

Phylogeography and Molecular Epidemiology of an epidemic strain of DENV1 in Sri Lanka.

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Background: In 2009, a severe dengue epidemic in Sri Lanka caused more mortality and morbidity than any previous recorded epidemic had done in that country. Each year since, dengue has reached epidemic proportions. The 2009 epidemic correlated with a major shift in the predominant disease-causing dengue virus strains: prior to 2009, DENV2 or 3 was isolated from most patients presenting with serious dengue disease in Sri Lanka, whereas DENV1 has persisted as the major causative agent since 2009.

Methods: Serum of patients hospitalized for dengue infection in the capital city of Sri Lanka, Colombo, during the 2012 epidemic. Samples confirmed as Dengue-1 positive by reverse transcription-PCR (RT-PCR) (using previously published methods) were inoculated onto cultured *Aedes albopictus* mosquito (C6/ 36) cells to expand the virus. RT-PCR of the envelope region was conducted using previously published methods. Sequencing was by the Sanger method. Closely related viral strains were identified using the NCBI BLAST tool. Phylogeographic relationships between strains were investigated using the BEAST software package.

Results: To determine whether the dengue epidemics in Sri Lanka since 2009 have been caused by the same or a new strain of dengue, we collected and expanded viruses from the serum of patients hospitalized in the Sri Lankan Government Teaching Hospital, Colombo North (Ragama) during the 2012 dengue epidemic. The envelope region, a part of the genome previously identified to contain variability

capable of distinguishing strains, was sequenced for viruses expanded from 5 patients. A query of all publically available nucleotide sequences (NCBI BLAST) revealed that the current viruses were genetically most similar to the previously sequenced Sri Lankan DENV1 2009 epidemic strain, suggesting that this strain remained the major causative agent of severe disease in Colombo, Sri Lanka during the 2012 epidemic (Figure 1). Phylogeographic analysis of the 100 most similar published DENV1 envelope sequences suggests that the viral strain may have traveled directly or indirectly from Thailand, to China, to Sri Lanka, and that additional traffic of the virus between Sri Lanka and Singapore may have occurred. These results were supported by analysis using the full-length sequence of the 2009 DENV1 strain (previously published) (Figure 2). One silent mutation was identified in the viral envelope region of the 2012 viruses that may have become fixed in the genome since 2009 (Figure 3). Additional work is underway to sequence the 2012 strains in full to confirm the phylogeographic findings and to further analyze the evolution of the epidemic virus.

Discussion: Our findings illustrate the dissemination route of a particularly virulent DENV1 strain. Rapid and wide spread occurred within but not beyond South and South-East Asia. This data could inform global control efforts. In addition, the unusual extended presence of a single strain of dengue at high levels in Colombo, Sri Lanka provides a unique opportunity to study the evolution of the virus within a small, relatively homogenous population. Mutations that have become fixed in the epidemic strain since 2009 may hint at new virulence factors and immune epitopes, and will be the subject of future study.

