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Research paper

Codon adaptation biases among sylvatic and urban genotypes of Dengue virus type 2



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ABSTRACT

Dengue virus (DENV) emerged from the sylvatic environment and colonized urban settings, being sustained in a human-*Aedes*-human transmission chain, mainly by the bites of females of the anthropophilic species *Aedes aegypti*. Herein, we sought evidence for fine-tuning in viral codon usage, possibly due to viral adaptation to human transmission. We compared the codon adaptation of DENV serotype 2 (DENV-2) genotypes from urban and sylvatic habitats and tried to correlate the findings with key evolutionary determinants. We found that DENV-2 codons of urban and sylvatic genotypes had a higher CAI to humans than to *Ae. aegypti*. Remarkably, we found no significant differences in codon adaptation to human between urban American/Asian and sylvatic DENV-2 genotypes. Moreover, CAI values were significantly different, when comparing all genotypes to *Ae. aegypti* codon preferences, with lower values for sylvatic than urban genotypes. In summary, our findings suggest the presence of a molecular signature among the genotypes that circulate in sylvatic and urban environments, and may help explain the trafficking of DENV-2 strains to an urban cycle.

1. Introduction

Dengue virus (DENV) is the world's most important mosquito-borne viral human pathogen. It is widespread throughout tropical and subtropical regions (Bhatt et al., 2013; Gubler, 1998). DENV has a natural sylvatic maintenance cycle, involving species of blood-feeding arboreal mosquitoes of the genus *Aedes* (*Ae. taylori*, *Ae. furcifer*, *Ae. vitattus*, and *Ae. luteocephalus*) and nonhuman primates, that develop viremic infections and serve to amplify transmission (Amarasinghe et al., 2011; Diallo et al., 2003). Urban cycle involve amplification hosts and peridomestic *Aedes* spp. vectors, mainly *Ae. aegypti* (Gubler, 1998; Vasilakis et al., 2011).

DENV is an enveloped virus of the genus *Flavivirus*, family *Flaviviridae*, classified in four phylogenetically and antigenically distinct serotypes (DENV-1-4). Its genome is made by a single-stranded, positive-sense RNA molecule of approximately 11 kb, which contains a single open reading frame encoding three structural [capsid (C), membrane (M) and envelope (E)] and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins (Chambers et al., 1990). Based on their genetic diversity and geographical distribution, distinct

groups have been also identified within each serotype, defined as genotypes (Cologna et al., 2005; Costa et al., 2012).

Six distinct DENV-2 genotypes are recognized (Costa et al., 2012; Twiddy et al., 2002; Wei and Li, 2017). The Sylvatic genotype circulates in a sylvatic cycle, while the American, American/Asian, Asian 1, Asian 2 and Cosmopolitan genotypes circulate in urban cycle (Wang et al., 2000). Inferred evolutionary relationships of DENV suggest that the urban genotypes evolved from sylvatic lineages due to several cross-species transmission events into humans, followed by the recent (*i.e.*, early 20th century) evolution of urban forms (Messina et al., 2014; Wang et al., 2000; Wei and Li, 2017). Notably, environmental and social factors such as the introduction of the anthropophilic vector, urbanization, deforestation and the increased human travel and trade have facilitated the emergence, spread and evolution of DENV into human populations (Mayer et al., 2017; Parvez and Parveen, 2017). Elements such as genetic composition and pathogen-host interaction are believed to be involved in different patterns of transmissibility (Jenkins and Holmes, 2003; Lara-Ramírez et al., 2014; Lequime et al., 2016; Lobo et al., 2009).

The degeneracy of the genetic code refers to the fact that a single

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amino acid can be coded by different codons. The redundancy in the genetic code has an important role in controlling metabolic processes, but not all species use its built-in codon redundancy in the same way. The unequal preference of specific codons over other synonymous codons during the translation process creates a bias in codon usage. Codon usage biases are common throughout the Tree of Life, and for viruses, the balance between mutation and natural selection allows for changes in codon usage biases (Morton, 2003). Genetic plasticity and the capacity to adapt to new hosts facilitate the emergence of RNA viruses in novel, unexplored environments. This process entails both, (i) adapting to different types of cellular machinery involved in viral replication and (ii) the evasion from different types of cellular antiviral responses. However, the mechanisms used for virus for trafficking among hosts remain poorly understood (Bahir et al., 2009; Longdon et al., 2014; Pal et al., 2014). Because DENV must infect successfully and alternate between mosquito and human hosts, nucleotide analyses support the notion that arboviruses use a restricted and balanced nucleotide composition as a compromise to be able to infect both hosts, thus successfully ensuring several processes such as translational efficiency and replication (Shen et al., 2015). In this context, the Codon adaptation index (CAI) correlates with gene expression levels and adaptation of viral genes to their hosts (Gustafsson et al., 2012; Neame, 2009; Pal et al., 2014). In the present study, we performed comprehensive analyses of codon adaptation of DENV-2 from different habitat settings and host systems.

