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RNA FINGERPRINTING AS A METHOD FOR DISTINGUISHING DENGUE 1 VIRUS STRAINS*

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Abstract. Virion RNAs of 12 geographically distinct dengue type 1 (DEN-1) virus isolates were clearly unique by RNA fingerprinting. Isolates from the same geographic area were very similar but differed from those of other areas, allowing us to establish three geographical groupings based upon percent shared oligonucleotides. Three Caribbean strains were virtually identical (85–91% homologous oligonucleotides) whereas Pacific/S.E. Asian strains exhibited considerably less homology to one another (44–49%). The Pacific/S.E. Asian strains exhibited little relationship (20–30%) to the Caribbean and African strains. A Sri Lankan isolate displayed a relatively high degree of homology to Nigerian isolates (60–66% homologous oligonucleotides), suggesting that the Sri Lanka DEN-1 infection originated from Africa. A 1978 Nigerian DEN-1 isolate and the 1969 Sri Lankan strain each exhibited greater than 50% homology with a 1977 Jamaican strain. The similarities observed between the African/Sri Lankan and Jamaican strains suggest that the DEN-1 virus which caused the 1977 Jamaican epidemic may have originated from Africa or Sri Lanka. The RNA fingerprint is a unique characteristic of DEN-1 strains from a particular geographic region, suggesting this technique as a useful tool for dengue epidemiological investigations.

Dengue (DEN) viruses types 1, 2, 3, and 4 are distinguishable serologically by neutralization and, in some circumstances, by complement-fixation tests.[1–3] More recently, oligonucleotide fingerprint analysis has shown that prototype viruses of each of the four DEN serotypes exhibits a unique RNA fingerprint. The fingerprints were quite dissimilar and, on average, only 7% of the large resolvable oligonucleotides were shared among them.[4] RNA fingerprint analysis has also been used to successfully differentiate viruses within a serotype, e.g., vesicular stomatitis viruses (Indiana and New Jersey serotypes),[5] La Crosse viruses,[6] influenza A H1N1 viruses,[7] St. Louis encephalitis viruses,[8] Rift Valley fever viruses,[9] and Pichinde viruses.[10]

The purpose of these investigations was to characterize DEN virus RNAs by RNA fingerprint analysis to determine whether different geographical and temporal isolates of DEN type 1 (DEN-1) virus display unique and distinguishable fingerprint patterns. Such capability should provide a powerful tool for the epidemiological study of dengue virus outbreaks.

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MATERIALS AND METHODS

Virus propagation and purification

The origins and passage histories of DEN virus strains used in this study are listed in Table 1. All viruses were originally isolated from human serum. Following the passages indicated in the table, each isolate was passaged two additional times in *Aedes albopictus* cell culture prior to RNA preparation. *Ae. albopictus* cells, derived from Igarashi clone C6/36[11] were grown at 28°C in the presence of CO_2 in 150-cm² flasks containing Dulbecco's modified Eagle medium (high glucose) (Gibco) supplemented with 5% heat-inactivated fetal bovine serum (FBS) and 0.1 mM non-essential amino acids (Gibco).

Dengue-1 virus strains were propagated by infecting *Ae. albopictus* cell monolayers (2×10^7 cells) at an input multiplicity of 0.08 to 0.3 plaque-forming unit (PFU) per cell. Virus was allowed to

TABLE 1
Dengue 1 strains used in this study

Designation	Original isolation		Passage history*
	Year	Location	
CV 1636/77	1977	Jamaica	5-sm, 1-LLC-MK_2 tc
777849	1977	Bahamas	1-AP-61 tc purified large plaque, 1-LLC-MK_2 tc
CV 58/81	1981	Jamaica	1-AP-61 tc, 1-C6/36 tc
IB-H 28326	1968	Nigeria	21-sm
IB-H 13689	1978	Nigeria	3-AP-61 tc, 1-C6/36 tc
HAWAIIAN	1945	Hawaii	131-sm, 1-LLC-MK_2 tc
17646	1974	Nauru Island	7-FRhL tc
17672	1974	Nauru Island	7-FRhL tc, 5-sm
D-74-061	1974	Bangkok	1-*Tx. splendens*
D-74-063	1974	Bangkok	1-*Tx. splendens*
D-75-001	1975	Bangkok	3-sm, 1-LLC-MK_2 tc, 1-sm
691475	1969	Sri Lanka	2-sm, 2-*Tx. splendens*, 2-LLC-MK_2 tc

* Abbreviations: sm, suckling mouse passage; tc, tissue culture passage; LLC-MK_2, continuous rhesus monkey kidney cells; AP-61, *Aedes pseudoscutellaris* cells; C6/36, *Aedes albopictus* cloned cells; *Toxorhynchites splendens* mosquitoes.

