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Evolutionary dynamics of the American African genotype of dengue type 1 virus in India (1962–2005)

J.A. Patil [a], S. Cherian [b], A.M. Walimbe [b], B.R. Patil [c], P.S. Sathe [c], P.S. Shah [a], D. Cecilia [a,*]

[a] Dengue Group, National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411001, India
[b] Bionformatics Group, National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411001, India
[c] Virus Repository, National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411001, India

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ABSTRACT

Dengue is a major health problem in India with all four serotypes represented. Recently there has been an increase in the occurrence of dengue-1 outbreaks. It is possible that there have been changes in the genetics of dengue virus-1 (DENV-1), either by fresh introductions or by evolution in situ. The studies on DENV-1 evolution so far have no Indian sequences included. To gain insight into the dynamics of DENV-1 in India, the envelope (E) gene of thirteen virus isolates representative of the period 1962–2005 were sequenced and analyzed together with the available sequences of 40 globally representative isolates.

All the Indian DENV-1 isolates were found to belong to the American African (AMAF) genotype. With the addition of 13 Indian isolates, the AMAF genotype can now be called Cosmopolitan. The Indian isolates were distributed into four lineages, India I, II, III and the Africa lineage, now called Afro-India. Of these, India III was the oldest and extinct lineage; the Afro-India was a transient lineage while India I, imported from Singapore and India II, evolving in situ, were the circulating lineages. Despite the extinction and introduction of lineages, no specific codon site was observed to be under selection pressure. The rate of nucleotide substitution estimated for DENV-1 was 6.5×10^{-4} substitutions/site/year, and the time to the most recent common ancestor (tMRCA) was estimated to be 78–180 years (1825–1925), similar to previous estimates. The tMRCA for the AMAF/Cosmopolitan genotype was 56–98 years (1907–1949), a period that covers World War I and II. The two imports from Africa (1953–1968) and Singapore (1964–1975) and an export to the Americas (1955–1965) prove that there have been changes in the lineage of the DENV-1 viruses circulating in India which has contributed to the global dynamics of DENV-1 evolution and perhaps to the changing epidemiology of dengue in India.

1. Introduction

Dengue is caused by one of four antigenically related dengue virus (DENV) serotypes (DENV-1 to 4) belonging to the family *Flaviviridae*. The viral genome is a non-segmented, single stranded, positive-sense RNA of about 10.7 kb, which has a cap structure at its 5′ end and lacks a poly (A) tract at its 3′ extremity (Deubel et al., 1990). It encodes three structural and seven non-structural proteins in the order 5′-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′ (Chambers et al., 1990). In recent years, there has been considerable interest in the genetic structures of DENV populations and the underlying evolutionary processes (Holmes and Twiddy, 2003; Rico-Hesse, 2003). Phylogenetic analyses of DENV-1 using partial E gene or E/NS1 junction sequences had defined three genotypes, I–III (Chungue et al., 1995; Hwang et al., 2003) or five genotypes, I–V (Rico-Hesse, 1990). Using the complete E gene sequence Goncalvez et al. (2002) also defined five genotypes, I–V. Phylogenetic studies on Indian isolates of DENV-1 are limited. The first report including Indian isolates using complete E gene sequence defined five genotypes (Domingo et al., 2006). The Indian isolates that were obtained from travelers clustered into the American African (AMAF) genotype. Subsequently reports from India (Kukreti et al., 2009; Anoop et al., 2010) confirmed the same using a short region of C–prM junction, which is a conserved region of the genome.

DENV-1 has been circulating in India since the 1940s. During the period the disease profile has changed from mild to severe (Kukreti et al., 2009). DENV-1 was associated with a DHF outbreak in Delhi in 1997 (Kurukumbi et al., 2001). DENV-1 was also implicated during recent outbreaks in Delhi in 2006 and 2008 (Kukreti et al., 2008; Bharaj et al., 2008; Chakravarti et al., 2010). The change in disease spectrum in all probability can be attributed to change in circulating viruses, caused by either evolution in situ

* Corresponding author at: National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411001, Maharashtra, India. Tel.: +91 20 2600219; fax: +91 20 26122669.
E-mail addresses: cecilia.dayaraj@gmail.com, ceciliadayaraj@gmail.com (D. Cecilia).

