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DIVERSIDADE INTRA-HOSPEDEIRO DO VÍRUS DA DENGUE TIPO 4 CIRCULANDO EM GUARUJÁ, SÃO PAULO

Dissertação apresentada ao programa de pós-graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Mestre em Ciências.

Área de concentração: Microbiologia

Orientador: Prof. Dr. Paolo Marinho de Andrade Zanotto

Versão original

São Paulo
2016

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INTRA-HOST GENETIC DIVERSITY OF DENGUE VIRUS TYPE 4 STRAINS FROM THE MUNICIPALITY OF GUARUJÁ, SÃO PAULO

Dissertation presented to the department of Microbiology, Graduate school of Biomedical Sciences at the University of São Paulo. In partial fulfillment of the requirements for the degree Master of Sciences.

Area of concentration: Microbiology

Advisor: Prof. Dr. Paolo Marinho de Andrade Zanotto

Original version

São Paulo
2016

RESUMO

Ortiz-Baez, AS. Diversidade intra-hospedeiro do vírus da dengue tipo 4 circulando em Guarujá, São Paulo. [Dissertação (Mestrado em Microbiologia)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo, 2016.

A caracterização da variabilidade genética intra-hospedeiro do vírus da dengue (DENV) é fundamental para a compreensão de sua evolução e dinâmica populacional no contexto atual como um importante patógeno viral humano. A diversidade viral acumulada em hospedeiros infectados influencia diretamente em outros aspectos como patogênese, transmissão e imunidade do hospedeiro. Contudo, apesar de existirem vários estudos sobre a diversidade genética intra-hospedeiro em dengue, nenhum tem sido relatado para DENV-4 até o presente momento. O ressurgimento e disseminação desse sorotipo foi associado com a sua co-circulação e o substituição dos sorotipos 1, 2 e 3 durante os recentes surtos no município do Guarujá-SP. Com base neste quadro epidemiológico, este estudo visou identificar a variação genética intra-hospedeiro do DENV-4 em amostras coletadas durante o surto de 2013, utilizando tecnologias de sequenciamento de nova geração. Portanto, nós caracterizamos a variabilidade genética de DENV-4 em diferentes níveis e as forcas evolutivas que afetam essa diversidade. Em adição, foram explorados os principais eventos na transmissão das variantes de DENV-4 identificadas. Nossos resultados revelaram uma baixa diversidade genética intra-hospedeiro para DENV-4. No entanto, mutação e pressões seletivas foram mecanismos importantes na variabilidade genética do vírus. A nível populacional, as variantes estão sujeitas á seleção natural negativa, não obstante identificamos seleção positiva atuando sob sítios específicos. Nenhuma evidência de recombinação foi detectada. Além disso, contra-intuitivamente, variantes de baixa frequência estão sendo transmitidas e contribuindo para diversidade genética do DENV-4 circulando em Guarujá. Nossos resultados fornecem novas evidências potencialmente úteis para futuros trabalhos focados em infecções mistas, escape imunológico, assim como o espalhamento e diversificação viral. Este estudo também e o primeiro trabalho a investigar a diversidade intra-hospedeiro do DENV-4.

Palavras-chave: Diversidade genética. Evolução molecular. Intra-hospedeiro. Vírus da Dengue tipo 4.

ABSTRACT

Ortiz-Baez, AS. Intra-host genetic diversity of dengue virus type 4 strains from the municipality of Guarujá, São Paulo. [Masters thesis (Microbiology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo, 2016.

Characterizing intra-host genetic variability in dengue virus (DENV) virus is paramount for understanding its evolution and population dynamics in the context of its current status as a major human viral pathogen. The extent to which viral diversity accrues in infected host influences aspects such as pathogenesis, transmission, and host immunity. Although there are several studies about intra-host genetic diversity in dengue, so far nothing has been revealed about DENV-4. In the Guarujá municipality in the State of São Paulo, the reemergence and spread of this serotype was associated with its co-circulation and the displacement of serotypes 1, 2 and 3 during recent outbreaks. Based on this epidemiological framework, we seek to identify the intra-host genetic variation of DENV-4 strains from samples collected during the 2013 outbreak by using deep sequencing technologies. We characterized the genetic variability of DENV-4 at different levels, and the forces shaping this diversity. Likewise, we explored major transmission events among DENV-4 variants. Our results revealed a low intra-host genetic diversity for DENV-4. However, we found selective and mutational pressures contributing to genetic diversity, while recombination did not seem play an important role. We further identified purifying selection at population level but sites subject to potential diversifying selection. Additionally, we observed low frequency haplotypes being transmitted among hosts and contributing to the viral diversity of DENV-4 circulating in Guarujá. Our findings provide preliminary insights for future studies in mixed infections, drug resistance, virus variant spread and immune scape. This study is the first effort to investigate the intra-host diversity of DENV-4.

Keywords: Genetic Diversity. Molecular Evolution. Intra-host. Dengue virus type 4.

1 INTRODUCTION

1.1 DENGUE VIRUS

Dengue virus (DENV) is a RNA virus, which belongs to the genus *Flavivirus* (family *Flaviviridae*) [1]. DENV structure consists of a 50 nm-diameter particle with an envelope of host-cell-derived lipids, associated with virus-derived proteins (envelope and membrane) (**Figure 1**). This structure encloses a ribonucleoprotein complex formed by the nucleocapsid protein and a single stranded, positive strand RNA of ≈11 kb in length [2]. Furthermore, the viral genome encodes a single open reading frame (ORF), flanked by untranslated regions (UTRs) at each end (95-450 nucleotides). The 5' UTR contain a cap structure while the 3' UTR lacks a poly (A) tail. Both 5'UTR and 3'UTR contain important elements critical for viral RNA replication and translation processes. Besides the UTRs, the viral RNA acts as an mRNA for the translation of ten viral proteins, starting with three structural proteins (C, prM, and E), and followed by seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [3] (**Figure 2**).

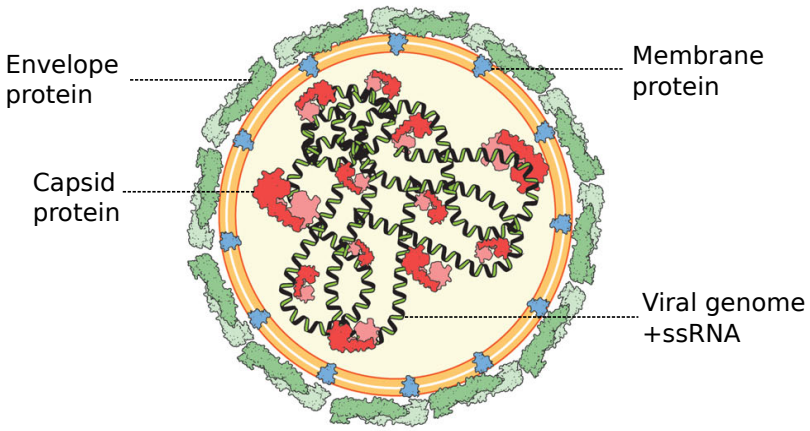

Figure 1 - Dengue virus structure. Schematic representation of dengue virus showing membrane and capsid structures organized around the viral genome. Figure adapted from: Cruz-Oliveira et al., 2015 [4].

The envelope glycoprotein (E) is considered as the major surface protein of DENV. It carries the main antigenic determinants that induces protective immunity, is essential for fusion activity and mediates receptor binding. Therefore, this protein presumably plays an important role in tropism, host range, and virulence [5]. A previous study also provided evidence of the

role of the E protein in assembly/disassembly process in flavivirus [6]. Crystal structures of the E protein in DENV have showed three structural and functional domains. Although antigenic regions have been reported for all the protein domains, it is possible to distinguish some domain-specific activities. Domain I (DI) is of particular importance for the organization of the protein structure and the DI-DII interface. Likewise, the domain II (DII) bears the fusion loop, providing an attachment point between the endosomal membrane and the viral membrane, thus triggering conformational changes in response to the acidic pH of the endosome. Finally, the domain III (DIII) participates in the recognition/ attachment to the cell surface, and harbors the receptor binding site [5,7,8] (**Figure 2**).

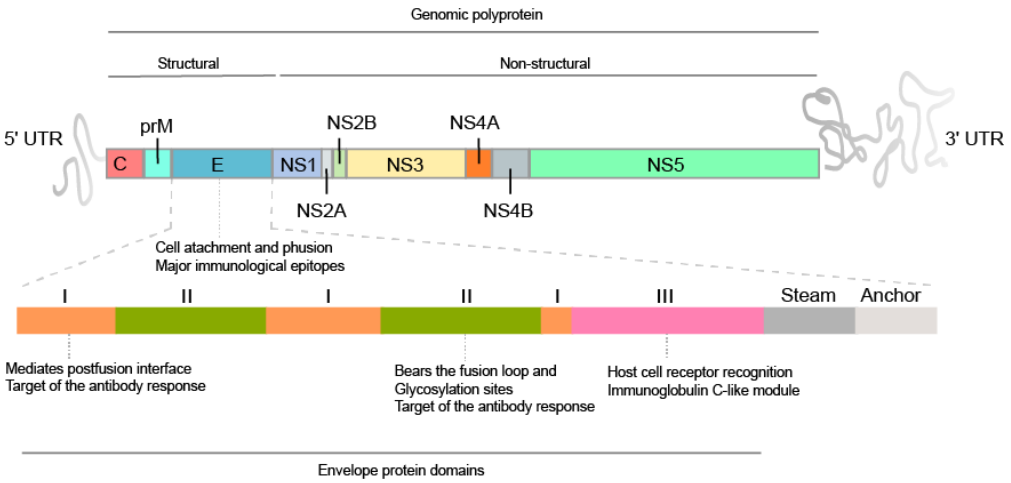

Figure 2 - Dengue virus genome. The viral genome is a positive-sense RNA that encodes ten genes flanked by the 5' and 3' untranslated regions. Genes encoding for structural proteins correspond to capside (C), pre-membrane (preM), and envelope (E). NS abbreviation represents genes encoding for non-stuctural proteins. The figure shows a zoom in the envelope protein, which is organized in three domains (DI-DIII), a stem and an anchor.