adsorb for 90 min at room temperature, followed by the addition of 30 ml per flask of growth medium containing 170 μCi (^{32}P) phosphate (NEN) per ml. Following incubation at 28°C for 7–8 days, the infected cell supernatants were clarified by centrifugation at 3,000 × *g* for 10 min. Virus suspensions were precipitated at 4°C for 6 hours by the addition of polyethylene glycol 6,000 and NaCl, 7% and 2.3% final concentrations, respectively. The precipitated virus was recovered by centrifugation at 6,000 × *g* for 30 min, resuspended in 4 ml TSE (0.01 Tris-HCl, pH 7.8, 0.12 M NaCl, 0.001 M EDTA) and purified by density gradient centrifugation at 208,000 × *g* for 18 hours in a 30% (w/v) glycerol to 50% (w/v) potassium tartrate gradient in TSE. Virus was recovered from visible bands, diluted, and repurified by rate zonal centrifugation at 270,000 × *g* for 90 min on a 20–70% (w/v) sucrose gradient in 1 M NaCl, 0.01 M Tris-HCl buffer, pH 8.5. Virus bands were recovered, diluted fourfold with HSB (0.4 M NaCl, 0.01 M Tris-HCl, pH 7.4) and the RNA was extracted.

Preparation and fingerprinting of viral RNA

For RNA extraction, sodium dodecyl sulfate (SDS) was added to 1% final concentration and the suspension was extracted with a phenol : cresol:8-hydroxyquinoline : chloroform mixture.[12] Following ethanol precipitation, the viral RNA was purified by centrifugation in a 15–30% (w/v) sucrose gradient in TSES buffer (0.02 M Tris-HCl, pH 7.4, 0.1 M NaCl, 0.002 M EDTA, 0.1% SDS) for 4 hours at 270,000 × *g* and 20°C. The 42S viral RNA was collected, diluted, and following the addition of 100 μg tRNA, was re-extracted with the phenol mixture as before, air-dried, resuspended in 20 μl of TE buffer (0.02 M Tris-HCl, pH 7.4, 0.002 M EDTA) and stored at −20°C. Final RNA samples contained 0.1–6 × 10^6 cerenkov cpm and were tested for size and purity by electrophoresis of an aliquot (2 to 5 × 10^4 cpm) on non-denaturing 2.4% polyacrylamide gels[4, 13] prior to digestion and fingerprint analysis.

Samples of 0.1–1.0 × 10^6 cpm of purified 42S DEN-1 viral RNAs were digested with RNase T1 and the resulting oligonucleotides were resolved by two-dimensional polyacrylamide gel electrophoresis[14] as modified by Clewley et al.[15] Following electrophoresis, the gels were autoradiographed at −70°C using Kodak X-Omat XAR-5 X-ray film.

Plaque assays and virus neutralization assays

Plaque assays were performed by infecting confluent BHK21-clone 15[16] cell monolayers (on 60-mm plastic petri dishes) with 0.2 ml of a virus dilution (in Eagle's MEM containing 5% FBS). Following adsorption for 1 hour at 36°C, the cells were overlaid with Eagle's MEM containing 5% FBS and 0.4 agarose (Seakem). Assays were incubated at 36°C in an atmosphere of 5% CO_2 for

6–7 days until plaques were visible. Agarose overlays were then removed and the monolayers stained with crystal violet using the procedure of Holland and McLaren.[17] Using this method, plaques could be counted immediately. Virus neutralization assays were performed using a plaque reduction neutralization test.[18]

RESULTS

The 12 DEN-1 isolates used in these studies (Table 1) represent three locations where DEN-1 is endemic or has been epidemic. These were the Caribbean (Jamaica and Bahamas), African (Nigeria), and Pacific/S.E. Asian [Hawaii, Nauru Island (Nauru), Bangkok and Sri Lanka] regions. In addition, certain of the isolates represented a temporal separation of 1 to 10 years (Bangkok—1 year, Jamaica—4 years, and Nigeria—10 years).

Caribbean strains

Two different human isolates from the 1977–1978 Caribbean epidemic were chosen for study—a Jamaican strain and a Bahamian strain, the latter being a plaque-purified virus isolated from *Ae. pseudoscutellaris* tissue culture harvests.[3] RNA fingerprints of the two 1977 strains and a recent 1981 Jamaican isolate are shown in Figure 1. These strains appear very similar; for clarification, schematic representations of coincident fingerprints are shown in Figure 2. Fingerprints of the two 1977 isolates, Jamaican and Bahamian are depicted in Figure 2A. The unique oligonucleotides numbered 1, 3, and 5 originated from the Jamaican strain, while Nos. 2, 4, and 6 were contributed by the large-plaque Bahamian isolate. It is very probable that one pair of oligonucleotides, Nos. 5 and 6, are homologous with the exception of a single base change (e.g., U → C) which would be capable of causing the observed lateral change. Aside from these minor differences, the two 1977 isolates were similar, sharing 63 of a total of 69 well-resolved oligonucleotides, or 91% homology. (Percent ho-

