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Evolutionary dynamics of dengue virus populations within the mosquito vector

Louis Lambrechts[1,2] and Sebastian Lequime[1,2,3]

To date, dengue virus evolution has mainly been addressed by studies conducted at the between-host level. Like other pathogens with high mutation rate and rapid replication, dengue viruses also evolve during the course of an infection. Over the last few years, the advent of deep-sequencing technologies has facilitated studies of dengue virus populations at the within-host level. Here, we review recent advances on the evolutionary dynamics of dengue virus populations within their mosquito vector. We discuss how identifying the evolutionary forces acting on dengue virus populations within the mosquito can shed light on the processes underlying vector–virus interactions and the evolution of epidemiologically relevant traits.

Addresses
[1] Insect-Virus Interactions Group, Department of Genomes and Genetics, Institut Pasteur, Paris, France
[2] Centre National de la Recherche Scientifique, Unité de Recherche Associée 3012, Paris, France
[3] Université Pierre et Marie Curie, Cellule Pasteur UPMC, Paris, France

Corresponding author: Lambrechts, Louis (louis.lambrechts@pasteur.fr)

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Introduction

Dengue viruses (DENV) are mosquito-borne RNA viruses in the genus *Flavivirus* (family Flaviviridae) that cause more human disease than any other arthropod-borne (arbo) virus [1]. They consist of four serotypes (DENV-1 to DENV-4) that are phylogenetically related [2] and loosely antigenically distinct [3]. Human DENV originate from sylvatic transmission cycles that occur between non-human primates and arboreal mosquitoes [4]. They emerged and became endemic in the human population in the last few hundred years [2]. Today, DENV are transmitted among human hosts by anthropophilic mosquito vectors, primarily *Aedes aegypti* [5].

Over the last three decades, phylogenetic and molecular evolution analyses based on viral genomic sequences have provided valuable insights into DENV evolutionary history and epidemiology [6]. It is noteworthy that this large body of work on various aspects of DENV biology such as the origin, spatio-temporal transmission dynamics, and pathogenesis has mainly been conducted at the between-host level. In other words, viral genomic sequences have generally been analyzed by considering a single genome per human or mosquito host sampled, which makes the implicit assumption that a single DENV genome adequately represents a given infection.

Because of their exceptionally high mutation rate — about a million times greater than that of vertebrates [7] — RNA viruses typically exist within their hosts as a heterogeneous population of related genetic variants, often referred to as a viral quasispecies [8]. The within-host genetic diversity of DENV was initially demonstrated by reverse transcription of viral RNA purified from individual human plasma samples, followed by cloning and sequencing of multiple PCR amplicons [9,10]. The majority of subsequent studies focused on the human host [11,12,13•,14], although the within-host genetic diversity of DENV populations was also confirmed in mosquitoes [15••,16]. Within-host DENV genetic diversity was initially reported to be lower in mosquitoes than in human patients [16], but this observation did not hold in later studies [15••,17•].

Early studies of within-host DENV genetic diversity based on clonal sequencing typically examined partial genome sequences, such as the viral envelope gene, or a limited number of complete genome sequences. With the advent of high-throughput sequencing technologies, genome-wide surveys progressively became the standard approach [13•,14,18,19], with some exceptions [20]. The primary aim of these studies was to estimate the extent of within-host DENV genetic diversity, and possibly correlate it with relevant clinical, epidemiological or phylogenetic features. For example, a comprehensive study of within-host DENV-2 genetic diversity in Nicaragua revealed non-uniform patterns across viral genes, variation across phylogenetic lineages, and some degree of relationship with between-host genetic diversity [13•]. One unresolved question is whether dengue disease severity in humans is associated with the level of within-host DENV genetic diversity, as was reported in some instances [12] but not others [13•,14].

Only during the last two years did studies begin to monitor the evolutionary dynamics of DENV populations

