54) and that one of these clades (clade A, genotype III) became extinct after 1998 to be replaced by genotype I viruses from two other clades (B and C). The replacement genotype I clades originated in Asia. In three other instances sudden changes in the genotype of dengue viruses have been observed; the changes also appeared to be due to stochastic events resulting in population bottlenecks (77, 81, 91).

**RESEARCH CHALLENGES**

Genetic analyses of dengue viruses suggest that the four dengue viruses evolved from a common ancestor in subhuman primates, presumably in populations separated for long periods (85). Although active and passive antibody enhancement of dengue viremias have been observed in monkeys (23, 35, 42), the phenomenon has been most thoroughly studied in humans. Exactly how enhancement phenomena contribute to the maintenance of the four dengue viruses in the urban cycle during the modern era is unclear. Given the massive number of infections, the tendency of RNA viruses to make transcriptional errors, pressure from heterotypic dengue antibodies, and stochastic events, it is a continuing mystery that there are still only four immunologically distinct dengue viruses. This review has merely scratched the surface of the complex interactions between host, mosquito, and viruses. Key unanswered questions abound. This cursory review suggests that the analytic tools available today are more powerful than the strength of the data derived from field studies. In the past, much attention has been directed at the visible spectrum of dengue virus infections, whereas a small number of ecological studies suggest that an entirely different universe exists—one composed of silent dengue infections. Badly needed are longitudinal multidisciplinary studies of both urban and sylvatic cycles, studies capable of tracking silent as well as overt dengue infections.
SUMMARY POINTS

1. The current distribution of dengue viruses in tropical countries results from the effect of temperature and rainfall. Dengue transmission is almost always seasonal, cases increase during warm, rainy seasons.

2. Important effects of temperature are the shortening of the extrinsic incubation period, increase in biting frequency and extension of the average life span of mosquitoes. In some settings, rainwater collections increase breeding sites.

3. While suspected, the data supporting an influence of interannual climate variations and of global warming on virus transmission are not convincing. Dengue transmission is closely related to urban poverty, the lack of universal piped water and air conditioning.

4. The importation and spread of dengue viruses are related to human determinants of dengue epidemic behavior. Crucial to successful transmission is the quantity of virus in blood. However, the precise threshold viremia needed for transmission of dengue viruses is unknown.

5. The maintenance of dengue viruses is controlled in large measure by the basic reproduction number, the number of new infections that derive from an index case. This number is related, in turn, to herd immunity, a number that when known, offers a vaccination target to stop transmission and protect the un-vaccinated.

6. A phenomenon that controls transmission of dengue viruses to mosquitoes and also determines the outcomes of human infections is sequential dengue infection that enhances viremia in possibly 10% of instances. Enhanced viremia during second dengue infections is controlled by the protective effect of heterotypic immunity.

7. It is widely held that severe disease is due to dengue virus virulence. While there are examples that different strains of dengue viruses differ in pathogenicity, notably in island outbreaks, severe dengue disease has been confined to settings in which antibody enhancement of infection is possible.

8. At the population level, clades of viruses or whole genotypes appear and disappear largely as a result of founder or stochastic events in which herd immunity or seasonal lows in vector populations result in loss of populations of viruses. The modern history of dengue is characterized by genetic diversity that contributes to the complex phenomena governing the survival of dengue viruses and the diseases they produce.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

21. Model demonstrates the dependence of the age at which Bangkok children are hospitalized for dengue hemorrhagic fever on the two-infection causal phenomenon.


Severe dengue disease was observed in adults infected with dengue 1 and then dengue 2 20 years later, demonstrating there is no upper limit to the dengue sensitization phenomenon.


15.16 Halstead
62. During the hot season in Thailand the gonotrophic cycle was 3 days but delayed by two days during the cool season, which reduced mosquito-man contact.

65. Histories of three mosquito-borne diseases in North America reveal climate is not a determinant of disease prevalence or range.

75. Classic study of dengue 4 in which extrinsic incubation period was measured for A. aegypti feeding on human volunteers.

86. Dengue viruses 1, 2, and 4 recovered from Malaysian and West African monkeys descended from a common ancestor and were transferred to humans within the past 500 years.