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Supplemental information

Loss of West Nile virus genetic diversity during mosquito infection due to species-dependent population bottlenecks

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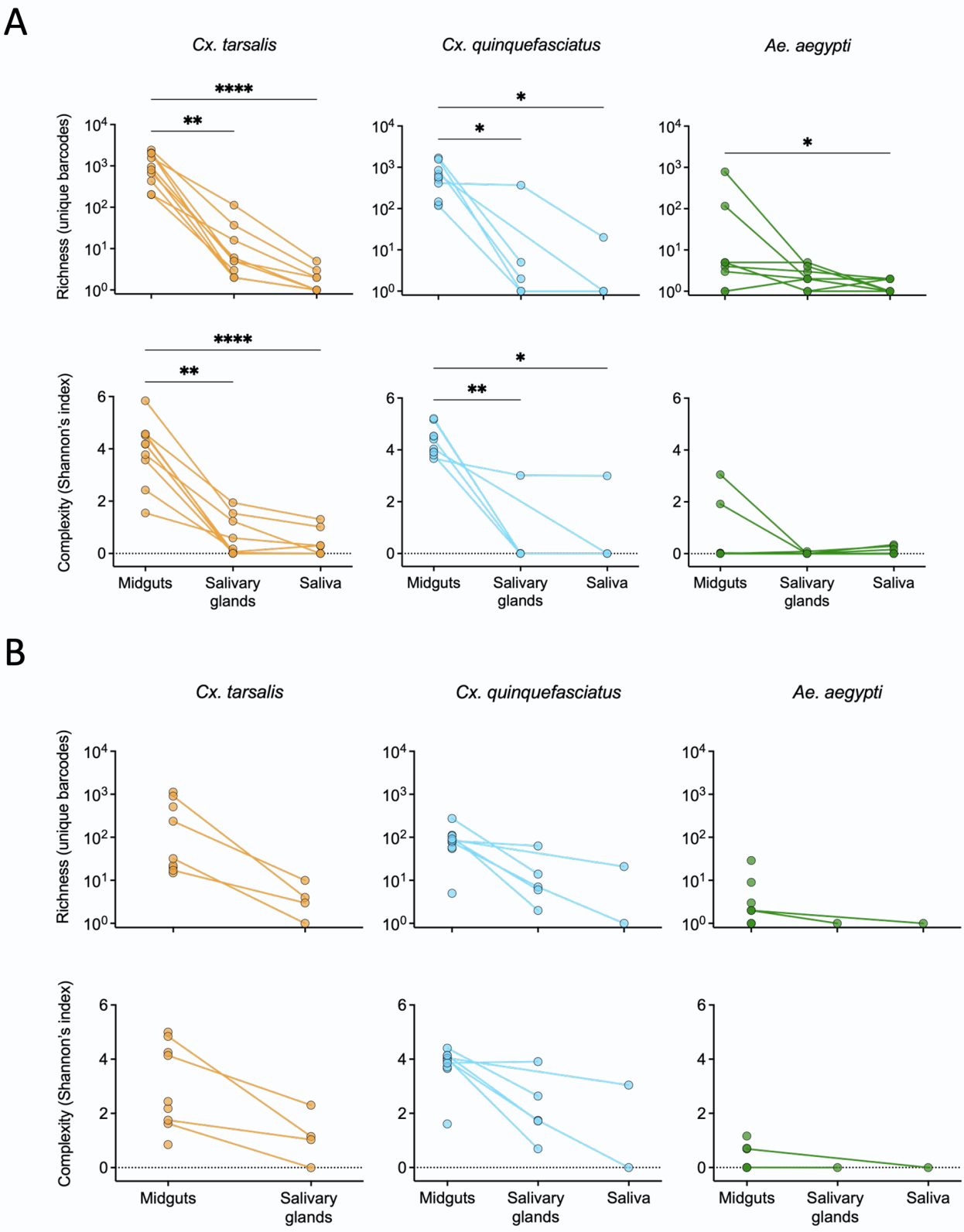
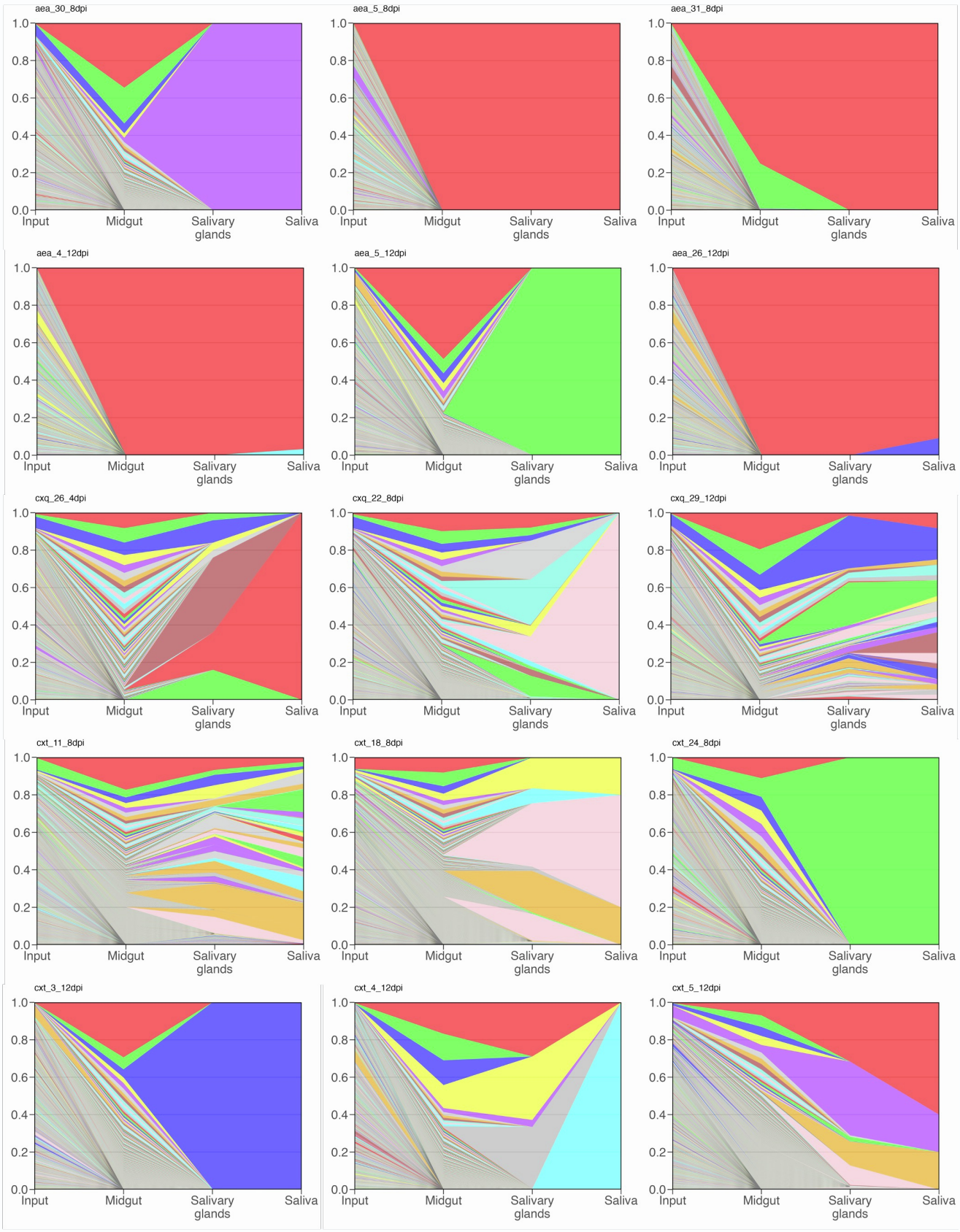
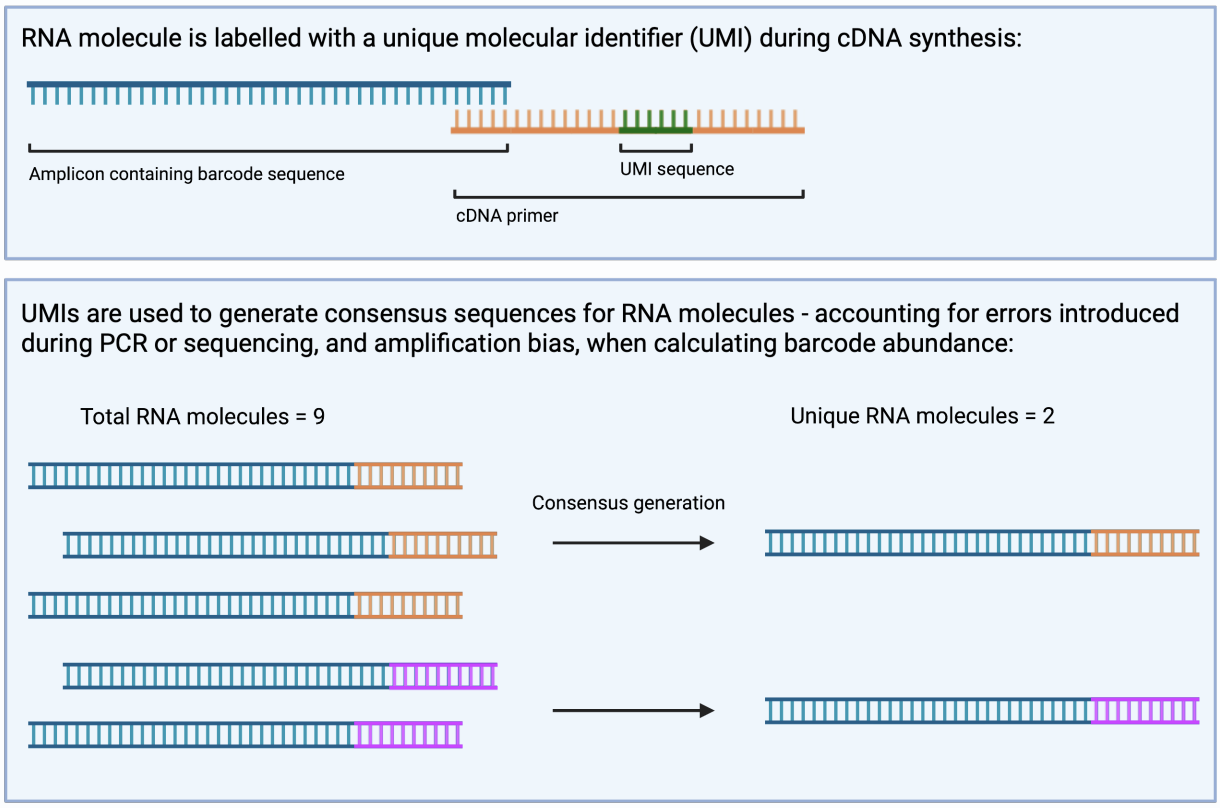