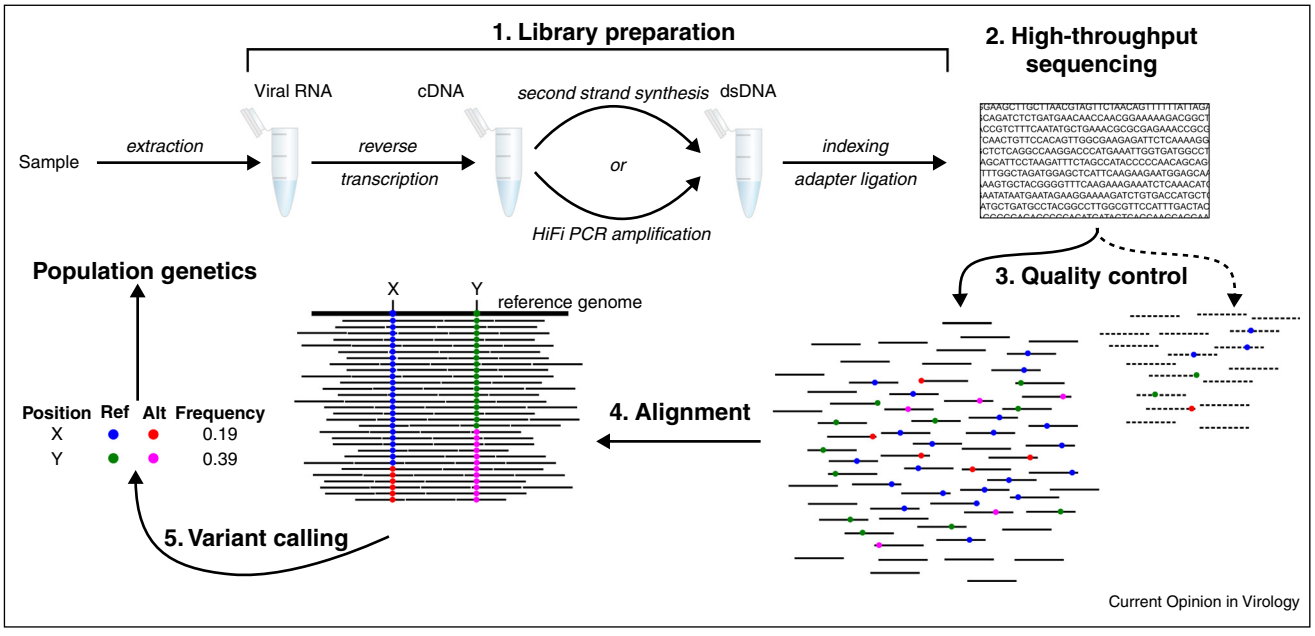
at the within-host level [17•,18,21••,22,23]. The abundant genetic diversity of RNA virus populations, coupled with short generation times, fuels their rapid evolution over the course of a single infection. Combining deep sequencing of within-host viral populations (Figure 1) with computational and statistical methods allows empirical analyses of their evolutionary dynamics [24]. Viral evolutionary analyses rely on the observed patterns of genetic diversity to infer the underlying evolutionary processes [25]. Evolutionary forces acting on within-host viral populations are not fundamentally different from those acting on any population. They include genetic mechanisms (i.e., mutation and recombination), demographic processes (i.e., genetic drift) and natural selection.

Here, we review our current knowledge on the within-host evolutionary dynamics of DENV, focusing on the infection of mosquito vectors because they represent the bulk of published studies to date. We illustrate how the analysis of within-host evolutionary dynamics can provide important insights into DENV–vector interactions and DENV epidemiology.

Within-host evolution of DENV populations

To our knowledge, only five studies published to date monitored DENV genetic diversity at the within-host level based on sequential observations of the same viral population. Rodriguez-Roche *et al.* examined patterns of within-host genetic diversity in human serum samples collected during a DENV-3 epidemic in Cuba [18]. Although this observation was based on a single dengue patient, they detected significant changes in the genetic composition of the within-host viral population sampled two and four days after fever onset. Plummer *et al.* monitored the evolution of DENV-2 genetic diversity during several passages in a mouse model to evaluate the effect of an antiviral molecule [22]. The antiviral treatment resulted in a higher number of viral genetic variants

Figure 1



Deep-sequencing analysis of within-host viral populations. (1) RNA is extracted from the sample, purified and reverse transcribed into complementary DNA (cDNA) using random hexamers or a specific reverse primer located at 3′ end of the viral genome. Reverse transcription is the most error-prone step of the library preparation because the reverse transcriptase (RT) lacks proofreading activity; however, recent advances in understanding RT activity may lead to the development of proofreading RT and increased fidelity of this step [50]. Double-stranded DNA (dsDNA) is obtained by synthesis of the second strand or by high-fidelity PCR amplification. Samples with low template concentration usually require PCR amplification using a variable number of overlapping amplicons to cover the viral genome [51]. dsDNA is prepared for sequencing through indexing (for multiplexing purposes) and ligation of sequencing adapters. This step usually includes or is followed by another round of PCR amplification. Minimizing the number of PCR cycles during library preparation is critical for data quality because each PCR cycle can introduce errors that are difficult to discriminate from true low-frequency variants, even when a high-fidelity DNA polymerase is used. (2) The dsDNA library is deeply sequenced (i.e., a given nucleotide position is sequenced hundreds to thousands of times per sample) on a high-throughput sequencing platform. Illumina has been the most popular sequencing technology in recent years [17•,21••,23]. (3) Sequencing reads are checked for quality (i.e., low-quality nucleotides are trimmed) and PCR duplicates may be removed. (4) Reads are aligned to the reference viral genome, which can be predetermined or assembled de novo using the newly generated sequence data. (5) On the basis of the alignment of sequencing reads and their quality score, variant-calling algorithms detect nucleotide variants for each position and estimate their relative frequency [52]. The variant detection threshold primarily depends on the sequencing depth, with deeper sequencing resulting in higher sensitivity. Finally, nucleotide variants and their frequencies are used for analyses of viral population genetics.