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Randomness and selection in the emergence of begomovirus recombinants

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Begomoviruses (Famille des Geminiviridae) have a circular single stranded DNA genome (2800 nt) and are transmitted by the whitefly *Bemisia tabaci*. Genomes are replicated with recombination prone mechanisms: rolling circle replication and recombination dependent replication. Consistently, recombinant genomes were frequently detected in begomoviruses and some of them were associated with emerging diseases. A screening of the recombination breakpoints detected in the sequence databases have revealed that they were significantly more present in some regions of the genome (hot-spots) [1] but the reason for this non random distribution is not yet clear. Firstly, it was thought that recombinants with breaks in cold spots are lethal or at least deleterious, a hypothesis that turned out to be non consistent with our recent test of 47 random recombinants generated in-vitro between two begomovirus species which all exhibited a within-host accumulation similar or intermediate to that of the parental clones [2]. Secondly it was thought that the cold spots correspond to regions in which breakpoints are not easily generated due to mechanical constraints. To test this second hypothesis we assessed the distribution of the break positions along the genome in naturally generated recombinants isolated from 9 plants coinoculated with the two begomovirus species which were previously used to generate the in-vitro library of recombinants. The parental viruses were the invasive Tomato yellow leaf curl virus (TYLCV), originating from the Middle East, and the Tomato leaf curl Mayotte virus (ToLCYTV) originating from Indian Ocean islands.

Recombinant genomes were isolated as early as possible before the emergence of dominant recombinants which would blur the full diversity of the generated breaks. Recombinants were isolated as early as 30 days post inoculation (dpi) at a frequency of 1%. Dominance of some recombinants was detectable in some of the plants at 150 dpi and in all those tested at 330 dpi. When all the breaks detected at all time points (30, 60, 150, 330 dpi) were plotted on the genome they were found to be distributed all along the genome suggesting that the possible mechanical constraints occurring at break generation, are limited. As generation of recombination breakpoints is relatively random and the within host viral accumulation is not significantly affected by random recombination (as shown with the in vitro generated recombinants [2]), it seems that the hot spots detected in the sequence database are due to selection at the agroecosystem level and particularly with respect to width of host range and vector transmissibility.

Finally, an emergence scenario of recombinant genomes could be derived from the comparison of the recombinant populations isolated at different dpi from the various plants coinfected under the same experimental conditions. The randomly generated populations of recombinants are shaped by several evolutionary factors. The dominant recombinant is not necessarily the same in the different plants which is indicative of the drift occurring at the different steps of plant colonization. Some of the plants however, hosted a similar dominant recombinant which can be explained by positive selection. Competition tests are in progress to confirm the selection hypothesis.

1. Lefeuvre, P., et al., *Widely Conserved Recombination Patterns among Single-Stranded DNA Viruses.* Journal of Virology, 2009. **83**(6): p. 2697-2707.

2. Vuillaume, F., et al., *Distribution of the Phenotypic Effects of Random Homologous Recombination between Two Virus Species.* PLoS Pathog, 2011. **7**(5): p. e1002028.