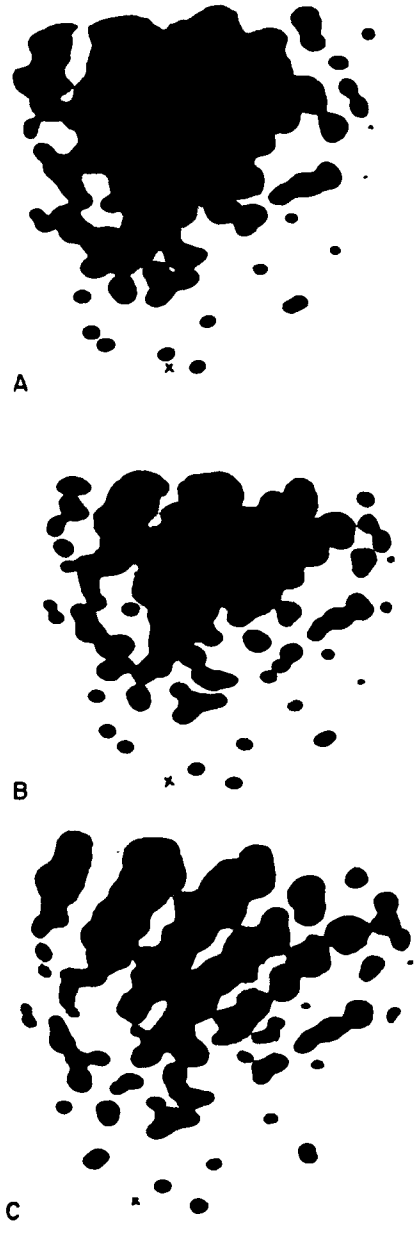

FIGURE 1. Oligonucleotide fingerprint analysis of three Caribbean DEN-1 strains. The ribonuclease T1-resistant oligonucleotides were derived from ^{32}P-labeled 42S virion RNA and separated by two-dimensional polyacrylamide gel electrophoresis. Migration in the first dimension is from left to right, and in the second dimension from bottom to top. The positions of the two dye markers, bromophenol blue (upper) and xylene cyanol FF (lower) are indicated (X). A, plaque-purified large-plaque isolate from the Bahamas, 1977; B, Jamaica isolate, 1977; C, Jamaica isolate, 1981.

mology in this and in all subsequent references is used to define the percentage of well-resolved oligonucleotides which migrated coincidentally upon co-electrophoresis.)

In Figure 2B, comparison is made between two DEN-1 viruses isolated from the same location but at different times—1977 and 1981 Jamaican strains. In this case, a greater number of unique oligonucleotides was detected. Six oligonucleotides appeared unique to the 1977 isolate, while five were contributed by the 1981 isolate. Again, one pair of oligonucleotides may in fact be homologous with the exception of a single base change, Nos. 3 and 4. Although less similar than the two Caribbean strains isolated in 1977 (Jamaican and Bahamian), the two Jamaican strains isolated 4 years apart share 70 well-separated oligonucleotides of a total of 81, or 86% homology.

African strains

RNA fingerprints of two DEN-1 strains from Africa (Ibadan, Nigeria) isolated over a 10-year interval are presented in Figure 3. To determine the extent of homology between the two viruses, RNA from each was simultaneously digested and co-electrophoresed. Origins of the large oligonucleotides in the co-electrophoresed samples were determined by comparing them with their respective single viral RNA digests. Co-electrophoresis of the 1968 and 1978 Nigerian isolates is shown in Figure 3C, together with a schematic representation (Fig. 3D). The 1978 strain retained 60 co-migrating oligonucleotides with the 1968 strain from a total of 76 well-resolved oligonucleotides, exhibiting oligonucleotide homology of 79%.

Pacific/S.E. Asian strains

Purified 42S virion RNAs of DEN-1 Pacific/S.E. Asian strains (from Nauru, Hawaii, Bangkok, and Sri Lanka) were similarly compared by oligonucleotide fingerprint analysis (Fig. 4). Each fingerprint pattern is clearly unique and distinguishable. Fingerprints of the three Bangkok isolates studied (two from 1974 and one from 1975) appeared identical (data not shown); therefore, only one Bangkok isolate fingerprint is shown (Fig. 4D). Interestingly, DEN virus isolated from two different patients during the same epidemic in the western Pacific (Nauru Island, 1974) did exhibit minor differences in their fingerprint patterns (Fig. 4, A, B). A schematic representation of coincident

