

Supplementary Information for

Surveillance Along the Rio Grande During the 2020 Vesicular Stomatitis Outbreak Reveals Spatio-temporal Dynamics of and Viral RNA Detection in Black Flies

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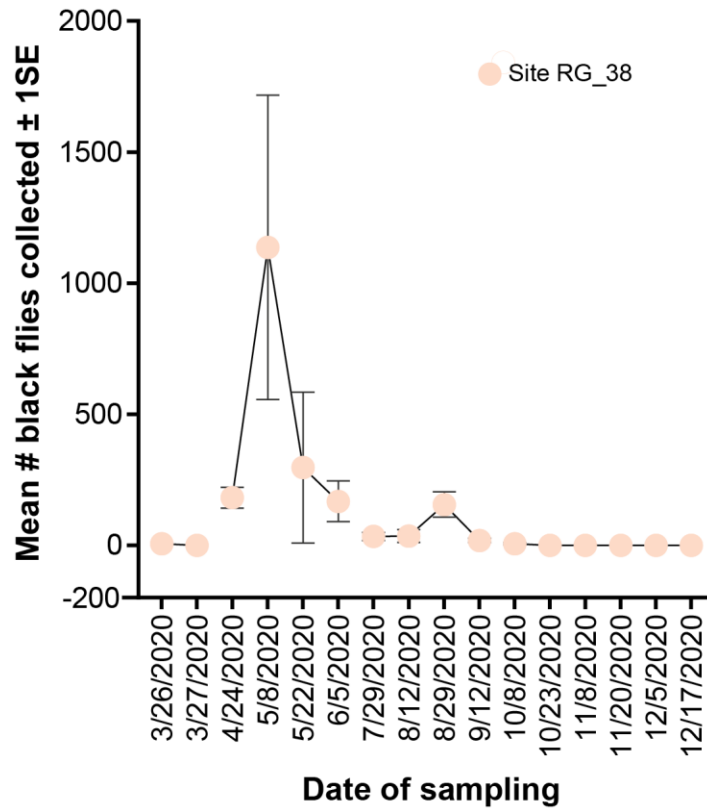


Figure S1. Mean number of black flies \pm 1SE collected on each sampling date at a continuously sampled site, RG_38. Site RG_38 was sampled approximately every two weeks except for three longer breaks that are indicated by gaps in the line connecting points.

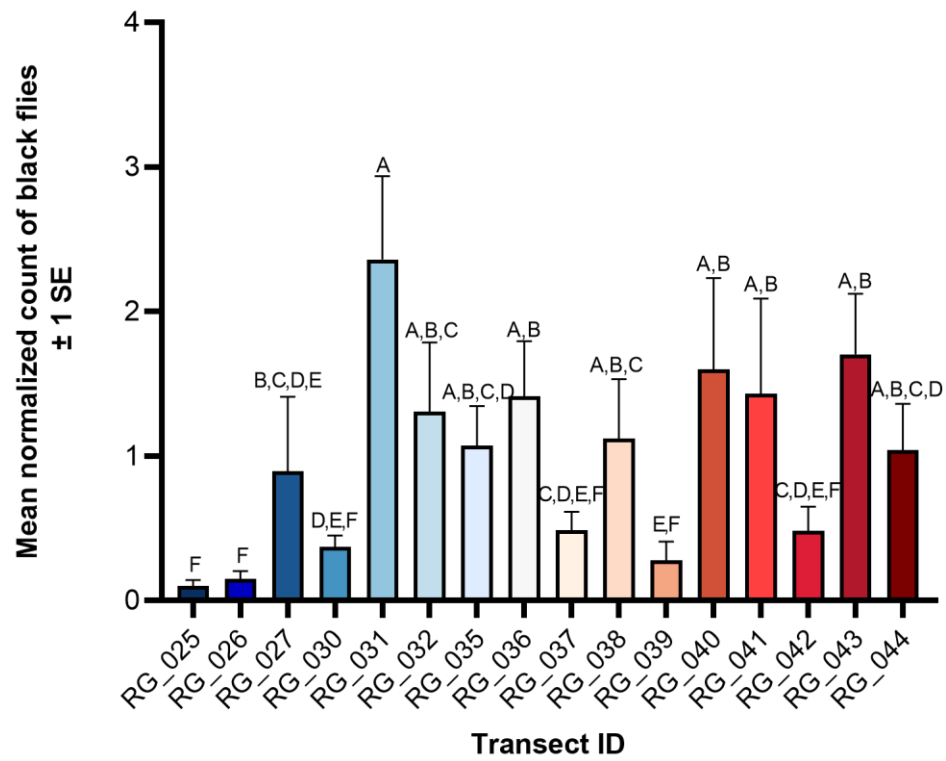


Figure S2. Mean normalized number of black flies ± 1 SE collected at each transect along the Rio Grande (Negative-binomial GLM: $df = 8$, $p = <0.001$). Differences between individual transects are specified by letters above each column (based on least-square means). Columns with the same letters are not statistically different.

