Invasion and Maintenance of Dengue Virus Type 2 and Type 4 in the Americas†

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Dengue virus type 4 (DENV-4) was first reported in the Americas in 1981, where it caused epidemics of dengue fever throughout the region. In the same year, the region's first epidemic of dengue hemorrhagic fever was reported, caused by an Asian strain of dengue virus type 2 (DENV-2) that was distinct from the American subtype circulating previously. 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. We employed a Bayesian coalescent approach to investigate the transmission histories of DENV-2 and DENV-4 since their introduction in 1981 and a parsimony method to assess patterns of strain migration. For both 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 far more rapidly for DENV-4 than DENV-2, indicative of a more rapid rate of exponential population growth in DENV-4 or a higher rate of geographic dispersal, allowing this virus to move more effectively among localities. We propose that these contrasting dynamics may reflect underlying differences in patterns of host immunity. Despite continued gene flow along particular transmission routes, the overall extent of viral traffic was less than expected under panmixis. Hence, DENV in the Americas has a clear geographic structure that maintains viral diversity between outbreaks.

Dengue viruses (DENVs) are single-stranded, positive-sense RNA viruses (family *Flaviviridae*) that exist as four antigenically distinct serotypes (DENV type 1 [DENV-1] to DENV-4), each of which harbors phylogenetically defined "subtypes" (21). Although the majority of infections (particularly primary infections) are asymptomatic (5, 45, 46), all four viruses are associated with dengue fever (DF), a febrile illness that in a minority of cases progresses to the life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Primary infection provides lifelong immunity against the infecting serotype but increases the risk of DHF/DSS upon secondary infection with another serotype (20, 23, 41). This increase in risk is most likely the result of cross-reactive antibodies (20, 23, 34) and memory T cells (14, 29, 30, 39) which enhance infectivity and are directly involved in the pathophysiology of DHF/DSS.

Dengue diseases are presently the most important arboviral infections of humans. Current estimates are that the virus is endemic in more than 100 countries, with some 50 million dengue infections recorded each year, including 500,000 cases of DHF/DSS that are serious enough to require hospital treatment (48). Disease burden is greatest in Southeast Asia, where DHF/DSS is a leading cause of death in children (15, 17) and where all four serotypes and the principal vector, the *Aedes aegypti* mosquito, are endemic. Worldwide population growth and urbanization, coupled with rapid and frequent global travel, have facilitated the emergence of epidemic dengue in regions outside Southeast Asia, with dramatic increases in DHF/DSS incidence.

This change in disease pattern accompanying a rise in hyperendemicity (i.e., the co-occurrence of multiple serotypes in the same locality) is particularly apparent in the Americas (18). Initially, dengue in this region occurred only as geographically restricted, largely self-limiting outbreaks of DF (16), and DENV-2 was the only serotype confirmed (first isolated in Trinidad in 1953 [1]). Between 1947 and 1963 there was no evidence of epidemic dengue, presumably a result of the Pan American Health Organization mosquito eradication campaign to control yellow fever. However, as support for the program waned, *A. aegypti* reinfestation occurred, and regional epidemics of DF associated with DENV-3 and DENV-1 took place in 1963 and 1977, respectively. The region's first major DHF/DSS epidemic occurred in 1981, when an Asian DENV-2 strain, designated either subtype III (32) or the "Asian-American" subtype (42), was imported into the region (9). The association of this virus with DHF/DSS led to the proposal that it is more virulent than the previously existing subtype V virus (or "American" subtype), which rarely caused severe disease (8, 38, 46). However, the 1981 DENV-2 epidemic followed an

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epidemic of DENV-1 in 1977 and was thus also associated with an increased number of secondary infections (19). The role of hyperendemicity in influencing disease dynamics in the Americas can therefore not be ignored.