2. Material and methods

2.1. Sequence datasets

The sequences used in this study were downloaded from the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/genbank/>) website in GenBank format. The dataset included 877 sequences from all six genotypes as follows: American (16), American/Asian (435), Asian 1 (228), Asian 2 (20), Cosmopolitan (163) and Sylvatic (15). Comparisons of codon usage preferences were performed against reference sets from *Homo sapiens* (3803 CDSs) (<https://github.com/CaioFreire/CUB>) and *Aedes aegypti* (585 CDSs) (<http://www.kazusa.or.jp/codon/>), using the standard genetic code. All the complete genomic sequences available for DENV-2 with information regarding the location and year of isolation were recovered, and later converted into FASTA format.

2.2. Molecular phylogenetic approach

FASTA sequences were aligned using Clustal Omega (Larkin et al., 2007) and recombinant sequences were screened using all algorithms implemented in RDP4 program (RDP, GENECONV, BootScan, MaxChi, Chimaera, Siscan and 3Seq) using the standard settings (Martin et al., 2015). The alignment of recombinant free sequences was manually inspected and edited using the program AliView v.1.18 (Larsson, 2014), resulting in a final dataset with 877 coding sequences (Table S1). Viral phylogenies based on full-length coding sequences were estimated using Maximum Likelihood (ML) implemented in FastTree 2 (Price et al., 2010). We first evaluated the best transition model to be GTR + Γ using JModelTest v. 0.1.1. The final tree was then visualized and plotted using FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk>). All sequences used in this work are presented in the format: genotype/accession number/country/year of isolation.

2.3. CAI of DENV to humans and *Aedes aegypti* mosquitoes

CAI is a measure of silent, synonymous codon usage bias based on the codon preference of a viral strain and a codon usage table for a given host (Sharp et al., 1986; Sharp and Li, 1987). We applied the CAI using a frequency table for housekeeping of human genes (Eisenberg

and Levanon, 2013) (available at <https://github.com/CaioFreire/CUB>) and for *Ae. aegypti* using the table available in the Codon Usage Database (Nakamura, 2000). The CAI values were calculated to measure the synonymous codon usage bias using the CAIcal program (Puigbò et al., 2008a). To evaluate the statistical support of the CAI values, we define a threshold value or expected CAI (e-CAI) (Puigbò et al., 2008b) by generating random sequences with GC content, amino acid composition and sequence length similar to the DENV-2 query sequences. CAI values above the e-CAI are interpreted as statistically significant, meaning that codon similarity arises from codon preferences rather than from internal biases (Puigbò et al., 2008b).

2.4. Statistical analysis

Statistical significance was determined by the Mann-Whitney *U* test to compare the difference between CAI values for humans and the mosquito, assuming a significance level of 0.05. Multiple comparisons among genotypes were performed using a Kruskal-Wallis test implemented in the pgirmess v1.6.5 package for the R statistical environment. To evaluate levels of independence, we further carried out a Dunn's *post hoc* test. Further, to avoid Type I error inflation, we applied a Bonferroni adjustment of *p*-values, using the PMCMR package, at a significance level of 0.05. Perl and R scripts were used to analyze most of the data in this study and are available from the authors upon request.

3. Results

As has been well documented, we recovered six phylogenetically structured groups or genotypes for DENV-2, based on the open reading frame of 877 non-recombinant sequences. The most basal group encompassed lineages from West Africa and Asia of Sylvatic genotype, which reinforced the notion that the urban genotypes emerged from the sylvatic cycle, as previously described (Vasilakis et al., 2011; Vasilakis et al., 2007a). Urban genotypes were also identified as: (i) American, (ii) American/Asian, (iii) Asian 1, (iv) Asian 2 and (v) Cosmopolitan genotypes (Fig. 1A, Fig. S1). Comparison of the CAI values obtained from human and *Ae. aegypti* codon usage tables, shown in Fig. 1B, revealed significant differences between hosts (*p*-value < 0.05), demonstrating that DENV-2 codon usage is more similar to that of humans than to *Ae. aegypti*.

In addition, we assessed the values of CAI for each genotype in comparison to human and *Ae. aegypti* hosts. Because CAI values for the polyprotein sequences were not normally distributed, as assessed using Shapiro-Wilk and Kolmogorov-Smirnov tests, we determined statistical significance with the Mann-Whitney *U* test to compare among CAI estimates. The distribution of CAI values for different genotypes showed that all the genotypes fell above the e-CAI estimated for each host. Furthermore, statistical analyses showed significant differences (*p*-value < 0.05) among urban genotypes for both hosts (Table 1).