1.2 ECOLOGY AND TRANSMISSION IN BRIEF

Dengue viruses circulate in both human and wild animals (e.g. monkeys and bats). In sylvatic enzootic cycles, transmission among non-human primates is mediated by arboreal canopy-dwelling Aedes spp. [1,3]. It is hypothesized that viruses circulating among humans, emerged multiple times independently over the course of evolution, from sylvatic strains circulating in Africa or Southeast Asia forests. DENV Sylvatic strains continue to circulate in these regions, increasing the risk of newly emerging strains involved in sustained transmission chains. In human populations, DENV is responsible for the most common arthropod-borne viral disease, namelly dengue fever (DF) [9]. Mosquito vectors of genus Aedes mediate the transmission of this arbovirus in endemic/epidemic cycles. The *Aedes* (Stegomya) *aegypti* subsp. *aegypti* is the main anthropophilic vector in urban cycles

throughout the tropical and subtropical regions of the world [10,11]. The peridomestic *Aedes* (Stegomya) *albopictus* is the main responsible by the transmission in subtropics and peri-urban settings. The global distribution of both vectors defines the worldwide establishment of the four DENV serotypes, reaching more than 100 countries, and putting in risk nearly half of the world's population (**Figure 3**) [3,12].

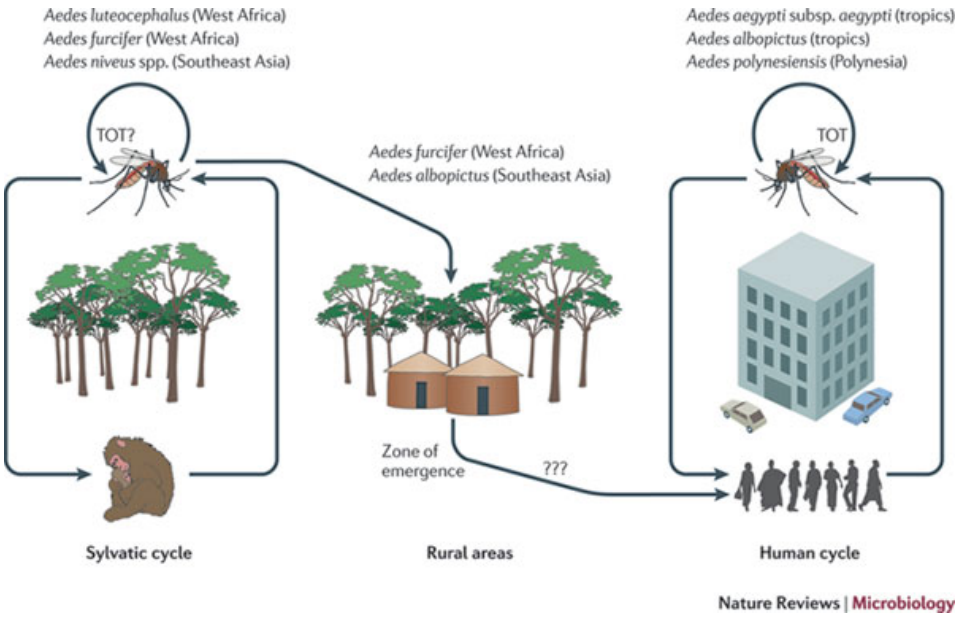

Figure 3 - Hypothetic origin and transmission of dengue virus in sylvatic, semi-urban and urban cycles. Source: Vasilakis et al., 2011 [3].

1.3 CLASSIFICATION

Four genetically and antigenically related serotypes of DENV are known (DENV 1-4) (**Figure 4**). Indeed, a recent study proposed the emergence of a fifth serotype circulating in a sylvatic transmission cycle [13]. The viral infection by any of them can result in a broad spectrum of clinical features, ranging from dengue without symptoms to overt severe manifestations, such as dengue haemorrhagic fever or shock syndrome [14]. Moreover, within each serotype it is possible to recognize a wide genetic diversity partitioned into monophyletic clusters of sequences termed genotypes, which also differ in their spatial-temporal distribution [15]. The absence of proof reading-repair activities contributes largely to the extensive genetic diversity within DENV populations [15,16].

Four major genotypes have been identified in DENV-4 [17,18]. Genotype I: Southeast Asia, genotype II: Southeast Asia and the Americas, genotype III: recent Thai strains isolated between 1997 and 2001 and, genotype IV: sylvatic DENV-4 strains isolated from sentinel monkeys in Malaysia. The genotype circulating in the Americas shows an additional spatiotemporal division, which segregates clade I (strains isolated in the Americas and Asian strains collected prior to 2000), from clade II (Asian strains collected after 2000) [1]. The

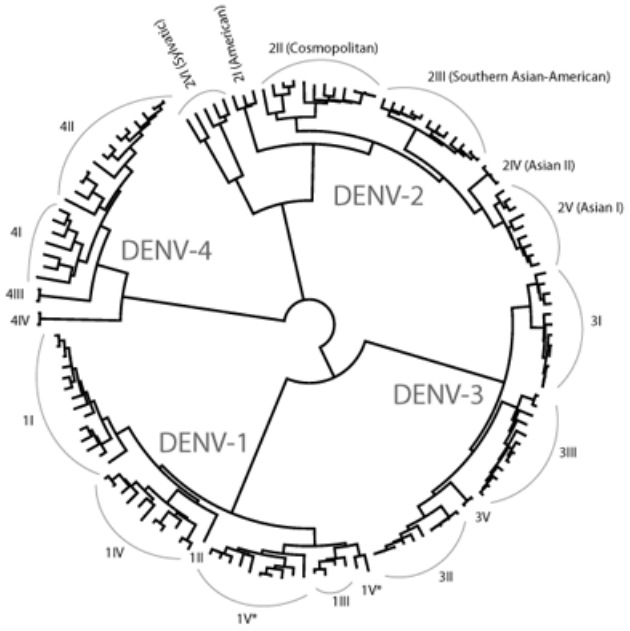

Figure 4 - Dengue virus phylogeny. The tree shows the classification of dengue viruses into serotypes and genotypes. Source: http://bioafrica2.mrc.ac.za.

Origin of DENV-4 in the Americas is currently unclear. Previous studies have suggested the introduction of this serotype (genotype II) from the Southeast Asia (French Polynesia) through the Caribbean [19,20], while other recent approach suggests the introduction through South America [21].

1.4 DENGUE IN THE WORLD AND BRAZIL

Currently, dengue is present in more than 125 countries in tropical and subtropical regions of the globe (**Figure 5**). More than 50% (3.6 billion people) of the global population is at risk, and an estimated 50 to over 200 million. DENV infections occur annually [22,23]. In Latin America, despite the implementation of an integrated management strategy for the prevention and control of dengue by the Pan American Health Organization (PAHO) [24], there have

been a progressive regional increase in the number of outbreaks and reported cases [9,25]. Co-circulation of multiple viral serotypes (hyperendemicity) and genotypes is considered the most common factor associated with the emergence of severe dengue, because of the heterologous DENV infections increase the probability of antibody-dependent enhancement (ADE), through enhancing the infection and replication of dengue virus in mononuclear cells [26–28].

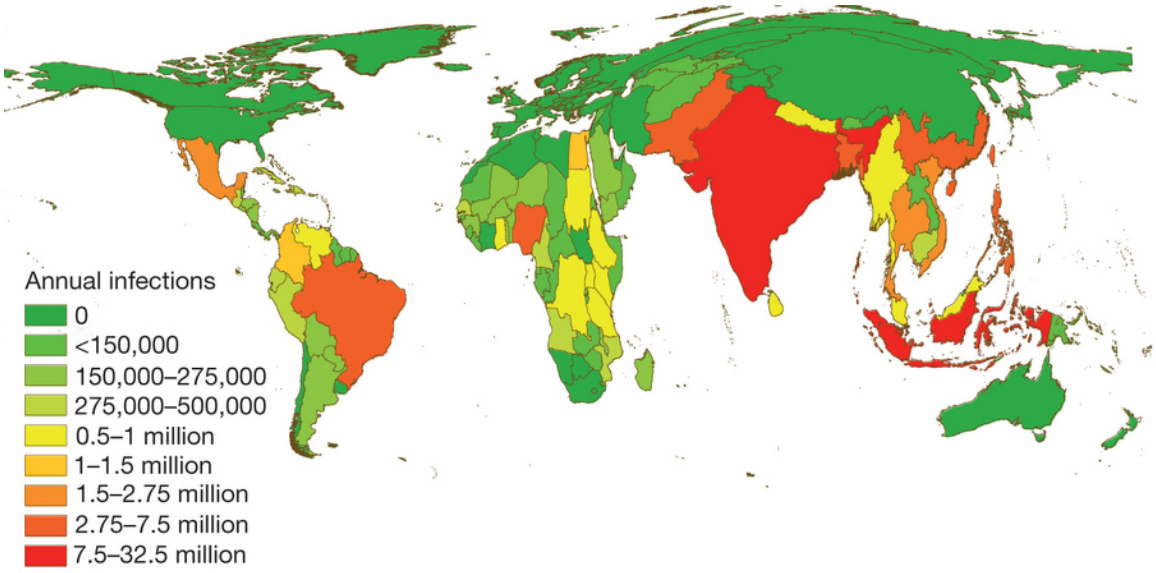

Figure 5 - Global distribution of dengue. World map representing the geographic distribution of dengue and the annual number of infections. Adapted from Bhatt et al., 2013.