The year 1981 was also when DENV-4 was first reported in the Americas. This invading strain, designated subtype II, was also of Asian origin (31). It spread rapidly throughout the region, causing DF but only sporadic cases of DHF/DSS (16). Compared to that of DENV-4, the progress of DENV-2 subtype III following its introduction was less easy to track, since serological testing would not have distinguished between the old (subtype V) and the new strains. By the late 1990s it was suggested that DENV-2 subtype III had displaced the American subtype V in regions where the two might compete (8), although the latter clearly has refugia in some regions of South and Central America (12, 46) and is probably more widespread but goes unreported due to an association with mild disease only (46). More recently, the spread of DENV-2 and DENV-4 in the Americas has been investigated through phylogenetic analysis (12, 13). This revealed a high degree of gene flow and temporally structured lineages, with any geographic patterning present most likely reflecting the strength of economic and cultural ties, which (in addition to geographic distance) influence the amount of viral movement among countries (12, 13).

During 2001, more than 600,000 cases of dengue infection were reported in the Americas, including 15,000 cases of DHF/DSS (48). Despite the renewed importance of dengue in this region, relatively little is known about the epidemiological dynamics of the virus in this region. However, this is central both to understanding the nature of any immune interplay between cocirculating serotypes and predicting future patterns of spread. We used Bayesian coalescent methods to compare the transmission dynamics of two dengue virus serotypes with very different epidemiological profiles in the Americas: DENV-4, which the host population would not have encountered before, and DENV-2, for which an apparently more virulent strain arrived into a region where the population had already been exposed to another member of its serotype. We considered a 20-year period from the time the viruses were first detected to the present. In addition, we undertook a thorough analysis of the phylogeography of DENV in the Americas, particularly to explore the process of viral gene flow and population subdivision in a mainland-island context and how this might influence the maintenance of genetic diversity.

MATERIALS AND METHODS

Data sets. Envelope (E) gene sequences of DENV-2 subtype III and DENV-4 subtype II isolated in the Americas were collected from GenBank and manually aligned using the Se-Al program (http://evolve.zoo.ox.ac.uk/software.html). Where multiple sequences were available from a particular country in a given year, a maximum of five unique sequences were included. Such sampling removed the bias toward particular countries and did not greatly affect parameter estimates (see below). One new DENV-4 sequence, D4.CRA1993, was also included (accession number AY934757; sequenced as described in reference 4). DENV-4 sequences comprised the complete E gene (1,485 bp). However, as the majority of DENV-2 subtype III E gene sequences consisted of 1,404 bp, longer sequences were trimmed to this length. Previous studies revealed no evidence of recombination in these data (4, 12, 13). In total, this resulted in data sets of 89 taxa representing 21 countries for DENV-2 and 62 taxa representing 16 countries in the case of DENV-4 (see Table S1 in the supplemental material). Figure S1 in the supplemental material shows the locations within the Americas of countries and regions represented. Finally, the numbers of countries reporting dengue

activity each year were taken from both the literature (6) and the Pan American Health Organization's dengue timeline (www.paho.org/english/hcp/hct/vbd/dengue_timeline.xls). Neither source distinguishes between different subtypes of DENV-2.

Phylogenetic analysis. Maximum-likelihood (ML) trees were estimated using the general time-reversible (GTR) $+\Gamma_4 + I$ model of base substitution. Parameter values for the GTR substitution matrix, base composition, gamma distribution of among-site rate variation (with four rate categories, Γ_4), and proportion of invariant sites (I) were estimated from the data (available from the authors on request). To assess the robustness of particular groupings, bootstrapping was performed using 1,000 replicate neighbor-joining trees under the ML substitution model. All analyses were performed using PAUP* (40).

Migration among geographic regions. Migration histories were inferred using a parsimony method (35) on ML trees (estimated as described above) for each American data set, rooted with an Asian member of the same subtype (D2.D80-3141/80 or D4.Indo/73) as an outgroup. This analysis was performed using the software program MacClade (33). Several analyses were undertaken for each serotype, considering migration between (i) mainland and islands, (ii) geographic regions (i.e., Central America, South America, Greater Antilles, and Lesser Antilles), (iii) economic groups (i.e., Caribbean Community and Common Market [CARICOM] member countries and nonmembers), and (iv) cultural/historical groups (i.e., Latin American, English-speaking West Indian, Dutch Antillean, and French Antillean).