Significant differences between urban and sylvatic genotypes were identified in *Ae. aegypti* (Table 1). Likewise, the genotypes with the highest CAI were Asian 1, Asian 2 and the American genotypes. We also observed that the CAI values for the sylvatic genotype were the lowest when comparing with urban genotypes in *A. aegypti* (*p*-value < 0.05) (Fig. 1B and Table 1). This suggested that genotypes from urban setting could have experienced some fine-tuning process in codon optimization to translation in *A. aegypti*.

For humans, the only pairwise comparison that was not significantly different between urban and sylvatic genotypes was that between the American/Asian and the Sylvatic genotype (Fig. 1B and Table 1), suggesting that they share similar silent selective pressures. The American, Asian 2 and Cosmopolitan genotypes had the highest CAI values for humans (Fig. 1B). Finally, we observed that these values were similar and roughly constant over time (Fig. 2). Crucially, values were higher than the e-CAI for all the genotypes in human and mosquito cells,

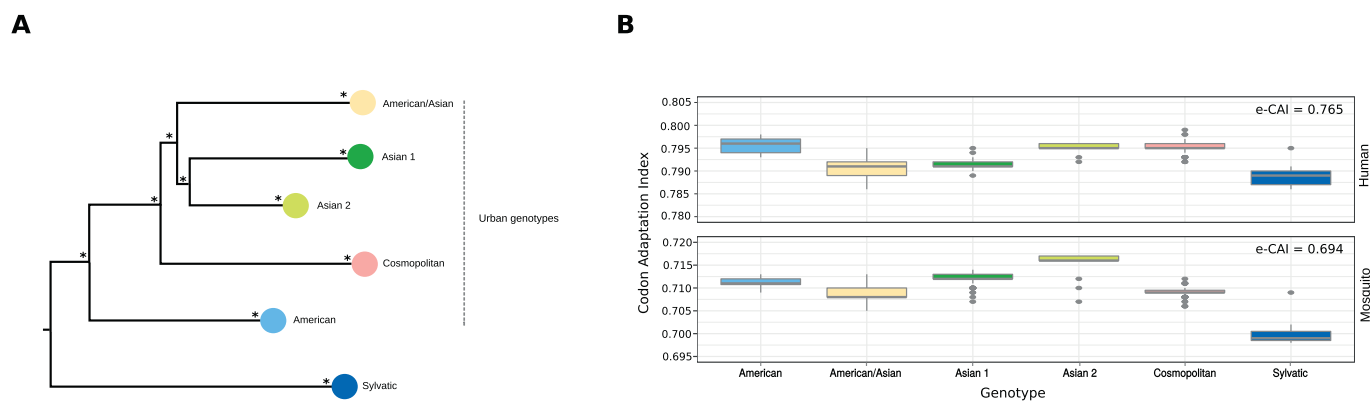


Fig. 1. DENV-2 genotypes. (A) Summary ML tree displaying the phylogenetic relationships between urban and sylvatic genotypes. (B) CAI values distribution of DENV-2 genotypes for human and vector hosts by using entire polyprotein sequences. The number of sequences for each genotype was distributed as follows: (i) American: 16 sequences; (ii) American/Asian: 435 sequences; (iii) Asian 1: 228 sequences; (iv) Asian 2: 20 sequences; (v) Cosmopolitan: 163 sequences; (vi) Sylvatic: 15 sequences. Genotypes are coded using different colors. The e-CAI is indicated for each host in the figure.

Table 1

Multiple comparison of CAI values among genotypes. CAI values estimated in human and *Ae. aegypti* cells are represented in gray and white cell background, respectively. Significant differences (**p*-value < .05) between pairs of genotypes are indicated by asterisk symbols.

Genotype	American	American/Asian	Asian 1	Asian 2	Cosmopolitan	Sylvatic
American	-	*	*	ns	ns	*
American/Asian	*	-	*	*	*	ns
Asian 1	ns	*	-	*	*	*
Asian 2	ns	*	ns	-	ns	*
Cosmopolitan	*	ns	*	*	-	*
Sylvatic	*	*	*	*	*	-

ns – Non statistical significance.

indicating that codon usage preferences were not random in DENV-2 genotypes (Fig. 2).