Dengue has been a serious health problem in Brazil for more than 30 years [29]. The complex interplay of several factors such as hyperendemicity, deficiencies in the vector management, lack of public awareness, environmental factors, and the breakdown in the healthcare policies and procedures, imposes a tough challenge in the control, and prevention of the disease in the country. Furthermore, the costs deriving from disease-associated treatment have a major impact on financial resources and the welfare of both governments and communities [30,31]. According to the PAHO, since 2006 to 2015, Brazil has contributed with around US $ 450 million in the regional dengue program[1], while the national dengue cost estimated is US$ 310 million per year on average, excluding vector control, loss in productivity and prevention campaigns [32].

In 2013, over 1.450.000 clinical cases were reported in Brazil, 3.749 with severe manifestations, and 201 deaths [33], hence reaching the highest record of cases ever in the

country **(**data up to August 20 17, 2016**)** [34,35] **(Figure 6)**. Likewise, in 2013, 39.52% of notified cases were registered in the Southeast region of the country [36], being São Paulo among the states reporting the highest number of dengue cases. Guarujá, a coastal city located around 63 km away from São Paulo (**Figure 7**), and with a population of 290.752 inhabitants (IBGE index), was one of the worst affected areas in the southeast region during the 2013 epidemic. A recent study conducted in this municipality, revealed the co-circulation of the four serotypes in the early days of the dengue epidemic [33]. Nevertheless, a shift in serotype during the season showed that DENV-4 becomes the dominant serotype in Guarujá during the 2013 epidemic [33,37]. The spreading of DENV-4 and the population susceptibility triggered a major dengue emergency situation.

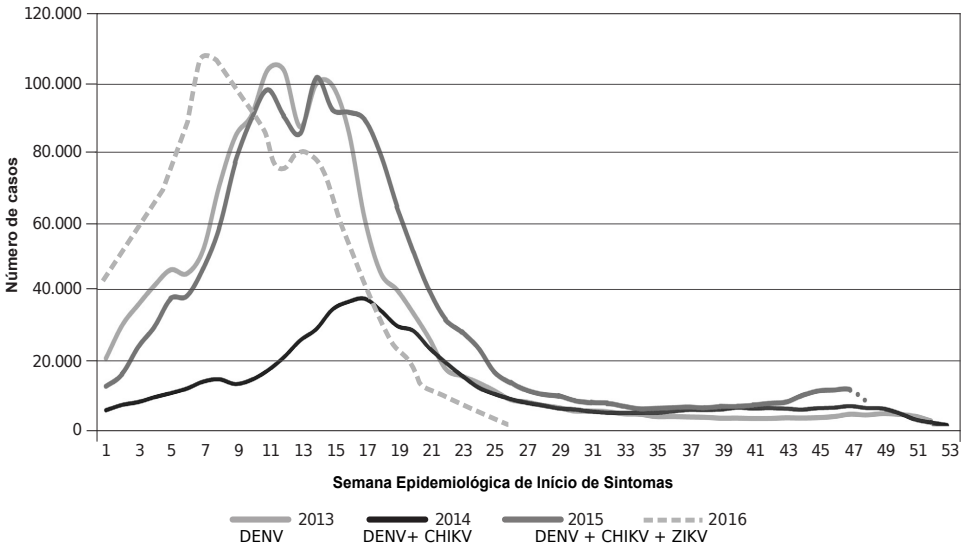

Figure 6 - Number of cases of CHIKV, DENV and ZIKV reported by epidemiological week. Adapted from Secretaria de vigilância em Saúde [34,38].

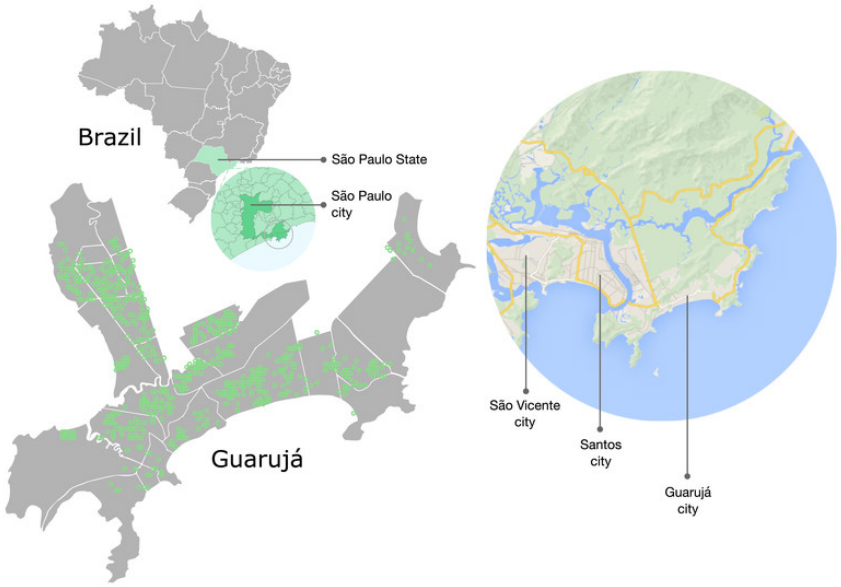

Figure 7 - Maps showing the geographic location of the municipality of Guarujá in São Paulo**.** Adapted from Villabona-Arenas et al., 2016 [37].

The early spread of DENV-4 started in 1953 in Philippines and Thailand. Then the virus spread to the French Polynesia and Southeast Asian countries, and was reported in the Americas until 1981 in several Caribbean countries and Brazil. Since then, the virus continued to expand its distribution, reaching several countries in South America and Asia (**Figure 8**) [39]. In Brazil, the reemergence of DENV-4 was documented in Boa Vista, Roraima State, 28 years after it was last detected in 1981 [40,41]. Afterwards, the virus was broadly associated with outbreaks in the Amazon, Bahia, Ceará, Goiás, Mato Grosso do Sul, Pernambuco, Pará, Piauí, Rio de Janeiro, Rio Grande do Sul, and São Paulo state. The spread of DENV-4 within Guarujá (São Paulo state) and the ensuing serotype turnover became into a dramatic epidemiological situation within a short time [33], providing an important opportunity to explore the intra-host diversity of DENV-4 into an urban population previously exposed to the remaining three serotypes.

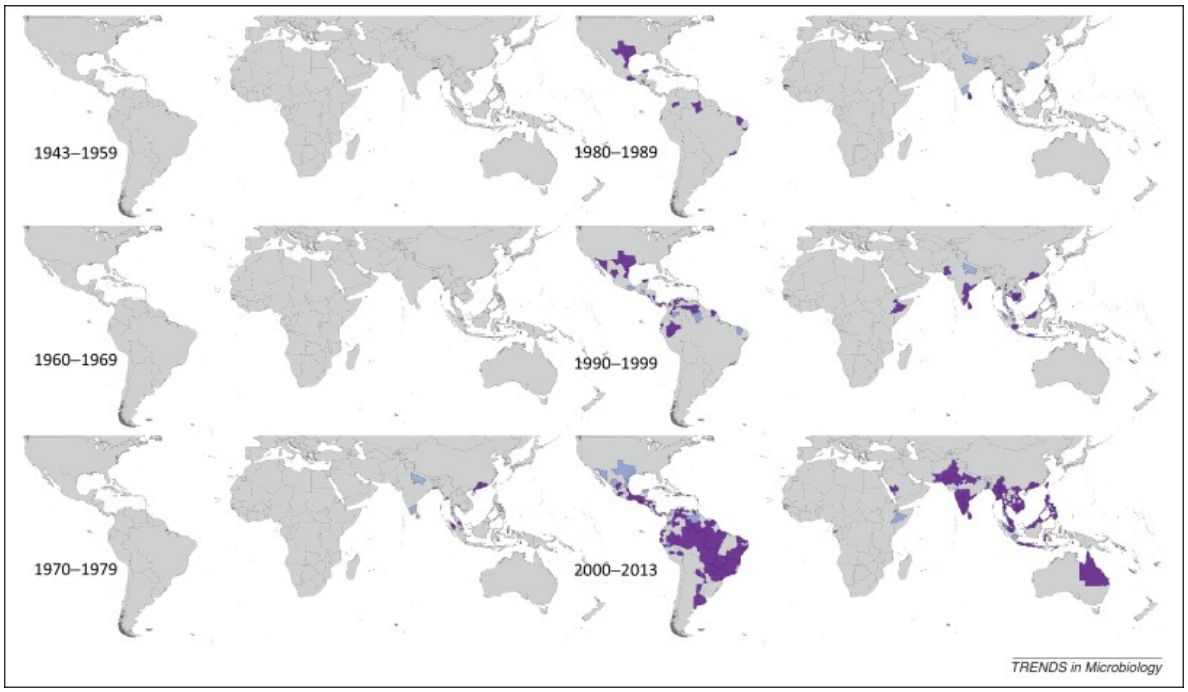

Figure 8 - Worldwide spread of DENV-4 from 1943 to 2013. Source: Messina et al., 2014 [39].