In each analysis, isolates were assigned a particular "state" dependent on the country of origin (see Table S2 in the supplemental material). Given the ML phylogeny and the defined isolate states, the minimum number of "state changes" needed to give rise to the observed distribution of states was estimated using parsimony. Since both trees contained polytomies, these calculations were performed on 1,000 randomly resolved trees, thereby giving an estimate of the number of state changes across 1,000 trees. Ambiguous changes were excluded. To determine the expected number of changes under the null hypothesis of complete mixing (panmixis) among states, the states of all isolates were randomized 500 times, and for each randomization the number of changes in state was calculated in exactly the same manner. The total number of changes was summed across all 500 randomizations and divided by the number of replicates. The difference between the mean number of observed and expected changes for each pair of states indicates the level of genetic isolation (difference of <0) or migration (difference of >0). This approach was repeated for each of the cultural, economic, and geographic categories designated. P values for each category were calculated by comparing the total number of observed state changes to the null distribution of the total number of state changes expected under panmixis (including ambiguous changes in both cases).

Substitution rates and population demography. Overall rates of evolutionary change (nucleotide substitutions per site per year), tree root ages, and demographic histories were estimated using the software program BEAST, which employs a Bayesian Markov chain Monte Carlo approach to infer population genetic histories from molecular sequences (10, 11). The method considers the genetic differences among viruses sampled at different times to produce a sampled set of probable trees. It then uses the distribution of coalescent events in the sampled trees to estimate relative genetic diversity. In the absence of population subdivision, this diversity equals $N_e\tau$, where N_e is the effective population size and τ is generation length. If population structure is present, then this value is inversely related to the rate at which population diversity is lost by genetic drift (both population size and population structure help to maintain genetic diversity through time). Statistical uncertainty in the data is reflected in the 95% high-probability density values. Analyses were carried out using the Bayesian skyline plot (11), which does not use a prespecified model of demographic history. The data were subsequently analyzed under constant, exponential, and logistic models of population growth. The likelihoods of these models were compared using Akaike's information criterion, and exponential growth rate (r) estimates were used to calculate population doubling times (λ) in years, using $\lambda = \ln(2)/r$, although it should be noted that the coalescent methods used cannot easily distinguish changing population size from population subdivision.