4. Discussion

The potential emergence of sylvatic strains into urban cycles has become an increasing focus of research and public concern (Moncayo et al., 2004; Vasilakis et al., 2011; Vasilakis et al., 2007b). Recently, this concern become more tangible after some studies have suggested human infection cases with presumably sylvatic DENV-2 strains. Phylogenetic analyses showed that the highly divergent strains (QML22/2015, D2Sab2015) of DENV-2 was basal and strikingly different from all other previously isolated strains (Liu et al., 2016; Pyke et al., 2017). In both cases, we excluded these sequences from our analyses, because including highly divergent sequences without knowing which genotype belong, may impact on the estimates for the whole dataset due to sampling bias and differences in the evolutionary rates.

Previous data suggest that the nature of selective pressures is broadly equivalent in urban and sylvatic strains (Vasilakis et al., 2011; Vasilakis et al., 2007b). This could mean that the successful emergence of a novel sylvatic DENV strains in an urban cycle is unlikely to require a major adaptive challenge (Vasilakis et al., 2007b).

Insects and mammals are separated by about 1 billion years of evolutionary history, and they differ at the organismal level and in many biochemical processes (Lobo et al., 2009; Shen et al., 2015). Since DENV needs to negotiate with different hosts (i.e. human and *Aedes*

mosquitoes), it is fair to assume that the efficiency in the viral translation process is a key factor sustaining the urban cycle.

Our results suggested that codon adaptation was higher for human cells than for *Ae. aegypti* (Fig. 1, Fig. 2). This was also reported for other flavivirus (Butt et al., 2016; Freire et al., 2015; Moratorio et al., 2013; Pal et al., 2014). In addition, similar CAI values recovered for urban and sylvatic genotypes suggests their ability to possibly make similar use of human cellular machinery. This notion is consistent with kinetic experiments comparing the replication of sylvatic and urban DENV-2 strains, which could explain that the emergence of urban DENV-2 from sylvatic progenitors may not have required adaptation to replicate more efficiently in humans (Vasilakis et al., 2007b). Notwithstanding, when using the CAI tables from *Ae. aegypti*, we observed a conspicuous difference between urban and sylvatic genotypes with lower CAI values for the sylvatic genotype (Fig. 2). This finding suggested that the emergence of urban genotypes into the urban cycle may have required viral adaptation towards increased competence in *Ae. aegypti*, which should then entail higher efficiency in viral replication. This is in good agreement with susceptibility experiments reported by Moncayo et al. (2004). Furthermore, competence studies with *Ae. aegypti* from regions where sylvatic genotype circulation occurs indicate its low vector capacity for Dengue virus (Amarasinghe et al., 2011; Diallo et al., 2008). Despite this, there was no previous attempt to establish an association with codon usage biases.

Fluctuations in CAI distribution across genotypes (Fig. 1B) indicated possible differences in codon adaptation for both hosts (Table 1).