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or introduction of new viruses. A change in the genotype of DENV-2 was shown to be associated with a change in disease profile in Cuba (Leitmeyer et al., 1999) and in India (Kumar et al., 2010).

Studying the evolutionary dynamics of DENV-1 in India over an extended period of time will be useful to understand the introduction and dissemination of the virus in the region. Earlier studies (Yang et al., 2000; Twiddy et al., 2002a,b; Bennett et al., 2006) have shown evidence of selection pressure and adaptive evolution in the E gene of dengue viruses. We sequenced the E gene of 13 Indian DENV-1 isolates obtained during 1962–2005 and looked for evidence of selection pressure and lineage reintroductions based on relatedness with publicly available global sequences of DENV-1 viruses.

2. Materials and methods

2.1. Viruses

The DENV-1 strains used in this study were isolated from different parts of India during epidemics spanning over ~40 years (1962–2005). Thirteen isolates were sequenced as part of this study (details of these virus strains are presented in Table 1). The earliest isolates, from 1962 to 1963, were from Vellore district, South India, while the rest of the isolates were from different regions of India. Eleven strains were isolated from human samples while two strains (NIV_62234_1962/M and NIV_623337_1962/M) were isolated from *A. aegypti* mosquitoes. All the viruses were isolated and archived by National Institute of Virology. Lyophilized viruses were procured from the Virus Repository and working stocks were prepared by inoculating Swiss albino suckling mice by the intracerebral route. Mice were maintained as per the guidelines of the Committee for Protection, Supervision and Control of Experiments on Animals (CPSCEA). Mice were observed for sickness; sick mice were euthanized and their brains were dissected out. A 10% suspension of mouse brain in 0.75% bovine albumin phosphate saline (BAPS) was prepared and stored at −70 °C in suitable aliquots until use.

2.2. RNA extraction, RT-PCR and sequencing

Viral RNA was isolated from 10% infected mouse brain suspension using QIAamp Viral RNA Mini kit (Qiagen), according to the manufacturer's instructions. cDNA was prepared with 5 μl RNA template using random hexamer and Avian Moloney Virus reverse transcriptase (Promega). The E gene was amplified using 5.0 μl cDNA, primers given in Table S1 and *Taq* polymerase (Invitrogen). PCR products were gel-purified using a QIAquick gel extraction kit (Qiagen). The purified PCR products were sequenced using BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) on an automatic sequencer (ABI PRISM Genetic Analyzer 3100; Applied Biosystems).

2.3. Molecular clock analysis

The dataset for the molecular clock analysis comprised seventeen Indian and forty global sequences representing the five different genotypes (sylvatic strains included). The E gene sequence were analyzed for nucleotide and amino acid diversity using CLUSTAL W implemented in MEGA v. 3.1 (Kumar et al., 2004). Sequences with >99.5% identity were removed from the analysis. Simultaneous estimation of phylogeny, rate of nucleotide substitution and divergence times (tMRCA) of DENV-1 strains of Indian origin and also of the different genotypes was carried out using the Bayesian MCMC approach as implemented in BEAST 1.5.3 (Drummond and Rambaut, 2007). The best-fit model of nucleotide substitution was selected by using Akaike Information Criterion (AIC) as implemented in MODELTEST 3.7 (Posada and Crandall, 1998). The GTR + G + I model (general time-reversible model with gamma-distributed rates of variation among sites and a proportion of invariable sites) was found to be the best-fit model for our dataset. We employed both strict and relaxed (uncorrelated exponential and uncorrelated lognormal) clock (Drummond et al., 2006) with different demographic models (constant size, exponential growth, logistic growth and expansion growth). The Bayesian skyline (BSL) plot was also constructed to study the genetic diversity of the population. Three independent MCMC analyses, each for 30, million steps, were performed for each combination of branch rate and demographic model, as well as for BSL and combined with a burn-in value set to 10% generations using LogCombiner program (implemented in BEAST). The convergence of the chain was evaluated by using Tracer 1.5 (Drummond and Rambaut, 2007). The effective sample size (ESS) values of >200 indicated sufficient level of sampling. The posterior probability and Bayes factor based on the marginal likelihoods of the models was used to choose the most suitable model for the data (Suchard et al., 2001). The maximum clade credibility tree was generated by using TreeAnnotator program (available in BEAST), and FigTree 1.2.3 (http://tree.bio.ed.ac.uk/) was used for visualization of the annotated tree. The 95% HPD intervals were used to ascertain the uncertainty in the parameter estimates.