RESULTS

Phylogeography of DENV in the Americas. ML trees for DENV-2 (subtype III; Asian-American strain) and DENV-4 (subtype II) were estimated (Fig. 1) and each taxon classified according to the geographic region from which it originated, whether its origin was island or mainland, the country's mem-
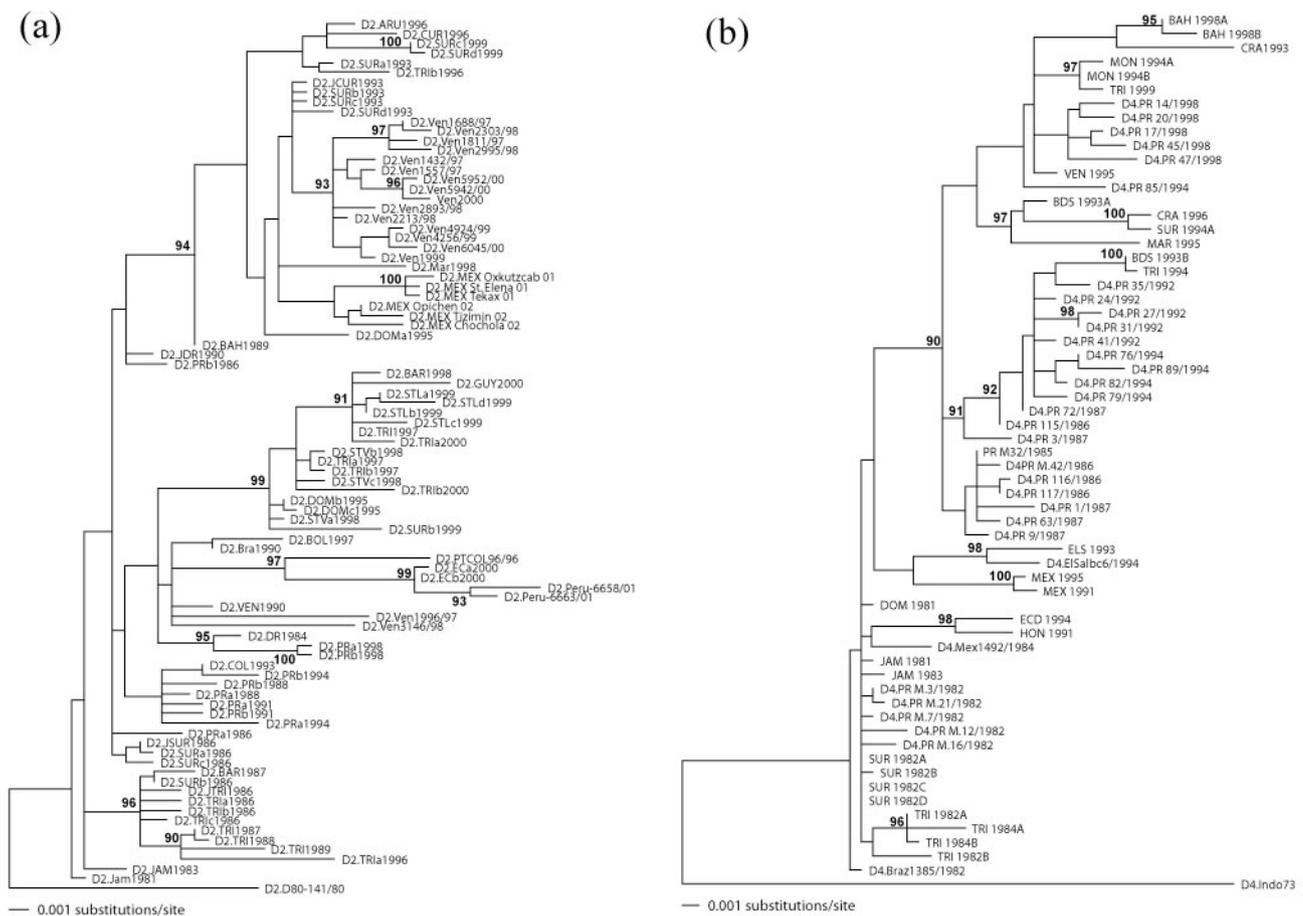
FIG. 1. ML trees of (a) DENV-2 subtype III and (b) DENV-4 subtype II from the Americas. Bootstrap support values above 90% are indicated, and all horizontal branch lengths are drawn to scale. In both cases an Asian outgroup sequence is included to root the trees. The names of American isolates include reference to country of origin and year of isolation. Abbreviations used for country names are given in Table S2 in the supplemental material.

bership in CARICOM, and whether the culture was best defined as West Indian, Latin American, Dutch Antillean, or French Antillean. A parsimony-based method revealed that for both serotypes the total number of migration events occurring in each category was less than expected under the null hypothesis of panmixis (Table 1) (differences between totals of <0; $P \leq 0.002$). Hence, there is population subdivision by location, and factors other than geographic proximity are important in determining the amount of gene flow between countries. Importantly, however, there were incidences of migration (differences of >0) between groups within categories. For DENV-2, migration occurred from the Lesser Antilles to South America and from the Greater Antilles to the Lesser Antilles and South America, but not in the opposite directions. Also, while there was less movement than expected between islands and mainland (total difference of <0 for this category), DENV-2 movement tended to be in the direction from island to mainland (difference of >0 in this direction but <0 for mainland to island). Conversely, the movement of DENV-4 tended to be in the opposite direction, from mainland to island. These data therefore suggest DENV-4 migrations from South America to the Lesser Antilles and Greater Antilles and from Central

America to South America, the differences indicating migration were smaller (closer to zero) than those for DENV-2.