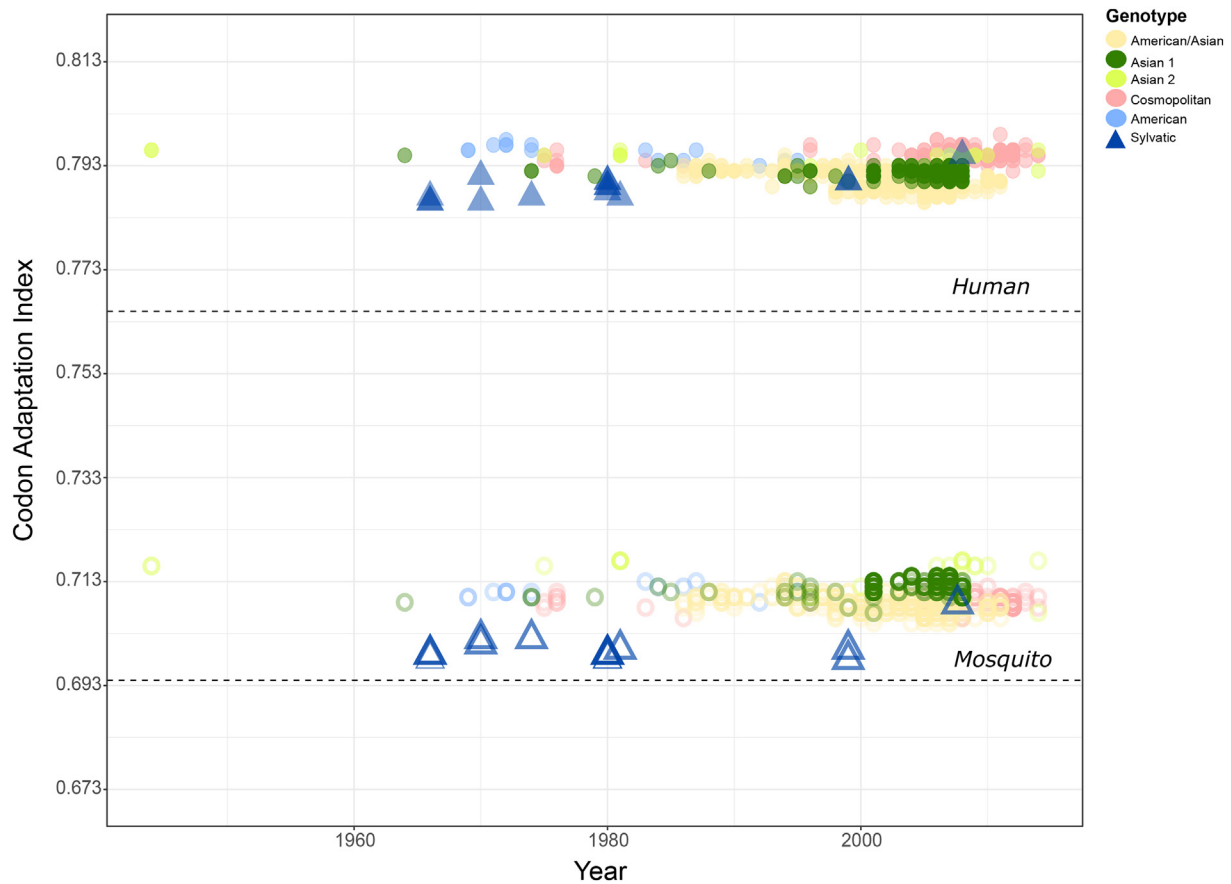


Fig. 2. Polyprotein CAI values distribution through time. Colors correspond to different genotypes. Symbols indicate strains circulating in urban (circle) and sylvatic (triangle) cycles. The color-filled symbols represent the CAI values in human cells while open symbols represent the adaptation in *Ae. aegypti* cells. Horizontal lines depict the expected CAI thresholds for both hosts.

Differential selection pressures exerted by the complex interplay of distinct virus factors, such as infection efficiency, population density and herd immunity (Plowright et al., 2017; Salazar et al., 2010). For example, a recent study reported by Salje et al. (2017) demonstrated the association between population density and the establishment of transmission chains. The increase in mosquito populations has also been identified as a potential determinant of the emergence and increased transmission of arbovirus, particularly in immunologically naïve human populations (Dudas et al., 2018; Pettersson et al., 2016). Our findings bring into question whether sylvatic strains could reach similar levels of transmission as the urban genotypes in Asian and American populations if the vector competence of *Ae. aegypti* is increased. In fact, recent outbreaks caused by other emerging viruses [e.g. Ebola virus, Influenza A virus (H1N1), Middle East respiratory syndrome coronavirus (MERS-CoV)], have occurred due to the zoonotic spillover and adaptation of the virus to replication in human cells (Dudas et al., 2018; Mänz et al., 2016; Plowright et al., 2017; Urbanowicz et al., 2016). In this context, as previous analyses have suggested (Moncayo et al., 2004), our findings support the notion that for the sylvatic strains to effectively colonize the urban environment, the virus needs a number of silent, adaptive nucleotide substitutions to optimize the codon usage to invertebrate host cells, while maintaining a compositional base balance suitable for efficient alternate spread among both human and insect hosts. Apparently, even when humans are susceptible to infection by DENV vectored by *Ae. aegypti*, its reduced vectorial competence ultimately constitutes a hurdle rather than an enabler of virus transmission.

5. Conclusions

In conclusion, our findings provide a comprehensive assessment of the codon adaptation of DENV-2 in different habitats (*i.e.* urban and sylvatic settings) and host systems (*i.e.* *Homo sapiens* and the mosquito vector *Aedes aegypti*). In this context, although the virus replicates in both human and mosquitoes, our analysis suggested that DENV-2 codon usage is better adapted to humans than to the main cosmopolitan vector (*Ae. aegypti*). This would imply that there is still room for adaptation and improved transmission among new DENV-2 strains for causing future pandemics.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2018.05.017>.

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References

Amarasinghe, A., Kuritsky, J.N., William Letson, G., Margolis, H.S., 2011. Dengue virus infection in Africa. *Emerg. Infect. Dis.* 17, 1349–1354. <http://dx.doi.org/10.3201/eid1708.101515>.