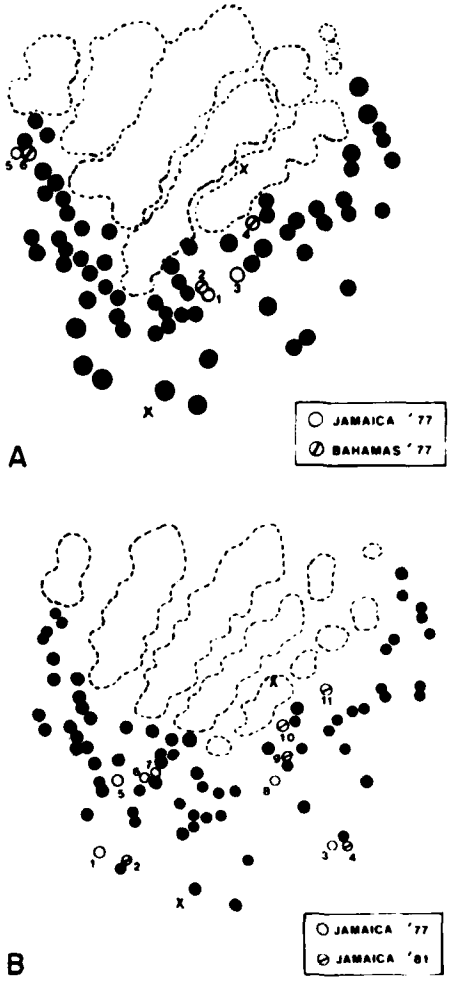

FIGURE 2. Schematic representation of oligonucleotide fingerprint analyses of mixtures of the three DEN-1 Caribbean strains. Oligonucleotides derived from the Jamaica 1977 isolate are indicated by open circles (○), while those from the large-plaque Bahamas 1977 or Jamaica 1981 isolates are indicated by hatched circles (◫). Filled circles (●) in this and in all subsequent schematics indicate oligonucleotide positions from the two RNA species which migrated coincidentally.

fingerprints of the two Nauru Island isolates is shown in Figure 5. The unique oligonucleotides number 1, 3, 5 originated from Isolate 1, while Nos. 2 and 4 were contributed by Isolate 2. Again, two pairs of oligonucleotides (No. 1–2, and 3–4)

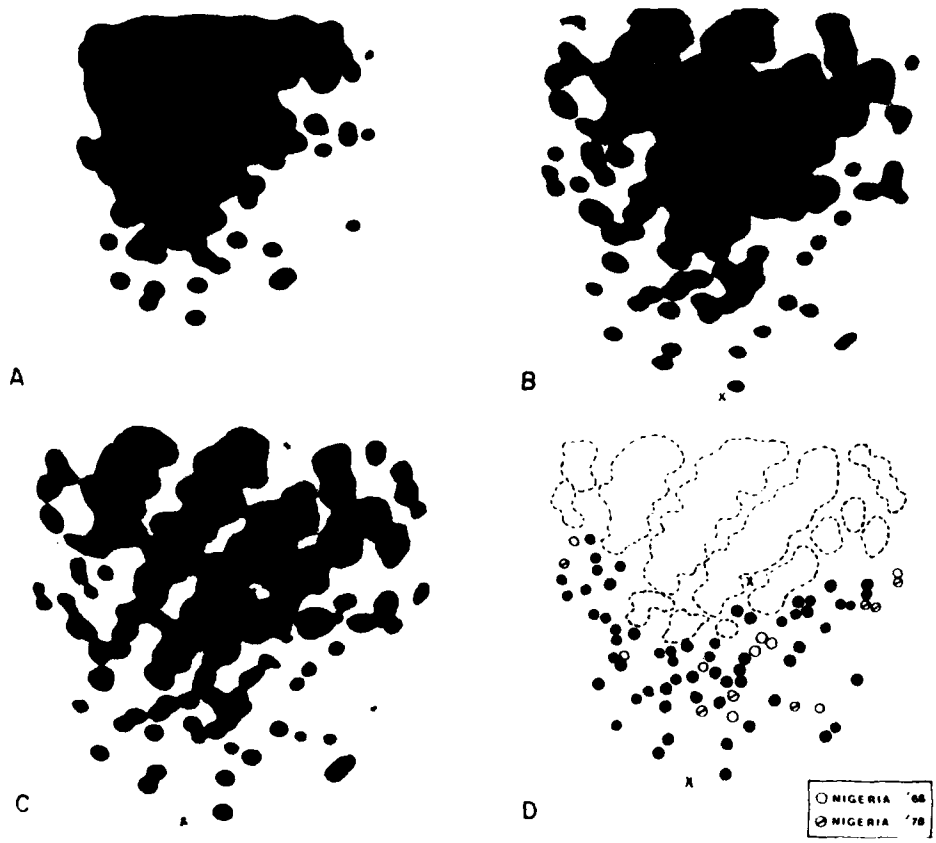

FIGURE 3. Oligonucleotide fingerprint analysis of two African (Nigerian) DEN-1 strains separated by a 10-year interval in isolation. **A**, 1968 isolate; **B**, 1978 isolate; **C**, mixture of the 1968 and 1978 isolates; **D**, schematic of (C).

may be homologous, with the exception of a single nucleotide change. Oligonucleotide No. 5, present in Isolate 1, is absent in Isolate 2. Aside from these minor changes, the two Nauru Island isolates are similar and share 53 of a total of 58 well-resolved oligonucleotides, or 91% homology.

To determine the extent of homology among other Pacific/S.E. Asian isolates, RNA from each of two isolates was simultaneously digested and co-electrophoresed. Co-electrophoresis of two Pacific isolates, Nauru Island No. 2 and prototype Hawaii (Fig. 6A) resulted in 49 co-migrating oligonucleotides from a total of 104, or 47% homology. The Nauru Island isolate contained 27 unique oligonucleotides while the Hawaiian contained 28. Superimposition of the two S.E. Asian isolates yielded a surprising result: only 22 oligonucleotides from the Sri Lankan and Bangkok isolates migrated coincidentally from a total of 107 oligonucleotides (21% homology) (Fig. 6B). The Sri Lankan isolate contained 46 unique oligonucleotides, whereas the Bangkok isolate contained 39. Upon closer inspection, the Sri Lankan isolate appeared to be much more similar to the Nigerian isolates. The Sri Lankan/Bangkok fingerprint comparison represents the only instance where isolates from a geographic proximity exhibited so little similarity.