When movement between groups based on socioeconomic factors was considered, similar patterns were observed for DENV-2 and DENV-4. In both cases movement from CARICOM to non-CARICOM members was closer to that expected under panmixis than movement in the opposite direction, which was much less than expected. Likewise, when cultural/historical factors were considered, both were suggestive of a relative isolation of Latin American countries, particularly with respect to movement from Latin American to West Indian countries.

Transmission histories. Using a Bayesian Markov chain Monte Carlo method, we estimated that the most recent common ancestors for DENV-2 (subtype III) and DENV-4 in the Americas existed in about 1980. Both viruses show a similar demographic pattern: an "invasion" phase, characterized by a rapid increase in the number of DENV lineages, followed by a "maintenance" phase, during which relative genetic diversity remains approximately constant (Fig. 2). Although the DENV-2 skyline plot (Fig. 2a) suggests a rapid increase in genetic diversity around 1982, this change is not statistically

TABLE 1. Differences between observed and expected numbers of migration events

Category	Origin	Difference[a] for the indicated destination							
		DENV-2				DENV-4			
		SA	CA	LA	GA	SA	CA	LA	GA
Region	South America (SA)		−2.05	−6.44	−4.55		−0.06	0.12	0.40
	Central America (CA)	−0.03		−0.05	−0.03	0.45		−0.10	−0.06
	Lesser Antilles (LA)	2.14	−0.22		−2.03	−0.16	−0.17		−0.20
	Greater Antilles (GA)	2.19	−0.15	2.11		−2.43	−2.25	−2.55	
	Total	−9.10				−7.00			
		I	M			I	M		
Island vs mainland	Island (I)		0.31				−5.63		
	Mainland (M)	−7.21				0.49			
	Total	−6.90				−5.14			
		C	O			C	O		
Economic	CARICOM (C)		−4.36				0.39		
	Other (O)	−8.16				−6.55			
	Total	−12.53				−6.15			
		WI	DA	FA	LA	WI	DA	FA	LA
Cultural/historical	West Indian (WI)		0.14	−0.14	−1.91		−0.01	−0.04	−0.29
	Dutch Antillean (DA)	−0.29		−0.02	0.30	0.15		0.00	0.12
	French Antillean (FA)	0.00	0.00		0.00	0.00	0.00		0.00
	Latin American (LA)	−8.33	−4.52	−0.35		−3.57	−0.95	0.29	
	Total	−15.13				−4.29			

[a] Differences less than zero indicate relative isolation, while differences greater than zero suggest migration: $P \leq 0.002$ for each category.

significant given the estimated confidence limits. For each Bayesian skyline plot analysis, the maximum a posteriori tree is presented on the same time scale as the skyline plot, along with a histogram representing the number of countries reporting DENV-2 or DENV-4 activity in each year. For both DENV-2 and DENV-4 the invasion phase began prior to the first reported outbreaks, suggesting that they had spread for some time before the number of infections became large enough to be noticed. In the case of DENV-4 this phase ended in 1981, after which genetic diversity remained constant, despite a dramatic drop and subsequent fluctuations in the number of countries reporting activity. This maintenance of diversity coincided with the emergence of population subdivision (illustrated in Fig. 2b by clades that are comprised mainly of sequences from islands or mainland countries and little mixing among them). In contrast, its invasion phase occurred during a period (up to 1982) when there was no population subdivision. The same pattern was true for DENV-2, although the initial invasion phase continued for 2 years after the first reports of activity, and the population subdivision during the maintenance phase is less clear-cut. This virus appears to have circulated among the islands before becoming subdivided between islands and mainland, although there was continued gene flow between the two.

