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Molecular Determinants of Epidemiological Fitness of Dengue Virus

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Aims: Mutations on the dengue virus (DENV) genome has been associated with major outbreaks without any change in the relative prevalence of the DENV serotypes in several places, including Singapore, suggesting the DENV genome encodes factors that define its epidemiological fitness. We recently identified that greater production of subgenomic viral RNA due to mutations in the non-coding 3' untranslated region (UTR) of the genome altered fitness by inhibition of type-I interferon signalling. However, these 3'UTR mutations only represent a small proportion of the total mutations we identified. Here, we test the possibility that the DENV genome encodes factors that interact epistatically to determine epidemiological fitness.

Methodology: We conducted phylogenetic analysis of DENV-2 using whole genome sequences. We mapped mutations in the coding region which founded the divergence of the different phylogenetic clades and compared it with mutations in the 3'UTR to determine co-evolution of the 2 regions.
We also employed the Gibson assembly (GA) method to synthesise infectious clones, and together with multi-site directed mutagenesis as a reverse genetic approach to understand epistatic interactions in DENV-2.

Result: The genome sequences obtained included viruses from two clade replacement events, one in Puerto-Rico (1994) and the other in Nicaragua (2005), both of which coincided with major outbreaks in the respective places. Our phylogenetic analysis suggests that the emergence of the dominant clades, PR2B and NI2B, occured when certain mutations in both the coding region and 3'UTR coincided.
We have also optimised a protocol to synthesise DENV infectious clones using GA and incorporated the mutations as a foundation to study the molecular mechanism of DENV-2 fitness.

Conclusion: Our results suggest that epistatic interaction between mutations in the viral genome is critical in defining DENV-2 fitness. The optimised GA method provides us with a useful approach to understand how epistatic interactions on the DENV genome influence epidemiological fitness.