To determine if either of the S.E. Asian strains was more closely related to the Pacific strains than

FIGURE 4. Oligonucleotide fingerprinting analysis of seven Pacific/S.E. Asian DEN-1 strains. Fingerprints of the three Bangkok isolates studied (two from 1974 and one from 1975) appeared identical; therefore, only one Bangkok isolate fingerprint is shown. **A**, Nauru Island No. 1, 1974; **B**, Nauru Island No. 2, 1974; **C**, Hawaii, 1945; **D**, Bangkok, 1974/1975; **E**, Sri Lanka, 1969.

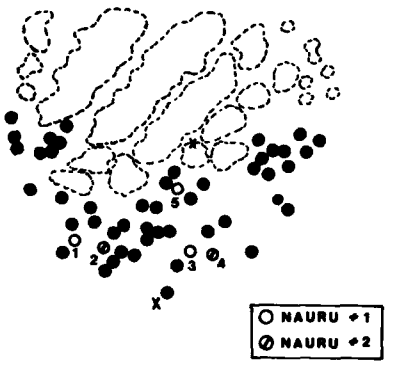

FIGURE 5. Schematic representation of RNA fingerprint analyses of a mixture of the two Nauru Island isolates studied, No. 1 and No. 2.

to each other, co-electrophoreses were performed between the Bangkok and Pacific strains as well as between the Sri Lankan and Pacific strains. Figure 6C depicts the result obtained from co-electrophoresis of the Bangkok and Hawaiian strains. Forty-nine percent of the well-separated oligonucleotides were common to both, while 17 oligonucleotides were unique to the Bangkok strain and 19, to the Hawaiian isolate. Co-electrophoresis of the Bangkok and Nauru Island No. 2 isolates resulted in the co-migration of 40/91 oligonucleotides or 44% homology (data not shown). Quite different results were obtained upon fingerprint comparison of the Sri Lankan with the Pacific strains. Only 27% oligonucleotide homology was detected between the Sri Lankan and Nauru Island No. 2 strains (Fig. 6D), but somewhat surprisingly oligonucleotide homology of 38% was exhibited between the Sri Lankan and prototype Hawaiian strain (data not shown). It therefore appears that the Pacific/S.E. Asian strains (Hawaiian, Nauru, and Bangkok) are all related to a similar degree (44 to 49% oligonucleotide homology), whereas the Sri Lankan strain appears not to belong to this group genetically. Even though a greater relationship appears to exist between the Sri Lankan and Hawaiian isolates, 38% homology, than between the isolates from Sri Lanka and Bangkok or Nauru, 21 to 27% oligonucleotide homology, the relationship is less than that observed among the other Pacific/S.E. Asia virus isolates.

Homologies between strains from different geographical areas

Co-electrophoreses (schematic representations) of several geographically separated strains of DEN-1 are presented in Figure 7. For the most part, the Pacific/S.E. Asian strains exhibited little resemblance (20–30% oligonucleotide homology) to the African and Caribbean strains (Fig. 7, A–C). The Sri Lankan strain shared a high degree of oligonucleotide homology with the African isolates. Co-electrophoresis of the Sri Lankan '69 and Nigerian '78 strains resulted in the co-migration of 59 of 89 oligonucleotides, or 66% homology (Fig. 7D).

Fingerprint comparisons between the 1977 Jamaican strain and all other intergeographic strains revealed relatively high homologies between only two other isolates, the Sri Lankan '69 and Nigerian '78 viruses (Fig. 7E, F). Co-electrophoresis of Jamaican '77 and Nigerian '78 isolates resulted in 45/85 co-migrating oligonucleotides (53% homology), while co-electrophoresis of Jamaican '77 and Sri Lankan '69 isolates resulted in an even greater number of co-migrating oligonucleotides, 55/93 (59% homology). These fingerprint comparisons clearly demonstrated that while these viruses were similar to one another they were not identical; therefore the 1977 Caribbean epidemic was probably not caused by either of these particular virus strains from Africa or Sri Lanka. However, the high degree of oligonucleotide homology displayed between the Jamaican and African/Sri Lankan isolates is striking.

A summary of the percent oligonucleotide homologies displayed between the various DEN-1 strains is presented in Table 2.

DISCUSSION

Virion RNAs of the four DEN prototype serotypes were previously shown to be distinct from one another by oligonucleotide fingerprint analysis.[4] On an average, only 7% of the large resolvable oligonucleotides were shared among the four prototype viruses. We similarly analyzed the virion RNAs of temporally and/or geographically separated DEN-1 strains and found them also to be unique, rendering the method of oligonucleotide mapping an important tool for distinguishing virus strains that are serologically indistinguishable.