2.4. Selection pressure analysis

Two data sets, one containing only Indian isolates and the other containing all isolates belonging to the AMAF genotype were included in the analysis. To identify the existence of positive selection pressure at individual codon sites in the E gene of DENV-

Table 1
Indian DENV-1 isolates sequenced in this study.

No.	Strain	Year	Location	Passage level	Accession No.
1	NIV_62227_1962	1962	Vellore	P-16	JF297571
2	NIV_62237_1962	1962/M[a]	Vellore	P-05	JF297572
3	NIV_62234_1962	1962/M[a]	Vellore	P-12	JF297573
4	NIV_63494_1963	1963	Vellore	P-15	JF297574
5	NIV_633760_1963	1963	Vellore	P-17	JF297575
6	NIV_631287_1963	1963	Vellore	P-15	JF297576
7	NIV_631288_1963	1963	Vellore	P-15	JF297577
8	NIV_703180_1970	1970	Delhi	Not known	JF297578
9	NIV_715393_1971	1971	Rajasthan	P-03	JF297579
10	NIV_826883_1982	1982	Delhi	P-06	JF297580
11	NIV_055290_2005	2005	Pune	P-02	JF297581
12	NIV_058741_2005	2005	Pune	P-02	JF297582
13	NIV_057516_2005	2005	Pune	P-02	JF297583

[a] Mosquito isolate.

1, three likelihood procedures were used: the SLAC and FEL methods and the more powerful REL method (Pond and Frost, 2005). The strength of selection pressure is determined on the basis of the ratio of non-synonymous (d*N*) to synonymous (d*S*) substitutions per site (ratio d*N*/d*S*). Sites were considered to be under positive selection if at least two of the methods indicated this with high statistical significance ($P < 0.1$/Bayes factor >50). The analysis was carried out using the online facility at the web server http://www.datamonkey.org, with a phylogenetic tree inferred by using the neighbor-joining method.

3. Results

3.1. Isolation and identification

The thirteen Indian isolates included in the study were obtained during outbreak investigations in different regions of India. Eleven were obtained by inoculation of mice with sera from infected individuals and two were obtained by inoculation of mosquito lysates. The serotype of all isolates was confirmed by multiplex RT-PCR and sequencing of the amplicon obtained. The entire E gene