1.5 BASIC OF VIRAL INTRA-HOST DIVERSITY

Intra-host genetic variation in RNA viruses is ultimately determined by the error-prone nature of the RNA polymerase and the lack of proofreading mechanisms during replication [42–44]. As a result, an infected organism can harbor a collection of closely related variants or haplotypes which are subject to selection pressures imposed by the host environment [43,45]. (**Figure 9**). It is well known that genetic variation is the fuel for natural selection [46,47]. The occurrence of natural selection is important to promote advantageous mutations and purge deleterious changes in virus populations. Similarly, recombination, complementation and stochastic processes contribute to shape the heterogeneity in viral populations within a single host [48]. Therefore, in view of the underlying viral diversity, some variants in the pool of mutants will be potentially able to quickly adapt and confront changing environments.

Thai et al. (2011) suggested that intra-host virus diversity could be potentially increased by mixed infections, which would provide the raw material for intra-serotype recombination [49]. Moreover, mixed infections can be caused by different combinations of serotypes or

genotypes [50–53]. Importantly, hyperendemic transmission in endemic countries have increased the likelihood of mixed infections with different serotypes [54,55].

Heterogeneity in RNA virus populations can be accurately characterized using Next-Generation Sequencing (NGS) [56–59]. In general, NGS provides an opportunity to study viral diversity beyond the consensus sequence with cost-effective options, high throughput and resolution [60]. Moreover, the development of these technologies has showed a great potential for the study of re-emerging RNA viruses with sustained transmission cycles in humans, like dengue (DENV) [56,61–63]. For example, using the resolution enabled by next generation sequencing (NGS) technologies, a recent study explored the genetic diversity of DENV populations during human and mosquito infections [64]. Likewise, Lequime et al., [62] showed the evolutionary forces acting on DENV populations in the mosquito vector. The revolution of NGS in many fields of virology suggests the potential of these tools to investigate and unravel a wide range of questions in clinical virology.

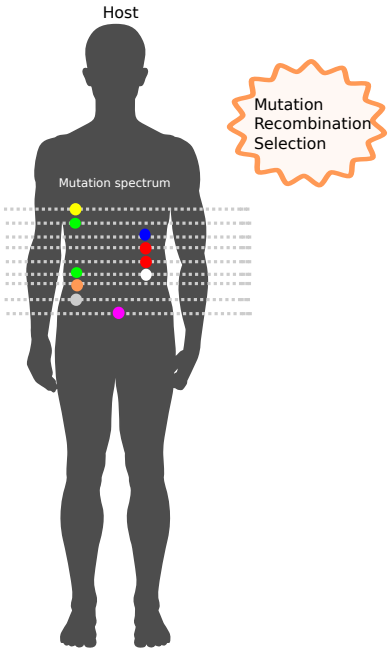

Figure 9 - Schematic representation of intra-host virus diversity and the most important factors shaping diversity in RNA viruses.

Understanding the intra-host genetic diversity of viruses is of critical importance to assess key aspects of disease pathogenesis, transmission, surveillance, and viral evolution in infected hosts. Despite, viral genetic diversity defines many evolutionary and epidemiological aspects

in DENV [65], there are not studies on the extent of the intra-host diversity of DENV-4. Related studies based on the remaining serotypes have focused on assessing the relationship between intra-host genetic variability and different issues such as disease severity [49,56,66,67], viral emergence [68], host's factors [69], clade identity [66], inter-host transmission [56,66,69], and selective pressures acting on viral populations [70–72]. Nevertheless, an important gap remains in our understanding of several aspects of DENV-4 evolution.

This work is part of an effort to assess the composition of DENV-4 populations at the intra-host level and improve our understanding about how this virus evolves and contributes to the extent of genetic diversity of DENV in Guarujá, which is ultimately a good indication of the viruses circulating near the largest metropolis of Brazil.

6 CONCLUSIONS

1) DENV-4 circulating in Guarujá corresponded to the most broadly distributed lineage in Brazil, which was introduced in 2005 into northern Brazil from South American countries and spread widely across several Brazilian locations.

2) Guarujá sequences were grouped in two lineages. Additionally, we identified one isolate from Guarujá closely related with strains from São José de Rio Preto and Mato Grosso.

3) This study provides the first DENV-4 genomes available for the 2013 urban outbreak in Guarujá, São Paulo.

4) Our results corroborate the co-circulation of DENV-1 and DENV-4 and suggest the occurrence of potential mixed infections during the DENV-4 outbreak, in the city of Guarujá, São Paulo.

5) DENV-4 evolves into heterogeneous populations composed of a few dominant haplotypes and a large number of low-frequency haplotypes. Although some of these viral variants can be maintained at the inter-host level their frequencies fluctuate among individuals.

6) Evolution of intra-patient populations of DENV-4 lies on low diversity levels during infection. However, envelope domains I and II display a high variability.

7) We did not find any evidence for intra-host recombination in DENV-4 populations circulating in infected hosts during the 2013 epidemic.

8) Preferred synonymous codons were dominated by A-ended codons, whilst charged amino acids played a major contribution in amino-acid usage in DENV-4 at the intra-host level.

9) Mutational bias and natural selection contributes to the codon usage bias in the envelope of DENV-4 at the intra-host level.

10) Selection pressures imposed on DENV-4 populations at the intra-host level differ between the entire envelope gene and individual sites. Negative selection operates on the gene-wide

scale, while positive selection acting at a per-site level would be influencing pH-dependent postfusion conformational changes.

REFERENCES*

1. Chen R, Vasilakis N. Dengue--quo tu et quo vadis? Viruses. 2011 Sep;3(9):1562–608.

2. Gebhard LG, Filomatori C V, Gamarnik A V. Functional RNA Elements in the Dengue Virus Genome. Viruses. 2011;3:1739–56.

3. Vasilakis N, Cardosa J, Hanley KA, Holmes EC, Weaver SC. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. Nat Rev Microbiol. 2011 Jun 13;9(7):532–41.

4. Cruz-Oliveira C, Freire JM, Conceição TM, Higa LM, Castanho MARB, Da Poian AT. Receptors and routes of dengue virus entry into the host cells. FEMS Microbiol Rev. 2015 Mar;39(2):155–70.

5. Crill WD, Chang G-JJ. Localization and Characterization of Flavivirus Envelope Glycoprotein Cross-Reactive Epitopes. J Virol. 2004 Dec 15;78(24):13975–86.

6. Heinz FX, Auer G, Stiasny K, Holzmann H, Mandl C, Guirakhoo F, et al. The interactions of the flavivirus envelope proteins: implications for virus entry and release. In: Positive-Strand RNA Viruses. Vienna: Springer Vienna; 1994. p. 339–48.

7. Modis Y, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. Nature. 2004 Jan 22;427(6972):313–9.

8. Nayak V, Dessau M, Kucera K, Anthony K, Ledizet M, Modis Y. Crystal Structure of Dengue Virus Type 1 Envelope Protein in the Postfusion Conformation and Its Implications for Membrane Fusion. J Virol. 2009 May 1;83(9):4338–44.

9. Murray NEA, Mikkel B Q, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299–309.

10. Weaver SC, Vasilakis N. Molecular evolution of dengue viruses: Contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. Infect Genet Evol. 2009;9(4):523–40.

11. Raghwani J, Rambaut A, Holmes EC, Hang VT, Hien TT, Farrar J, et al. Endemic Dengue Associated with the Co-Circulation of Multiple Viral Lineages and Localized Density-Dependent Transmission. PLoS Pathog. 2011;7(6):e1002064.

12. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. Elife. 2015;4:e08347.

*De acordo com: International Committee of Medical Journal Editors. [Internet]. Uniform requirements for manuscripts submitted to biomedical journals. [2011 Jul 15]. Available from: http://www.nlm.nih.gov/bsd/uniform_requirements.htlm

13. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Med journal, Armed Forces India. 2015 Jan;71(1):67–70.

14. WHO. Dengue guidelines for diagnosis, treatment, prevention and control. New. Geneva: World Health Organization; 2009. 147 p.

15. Holmes EC. The evolution and emergence of RNA viruses. Oxford University Press; 2009. 288 p. (Oxford series in ecology and evolution).

16. Holmes EC, Burch SS. The causes and consequences of genetic variation in dengue virus. Trends Microbiol. 2000;8:74–7.

17. Villabona-Arenas CJ, de Andrade Zanotto PM. Evolutionary history of Dengue virus type 4: Insights into genotype phylodynamics. Infect Genet Evol. 2011;11(5):878–85.

18. Vasilakis N, Weaver SC. Chapter 1 The History and Evolution of Human Dengue Emergence. In: Karl Maramorosch AJS, Murphy FA, editors. Academic Press; 2008. p. 1–76. (Advances in Virus Research; vol. 72).