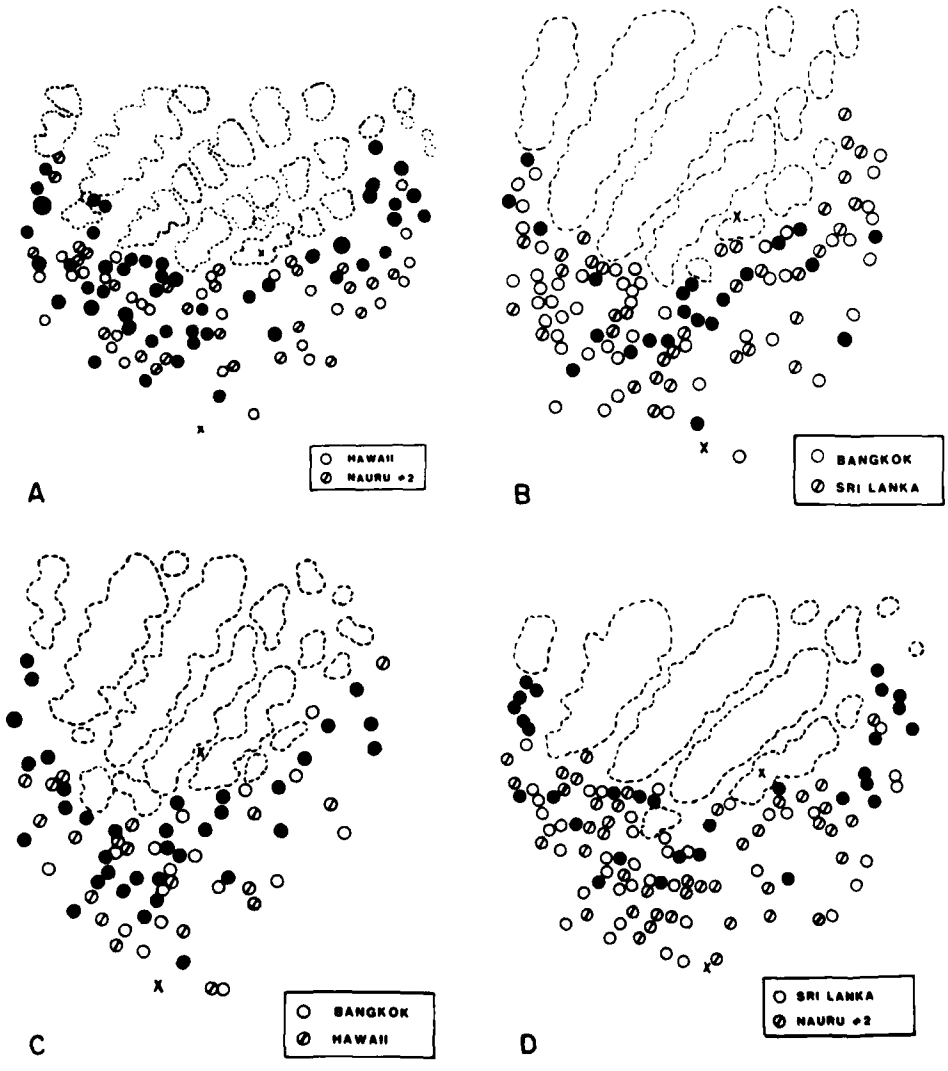

FIGURE 6. Schematic representations of oligonucleotide fingerprint analyses of mixtures of DEN-1 Pacific/S.E. Asian strains.

The DEN-1 virus genome does not appear to be as stable as either the VSV-Indiana or influenza virus genomes.[5,7] The Bangkok DEN-1 strains isolated 1 year apart were indistinguishable from one another by fingerprint analysis (100% homology). However, oligonucleotide homology dropped to 86% when isolations were made 4 years apart (Jamaica, '77 and Jamaica, '81 strains), and dropped further still to 79% over a 10-year interval in isolation (Nigeria, '68 and Nigeria, '78 strains). Since some of the virus isolates used as examples of temporal genetic evolution were also separated geographically, the possibility that the rate of genetic change had varied in different endemic areas could not be excluded; however, the DEN-1 genome appeared to be undergoing a slow