- Bahir, I., Fromer, M., Prat, Y., Linial, M., 2009. Viral adaptation to host: a proteome-based analysis of codon usage and amino acid preferences. *Mol. Syst. Biol.* 5 (311). <http://dx.doi.org/10.1038/msb.2009.71>.
- Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. *Nature* 496, 504–507. <http://dx.doi.org/10.1038/nature12060>.
- Butt, A.M., Nasrullah, I., Qamar, R., Tong, Y., 2016. Evolution of codon usage in Zika virus genomes is host and vector specific. *Emerg. Microbes Infect.* 5, e107. <http://dx.doi.org/10.1038/emi.2016.106>.
- Chambers, T.J., Hahn, C.S., Galler, R., Rice, C.M., 1990. Flavivirus genome organization, expression, and replication. *Annu. Rev.* 44, 649–688.
- Cologna, R., Armstrong, P.M., Rico-Hesse, R., 2005. Selection for virulent dengue viruses occurs in humans and mosquitoes. *J. Virol.* 79, 853–859. <http://dx.doi.org/10.1128/JVI.79.2.853-859.2005>.
- Costa, R.L., Voloch, C.M., Schrago, C.G., 2012. Comparative evolutionary epidemiology of dengue virus serotypes. *Infect. Genet. Evol.* 12, 309–314. <http://dx.doi.org/10.1016/j.meegid.2011.12.011>.
- Diallo, M., Ba, Y., Sall, A.A., Diop, O.M., Ndiene, J.A., Mondo, M., Girault, L., Mathiot, C., 2003. Amplification of the sylvatic cycle of dengue virus type 2, Senegal, 1999–2000: entomologic findings and epidemiologic considerations. *Emerg. Infect. Dis.* 9, 362–367. <http://dx.doi.org/10.3201/eid0903.020219>.
- Diallo, M., Ba, Y., Faye, O., Soumare, M.L., Dia, I., Sall, A.A., 2008. Vector competence of *Aedes aegypti* populations from Senegal for sylvatic and epidemic dengue 2 virus isolated in West Africa. *Trans. R. Soc. Trop. Med. Hyg.* 102, 493–498. <http://dx.doi.org/10.1016/j.trstmh.2008.02.010>.
- Dudas, G., Carvalho, L.M., Rambaut, A., Bedford, T., 2018. MERS-CoV spillover at the camel-human interface. *elife* e31257, 7. <http://dx.doi.org/10.1101/173211>.
- Eisenberg, E., Levanon, E.Y., 2013. Human housekeeping genes, revisited. *Trends Genet.* 29, 569–574. <http://dx.doi.org/10.1016/j.tig.2013.05.010>.
- Freire, C. de M., Iamarino, A., Neto, Lima, de D.F., Sall, A.A., Zanotto, P.M. de A., 2015. Spread of the pandemic Zika virus lineage is associated with NS1 codon usage adaptation in humans. In: *bioRxiv* 1–8, <http://dx.doi.org/10.1101/032839>.
- Gubler, D.J., 1998. Dengue and dengue hemorrhagic fever. *Clin. Microbiol. Rev.* 11, 480–496.
- Gustafsson, C., Minshull, J., Govindarajan, S., Ness, J., Villalobos, A., Welch, M., 2012. Engineering genes for predictable protein expression. *Protein Expr. Purif.* 83, 37–46. <http://dx.doi.org/10.1016/j.pep.2012.02.013>.
- Jenkins, G.M., Holmes, E.C., 2003. The extent of codon usage bias in human RNA viruses and its evolutionary origin. *Virus Res.* 92, 1–7. [http://dx.doi.org/10.1016/S0168-1702\(02\)00309-X](http://dx.doi.org/10.1016/S0168-1702(02)00309-X).
- Lara-Ramírez, E.E., Salazar, M.I., López-López, M.D.J., Salas-Benito, J.S., Sánchez-Varela, A., Guo, X., 2014. Large-scale genomic analysis of codon usage in dengue virus and evaluation of its phylogenetic dependence. *Biomed. Res. Int.* 2014, 1–9. <http://dx.doi.org/10.1155/2014/851425>.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.a., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, a., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948. <http://dx.doi.org/10.1093/bioinformatics/btm404>.
- Larsson, A., 2014. AliView : a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30, 3276–3278. <http://dx.doi.org/10.1093/bioinformatics/btu531>.
- Lequime, S., Paul, R.E., Lambrechts, L., 2016. Determinants of arbovirus vertical transmission in mosquitoes. *PLoS Pathog.* 12, 1–14. <http://dx.doi.org/10.1371/journal.ppat.1005548>.