19. Foster JE, Bennett SN, Vaughan H, Vorndam V, McMillan WO, Carrington CVF. Molecular evolution and phylogeny of dengue type 4 virus in the {C}aribbean. Virology. 2003;306:129–34.

20. Henchal EA, Repik PM, McCown JM, Brandt WE. Identification of an antigenic and genetic variant of dengue-4 virus from the Caribbean. Am J Trop Med Hyg. 1986 Mar;35(2):393–400.

21. Carrington CVF, Foster JE, Pybus OG, Bennett SN, Holmes EC. Invasion and Maintenance of Dengue Virus Type 2 and Type 4 in the Americas. J Virol. 2005 Dec;79(23):14680–7.

22. Gubler DJ. Dengue, Urbanization and Globalization: The Unholy Trinity of the 21(st) Century. Trop Med Heal. 2011;39(4 Suppl):3–11.

23. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr 25;496(7446):504–7.

24. JL SM, Brathwaite-Dick O. Integrated strategy for dengue prevention and control in the Region of the Americas. Rev Panam Salud Publica. 2007;21:55–63.

25. Mart'in S, Luis J, Brathwaite, Olivia, Zambrano, Betzana, et al. The Epidemiology of Dengue in the Americas Over the Last Three Decades: A Worrisome Reality. Am J Trop Med Hyg. 2010;82(1):128–35.

26. Anoop M, Issac A, Mathew T, Philip S, Kareem NA, Unnikrishnan R, et al. Genetic characterization of dengue virus serotypes causing concurrent infection in an outbreak in Ernakulam, Kerala, South India. Indian J Exp Biol. 2010;48:849–57.

27. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a

continuing global threat. Nat Rev Microbiol. 2010 Dec;8:S7–16.

28. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med. 1977;146(1):201–17.

29. Osanai CH, Travassos da Rosa AP, Tang AT, do Amaral RS, Passos AD TP. Dengue outbreak in Boa Vista, Roraima [in Portuguese]. Rev Inst Med Trop Sao Paulo. 1983;(25):53–4.

30. Guzmán MG, Kour G. Dengue diagnosis, advances and challenges. Int J Infect Dis. 2004;8(2):69–80.

31. PAHO W. State of the art in the Prevention and Control of Dengue in the Americas Meeting report. Washington, D.C.; 2014.

32. Instituto de Avaliação de Tecnologia em Saúde (IATS) [Internet]. 2015. Available from: http://www.iats.com.br/?p=noticias&id=388

33. Villabona-Arenas CJ, de Oliveira JL, Capra CDS, Balarini K, Loureiro M, Fonseca CRTP, et al. Detection of four dengue serotypes suggests rise in hyperendemicity in urban centers of Brazil. PLoS Negl Trop Dis. 2014 Mar;8(2):e2620.

34. Vigilância S De. Boletim epidemiológico. São Paulo, Brasil; 2016.

35. Brasil S. Casos de Dengue. Brasil, Regiões e Unidades Federadas, 1990 a 2014. 2015.

36. Organização Pan-Americana da Saúde - Brasil. Dados da dengue no Brasil, 2013.

37. Villabona-Arenas CJ, de Oliveira JL, de Sousa-Capra C, Balarini K, Pereira da Fonseca CRT, Zanotto PM de A. Epidemiological dynamics of an urban Dengue 4 outbreak in São Paulo, Brazil. PeerJ. 2016 Apr 5;4:e1892.

38. Vigilância S De. Boletim epidemiológico. Vol. 46. São Paulo, Brasil; 2015.

39. Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol. 2014;22(3):138–46.

40. Pybus OG, Rambaut A. Evolutionary analysis of the dynamics of viral infectious disease. Nat Rev Genet. 2009 Aug;10(8):540–50.

41. Temporão JG, Penna GO, Carmo EH, Coelho GE, do Socorro Silva Azevedo R, Nunes MRT, et al. Dengue Virus Serotype 4, Roraima State, Brazil. Vol. 17, Emerg Infect Dis. 2011.

42. Sim S, Hibberd ML, Bhatt S, Gething P, Brady O, Messina J, et al. Genomic approaches for understanding dengue: insights from the virus, vector, and host. Genome Biol. 2016 Dec 2;17(1):38.

43. Andino R, Domingo E. Viral quasispecies. Virology. 2015 Mar 27;479:46–51.

44. Domingo E, Sheldon J, Perales C. Viral quasispecies evolution. Microbiol Mol Biol Rev. 2012 Jun;76(2):159–216.

45. Kurosu T. Quasispecies of dengue virus. 2011;39(4 Suppl):29–36.

46. Moya A, C.Holmes E, González-Candelas F. The population genetics and evolutionary epidemiology of rna viruses. Nat Rev Microbiol. 2004;2:1–11.

47. Lauring AS, Frydman J, Andino R. The role of mutational robustness in RNA virus evolution. Nat Rev Microbiol. 2013 May;11(5):327–36.

48. Andino R, Domingo E. Viral quasispecies. Virology. 2015 May;479–480:46–51.

49. Thai KTD, Henn MR, Zody MC, Tricou V, Nguyet NM, Charlebois P, et al. High-Resolution Analysis of Intrahost Genetic Diversity in Dengue Virus Serotype 1 Infection Identifies Mixed Infections. J Virol. 2012;86(2):835–43.

50. Gubler DJ, Kuno G, Sather GE, Waterman SH. A case of natural concurrent human infection with two dengue viruses. Am J Trop Med Hyg. 1985;34:170–3.

51. Laille M, Deubel V, Sainte-Marie FF. Demonstration of concurrent dengue 1 and dengue 3 infection in six patients by the polymerase chain reaction. J Med Virol. 1991;34(1):51–4.

52. Lorono-Pino MA, Cropp CB, Farfan JA, Vorndam A V, RodriGuez-Angulo EM, Rosado-Paredes EP, et al. Common Occurrence Of Concurrent Infections By Multiple Dengue Virus Serotypes. Am J Trop Med Hyg. 1999;61(5):725–30.

53. Fatima Z, Idrees M, Bajwa M, Tahir Z, Ullah O, Zia M, et al. Serotype and genotype analysis of dengue virus by sequencing followed by phylogenetic analysis using samples from three mini outbreaks-2007-2009 in Pakistan. BMC Microbiol. 2011;11(1):200.

54. VinodKumar CS, Kalapannavar NK, Basavarajappa KG, Sanjay D, Gowli C, Nadig NG, et al. Episode of coexisting infections with multiple dengue virus serotypes in central Karnataka, India. J Infect Public Health. 2013;6(4):302–6.

55. Wang WK, Chao DY, Lin SR, Chang SC. Concurrent infections by two dengue virus serotypes among dengue patients in Taiwan. J Microbiol Immunol Infect. 2003;36:89–95.

56. Romano CM, Lauck M, Salvador FS, Lima CR, Villas-Boas LS, Araújo ESA, et al. Inter- and Intra-Host Viral Diversity in a Large Seasonal DENV2 Outbreak. PLoS One. 2013;8(8):e70318.

57. Alfson KJ, Beadles MW, Griffiths A. A new approach to determining whole viral genomic sequences including termini using a single deep sequencing run. J Virol Methods. 2014 Nov;208:1–5.

58. Acevedo A, Andino R. Library preparation for highly accurate population sequencing

of RNA viruses. Nat Protoc. 2014 Jun;9(7):1760–9.

59. Archer J, Baillie G, Watson S, Kellam P, Rambaut A, Robertson D. Analysis of high-depth sequence data for studying viral diversity: a comparison of next generation sequencing platforms using Segminator II. BMC Bioinformatics. 2012;13(1):47.

60. Wright CF, Morelli MJ, Thebaud G, Knowles NJ, Herzyk P, Paton DJ, et al. Beyond the Consensus: Dissecting Within-Host Viral Population Diversity of Foot-and-Mouth Disease Virus by Using Next-Generation Genome Sequencing. J Virol. 2011 Mar 1;85(5):2266–75.

61. Hapuarachchi HC, Koo C, Kek R, Xu H, Lai YL, Liu L, et al. Intra-epidemic evolutionary dynamics of a Dengue virus type 1 population reveal mutant spectra that correlate with disease transmission. Sci Rep. 2016 Mar 4;6:22592.

62. Lequime S, Fontaine A, Ar Gouilh M, Moltini-Conclois I, Lambrechts L, Lauring A, et al. Genetic Drift, Purifying Selection and Vector Genotype Shape Dengue Virus Intra-host Genetic Diversity in Mosquitoes. Malik HS, editor. PLOS Genet. 2016 Jun 15;12(6):e1006111.

63. Aw P, de Sessions P, Wilm A, Hoang L, Nagarajan N, Sessions O, et al. Next-Generation Whole Genome Sequencing of Dengue Virus. In: Padmanabhan R, Vasudevan SG, editors. Dengue SE - 12. Springer New York; 2014. p. 175–95. (Methods in Molecular Biology; vol. 1138).

64. Sessions OM, Wilm A, Kamaraj US, Choy MM, Chow A, Chong Y, et al. Analysis of Dengue Virus Genetic Diversity during Human and Mosquito Infection Reveals Genetic Constraints. Armstrong PM, editor. PLoS Negl Trop Dis. 2015 Sep 1;9(9):e0004044.

