Vectors as motors (of virus evolution)

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A recommendation of

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Many viruses are transmitted by biological vectors, i.e. organisms that transfer the virus from one host to another. Dengue virus (DENV) is one of them. Dengue is a mosquito-borne viral disease that has rapidly spread around the world since the 1940s. One recent estimate indicates 390 million dengue infections per year [1]. As many arthropod-borne vertebrate viruses, DENV has to cross several anatomical barriers in the vector, to multiply in its body and to invade its salivary glands before getting transmissible. As a consequence, vectors are not passive carriers but genuine hosts of the viruses that potentially have important effects on the composition of virus populations and, ultimately, on virus epidemiology and virulence. Within infected vectors, virus populations are expected to acquire new mutations and to undergo genetic drift and selection effects. However, the intensity of these evolutionary forces and the way they shape virus genetic diversity are poorly known.

In their study, Lequime *et al.* [2] finely disentangled the effects of genetic drift and selection on DENV populations during their infectious cycle within mosquito (*Aedes aegypti*) vectors. They evidenced that the genetic diversity of viruses within their vectors is shaped by genetic drift, selection and vector genotype. The experimental design consisted in artificial acquisition of purified virus by mosquitoes during a blood meal. The authors monitored the diversity of DENV populations in *Ae. aegypti* individuals at different time points by high-throughput sequencing (HTS). They estimated the intensity of genetic drift and selection effects exerted on virus populations by comparing the DENV diversity at these sampling time points with the diversity in the purified virus stock (inoculum).

Disentangling the effects of genetic drift and selection remains a methodological challenge because both evolutionary forces operate concomitantly and both reduce genetic diversity. However, selection reduces diversity in a reproducible manner among experimental replicates (here, mosquito individuals): the fittest variants are favoured at the expense of the weakest ones. In contrast, genetic drift reduces diversity in a stochastic manner among replicates. Genetic drift acts equally on all variants irrespectively of their fitness. The strength of genetic drift is frequently evaluated with the effective population size Ne: the lower Ne, the stronger the genetic drift [3]. The estimation of the effective population size of DENV populations by Lequime *et al.* [2] was based on single-nucleotide polymorphisms (SNPs) that were (i) present both in the inoculum and in the virus populations sampled at the different time points and (ii) that were neutral (or nearly-neutral) and therefore subjected to genetic drift only and insensitive to selection. As expected for viruses that possess small and constrained genomes, such neutral SNPs are extremely rare. Starting from a set of >1800 SNPs across the DENV genome, only three SNPs complied with the neutrality criteria and were enough represented in the sequence dataset for a precise Ne estimation. Using the method described by Monsion *et al.* [4], Lequime *et al.* [2] estimated Ne values ranging from 5 to 42 viral genomes (95% confidence intervals ranged from 2 to 161 founding viral genomes). Consequently, narrow bottlenecks occurred at the virus acquisition step, since the blood meal had allowed the ingestion of ca. 3000 infectious virus particles, on average. Interestingly, bottleneck sizes did not differ between mosquito genotypes. Monsion *et al.*'s [4] formula provides only an approximation of Ne. A corrected formula has been recently published [5]. We applied this exact Ne formula to the means and variances of the frequencies of the three neutral markers estimated before and after the bottlenecks (Table 1 in [2]), and nearly identical Ne estimates were obtained with both formulas.

Selection intensity was estimated from the dN/dS ratio between the nonsynonymous and synonymous substitution rates using the HTS data on DENV populations. DENV genetic diversity increased following initial infection but was restricted by strong purifying selection during virus expansion in the midgut. Again, no differences were detected between mosquito genotypes. However and importantly, significant differences in DENV genetic diversity were detected among mosquito genotypes. As they could not be related to differences in initial genetic drift or to selection intensity, the authors raise interesting alternative hypotheses, including varying rates of *de novo* mutations due to differences in replicase fidelity or differences in the balancing selection regime. Interestingly, they also suggest that this observation could simply result from a methodological issue linked to the detection threshold of low-frequency SNPs.

References

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