As might be expected given the skyline plots, the logistic model of population growth was the best fit to the data for both viruses (Table 2). If we assume no significant population structure, then we can interpret the changing genetic diversity as changing effective population size (see Materials and Methods). In this case the two serotypes show a very large difference in mean growth rate during the initial phase of the epidemics (Table 2); the doubling time for DENV-2 was estimated at approximately 32 weeks (95% confidence interval [CI], 15.6

weeks to 2.5 years), in comparison to just over 2 weeks (95% CI, 1.6 to 5.7 weeks) for DENV-4. This pattern is not due to differences in substitution rates, since these are remarkably similar among the serotypes (8.0×10^{-4} and 8.3×10^{-4} substitutions/site/year for DENV-2 and DENV-4, respectively). However, this difference may be partly due to the emergence of population structure (see Discussion for details). The relative genetic diversity was higher for DENV-2 than for DENV-4, but this difference was not significant (confidence intervals overlap). The observed difference in transmission dynamics between the serotypes during the invasion phase was also obtained when all American data available for these viruses (DENV-2, 103 sequences; DENV-4, 99 sequences) were used, i.e., when the data were not limited to five sequences per country (results not shown).

DISCUSSION

Our results suggest that DENV-2 subtype III and DENV-4 subtype II, which currently cocirculate in the Americas, evolved from ancestors that existed around 1980, very shortly before the first reports of the dengue cases in the eastern Caribbean (7) and Cuba (27). Despite large geographic distances and marine barriers, phylogenetic trees of these viruses show that they quickly spread throughout the region. However, their directions of spread were markedly different. DENV-2 appears to have migrated from the Greater Antilles to South America and the Lesser Antilles and from the latter to South America. The movement from the Greater Antilles to South America may represent the sum of the other two routes, such that the Caribbean archipelago acts as a link between the northern Caribbean and South America, along which DENV-2 migrated south. Although the evidence for migration is weaker

FIG. 2. Genealogies and corresponding Bayesian skyline plots showing the transmission histories of (a) DENV-2 subtype III and (b) DENV-4 subtype II, drawn on the same time scale. The *y* axes of the skyline plots represent relative genetic diversity, which is equal to the product of effective population size and generation length in the absence of population structure (see Materials and Methods). For both viruses the maximum a posteriori tree is presented on the same time scale as the skyline plot, with tip times corresponding to sampling times. The thick black lines are the median estimates, and the areas between the 95% CIs are shaded gray. Isolates on the trees are identified by their country of origin; mainland countries are labeled in red and islands in blue, and the tips of the phylogenies correspond to their sampling times. Abbreviations used for country names are given in Table S2 in the supplemental material. The numbers of countries reporting DENV-2 and DENV-4 activity in each year are summarized in the histograms shown. In the case of DENV-2, this represents the activities of both subtype III and V, which are not distinguished in epidemiological reports.

in the case of DENV-4, there is an apparent dispersal along the same transmission route but in the opposite direction. The islands, particularly the Greater Antilles, appear to have acted as a sink for DENV-4, even though the first report of DENV-4 in the region came from the islands of St. Barthelemy and St. Martin (French Antilles) (7). Consequently, although the

earliest epidemiological reports and isolates for both DENV-4 and DENV-2 came from islands, we propose that the founding populations within the Americas differed and that DENV-4 may have originated on the American mainland. Although the two viruses moved in different directions along the same trans-mission routes, their patterns of segregation between "eco-

TABLE 2. Transmission dynamics estimates for DENV-2 and DENV-4 under various population growth models