65. Aaskov J, Buzacott K, Field E, Lowry K, Berlioz-Arthaud A, Holmes EC. Multiple recombinant dengue type 1 viruses in an isolate from a dengue patient. J Gen Virol. 2007;88(12):3334–40.

66. Parameswaran P, Charlebois P, Tellez Y, Nunez A, Ryan EM, Malboeuf CM, et al. Genome - Wide Patterns of Intrahuman Dengue Virus Diversity Reveal Associations with Viral Phylogenetic Clade and Interhost Diversity. J Virol. 2012;86(16):8546–58.

67. Descloux E, La Fuentez C, Roca Y, De Lamballerie X. Clinical significance of intra-host variability of Dengue-1 virus in venous and capillary blood. Clin Microbiol Infect. 2014 Mar;20(3):O167-75.

68. Ke R, Aaskov J, Holmes EC, Lloyd-Smith JO. Phylodynamic Analysis of the Emergence and Epidemiological Impact of Transmissible Defective Dengue Viruses. PLoS Pathog. 2013;9(2):e1003193.

69. Lin S-R, Hsieh S-C, Yueh Y-Y, Lin T-H, Chao D-Y, Chen W-J, et al. Study of Sequence Variation of Dengue Type 3 Virus in Naturally Infected Mosquitoes and Human Hosts: Implications for Transmission and Evolution. J Virol. 2004;78(22):12717–21.

70. Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V, et al. Molecular evolution of dengue 2 virus in Puerto Rico: positive selection in the viral envelope accompanies clade reintroduction. J Gen Virol. 2006;87:885–93.

71. Holmes EC. Patterns of Intra- and Interhost Nonsynonymous Variation Reveal Strong Purifying Selection in Dengue Virus. J Virol. 2003;77(20):11296–8.

72. Descloux E, Cao-Lormeau V-M, Roche C, Lamballerie X De. Dengue 1 diversity and microevolution, French Polynesia 2001-2006: connection with epidemiology and clinics. {PLoS} Neglected Trop Dis. 2009;3(8):e493.

73. Edgar R. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics. 2004;5(1):113.

74. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1.

75. Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics. 2010 Oct;26(19):2462–3.

76. Schmidt HA, Strimmer K, Vingron M, von Haeseler A. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics. 2002 Mar 1;18(3):502–4.

77. Strimmer K, von Haeseler A. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc Natl Acad Sci U S A. 1997 Jun 24;94(13):6815–9.

78. Rambaut A, Lam TT, Carvalho LM, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol. 2016;2.

79. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012 Aug;29(8):1969–73.

80. Posada D. jModelTest: phylogenetic model averaging. Mol Biol Evol. 2008 Jul;25(7):1253–6.

81. Minin VN, Bloomquist EW, Suchard MA. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. Mol Biol Evol. 2008 Jul;25(7):1459–71.

82. Rambaut A, Drummond AJ. Tracer v1.4. Available from http://beast.bio.ed.ac.uk/Tracer. Available from http://beast.bio.ed.ac.uk/Tracer. 2007.

83. Rambaut A, Drummond A. FigTree. 2009.

84. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam A V. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-

polymerase chain reaction. J Clin Microbiol. 1992 Mar;30(3):545–51.

85. Andrews S. FastQC: a quality control tool for high throughput sequence data. [Internet]. 2010. Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

86. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. J Comput Biol. 2012 May;19(5):455–77.

87. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990 Oct;215(3):403–10.

88. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Meth. 2012 Apr;9(4):357–9.

89. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, del Angel G, Levy-Moonshine A, et al. From fastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. Current Protocols in Bioinformatics. 2013. 1-33 p.

90. Wilm A, Aw PPK, Bertrand D, Yeo GHT, Ong SH, Wong CH, et al. LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res. 2012 Dec;40(22):11189–201.

91. Töpfer A, Zagordi O, Prabhakaran S, Roth V, Halperin E, Beerenwinkel N. Probabilistic Inference of Viral Quasispecies Subject to Recombination. J Comput Biol. 2013 Feb 1;20(2):113–23.

92. Huson DH, Bryant D. Application of Phylogenetic Networks in Evolutionary Studies. Mol Biol Evol. 2006; 23(2):254-267.

93. Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics. 2010;26(19):2462–3.

94. Jombart T, Cori A, Didelot X, Cauchemez S, Fraser C, Ferguson N, et al. Bayesian Reconstruction of Disease Outbreaks by Combining Epidemiologic and Genomic Data. Tanaka MM, editor. PLoS Comput Biol. 2014 Jan 23;10(1):e1003457.

95. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: The R foundation for Statistical Computing; 2011.

96. Peden JF. Analysis of Codon Usage. University of Nottingham; 1999.

97. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011 Aug 15;27(16):2194–200.

98. Pond SLK, Frost SDW, Muse S V. HyPhy: hypothesis testing using phylogenies. Bioinformatics. 2005;21:676–9.

99. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010 May;59(3):307–21.

100. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007 Aug;24(8):1596–9.

101. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989 Nov;123(3):585–95.

102. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009 Jun 1;25(11):1451–2.

103. Jombart T, Bateman A. adegenet: a R package for the multivariate analysis of genetic markers. 2008;24(11):1403–5.

104. Amarilla AA, de Almeida FT, Jorge DM, Alfonso HL, de Castro-Jorge LA, Nogueira NA, et al. Genetic diversity of the E Protein of Dengue Type 3 Virus. Virol J. 2009;6(1):113.

105. Anderson R, King AD, Innis BL. Correlation of E protein binding with cell susceptibility to dengue 4 virus infection. J Gen Virol. 1992 Aug 1;73(8):2155–9.

106. Monitoramento dos casos de dengue e febre de chikungunya até a semana epidemiológica 15. 2015.

107. Lemey P, Rambaut A, Pybus OG. HIV evolutionary dynamics within and among hosts. AIDS Rev. 8(3):125–40.

108. Stack JC, Murcia PR, Grenfell BT, Wood JLN, Holmes EC, Chen R, et al. Inferring the inter-host transmission of influenza A virus using patterns of intra-host genetic variation. Proc Biol Sci. 2013 Jan 7;280(1750):20122173.

109. Allicock OM, Lemey P, Tatem AJ, Pybus OG, Bennett SN, Mueller BA, et al. Phylogeography and Population dynamics of Dengue viruses in the Americas. Mol Biol Evol. 2012;29(6):1533–43.

110. Teixeira Nunes RM, Nuno Rodrigues F, Vasconcelos Baldez H, Barbosa de Almeida Medeiros, Daniele; Pereira Silva de Lima, Clayton; Carvalho Lima V, Vieira Pinto da Silva E, Ferreira Cardoso, Jedson; costa Sousa Jr., Edivaldo; Nascimento Barbosa Nunes K, et al. Phylogeography of Dengue Virus Serotype 4, Brazil, 2010–2011. Emerg Infect Dis. 2012;18(November):1858–64.

111. de Melo FL, Romano CM, de Andrade Zanotto PM. Introduction of dengue virus 4 (DENV-4) genotype I into Brazil from Asia? PLoS Negl Trop Dis. 2009 Jan;3(4):e390.

112. de Figueiredo RMP, Naveca FG, de Souza Bastos M, do Nascimento Melo M, de Souza Viana S, Mourão MPG, et al. Dengue Virus Type 4, Manaus, Brazil. Emerg Infect Dis. 2008;14:667–9.

113. de Figueiredo MLG, Alfonso HL, Amarilla AA, Figueiredo LTM, Aquino VH, da Costa CA, et al. Detection of DENV-4 genotype I from mosquitoes collected in the city of Manaus, Brazil. Virol J. 2013;10(1):60.

114. de Souza RP, Rocco IM, Maeda AY, Spenassatto C, Bisordi I, Suzuki A, et al. Dengue Virus Type 4 Phylogenetics in {B}razil 2011: Looking beyond the Veil. PLoS Negl Trop Dis. 2011;5(12):e1439.

115. T Nunes MR, Palacios G, Rodrigues Faria N, Costa Sousa Jr E, Pantoja JA, Rodrigues SG, et al. Air Travel Is Associated with Intracontinental Spread of Dengue Virus Serotypes 1–3 in Brazil. PLoS Negl Trop Dis. 2014;8(4).

116. Carvalho LM, Faria NR, Perez AM, Suchard MA, Lemey P, Silveira W de C, et al. Spatio-temporal Dynamics of Foot-and-Mouth Disease Virus in South America. 2015.

117. Azevedo R do S da S, Oliveira CS, Vasconcelos PF da C, Azevedo R do S da S, Oliveira CS, Vasconcelos PF da C. Chikungunya risk for Brazil. Rev Saude Publica. 2015;49:1–6.

118. Araújo JMG de, Bello G, Romero H, Nogueira RMR. Origin and Evolution of Dengue Virus Type 3 in Brazil. PLoS Negl Trop Dis. 2012;6(9):e1784.

119. Bertolacci-Rocha LG, Cunha RV da, Lichs GG de C, Fabbro MMFJD, Motta-Castro ARC, Bertolacci-Rocha LG, et al. Introduction of the dengue virus type 4 in the State of Mato Grosso do Sul, Brazil. Cad Saude Publica. 2014 Aug;30(8):1789–92.