- Liu, W., Pickering, P., Duchêne, S., Holmes, E.C., Aaskov, J.G., 2016. Highly divergent dengue virus type 2 in traveler returning from Borneo to Australia. *Emerg. Infect. Dis.* 22, 2146–2148. <http://dx.doi.org/10.3201/eid2212.160813>.
- Lobo, F.P., Mota, B.E.F., Pena, S.D.J., Azevedo, V., Macedo, A.M., Tauch, A., Machado, C.R., Franco, G.R., 2009. Virus-host coevolution: common patterns of nucleotide motif usage in Flaviviridae and their hosts. *PLoS One* 4, e6282. <http://dx.doi.org/10.1371/journal.pone.0006282>.
- Longdon, B., Brockhurst, M.A., Russell, C.A., Welch, J.J., Jiggins, F.M., 2014. The evolution and genetics of virus host shifts. *PLoS Pathog.* 10, e1004395. <http://dx.doi.org/10.1371/journal.ppat.1004395>.
- Mänz, B., de Graaf, M., Mögling, R., Richard, M., Bestebroer, T.M., Rimmelzwaan, G.F., Fouchier, R.A.M., 2016. Multiple natural substitutions in avian influenza A virus PB2 facilitate efficient replication in human cells. *J. Virol.* 90, 5928–5938. <http://dx.doi.org/10.1128/JVI.00130-16>.
- Martin, D.P., Murrell, B., Golden, M., Khoosal, A., Muhire, B., 2015. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol.* 1, 1–5. <http://dx.doi.org/10.1093/ve/vev003>.
- Mayer, S.V., Tesh, R.B., Vasilakis, N., 2017. The emergence of arthropod-borne viral diseases: a global prospective on dengue, chikungunya and Zika fevers. *Acta Trop.* 166, 155–163. <http://dx.doi.org/10.1016/j.actatropica.2016.11.020>.
- Messina, J.P., Brady, O.J., Scott, T.W., Zou, C., Pigott, D.M., Duda, K. a, Bhatt, S., Katzelnick, L., Howes, R.E., Battle, K.E., Simmons, C.P., Hay, S.I., 2014. Global spread of dengue virus types: mapping the 70 year history. *Trends Microbiol.* 22, 138–146. <http://dx.doi.org/10.1016/j.tim.2013.12.011>.
- Moncayo, A.C., Fernandez, Z., Ortiz, D., Diallo, M., Sall, A., Hartman, S., Davis, C.T., Coffey, L., Mathiot, C.C., Tesh, R.B., Weaver, S.C., 2004. Dengue emergence and adaptation to peridomestic mosquitoes. *Emerg. Infect. Dis.* 10, 1790–1796. <http://dx.doi.org/10.3201/eid1010.030846>.
- Moratorio, G., Iriarte, A., Moreno, P., Musto, H., Cristina, J., 2013. A detailed comparative analysis on the overall codon usage patterns in West Nile virus. *Infect. Genet. Evol.* 14, 396–400. <http://dx.doi.org/10.1016/j.meegid.2013.01.001>.
- Morton, B.R., 2003. The role of context-dependent mutations in generating compositional and codon usage bias in grass chloroplast DNA. *J. Mol. Evol.* 56, 616–629. <http://dx.doi.org/10.1007/s00239-002-2430-1>.
- Nakamura, Y., 2000. Codon usage tabulated from international DNA sequence databases: status for the year 2000. *Nucleic Acids Res.* 28, 292. <http://dx.doi.org/10.1093/nar/28.1.292>.
- Neame, E., 2009. Gene expression: structure versus codon bias. *Nat. Rev. Genet.* 7 (406). <http://dx.doi.org/10.1038/nrmicro2153>.
- Pal, S., Banerjee, A., Das, P.S., Pati, B.R., Mandal, K.C., Chakraborty, B., Sen, A., Mohapatra, P.K. Das, 2014. Dengue virus type 4 evolution and genomics: a bioinformatic approach. *Indian J. Biotechnol.* 13, 330–336.
- Parvez, M.K., Parveen, S., 2017. Evolution and emergence of pathogenic viruses: past, present, and future. *Intervirology* 60, 1–7. <http://dx.doi.org/10.1159/000478729>.
- Pettersson, J.H.O., Eldholm, V., Seligman, S.J., Lundkvist, Å., Falconar, A.K., Gaunt, M.W., Musso, D., Nougairède, A., Charrel, R., Gould, E.A., De Lamballerie, X., 2016. How did Zika virus emerge in the Pacific Islands and Latin America? *MBio* 7, e01239-16. <http://dx.doi.org/10.1128/mBio.01239-16>.
- Plowright, R.K., Parrish, C.R., McCallum, H., Hudson, P.J., Ko, A.I., Graham, A.L., Lloyd-Smith, J.O., 2017. Pathways to zoonotic spillover. *Nat. Rev. Microbiol.* 15, 502–510. <http://dx.doi.org/10.1038/nrmicro.2017.45>.
- Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One* 5, e9490. <http://dx.doi.org/10.1371/journal.pone.0009490>.