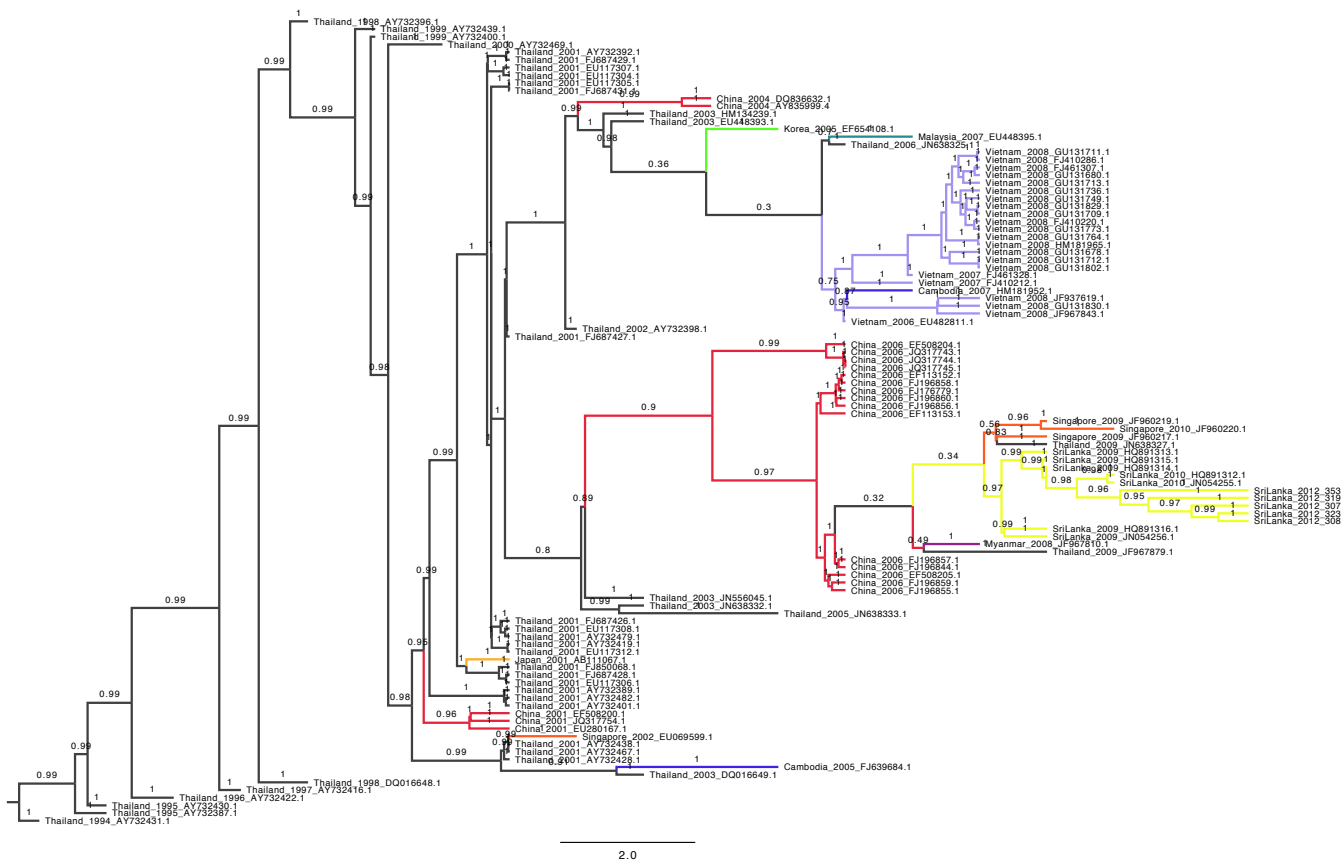


Figure 1: Origins of Sri Lankan 2012 DENV1: A consensus of a 350 bp region of Env sequenced from 5 2012 DENV1 viruses was used to query the NCBI database for the 100 most similar sequences. Phylogeographic analysis of these sequences was done using Bayesian methodology encompassed in the BEAST software package assuming symmetric dissemination between countries. Line and node colors indicate viral origin (country): Yellow: Sri Lanka, Orange: Singapore, Black: Thailand, Red: China, Lavendar: Vietnam, Green: Korea, Gold: Japan, Blue: Cambodia, Teal: Malaysia, Purple: Myanmar). Length of line represents predicted time of evolution since diversion from the most recent common ancestor (node). Number above line represents probability of country of origin assignment of the ancestral node calculated using Bayesian methods.