120. Superintendência de Vigilância em Saúde (SUVISA). Isolamento do vírus DENV 4 no estado de Goiás. Goiás; 2011.

121. Nogueira RM, Eppinghaus AL. Dengue virus type 4 arrives in the state of Rio de Janeiro: a challenge for epidemiological surveillance and control. Mem Inst Oswaldo Cruz. 2011 May;106(3):255–6.

122. Gonçalves De Castro M, Maria R, Nogueira R, Bispo De Filippis AM, Ferreira AA, Da M, et al. Dengue virus type 4 in Niterói, Rio de Janeiro: the role of molecular techniques in laboratory diagnosis and entomological surveillance. Mem Inst Oswaldo Cruz Rio Janeiro. 2012;107(7):940–5.

123. Amâncio FF, Pereira MA, Iani FC de M, D'anunciação L, Almeida JLC de, Soares JAS, et al. Fatal outcome of infection by dengue 4 in a patient with thrombocytopenic purpura as a comorbid condition in Brazil. Rev Inst Med Trop Sao Paulo. 2014 Jun;56(3):267–70.

124. Ho T, Tzanetakis IE. Development of a virus detection and discovery pipeline using next generation sequencing. Virology. 2014;471–473:54–60.

125. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet. 2016 May 17;17(6):333–51.

126. Marston D, McElhinney L, Ellis R, Horton D, Wise E, Leech S, et al. Next generation sequencing of viral RNA genomes. BMC Genomics. 2013;14(1):444.

127. Sim S, Aw PPK, Wilm A, Teoh G, Hue KDT, Nguyen NM, et al. Tracking Dengue Virus Intra-host Genetic Diversity during Human-to-Mosquito Transmission. PLoS Negl Trop Dis. 2015;9(9):e0004052.

128. Walker JM. Dengue: Methods and Protocols. Methods in Molecular Biology. 2014. 403 p.

129. Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, et al. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010 Feb;7(2):111–8.

130. Djikeng A, Halpin R, Kuzmickas R, DePasse J, Feldblyum J, Sengamalay N, et al. Viral genome sequencing by random priming methods. BMC Genomics. 2008;9(1):5.

131. Amâncio FF, Ferraz ML, de M. Almeida MC, Pessanha JEM, Iani FCM, Fraga GL, et al. Dengue virus serotype 4 in a highly susceptible population in Southeast Brazil. J Infect Public Health. 2014;7(6):547–52.

132. Heinen LB da S, Zuchi N, Cardoso BF, dos Santos MAM, Nogueira ML, Dezengrini-Slhessarenko R. Dengue outbreak in Mato Grosso state, midwestern Brazil. Rev Inst Med Trop Sao Paulo. 2015 Dec;57(6):489–96.

133. Granberg F, Vicente-Rubiano M, Rubio-Guerri C, Karlsson OE, Kukielka D, Belák S, et al. Metagenomic Detection of Viral Pathogens in Spanish Honeybees: Co-Infection by Aphid Lethal Paralysis, Israel Acute Paralysis and Lake Sinai Viruses. Kapoor A, editor. PLoS One. 2013 Feb 27;8(2):e57459.

134. Colombo TE, Vedovello D, Mondini A, Reis AFN, Cury AAF, Oliveira FH de, et al. Co-infection of dengue virus by serotypes 1 and 4 in patient from medium sized city from Brazil. Rev Inst Med Trop Sao Paulo. 2013 Jul;55(4):275–81.

135. Andrade EHP, Figueiredo LB, Vilela APP, Rosa JCC, Oliveira JG, Zibaoui HM, et al. Spatial-Temporal Co-Circulation of Dengue Virus 1, 2, 3, and 4 Associated with Coinfection Cases in a Hyperendemic Area of Brazil: A 4-Week Survey. Am J Trop Med Hyg. 2016 May 4;94(5):1080–4.

136. Vigilância S De. Boletim epidemiológico. Vol. 45. São Paulo, Brasil; 2014.

137. Zhang C, Mammen Jr. MP, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P, et al. Clade Replacements in Dengue Virus Serotypes 1 and 3 Are Associated with Changing Serotype Prevalence. J Virol. 2005;79(24):15123–30.

138. Adams B, Holmes EC, Zhang C, M. P. Mammen J, Nimmannitya S, Kalayanarooj S, et al. Cross-protective immunity can account for the alternating epidemic pattern of dengue virus serotypes circulating in Bangkok. PNAS. 2006;103 (38):14234–9.

139. Kasibhatla SM, Waman VP, Kale MM, Kulkarni-Kale U. Analysis of Next-generation

Sequencing Data in Virology - Opportunities and Challenges. In: Next Generation Sequencing - Advances, Applications and Challenges. InTech; 2016.

140. Quiñones-Mateu ME, Avila S, Reyes-Teran G, Martinez MA, Holley RW, Apgar J, et al. Deep sequencing: becoming a critical tool in clinical virology. J Clin Virol. 2014 Sep;61(1):9–19.

141. Klein DE, Choi JL, Harrison SC. Structure of a Dengue Virus Envelope Protein Late-Stage Fusion Intermediate. J Virol. 2013 Feb 15;87(4):2287–93.

142. Rey FA. Dengue virus envelope glycoprotein structure: new insight into its interactions during viral entry. Proc Natl Acad Sci U S A. 2003 Jun 10;100(12):6899–901.

143. Chilamakuri CS, Lorenz S, Madoui M-A, Vodák D, Sun J, Hovig E, et al. Performance comparison of four exome capture systems for deep sequencing. BMC Genomics. 2014;15(1):449.

144. Dohm JC, Lottaz C, Borodina T, Himmelbauer H. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. Nucleic Acids Res. 2008 Sep;36(16):e105.

145. Van den Hoecke S, Verhelst J, Saelens X, Baets S De, Eckert N, Kim JI, et al. Illumina MiSeq sequencing disfavours a sequence motif in the GFP reporter gene. Sci Rep. 2016 May 19;6:26314.

146. Sanjuan R, Nebot MR, Chirico N, Mansky LM, Belshaw R. Viral Mutation Rates. J Virol. 2010 Oct 1;84(19):9733–48.

147. Mangul S, Wu NC, Mancuso N, Zelikovsky A, Sun R, Eskin E. Accurate viral population assembly from ultra-deep sequencing data. Bioinformatics. 2014 Jun 15;30(12):i329–37.

148. Töpfer A, Höper D, Blome S, Beer M, Beerenwinkel N, Ruggli N, et al. Sequencing approach to analyze the role of quasispecies for classical swine fever. Virology. 2013 Mar 30;438(1):14–9.

149. Metzner KJ, Giulieri SG, Knoepfel SA, Rauch P, Burgisser P, Yerly S, et al. Minority Quasispecies of Drug-Resistant HIV-1 That Lead to Early Therapy Failure in Treatment-Naive and -Adherent Patients. Clin Infect Dis. 2009 Jan 15;48(2):239–47.

150. Capobianchi MR, Giombini E, Rozera G. Next-generation sequencing technology in clinical virology. Clin Microbiol Infect. 2013;19(1):15–22.

151. Chabria SB, Gupta S, Kozal MJ. GG15CH13-Kozal Deep Sequencing of HIV: Clinical and Research Applications. Annu Rev Genomics Hum Genet. 2014;15:295–325.

152. Forrester NL, Coffey LL, Weaver SC. Arboviral bottlenecks and challenges to maintaining diversity and fitness during mosquito transmission. Viruses. 2014 Oct;6(10):3991–4004.

153. Villabona-Arenas CJ, de Brito AF, de Andrade Zanotto PM. Genomic mosaicism in two strains of dengue virus type 3. Infect Genet Evol. 2013;18(0):202–12.

154. AbuBakar S, Wong P-F, Chan Y-F. Emergence of dengue virus type 4 genotype IIA in Malaysia. J Gen Virol. 2002;83(10):2437–42.

155. Worobey M, Rambaut A, Holmes EC. Widespread intraserotype recombination in natural populations of dengue virus. Proc Natl Acad Sci USA. 1999;96:7352–7.

156. Chao D-Y. Inter- and Intra-Host Evolution of Dengue Viruses and the Inference to the Pathogenesis. Viral Genomes - Mol Struct Divers Gene Expr Mech Host-Virus Interact. 2012;195–216.

157. Modis Y, Ogata S, Clements D, Harrison SC. A ligand-binding pocket in the dengue virus envelope glycoprotein. Proc Natl Acad Sci USA. 2003;10(100):6986–91.

158. Descloux E, Cao-Lormeau V-M, Roche C, De Lamballerie X. Dengue 1 Diversity and Microevolution, French Polynesia 2001–2006: Connection with Epidemiology and Clinics. PLoS Negl Trop Dis. 2009;3(8):e493.

159. Descloux E, Cao-Lormeau V-M, Roche C, De Lamballerie X. Dengue 1 diversity and microevolution, French Polynesia 2001-2006: connection with epidemiology and clinics. PLoS Negl Trop Dis. 2009;3(8):e493.

160. Aaskov J, Buzacott K, Thu HM, Lowry K, Holmes EC. Long-Term Transmission of Defective RNA Viruses in Humans and Aedes Mosquitoes. Science (80-). 2006 Jan;311:236–8.

161. Beerenwinkel N, Günthard HF, Roth V, Metzner KJ. Challenges and opportunities in estimating viral genetic diversity from next-generation sequencing data. Front Microbiol. 2012;3(329).