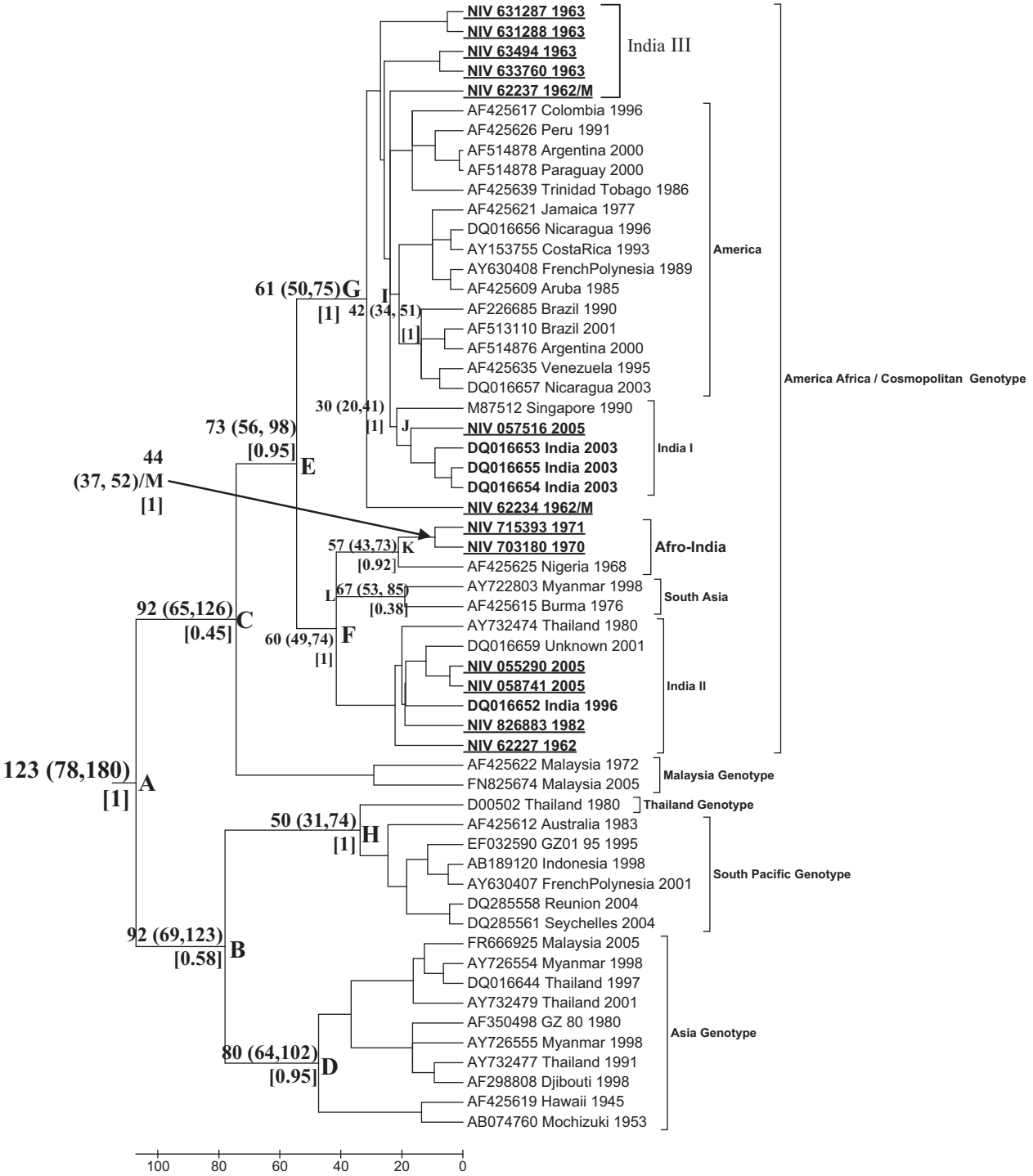

Fig. 1. Maximum clade credibility (MCC) tree of E gene sequences of DENV-1 with relaxed uncorrelated exponential clock with exponentially growing population size. The tMRCA estimates of the key nodes (labeled A–M) indicate time of divergence. Indian sequences are shown in bold and isolates sequenced in this study are bold and underlined.

was sequenced for the 13 isolates using RNA obtained from infected mouse brain stocks.

3.2. Molecular clock analysis

3.2.1. Phylogenetic analysis

The E gene sequences of the 17 Indian isolates (13 sequenced as a part of this study and 4 sequences retrieved from Genbank) were analyzed along with those of 40 publicly available global isolates. The viruses were distributed into five genotypes the America–Africa (AMAF), Malaysia (MAL), Thailand (THAI), Asia (ASIA), and South Pacific (SP) as previously reported by Domingo et al. (2006).

All the Indian isolates of DENV-1 representing the period from 1962 to 2005 fell into the AMAF genotype (Fig. 1). The genotype was earlier reported to contain five well-defined lineages America, Africa, India I, II and South Asia (Domingo et al., 2006). The Indian isolates, sequenced in the present study clustered with lineages India I, II and Africa. A group of five isolates formed a new lineage (India III), and one mosquito isolate (NIV_62234_1962/M) formed an outer branch. None of the Indian isolates clustered with lineage America. The India I lineage included Indian isolates of 2003 and 2005 and one isolate from Singapore, which has been reported to be a recombinant (Tolou et al., 2001). The India-II lineage included Indian isolates from 1982 to 2005. The isolates of 2005 represented in lineage India-I and II were obtained during a single outbreak in Pune. The India-III lineage contained exclusively viruses isolated in the 1960s. Two isolates obtained during 1970–1971 clustered with the Africa lineage which can therefore be referred to as the Afro-Indian lineage. Analysis of nucleotide diversity revealed that the intra-lineage divergence ranged from 1.4–2.8% while the inter-lineage divergence ranged from 2.9–5.4%. On the other hand the amino acid divergence was not very different, intra-lineage divergence being 0.8–2.45% and inter-lineage 1.28–2.45%. Comparison of the consensus amino acid sequences of the six AMAF lineages with the consensus sequence of the AMAF isolates used in this study showed substitutions at eight sites (Table S2). Of the six amino acid substitutions in lineage India-I, four were specific to the lineage while T339I was shared with the Afro-India lineage and T386A was shared with America and SA lineages. Furthermore, of the four specific changes, I129V, V436I and A481T were present exclusively in the Indian isolates and not in the Singapore isolate. In lineage India-II M297V/A was exclusive while V380I was shared with majority of SA strains. There was one change in the Afro-India lineage and no change in the India III lineage.

