Two Distinct Genomic Lineages of Sinaivirus Detected in Guyanese Africanized Honey Bees

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Abstract

Over the past decade or so, PCR-based screening programs have reported that Africanized honey bees (AHB) are also hosts to viruses commonly found in European honey bees. Very little is known about the genomic variants found in AHB. Here, we present two distinct lineages of sinaiviruses in AHB.

Lake Sinai virus (LSV), family Sinhaliviridae within the order Nodamuvirales, was first discovered in European honey bee (EHB) colonies in the United States (1). Since then, LSV variants have been detected in multiple bee species across the world (2, 3). LSV has also been detected in African honey bees (4), and the only African LSV genomes published to date are from those found in honey bees collected in South Africa (5). Here, we sequenced and identified two distinct lineages of LSV (Fig. 1) in Guyanese Africanized honey bees (AHB).

Samples made up of 30 whole worker AHB per apiary (Table 1) were pooled and liquefied using the gentleMACS dissociator (RNA 02.01 program; Miltenyi Biotec). The samples represented 3 regions across Guyana, namely, regions 6, 4, and 3. Total RNA was extracted per the manufacturer’s instructions using the NucleoSpin virus RNA-DNA isolation kit (TaKaRa Bio USA). Sequencing was carried out as previously described (6), except that cDNA synthesis was carried out using the template switching (TS) RT enzyme mix (New England Biolabs) with an N6 TS modified random primer (Thermo Fisher Scientific). RNA and DNA quantifications were carried out using the Qubit 4 fluorometer (Thermo Fisher Scientific). Region- and apiary-specific Oxford Nanopore Technologies (ONT) libraries (8 in total; Table 1) were prepared using the rapid barcoding SQK-RBK004 sequencing kit (Oxford Nanopore Technologies). All libraries were sequenced in a single sequencing run using the high-accuracy base-calling model with a minimum Q score of 7 set on an ONT MinION device using one FLO-MIN106 R9 flow cell.

Consensus genome assemblies and phylogenies were created using default parameters for all software. The reads obtained were reference assembled against the top Epi2Me WIMP hit for each genome (Oxford Nanopore Technologies), which was either the genome submitted under GenBank accession number NC_035112.1 or NC_035113.1, using Minimap 2.17 in Geneious Prime 2021.1.1 and manually curated to correct ambiguities where possible. For each Guyanese LSV consensus genome in lineages NE and 8, reads ranging from 115 to 1,248 bases (Table 1) were used to assemble the near-complete consensus genomes of LSV-8- and LSV-NE-like variants (Fig. 1). All Guyanese LSV genomes were assigned a label to indicate the region of origin, beekeeper code, library, barcode, and finally, the best Epi2Me LSV
genome match (Table 1; Fig. 1). The coding regions (5’ and 3’ regions removed) of the new consensus genomes were aligned to available LSV genomes found in NCBI (downloaded March 2022) using Muscle 3.8.425 (7) and visualized using FastTree 2.1.11 (8) in Geneious Prime 2021.1.1.

LSV variants can be subdivided into lineages based on the RNA-dependent RNA polymerase (RdRp) gene and their whole-genome sequences (9, 10). Phylogenetic analysis revealed that two distinct LSV lineages exist in Guyanese AHB (Fig. 1). One clade of Guyanese LSV variants clustered with a variant in lineage 8, named LSV-SA2 (Fig. 1). The second clade of Guyanese LSV variants clustered with variants previously assigned to LSV lineage 1 (10). Here, we show how LSV lineage 1 can be split into two lineages, creating a new lineage, namely, lineage NE (Fig. 1).

Data availability. The genome sequences for this project have been deposited at GenBank under the following accession numbers: ON108628 to ON108639. The Oxford Nanopore Technology reads are available under BioProject accession number PRJNA820891. Links to the read files in the SRA for all the new LSV genomes can be found in Table 1.