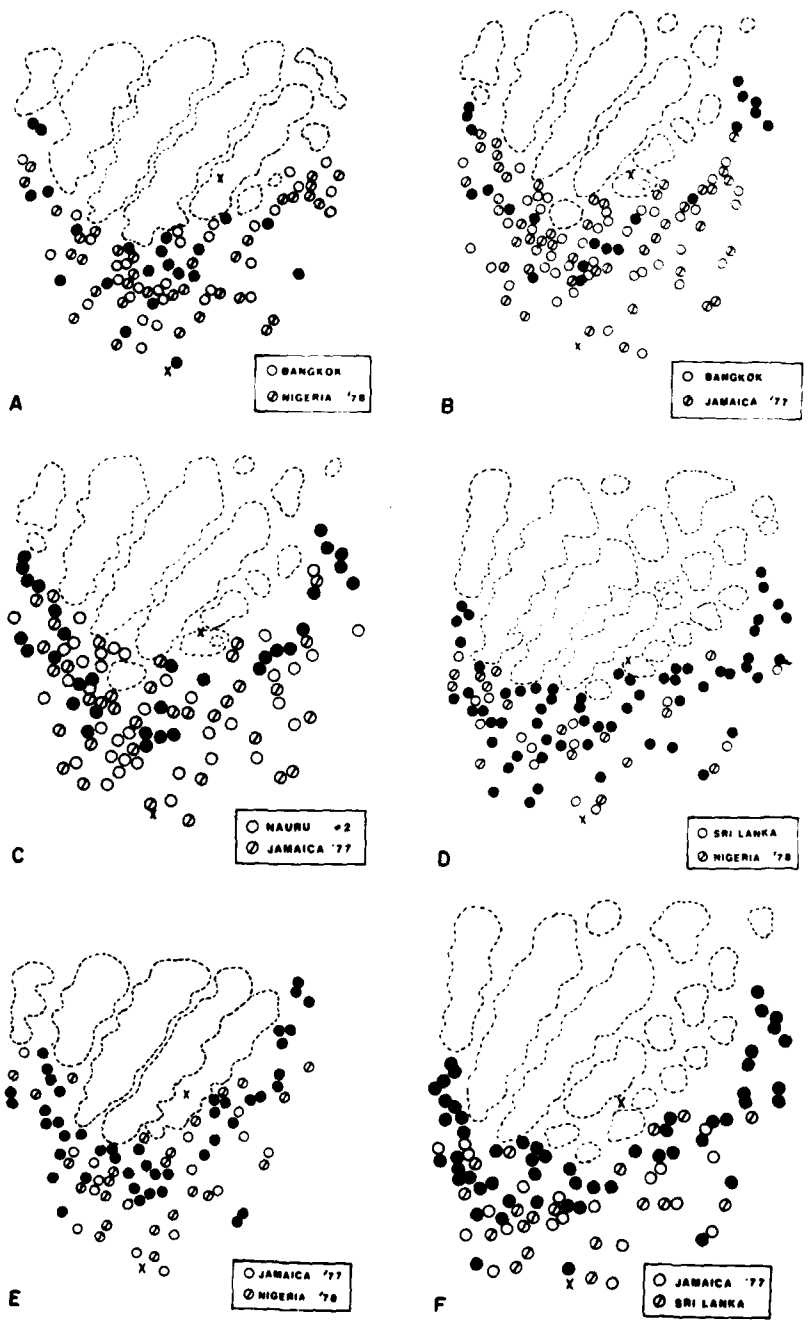

FIGURE 7. *Schematic* representations of RNA fingerprint analyses (co-electrophoreses) of several intergeographical strains of DEN-1.

TABLE 2

Summary of oligonucleotide homologies between Dengue 1 virus strains

Geographic group	Strains (Year)		% homology
Caribbean	Jamaica	('77) + *Bahamas ('77)	91
	Jamaica	('77) + Jamaica ('81)	86
African	Nigeria	('68) + Nigeria ('78)	79
Pacific/S.E. Asian	Bangkok	('74) + Bangkok ('75)	100
	Nauru No. 1	('74) + Nauru No. 2 ('74)	91
	Nauru No. 2	('74) + Hawaii ('45)	47
	Hawaii	('45) + Bangkok ('74)	49
	Bangkok	('74) + Nauru No. 2 ('74)	44
	Sri Lanka	('69) + Hawaii ('45)	38
	Sri Lanka	('69) + Nauru No. 2 ('74)	27
	Sri Lanka	('69) + Bangkok ('74)	21
Intrageographic comparisons	Hawaii	('45) + Jamaica ('77)	20
	Hawaii	('45) + Nigeria ('78)	20
	Bangkok	('74) + Jamaica ('77)	20
	Bangkok	('74) + Nigeria ('78)	24
	Nauru No. 2	('74) + Jamaica ('77)	30
	Nauru No. 2	('74) + Nigeria ('78)	30
	Sri Lanka	('69) + Nigeria ('78)	66
	Sri Lanka	('69) + Nigeria ('68)	60
	Sri Lanka	('69) + Jamaica ('77)	59
	Sri Lanka	('69) + Jamaica ('81)	55
	Jamaica	('77) + Nigeria ('78)	53
	Jamaica	('77) + Nigeria ('68)	49

* Plaque-purified large plaque virus.

but continuous evolution. Although it would have been more desirable to determine genetic stability over time using only isolates from a single region, such isolates were not available.

In the course of our analyses, it was observed that a given DEN-1 fingerprint pattern appeared characteristic of a particular geographical area, allowing us to establish three geographical groupings based upon percent shared oligonucleotides. With one exception, virus isolates from each of these three groups (i.e., Africa, the Caribbean, and Pacific/S.E. Asia) retained 45–100% oligonucleotide homology with other isolates within that region.