3.2.2. Nucleotide substitution rate and divergence times

Estimates of the nucleotide substitution rates and the time to most recent common ancestor (tMRCA) of all genotypes as well as Indian DENV-1 strains were inferred from the 57 dated E gene sequences. The exponential growth rate was found to be significant (1.8×10^{-2} 95% HPD limits: 1.4×10^{-3} to 3.6×10^{-2}) and was supported by posterior probability and BSL plot (Table S3, Fig. S1). Hence uncorrelated exponential clock with exponential growth demography was selected as the most suitable model. Under this model, the mean substitution rate was 6.5×10^{-4} substitutions/site/year [95% HPD limits: 4.8×10^{-4} to 8.3×10^{-4}] and estimate of the tMRCA for all genotypes of DENV-1 was around 123 years (node A) (95% HPD: 78,180 years) with respect to the most recent isolate of 2005 (Table S3).

Interestingly the BSL analysis provided similar estimates for the substitution rate (6.5×10^{-4}; 95%HPD 4.6×10^{-4} to 8.5×10^{-4}) and tMRCA (119.3; 95% HPD 72.7, 186.7) for all genotypes. The maximum clade credibility tree generated under the best fit model is shown in Fig. 1. Node A branched into two independently evolving nodes; B (95% HPD: 1882–1936) providing a common ancestor for SP and Asian genotypes and C (95% HPD: 1879–1940) providing common ancestor for Malaysian sylvatic and AMAF genotypes. The Asian genotype emerged during 1903–1941 (node D) whereas the SP genotype emerged later during 1931–1974 (node H). The lineages of the AMAF genotype shared a common ancestor which emerged during 1907–1949 (node E).

The AMAF genotype branched into nodes F (1920–1952) and G (1930–1955). Node F provided common ancestor for lineages Afro-India/SA/India II and node G provided common ancestor for India III/America/India I. The mosquito isolate NIV_62234_1962/M seemed to be the root of lineage India III which represented a common ancestor for India-I and America lineages (95% HPD: 1954–1971). The India-I lineage, shared a common ancestor with the Singapore strain (node J) (95% HPD: 1964–1985). In the Afro-Indian lineage, the two Indian isolates of 1970 and 1971 shared a common ancestor, node M (95% HPD: 1953–1968) which was probably derived from the African isolate (node K) (95% HPD: 1932–1962). The India-II lineage shared a common ancestor (node L) with the South Asia lineage (95% HPD: 1931–1956).

3.3. Selection pressure analysis

Different datasets were used, one consisting of only Indian sequences ($n = 17$) and one consisting of all AMAF genotype sequences ($n = 38$) used in this study. No selection pressure was noted within the Indian isolates (data not shown) but analysis of the AMAF genotype strains indicated weak evidence of positive selection at amino acid position 287. The lack of selection pressure within the group of Indian isolates cannot be attributed to the probable masking effect of varying passage levels, as we did observe variability of 1.6–2.0% in India I/II lineage, with isolates having minimum passage history.

4. Discussion

Phylogenetic analysis of DENV-1 isolates from India obtained over a period of about 40 years using the E region revealed that all Indian isolates belonged to genotype AMAF. With the addition 17 of Indian isolates and the already included isolates from Myanmar, the AMAF genotype could be referred to as 'Cosmopolitan' genotype. A total of six lineages were present in this AMAF/Cosmopolitan genotype, three India, one Afro-India, one South Asia and one America. The existence of four phylogenetically distinct lineages in India suggests that there may have been independent entries of the virus into the country. An earlier report by Anoop et al. (2010), which included three of the isolates sequenced in the present study, had also reported the categorization of Indian viruses into four Indian lineages on the basis of core/prM region.