Parameter	Value for the indicated model and virus[a]					
	Constant		Exponential		Logistic	
	DENV-2	DENV-4	DENV-2	DENV-4	DENV-2	DENV-4
Log likelihood	−4,956.5	−4,201.5	−4,951.4	−4,181.5	−4,939.4	−4,162.0
Substitutions/site/yr (10^{-4})	7.9 (6.6–9.3)	8.0 (6.2–9.8)	8.0 (6.6–9.4)	8.2 (6.6–10.0)	8.0 (6.6–9.5)	8.3 (6.8–10)
Population size ($N_e\tau$)	32.2 (23.2–41.7)	23.2 (14.9–31.9)	86.0 (37.5–143.3)	207.3 (49.8–437.8)	41.5 (28.2–56.9)	29.8 (19.1–41.3)
Root age (yr)	24.4 (22.3–26.9)	22.6 (19.7–25.8)	24.0 (21.9–26.1)	21.4 (19.1–23.9)	22.4 (21.0–24.2)	19.3 (18.4–20.3)
MRCA[b]	1978	1976	1978	1978	1980	1980
Growth rate (no. of new infections/individual/yr)			0.09 (0.04–0.15)	0.19 (0.11–0.26)	1.13 (0.28–2.25)	16.44[d] (6.40–25.00)
Doubling time			7.7 yr (4.6–17.3 yr)	3.7 yr (2.7–6.3 yr)	0.6 yr = 31.7 wk (0.3–2.5 yr)	0.04 yr = 2.2 wk (0.0–0.1 yr)
T50 (yr)[c]					17.8 (15.3–20.3)	18.8 (18.0–19.8)

[a] In all cases, values for the mean posterior distribution and the 95% high-probability values (in parentheses) are shown.
[b] MRCA, year of most recent common ancestor.
[c] T50, number of years before date of most recent isolate that population size was 50% of maximum.
[d] Mean growth rate for DENV-4 is at least 16.44 (upper limit for growth rate was set at 25).

nomic" and "historical/cultural" groups were essentially the same. CARICOM members are predominantly islands and the Latin American countries are predominantly on the mainland, so the fact that these results do not mirror the results of the "region" and "mainland-island" analyses suggests that factors other than geographical location play an important role in shaping the phylogeography of dengue virus.

Despite considerable gene flow within the Americas, the overall extent of viral traffic is less than expected under panmixis so that some population subdivision has arisen. This also seems to have shaped patterns of genetic diversity as reflected in the skyline plots. This effect is particularly apparent for DENV-4; the initial increase in genetic diversity corresponds to a period when there was no population subdivision (or the period during which subdivision was created) and thus reflects rapid DENV-4 transmission throughout the region, as shown by the sudden increase in the number of countries reporting this virus. After this point, the viral population becomes subdivided so that genetic diversity is subsequently retained by geographic structure, despite the decrease in population size expected given the drop in the number of countries reporting DENV-4 activity. DENV-2 shows a similar pattern of a growth phase followed by a phase in which genetic diversity is maintained, but since the epidemiological data do not distinguish between different subtypes of DENV-2, the relationship between the two is more difficult to assess.

Although both viruses arrived at the same time and dispersed throughout the region, their initial stages of invasion and expansion were very different, with the number of DENV lineages increasing far more rapidly for DENV-4 than for DENV-2. There are two interpretations of this difference in genetic diversity. First, if we assume that population subdivision has had only a minor effect on our coalescent estimates of demographic history, then the estimated growth rate for DENV-4 was approximately 16-fold higher than that for DENV-2; the population size of DENV-2 was estimated to have doubled every 32 weeks, with DENV-4 having a doubling time of only 2 weeks. The latter is the highest viral population growth rate measured by coalescent methods to date (37, 44). The growth rate for DENV-2 subtype III is also much higher

than those estimated for other viruses, with the exception of DENV-2 in Venezuela between 1997 and 2000, which had an estimated doubling time of 25 weeks (44). Alternatively, it is possible that the more rapid initial increase in DENV-4 sampled lineages reflects a more rapid geographic dispersal within the Americas and therefore a more rapid implementation of population structure during the invasion phase. Looking backwards in time, this produces a dramatic drop in relative genetic diversity around 1980 coinciding with the loss of population structure. A higher rate of DENV-4 dispersal might also lead to more gene flow after the invasion phase, although this is not supported by our parsimony analysis of migration patterns. Indeed, it is possible that both faster population growth and greater population dispersal contributed to the more vigorous initial transmission dynamics of DENV-4, although the methods used here cannot uncover the relative importance of these two factors.