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| Isolate | Top Epi2Me match | Sample description (30 pooled adult bees) | Region | Community  | Sample no. | Barcode | No. of mapped reads | Total no. of reads | Min length (bp) | Max length (bp) | No. of bases | Genome length (bp) | Coverage (x) | SRA accession no. | GenBank accession no. |
|---------|-----------------|-------------------------------------------|--------|----------|------------|---------|-------------------|-----------------|----------------|----------------|-------------|----------------|--------------|----------------|------------------|
| LSV_Guyana_R6_OS_3_01_NE | NE (NC_035113.1) | 5 bees each from 6 hives | 6 | Sandvoort | 3 | 1 | 533 | 1,1078 | 199 | 3,209 | 4,014,040 | 5,533 | 73.27 | ON108632.1 | SRX14642749 |
| LSV_Guyana_R6_OS_3_01_SA2 | SA2 (NC_035112.1) | 15 bees each from 2 hives | 4 | 2 | 115 | 14,165 | 216 | 1,81 | 60,752 | 5,737 | 10.91 | ON108637.1 | SRX14642753 |
| LSV_Guyana_R6_OS_5_03_SA2 | SA2 (NC_035112.1) | 5 bees each from 6 hives | 5 | 3 | 1,248 | 18,069 | 190 | 3,49 | 99,451 | 5,737 | 173.35 | ON108633.1 | SRX14642754 |
| LSV_Guyana_R6_FL_6_04_SA2 | SA2 (NC_035112.1) | 6 bees each from 5 hives | 6 | 4 | 463 | 1,303 | 187 | 2,55 | 37,163 | 5,737 | 64.84 | ON108634.1 | SRX14642755 |
| LSV_Guyana_R3_RW_9_05_NE | NE (NC_035113.1) | 5 bees each of 6 hives | 3 | La Grange | 9 | 5 | 557 | 1,2021 | 181 | 3,88 | 43,776 | 5,596 | 78.23 | ON108631.1 | SRX14642756 |
| LSV_Guyana_R3_RW_9_05_SA2 | SA2 (NC_035112.1) | Single feral colony | 12 | 6 | 200 | 1,483 | 235 | 2,55 | 15,392 | 5,737 | 62.43 | ON108638.1 | SRX14642757 |
| LSV_Guyana_R3_feral_12_06_NE | NE (NC_035113.1) | Land of Caanan | 16 | 8 | 232 | 1,236 | 183 | 2,12 | 19,828 | 5,740 | 34.54 | ON108639.1 | SRX14642752 |
| LSV_Guyana_R3_feral_12_06_SA2 | SA2 (NC_035112.1) | Harmonie | 12 | 6 | 200 | 1,483 | 235 | 2,55 | 15,392 | 5,737 | 62.43 | ON108638.1 | SRX14642757 |
| LSV_Guyana_R4_FB_13_07_NE | NE (NC_035113.1) | 5 bees each from 6 hives | 4 | Beehive | 13 | 7 | 274 | 2,172 | 182 | 2,52 | 199,407 | 5,737 | 34.76 | ON108635.1 | SRX14642760 |
| LSV_Guyana_R4_FB_13_07_SA2 | SA2 (NC_035112.1) | Harmonie | 12 | 6 | 200 | 1,483 | 235 | 2,55 | 15,392 | 5,737 | 62.43 | ON108638.1 | SRX14642757 |
| LSV_Guyana_R4_FB_16_08_SA2 | SA2 (NC_035112.1) | 7 bees each from 2 hives and 8 bees each from another 2 hives | 16 | 8 | 1,066 | 2,172 | 168 | 3,10 | 84,440 | 5,783 | 146.01 | ON108629.1 | SRX14642751 |
| LSV_Guyana_R4_FB_16_08_NE | NE (NC_035113.1) | Land of Caanan | 16 | 8 | 232 | 1,236 | 183 | 2,12 | 19,828 | 5,740 | 34.54 | ON108639.1 | SRX14642752 |
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