Indian isolates of lineage I were closely related with an isolate from Singapore and may represent an independent entry of DENV-1 into India from Singapore, which is a major economic and travel hub in South-East Asia and may play an important role in the local dissemination of DENV. Lineage India II had viruses from 1962, 1982 and 2005 and therefore represents an evolving lineage. The 2005 isolates from Pune belonged to lineages I and II indicating that there was co-circulation of two lineages during a single outbreak. India III lineage contained isolates exclusively from the 1960s and therefore is representative of an extinct lineage. Isolates from 1970 and 1971 clustered with the previously identified lineage Africa (Domingo et al., 2006).

The diversity observed within and between lineages for DENV-1 was higher than that observed for DENV-2 (Kumar et al., 2010). Comparison of consensus amino acid sequences of the E gene of the six lineages revealed occurrence of substitutions in the four circulating lineages with lineage India I being the most evolving with 6 changes. The lack of substitutions in India-III indicates that it could be the parent lineage of the AMAF/Cosmopolitan genotype.

The rate of nucleotide substitution based on the molecular clock analysis, was estimated to be 6.5×10^{-4} subs/site/year and the tMRCA was 123 years for all DENV-1 genotypes, which is in agreement with previous estimates (Twiddy et al., 2003). The Malaysian sylvatic genotype shared a common node (node C) with the AMAF/Cosmopolitan genotype corresponding to the time period 1879–1913. However, the time estimate obtained is of low reliability because of low posterior support. Similar findings were reported by Teoh et al. (2010). Considering that the sylvatic genotype strains rooted from within the human genotypes, makes its origin somewhat uncertain.

The estimated tMRCA of the AMAF/Cosmopolitan genotype (node E) which encompassed all the Indian isolates was ~77 years old (between 1901 and 1949). The cosmopolitan characteristic of the genotype reflects the movement of laborers between the 3 continents of Africa, Asia and America in the early 1900s. Within the AMAF/Cosmopolitan genotype, the source of India III lineage is not clear, however the period is coincident with the introduction of the American genotype of DENV-2 into India (Kumar et al., 2010). The divergence of America and India I lineage at node I (1954–1963) within India III coincides with the increased global movements in the aftermath of World War II and suggests the export of DENV-1 from India to the Americas. The export of Indian viruses belonging to the Cosmopolitan genotype of DENV-2 was also reported earlier (Kumar et al., 2010). An interesting observation is that two mosquito isolates (NIV_62234_1962/M and NIV_62237_1962/M) formed the outer branches of the cluster of India III and India I/America respectively, further lending credence to the role of mosquitoes in the propagation and dissemination of dengue viruses. The node of divergence shared by two Indian isolates (1970, 1971) and the Nigerian isolate within the Afro-India lineage indicated an introduction coincidental with the influx of refugees from Uganda during 1953–1968 (Patel, 1972). The currently circulating lineages are India I and II. Lineages India II and South Asia shared a common ancestor at node L (1931–1945), which also coincides with the 1940s trafficking involved in World War II. Lineage India I appears to be one of the recent introductions into the country, from Singapore and may have an ancestor of the period 1964–1975. Unfortunately the non availability of additional Indian isolates before the 1990s does not allow for more robust analysis.

The role of natural selection pressure has been ascribed for strain extinction and/or replacement (Sittisombut et al., 1997; Wittke et al., 2002). Despite the extinction of two lineages (African and India III) and the survival of two lineages (India I and II), no strong selection pressure could be observed as was previously reported (Twiddy et al., 2002a,b). Residue 287 at which weak statistical signal was observed falls into domain II in a region that is important for post-fusion trimerization (Fritz et al., 2008).

5. Conclusion

In conclusion, all the 17 Indian viruses belonged to four of the six lineages of the AMAF/Cosmopolitan genotype of DENV-1. There is a strong suggestion of the import of viruses to India during 1953–1968 from Africa and later from Singapore and export of viruses to the Americas during 1955–1965. The extinction and continuation of lineages may be dependent on various selecting factors including immune response and transmissibility which could become more evident after full genome analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.05.011.