Supplemental Figure 1. Impact of intrahost bottlenecks on bcWNV populations in *Cx. tarsalis*, *Cx. quinquefasciatus*, and *Ae. aegypti* at days 4 and 12 post infection, related to Figure 3.

bcWNV population richness and complexity in midgut, salivary gland, and saliva samples from *Cx. tarsalis*, *Cx. quinquefasciatus*, and *Ae. aegypti* mosquitoes at days 4 **(A)** and 12 **(B)** post-infection. Lines connecting points indicate that samples were collected from the same mosquito. Diversity indices were generated by applying the Shannon function from the QSutils package in R to barcode abundance vectors. Dashed line represents 0. Statistical significance determined using Kruskal-Wallis with Dunn's multiple comparisons test, * = p<0.05, ** = p<0.005, *** = p<0.0005, **** = p<0.0001. Only significant comparisons shown.



Supplemental Figure 2. Visualization of bcWNV dynamics in individual mosquitoes not selected for Figure 4A.
bcWNV barcode dynamics during mosquito infection in individual *Cx. tarsalis*, *Cx. quinquefasciatus*, and *Ae. aegypti* mosquitoes at days 4, 8 and 12 post-infection. Species and timepoint are indicated in plot header; aea = *Ae. aegypti*, cxq = *Cx. quinquefasciatus*, cxt = *Cx. tarsalis.* Barcodes were ranked from most to least frequent by each midgut sample. Color represents barcode rank and is not consistent across graphs.



Supplemental Figure 3. Inclusion of a unique molecular identifier accounts for amplification bias and sequencing error, related to Methods (created with BioRender.com).

Supplemental Table 1. Infection rates of relevant compartments in *Cx. tarsalis*, *Cx. quinquefasciatus*, and *Ae. aegypti,* related to Figure 3B.

Species:	DPI:	Rate of infection in tissue/secretion:		
		Midguts	Salivary Glands	Saliva
Cx. tarsalis	4	29/36 (80.5%)	27/36 (75.0%)	2/36 (5.5%)
	8	34/36 (94.4%)	34/36 (94.4%)	14/36 (38.9%)
	12	35/36 (97.2%)	35/36 (97.2%)	35/36 (97.2%)
	Total	98/108 (90.7%)	96/108 (88.9%)	51/108 (47.2%)
Cx. quinquefasciatus	4	36/36 (100%)	20/36 (55.6%)	5/36 (13.9%)
	8	36/36 (100%)	15/36 (41.7%)	3/36 (8.3%)
	12	36/36 (100%)	24/36 (66.7%)	9/36 (25.0%)
	Total	108/108 (100%)	59/108 (54.6%)	17/108 (15.7%)
Ae. aegypti	4	10/40 (25.0%)	2/40 (5.0%)	2/40 (5.0%)
	8	24/39 (61.5%)	22/39 (56.4%)	10/39 (25.6%)
	12	15/39 (38.5%)	19/39 (48.7%)	12/39 (30.8%)
	Total	49/118 (41.5%)	43/118 (36.4%)	24/108 (22.2%)

Supplemental table 2. Primers and probes used for qRT-PCR and library preparation, related to Methods.

Primer:	**Sequence:**
WNV_ENV_F70	TCAGCGATCTCTCCACCAAAG
WNV_ENV_R70	GGGTCAGCACGTTTGTCATTG
WNENV-prob	FAM/TGCCCGACC/ZEN/ATGGGAGAAGCTC/3|ABkFQ/
ID_cDNAWNV_7374_Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNNNNcagtGCCATCCACTACAGCGTTCT
5'_ID_Primer_Rev	GTCTCGTGGGCTCGGAGATGTGTAT
5'_ID_WNV_7171_For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNTTCCCCTTCGTCGATGTTGG
2nd round FP (N5XX)	AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC
2nd round RP (N7XXX)	CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

*Nextera sequencing primer binding sites