- Puigbò, P., Bravo, I.G., Garcia-Vallve, S., 2008a. CAIcal: a combined set of tools to assess codon usage adaptation. *Biol. Direct* 3, 38. <http://dx.doi.org/10.1186/1745-6150-3-38>.
- Puigbò, P., Bravo, I.G., Garcia-Vallve, S., 2008b. E-CAI: a novel server to estimate an expected value of codon adaptation index (eCAI). *BMC Bioinformatics* 9, 65. <http://dx.doi.org/10.1186/1471-2105-9-65>.
- Pyke, A.T., Huang, B., Warrilow, D., Moore, P.R., McMahon, J., Harrower, B., 2017. Complete genome sequence of a highly divergent dengue virus type 2 strain, imported into Australia from Sabah, Malaysia. *Genome Announc.* 5, e00546-17. <http://dx.doi.org/10.1128/genomeA.00546-17>.
- Salazar, M.I., Loroño-Pino, M. a, Farfán-Ale, J. a, Olsen, K.E., Beaty, B.J., 2010. American and American/Asian genotypes of dengue virus differ in mosquito infection efficiency: candidate molecular determinants of productive vector infection. *Rev. Biomédica* 21, 121–135.
- Salje, H., Lessler, J., Berry, I.M., Melendrez, M.C., Endy, T., Kalayanaraj, S., A-nue-goonpipat, A., Chanama, S., Sangkijporn, S., Klungthong, C., Thaisomboonsuk, B., Nisalak, A., Gibbons, R.V., Iamsrithaworn, S., Macareo, L.R., Yoon, I., Sangarsang, A., Jarman, R.G., Cummings, D.A.T., 2017. Dengue diversity across spatial and temporal scales: local structure and the effect of host population size. *Science* (80-). 355, 1302–1306.
- Sharp, P.M., Li, W.-H., 1987. The codon adaptation index—a measure of directional synonymous codon usage bias, and its potential applications. *Nucleic Acids Res.* 15, 1281–1295.
- Sharp, P.M., Tuohy, T.M.F., Mosurski, K.R., 1986. Codon usage in yeast: cluster analysis clearly differentiates highly and lowly expressed genes. *Nucleic Acids Res.* 14, 5125–5143.
- Shen, S.H., Stauff, C.B., Gorbatshevych, O., Song, Y., Ward, C.B., Yurovsky, A., Mueller, S., Fitcher, B., Wimmer, E., 2015. Large-scale recoding of an arbovirus genome to re-balance its insect versus mammalian preference. *Proc. Natl. Acad. Sci. U. S. A.* 112, 4749–4754. <http://dx.doi.org/10.1073/pnas.1502864112>.
- Twiddy, S.S., Farrar, J.J., Chau, N.V., Wills, B., Gould, E.A., Gritsun, T., Lloyd, G., Holmes, E.C., 2002. Phylogenetic relationships and differential selection pressures among genotypes of Dengue-2 virus. *Virology* 72, 63–72. <http://dx.doi.org/10.1006/viro.2002.1447>.
- Urbanowicz, R.A., McClure, C.P., Sakuntabhai, A., Sall, A.A., Kobinger, G., Muller, M.A., Holmes, E.C., Rey, F.A., Simon-Loriere, E., Ball, J.K., 2016. Human adaptation of Ebola virus during the west African outbreak. *Cell* 167, 1079–1087. <http://dx.doi.org/10.1016/j.cell.2016.10.013>.
- Vasilakis, N., Holmes, E.C., Fokam, E.B., Faye, O., Diallo, M., Sall, A.A., Weaver, S.C., 2007a. Evolutionary processes among sylvatic dengue type 2 viruses. *J. Virol.* 81, 9591–9595. <http://dx.doi.org/10.1128/JVI.02776-06>.
- Vasilakis, N., Shell, E.J., Fokam, E.B., Mason, P.W., Hanley, K.A., Estes, D.M., Weaver, S.C., 2007b. Potential of ancestral sylvatic dengue-2 viruses to re-emerge. *Virology* 358, 402–412. <http://dx.doi.org/10.1016/j.virol.2006.08.049>.
- Vasilakis, N., Cardoso, J., Hanley, K.A., Holmes, E.C., Weaver, S.C., 2011. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat. Rev. Microbiol.* 9, 532–541. <http://dx.doi.org/10.1038/nrmicro2595.Fever>.
- Wang, E., Ni, H., Xu, R., Barrett, A.D.T., Watowich, S.J., Gubler, D.J., Weaver, S.C., 2000. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *J. Virol.* 74, 3227–3234. <http://dx.doi.org/10.1128/JVI.74.7.3227-3234.2000>. Updated.
- Wei, K., Li, Y., 2017. Global evolutionary history and spatio-temporal dynamics of dengue virus type 2. *Sci. Rep.* 7, 45505. <http://dx.doi.org/10.1038/srep45505>.