162. Pirooznia M, Kramer M, Parla J, Goes FS, Potash JB, McCombie W, et al. Validation and assessment of variant calling pipelines for next-generation sequencing. Hum Genomics. 2014;8(1):14.

163. Lauring AS, Acevedo A, Cooper SB, Andino R. Codon Usage Determines the Mutational Robustness, Evolutionary Capacity, and Virulence of an RNA Virus. Cell Host Microbe. 2012;12(5):623–32.

164. Lara-Ramírez EE, Salazar MI, López-López M de J, Salas-Benito JS, Sánchez-Varela A, Guo X, et al. Large-scale genomic analysis of codon usage in dengue virus and evaluation of its phylogenetic dependence. Biomed Res Int. 2014;2014:851425.

165. Schubert AM, Putonti C. Evolution of the sequence composition of Flaviviruses. Infect Genet Evol. 2010;10(1):129–36.

166. Xiang H, Zhang R, Butler RR, Liu T, Zhang L, Pombert J-F, et al. Comparative Analysis of Codon Usage Bias Patterns in Microsporidian Genomes. Ling E, editor. PLoS One. 2015 Jun 9;10(6):e0129223.

167. Carbone A. Codon Bias is a Major Factor Explaining Phage Evolution in Translationally Biased Hosts. J Mol Evol. 2008;66(3):210–23.

168. Plotkin JB, Kudla G. Synonymous but not the same: the causes and consequences of codon bias. Nat Rev Genet. 2011 Jan;12(1):32–42.

169. Butt AM, Nasrullah I, Tong Y. Genome-wide analysis of codon usage and influencing factors in chikungunya viruses. PLoS One. 2014;9(3):e90905.

170. Jenkins GM, Holmes EC. The extent of codon usage bias in human RNA viruses and its evolutionary origin. Virus Res. 2003;92:1–7.

171. Pal S, Banerjee A, Das PS, Pati BR, Mandal KC, Chakraborty B, et al. Dengue virus type 4 evolution and genomics: A bioinformatic approach. Indian J Biotechnol. 2014;13:330–6.

172. Shi S-L, Jiang Y-R, Liu Y-Q, Xia R-X, Qin L. Selective pressure dominates the synonymous codon usage in parvoviridae. Virus Genes. 2013 Feb 21;46(1):10–9.

173. Behura SK, Severson DW, Kyle J, Harris E, Gubler D, Ramanathan M, et al. Nucleotide substitutions in dengue virus serotypes from Asian and American countries: insights into intracodon recombination and purifying selection. BMC Microbiol. 2013;13(1):37.

174. Zhou J, Zhang J, Sun D, Ma Q, Chen H, Ma L, et al. The distribution of synonymous codon choice in the translation initiation region of dengue virus. PLoS One. 2013;8(10):e77239.

175. Das S, Nandy A. Phylogenetic and Genetic Analysis of Envelope Gene of the Prevalent Dengue Serotypes in India in Recent Years. Proceedings. 2015;1(Section B):1–9.

176. Behura S, Severson D. Nucleotide substitutions in dengue virus serotypes from Asian and American countries: insights into intracodon recombination and purifying selection. BMC Microbiol. 2013;13(1):37.

177. Dowd KA, DeMaso CR, Pierson TC. Genotypic Differences in Dengue Virus Neutralization Are Explained by a Single Amino Acid Mutation That Modulates Virus Breathing. MBio. 2015 Dec 31;6(6):e01559-15.

178. Schuh AJ, Tesh RB, Barrett ADT. Genetic characterization of Japanese encephalitis virus genotype II strains isolated from 1951 to 1978. J Gen Virol. 2011 Mar;92(Pt 3):516–27.

179. Shah M, Wadood A, Rahman Z, Husnain T, Erum K, Mehreen K, et al. Interaction and Inhibition of Dengue Envelope Glycoprotein with Mammalian Receptor DC-Sign, an In-Silico Approach. Agarwal PK, editor. PLoS One. 2013 Mar 18;8(3):e59211.

180. Grubaugh ND, Weger-Lucarelli J, Murrieta RA, Fauver JR, Garcia-Luna SM, Prasad AN, et al. Genetic Drift during Systemic Arbovirus Infection of Mosquito Vectors

Leads to Decreased Relative Fitness during Host Switching. Cell Host Microbe. 2016 Apr;19(4):481–92.

181. Romano CM, de Matos AM, Araújo ESA, Villas-Boas LS, da Silva WC, Oliveira OMNPF, et al. Characterization of Dengue Virus Type 2: New Insights on the 2010 Brazilian Epidemic. PLoS One. 2010;5(7):e11811.

182. Twiddy SS, Farrar JJ, Vinh Chau N, Wills B, Gould EA, Gritsun T, et al. Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. Virology. 2002;298(1):63–72.

183. Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V, et al. Selection-driven evolution of emergent dengue virus. Mol Biol Evol. 2003;20(10):1650–8.

184. Kosakovsky Pond SL, Frost SDW. Not So Different After All: A Comparison of Methods for Detecting Amino Acid Sites Under Selection. Mol Biol Evol. 2005 Feb 9;22(5):1208–22.

185. Sironi M, Cagliani R, Forni D, Clerici M. Evolutionary insights into host–pathogen interactions from mammalian sequence data. Nat Rev Genet. 2015 Mar 18;16(4):224–36.

186. Pond SLK, Frost SDW, Muse S V. HyPhy: hypothesis testing using phylogenies. Bioinformatics. 2005 Mar 1;21(5):676–9.

187. Lee AJ, Das SR, Wang W, Fitzgerald T, Pickett BE, Aevermann BD, et al. Diversifying Selection Analysis Predicts Antigenic Evolution of 2009 Pandemic H1N1 Influenza A Virus in Humans. García-Sastre A, editor. J Virol. 2015 May 15;89(10):5427–40.

188. Ross HA, Rodrigo AG. Immune-Mediated Positive Selection Drives Human Immunodeficiency Virus Type 1 Molecular Variation and Predicts Disease Duration. J Virol. 2002 Nov 15;76(22):11715–20.

189. Koo C, Nasir A, Hapuarachchi H, Lee K-S, Hasan Z, Ng L-C, et al. Evolution and heterogeneity of multiple serotypes of Dengue virus in Pakistan, 2006–2011. Virol J. 2013;10(1):275.

190. Sessions OM, Barrows NJ, Souza-Neto JA, Robinson TJ, Hershey CL, Rodgers MA, et al. Discovery of insect and human dengue virus host factors. Nature. 2009;458(7241):1047–50.

191. Bordería A V, Isakov O, Moratorio G, Henningsson R, Agüera-González S, Organtini L, et al. Group Selection and Contribution of Minority Variants during Virus Adaptation Determines Virus Fitness and Phenotype. PLoS Pathog. 2015 May;11(5):e1004838.

192. Choudhury MA, Lott WB, Banu S, Cheng AY, Teo Y-Y, Twee R, et al. Nature and Extent of Genetic Diversity of Dengue Viruses Determined by 454 Pyrosequencing.

PLoS One. 2015;10(11):e0142473.

193. Tang C-T, Liao M-Y, Chiu C-Y, Shen W-F, Chiu C-Y, Cheng P-C, et al. Generation of Monoclonal Antibodies against Dengue Virus Type 4 and Identification of Enhancing Epitopes on Envelope Protein. Mantis NJ, editor. PLoS One. 2015 Aug 26;10(8):e0136328.

194. Howard CB. Identification of epitopes on the Dengue virus type 4 envelope glycoprotein involved in neutralisation by antibodies. [Ph. D. thesis]. Fac Sci Technol Sch Life Sci. Queensland University of Technology; 2006.

195. Morizono K, Chen ISY. Receptors and tropisms of envelope viruses. Curr Opin Virol. 2011;1(1):13–8.

196. Mair CM, Ludwig K, Herrmann A, Sieben C. Receptor binding and pH stability - How influenza A virus hemagglutinin affects host-specific virus infection. Biochim Biophys Acta - Biomembr. 2014;1838(4):1153–68.

197. Schrauwen EJ, Fouchier RA. Host adaptation and transmission of influenza A viruses in mammals. Emerg Microbes Infect. 2014;3(0):e9.

198. Villabona-Arenas C. Phylogeography of the 2013 urban outbreak of dengue virus in Guarujá, São Paulo. São Paulo. [Ph. D. thesis (Microbiology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2014.

199. Bull RA, Eden J-S, Luciani F, McElroy K, Rawlinson WD, White PA. Contribution of Intra- and Interhost Dynamics to Norovirus Evolution. J Virol. 2012 Mar 15;86(6):3219–29.

200. Kinoshita H, Mathenge EGM, Hung NT, Huong VTQ, Kumatori A, Yu F, et al. Isolation and characterization of two phenotypically distinct dengue type-2 virus isolates from the same dengue hemorrhagic Fever patient. Jpn J Infect Dis. 2009 Sep;62(5):343–50.

201. Rodriguez-Roche R, Blanc H, Bordería A V, Díaz G, Gonzalez D, Santana E, et al. Increasing clinical severity during a dengue virus type 3 Cuban epidemic: deep 1 sequencing of evolving viral populations 2 Running title: Evolving DENV-3 populations, Cuba, 2001-2002 3. J Virol. 2016;14(90):4320–33.

202. García-Arenal F, Fraile A, Malpica J. Variability and genetic structure of plant virus populations. Annu rev Phytopathol. 2001;39:157–86.