Why might DENV-2 and DENV-4 have such different transmission dynamics? The difference does not correspond to underlying differences in rates of evolutionary change, as these were essentially equivalent and similar to those previously estimated for dengue virus (12, 24, 43). Changes in genetic diversity through time are also sometimes determined by transmission mechanism. For example, rates of population growth for hepatitis C virus transmitted by blood products or injecting drug use are significantly higher than those in Asian and African populations with endemic hepatitis C virus infection (37). It is also possible that differences in mosquito susceptibility affect growth rates (3), although our finding that the two serotypes reached equivalent population sizes in the Caribbean argues against this.

Another possible explanation for the difference in relative genetic diversity involves differences in the level of immunity within host populations, itself reflecting the past history of dengue virus infection. Specifically, in 1981 there would have been no herd immunity to DENV-4, as this serotype was not reported in the Americas until this time. As a consequence, DENV-4 would have been able to spread very rapidly. In contrast, DENV-2, in the guise of subtype V, had been documented for many decades prior to 1981, with outbreaks having

occurred as recently as 1968 to 1969 in Curacao, Haiti, Jamaica, Puerto Rico, and the Lesser Antilles and between 1970 and 1974 in Colombia, French Guyana, and Puerto Rico. More recently, subtype V was associated with a large epidemic of DF in Peru (46). The proportion of DENV-2-susceptible individuals in the Americas would therefore have been much smaller, resulting in a lower growth rate. Although little is known about cross-neutralization between DENV serotypes, studies have shown that antibodies against DENV-1 neutralize DENV-2. This phenomenon is more notable between DENV-1 and DENV-2 subtype V than subtype III (26), presumably because the former shares one or more neutralizing epitopes with DENV-1 (25). As DENV-4 is the most phylogenetically divergent of the four serotypes (22, 28), it is possible that it is subject to weaker cross-reactive immune responses that would further enhance its growth potential (although the relative effect of this is difficult to assess, since a weaker cross-reactive immune response would also presumably reduce the effect of antibody-dependent enhancement, resulting in lower viremia and thus transmissibility).

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 1977 (36) and within only 1 year spread throughout the region, with at least 30 countries reporting activity (6, 47; www.paho.org/english/hcp/hct/vbd/dengue_timeline.xls). Preliminary data on DENV-3 isolates from the Americas suggest that this virus, which was absent from the region for 17 years before being reintroduced in 1994, has expanded at a higher rate than DENV-2 but not as rapidly as DENV-4 (J. E. Foster et al., unpublished data). Hence, some protective immunity against DENV-3 may remain, although this conclusion is tentative since the time frame and overall host population size differ.

Finally, the phylogenetic data suggest that both DENV-2 and DENV-4 have evolved in situ in the Americas without multiple introductions from other regions after 1981 (12, 13). This is in contrast to the findings of recent studies on DENV-1 in the Pacific, where there were repeated introductions from Southeast Asia (2). This was linked to the large volume of Southeast Asian tourists visiting the Pacific. In contrast, the Caribbean basin receives over 95% of its visitors from Europe, North America, and other countries within the Americas. It is therefore less susceptible to repeated introductions from Asia.

In addition to providing insight into the nature of dengue virus colonization in a mainland-island situation, our elucidation of the demographic and migration histories of DENV-2 and DENV-4 should contribute to efforts to control and monitor the spread of dengue in the Americas, particularly given their very rapid rates of spread. For example, our data indicate a relatively weak connection between Central America and the Antilles, such that one might predict that an epidemic beginning in Central America would spread south before making its way to the islands via South America. Likewise, reports of an outbreak in the northern part of South America should put the Antilles on alert, and vice versa. The results also justify the roles of bodies such as the Pan American Health Organization through which regional and country-based organizations can coordinate their efforts, particularly among those belonging to different "economic" or "cultural/historical" groups with which they might normally have less contact.

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