References

Anoop, M., Issac, A., Mathew, T., Philip, S., Kareem, N.A., Unnikrishnan, R., Sreekumar, E., 2010. Genetic characterization of dengue virus serotypes causing concurrent infection in an outbreak in Ernakulam, Kerala, South India. Indian J. Exp. Biol. 48 (Aug (8)), 849–857.

Bennett, S.N., Holmes, E.C., Chirivella, M.D., Rodriguez, M., Beltran, M., Vorndam, V., Gubler, D.J., McMillan, W.O., 2006. Molecular evolution of dengue 2 virus in Puerto Rico: positive selection in the viral envelope accompanies clade reintroduction. J. Gen. Virol. 87, 885–893.

Bharaj, P., Chahar, H.S., Pandey, A., Diddi, K., Dar, L., Guleria, R., Kabra, S.K., Broor, S., 2008. Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. Virol. J. 5, 1.

Chakravarti, A., Kumar, A., Matlani, M., 2010. Displacement of dengue virus type 3 and type 2 by dengue virus type 1 in Delhi during 2008. Indian J. Med. Microbiol. 28 (Oct–Dec (4)), 412.

Chambers, T.J., Hahn, C.S., Galler, R., Rice, C.M., 1990. Flavivirus genome organization, expression and replication. Annu. Rev. Microbiol. 44, 649–688.

Chungue, E., Cassar, O., Drouet, M.T., Guzman, M.G., Laille, M., Rosen, L., 1995. Molecular epidemiology of dengue-1 and dengue-4 viruses. J. Gen. Virol. 76 (Pt 7), 1877–1884.

Deubel, V., Laille, M., Hugnot, J.P., Chungue, E., Guesdon, J.L., Drouet, M.T., Bassot, S., Chevrier, D., 1990. Identification of dengue sequences by genomic amplification: rapid diagnosis of dengue virus serotypes in peripheral blood. J. Virol. Methods 30 (Oct (1)), 41–54.

Domingo, C., Palacios, G., Jabado, O., Reyes, N., Niedrig, M., Gascón, J., Cabrerizo, M., Lipkin, W.I., Tenorio, A., 2006. Use of a short fragment of the C-terminal E gene for detection and characterization of two new lineages of dengue virus 1 in India. J. Clin. Microbiol. 44 (Apr (4)), 1519–1529.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.

Fritz, R., Stiasny, K., Heinz, F.X., 2008. Identification of specific histidines as pH sensors in flavivirus membrane fusion. J. Cell Biol. 183, 353–361.

Goncalvez, A.P., Escalante, A.A., Pujol, F.H., Ludert, J.E., Tovar, D., Salas, R.A., Liprandi, F., 2002. Diversity and evolution of the envelope gene of dengue virus type 1. Virology 303, 110–119.

Patel, H., 1972. General amin and the Indian exodus from Uganda. J. Opin. 2 (Winter (4)), 12–22.

Holmes, E.C., Twiddy, S.S., 2003. The origin, emergence and evolutionary genetics of dengue virus. Infect. Genet. Evol. 3, 19–28.

Hwang, K.P., Chu, P.Y., Tung, Y.C., Wang, H.L., Yueh, Y.Y., Wu, Y.C., Chin, C., Lin, K.H., 2003. Molecular epidemiological study of dengue virus type 1 in Taiwan. J. Med. Virol. 70 (Jul (3)), 404–409.

Kukreti, H., Chaudhary, A., Rautela, R.S., Anand, R., Mittal, V., Chhabra, M., Bhattacharya, D., Lal, S., Rai, A., 2008. Emergence of an independent lineage of dengue virus type 1 (DENV-1) and its co-circulation with predominant DENV-3 during the 2006 dengue fever outbreak in Delhi. Int. J. Infect. Dis. 12 (5), 542–549.

Kukreti, H., Dash, P.K., Parida, M., Chaudhary, A., Saxena, P., Rautela, R.S., Mittal, V., Chhabra, M., Bhattacharya, D., Lal, S., Rao, P.V., Rai, A., 2009. Phylogenetic studies reveal existence of multiple lineages of a single genotype of DENV-1 (genotype III) in India during 1956–2007. Virol. J. Jan 6 (6), 1.