Unlike viruses in the African and Caribbean groups, the seven viruses studied from the Pacific and Southeast Asian regions displayed a substantially lesser degree of intragroup oligonucleotide homology. Only two or more viruses isolated from specific areas within these regions exhibited a very high degree of homology, i.e., the three 1974–1975 Bangkok isolates were identical (100% homology) while the two 1974 Nauru Island isolates were very similar (91% homology). However, the Pacific isolates from Hawaii and Nauru Island displayed only 47% homology to each other, and these in turn showed 49% and 44% oligonucleotide homologies, respectively, with the Bangkok isolates. We believe that the reduced oligonucleotide homologies displayed among viruses within the Pacific/S.E. Asian group are due in part to the large geographical area encompassed by this grouping as compared to the more geographically limited Caribbean and Nigerian groups.

The Sri Lankan isolate displayed a relatively high degree of homology (60 to 66%) with Nigerian isolates rather than with those isolates from its geographically proximal neighbor, Bangkok (21% homology). Because normally only 20–30% oligonucleotide homology could be detected between the three geographical virus groups, the Sri Lankan isolate, although recovered from the S.E. Asia region, appeared more like a member of the African group. Because Africa is the ancestral home of *Ae. aegypti* and it is thought that endemic dengue in Africa may have been the origin of various Asian and American strains,[19-22] it seems probable that the Sri Lanka DEN-1 virus could have been imported from Africa either directly or indirectly.

Because the fingerprint technique was able to distinguish between genomes of viruses isolated

from geographically different areas, it was applied to answer epidemiological questions concerning the origin of the 1977 Caribbean epidemic. Epidemiological investigations of the sudden appearance of DEN-1 in Jamaica suggested an African or Asian origin.[23] Analysis of pertinent RNA fingerprints revealed that the 1969 Sri Lankan and the 1978 Nigerian isolates exhibited 59% and 53% oligonucleotide homologies, respectively, with the 1977 Jamaican strain. Although none of these viruses approached identity, the striking homologies displayed between the Sri Lankan or African strain and the Jamaican strain suggest that the 1977 Jamaican epidemic may have resulted from a virus introduced from a region near either Sri Lanka or Africa. Although it might appear that virus from the Sri Lanka region was the more suspect source of the Jamaican epidemic, the Sri Lankan and Jamaican isolations were also separated by a span of 8 years. During those years the 1969 Sri Lankan strain may have evolved, but without a later Sri Lankan isolate this cannot be determined.

In similar studies, separate geographical isolates of DEN-2 virus strains also exhibited unique RNA fingerprints (P. Repik, unpublished data; D. Trent, personal communication), as have separate isolates of DEN-3 strains (D. Gubler, personal communication). These results all suggest that the technique of RNA fingerprinting can be a useful tool for flavivirus epidemiologists, allowing more precise identification of dengue isolates belonging to a particular subtype as well as monitoring genome changes that can occur over time and geographic distribution. Such studies would require numerous additional dengue virus strains from all endemic as well as epidemic areas.

The technique of oligonucleotide mapping or RNA fingerprinting is within the capabilities of many arbovirus laboratories and does measure certain changes in nucleotide sequence; however, there are rather serious limitations and it is by no means as precise as nucleotide sequence analysis. Oligonucleotide fingerprints are highly reproducible in duplicate analyses, and oligonucleotides found to be common by their migration on polyacrylamide gels actually do have identical nucleotide sequences.[24] In general, only the larger oligonucleotides (10 to ≈40 nucleotides in length) can be compared since they presumably represent unique RNA sequences. Such oligonucleotides had been thought to comprise approximately 10–15% of the viral genome.[5, 25] More recently, theoretical analysis of the RNA fingerprinting technique has been performed by computer-simulation,[26, 27] and has provided a means of estimating statistical confidence limits to be used when quantitatively comparing fingerprints of different RNA molecules. Large oligonucleotides (greater than 11 nucleotides in length) apparently represent approximately 30% of the entire genome, and on an average, only 85, 50, or 25% of large oligonucleotides remain in common when two overall RNA base sequences (full-length genomes) differ by 1, 5 or 10%, respectively. RNA fingerprinting is sensitive to small genome changes and is particularly useful only when comparing closely related RNAs or regions of RNAs that have overall base sequence homologies greater than 90%. Through extrapolation of data, computer-simulation can also be applied to help estimate the overall sequence homology of two RNAs based upon percent oligonucleotide homologies. Thus, viruses belonging to the Caribbean and African groups were estimated to have greater than 99% and 98.5% overall RNA genome homologies, respectively, while those in the Pacific/S.E. Asian group (Nauru Island, Hawaii, Bangkok) were estimated to have 93–95% genome homologies. Estimated total genome homologies between viruses of the three different geographic groupings appeared to be in the 85–89% range.

An additional observation from these studies was that the majority of oligonucleotides which appear to be conserved are those enriched in cytosine and adenine (left side of fingerprint), while those oligonucleotides which appear more subject to change contain a higher proportion of uracil (right side of fingerprint). No portion of the dengue genome has been sequenced aside from the 5′ terminus and penultimate base of DEN-3 virion RNA.[4] Therefore, the spatial organization of the oligonucleotides within the viral genome and subsequent coding assignments are unknown. The importance of oligonucleotide composition in resistance or susceptibility to change awaits detailed genetic characterization of the viral particle and a better understanding of replication strategies under both natural and laboratory pressures.

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