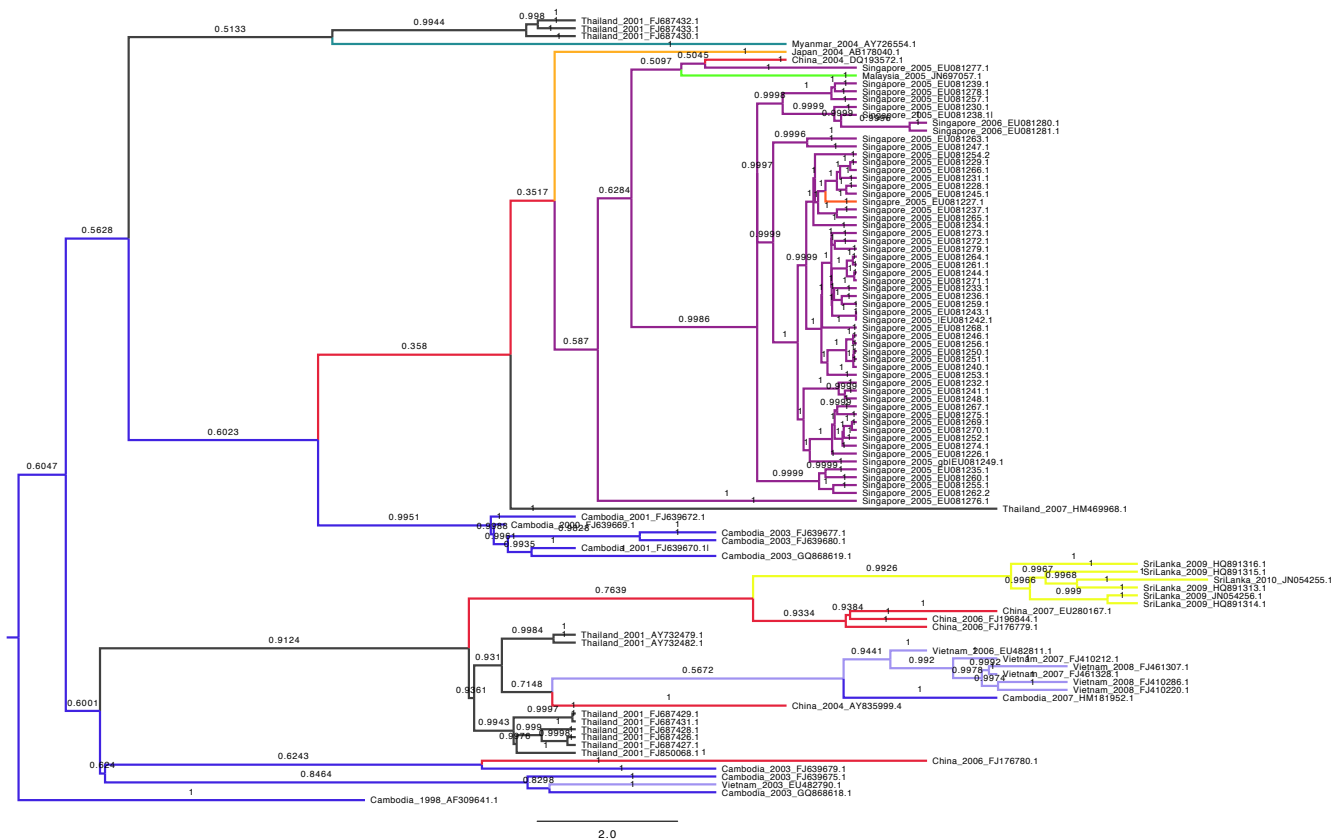


Figure 2: Origins of Sri Lankan 2009 DENV1: A consensus of full length sequences of DENV1 isolated during the 2009 epidemic used to query the NCBI database for the 100 most similar sequences. Phylogeographic analysis of these sequences was done using Bayesian methodology encompassed in the BEAST software package assuming symmetric dissemination between countries. Line and node colors indicate viral origin (country): Yellow: Sri Lanka, Purple: Singapore, Black: Thailand, Red: China, Lavendar: Vietnam, Green: Malaysia, Orange: Japan, Blue: Cambodia, Teal: Myanmar). Length of line represents predicted time of evolution since diversion from the most recent common ancestor (node). Number above line indicates probability of country of origin assignment of the ancestral node calculated using Bayesian methods.

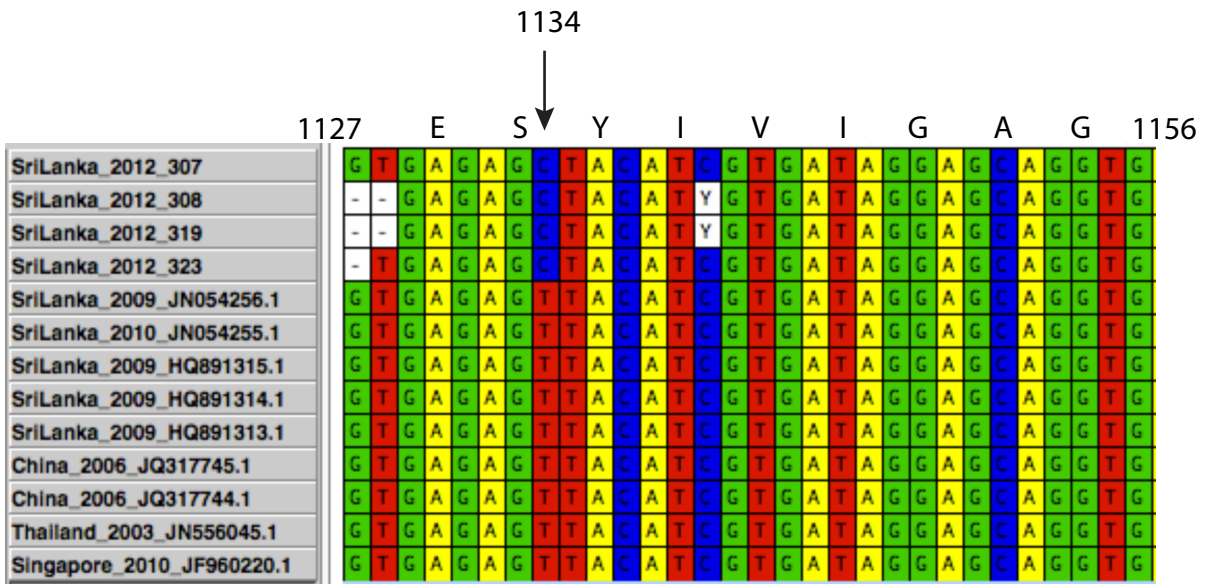


Figure 3: Evolution of Sri Lankan epidemic DENV1. The sequences of the envelope-encoding region of DENV1 for the Sri Lankan 2012 viruses were aligned with viruses sequenced from Sri Lankan patients in 2009 and 2010 as well as other related viruses from China, Thailand, and Singapore. Numbering is relative to the first base of the DENV1 envelope genetic sequence. Amino acid translation is indicated. T->G mutation is found at the wobble base nucleotide 1134 for all sequenced 2012 viruses from Sri Lanka.