Kumar, M., Pasha, S.T., Mittal, V., Rawat, D.S., Arya, S.C., Agarwal, N., Bhattacharya, D., Lal, S., Rai, A., 2004. Unusual emergence of Guate98-like molecular subtype of DEN-3 during 2003 dengue outbreak in Delhi. Dengue Bull. 28, 161–167.

Kumar, S.R., Patil, J.A., Cecilia, D., Cherian, S.S., Barde, P.V., Walimbe, A.M., Yadav, P.D., Yergolkar, P.N., Shah, P.S., Padbidri, V.S., Mishra, A.C., Mourya, D.T., 2010. Evolution, dispersal and replacement of American genotype dengue type 2 viruses in India (1956–2005): selection pressure and molecular clock analyses. J. Gen. Virol. Mar (91 Pt 3), 707.

Kurukumbi, M., Wali, J.P., Broor, S., Aggarwal, P., Seth, P., Handa, R., Dhar, L., Vajapayee, M., 2001. Seroepidemiology and active surveillance of dengue fever/dengue haemorrhagic fever in Delhi. Indian J. Med. Sci. 55 (Mar (3)), 149–156.

Leitmeyer, K.C., Vaughn, D.W., Watts, D.M., Salas, R., De Chacon, I.V., Ramos, C., Rico-Hesse, R., 1999. Dengue virus structural differences that correlate with pathogenesis. J. Virol. 73, 4738–4747.

Pond, S.L., Frost, S.D., 2005. Datamonkey: rapid detection of selective pressure on individual sites of codon alignments. Bioinformatics 21, 2531–2533.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rico-Hesse, R., 1990. Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. Virology 174, 479–493.

Rico-Hesse, R., 2003. Microevolution and virulence of dengue viruses. Adv. Virus Res. 59, 315–341.

Sittisombut, N., Sistayanarain, A., Cardosa, M.J., Salminen, M., Damrongdachakul, S., Kalayanarooj, S., Rojanasuphot, S., Supawadee, J., Maneekarn, N., 1997. Possible occurrence of a genetic bottleneck in dengue serotype 2 viruses between the 1980 and 1987 epidemic seasons in Bangkok, Thailand. Am. J. Trop. Med. Hyg. 57, 100–108.

Suchard, M.A., Weiss, R.E., Sinsheimer, J.S., 2001. Bayesian selection of continuous-time Markov chain evolutionary models. Mol. Biol. Evol. 18, 1001–1013.

Teoh, B.T., Sam, S.S., Abd-Jamil, J., AbuBakar, S., 2010. Isolation of ancestral sylvatic dengue virus type 1, Malaysia. Emerg. Infect. Dis. 16 (Nov (11)), 1783–1785.

Tolou, H.J., Couissinier-Paris, P., Durand, J.P., Mercier, V., de Pina, J.J., De Micco, P., Billoir, F., Charrel, R.N., de Lamballerie, X., 2001. Evidence for recombination in natural populations of dengue virus type 1 based on the analysis of complete genome sequences. J. Gen. Virol. 82 (Jun (Pt 6)), 1283–1290.

Twiddy, S.S., Farrar, J.J., Vinh, C.N., Wills, B., Gould, E., Gustin, T., Lloyd, G., Holmes, E.C., 2002a. Phylogenetic relationships and differential selection pressures among genotypes of dengue 2 virus. Virology 298, 63–72.

Twiddy, S.S., Woelk, C.H., Holmes, E.C., 2002b. Phylogenetic evidence for adaptive evolution of dengue viruses in nature. J. Gen. Virol. 83, 1679–1689.

Twiddy, S.S., Holmes, E.C., Rambaut, A., 2003. Inferring the rate and time scale of dengue virus evolution. Mol. Biol. Evol. 20, 122–129.

Wittke, V., Robb, T.E., Thu, H.M., Nimmannitya, S., Kalayanrooj, S., Vaughn, D.W., Endy, T.P., Holmes, E.C., Aaskov, J.G., 2002. Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. Virology 301, 148–156.

Yang, J., Yang, P., Qin, E., 2000. Sequence analysis of the 5′ and 3′ terminal regions of dengue type 2 virus 04 strain. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 14 (Mar (1)), 24–26.