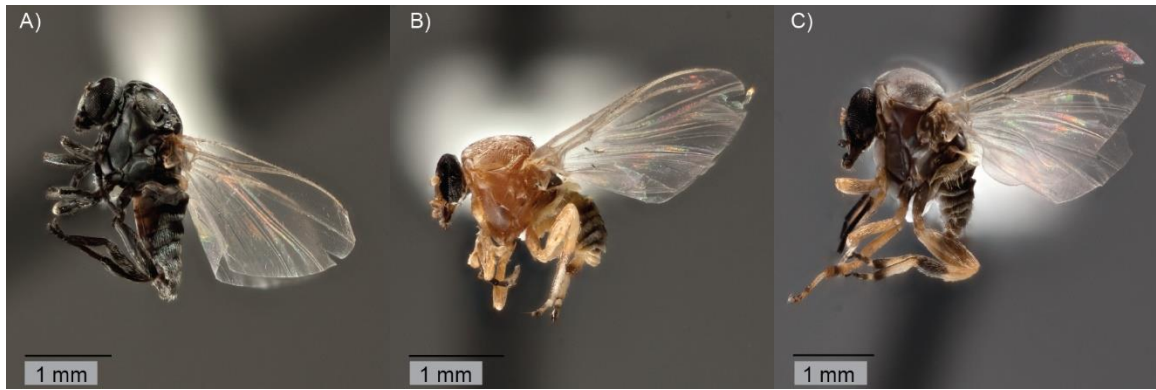


Figure S3. Representative images of the three morphogroup categories, A, B, and C, for black fly identification.

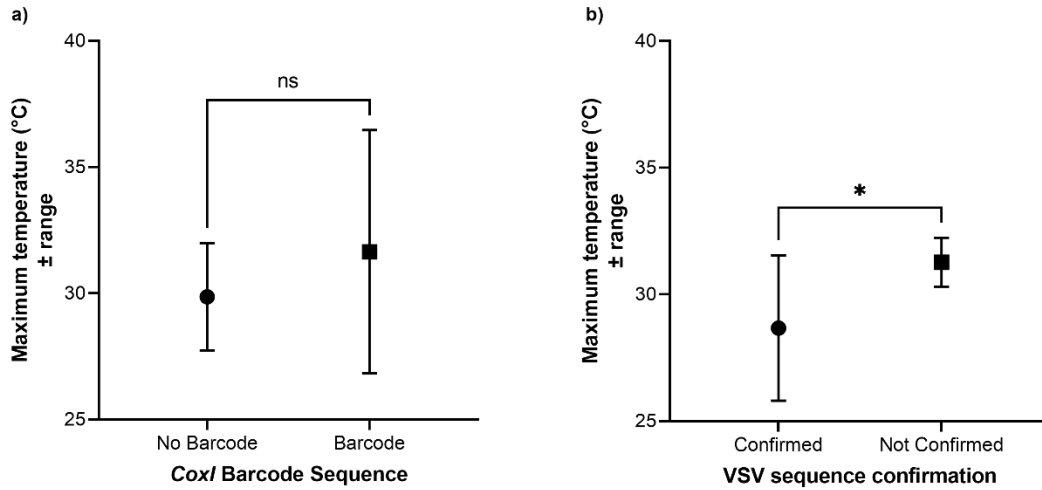


Figure. S4. Relationship between maximum temperature on the day of black fly collection and CoxI barcode and VSV sequence acquisition. **a)** Mean maximum temperature and maximum temperature range on the day of black fly collection for pools with CoxI barcode sequence confirmation and those without sequence confirmation. There was no difference in the maximum temperature with pools that did not produce CoxI barcode sequence and those that did (t-test: $t = 1.62$, $df = 117$, $p = 0.11$). **b)** Mean maximum temperature and maximum temperature range on the day of black fly collection for pools with sequence confirmation of VSIV and those without sequence confirmation. There was a significant difference in the maximum temperature with pools that did not produce a VSIV sequence being hotter than those that did (t-test: $t = 2.41$, $df = 11$, $p = 0.03$).

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1. VSIV152NM0520.ab1      1      10      20      30      40      50
2. VSIV173NM0420.ab1      CTCCTTCGTCAATCACTGTACGAAGTACAAGATAAGATCCCGGGTTTGCACAGCTCT
3. VSIV204NM0420.ab1      CACTGTACGAAGTACAAGATAAGATCCCGGGTTTGCACAGCTCT
4. VSIV205NM0420.ab1      ACAAGATAAGATCCCGGGTTTGCACAGCTCT
5. VSIV270NM0520.ab1      TGCTCTTCGTCAATCACTGTACGAAGTACAAGATAAGATCCCGGGTTTGCACAGCTCT
6. MT437283                TGCTCTTCGTCAATCAATCAATGTAAGAGTCAAGATAAGATCCCGGGTTTGCACAGCTCT

1. VSIV152NM0520.ab1      60      70      80      90      100     110
2. VSIV173NM0420.ab1      ACATTTAAATATGCTATGTTGTATTTGGATCCTTCTATCGGAGGTGTCTCTGGCATGTC
3. VSIV204NM0420.ab1      ACATTTAAATATGCTATGTTGTATTTGGATCCTTCTATCGGAGGTGTCTCTGGCATGTC
4. VSIV205NM0420.ab1      ACATTTAAATATGCTATGTTGTATTTGGATCCTTCTATCGGAGGTGTCTCTGGCATGTC
5. VSIV270NM0520.ab1      ACATTTAAATATGCTATGTTGTATTTGGATCCTTCTATCGGAGGTGTCTCTGGCATGTC
6. MT437283                ACATTTAAATATGCTATGTTGTATTTGGATCCTTCTATCGGAGGTGTCTCTGGCATGTC

1. VSIV152NM0520.ab1      120     130     140     150     160     170     176
2. VSIV173NM0420.ab1      ATTGTCTAGGTTTTTTGATTCGAGCATTCCCAGATCCCGTAACAGAAAGTCT
3. VSIV204NM0420.ab1      ATTGTCTAGGTTTTTTGATTCGAGCATTCCCAGATCCCGTAACAGAAAGTCT
4. VSIV205NM0420.ab1      ATTGTCTAGGTTTTTTGATTCGAGCATTCCCAGATCCCGTAACAGAAAGTCT
5. VSIV270NM0520.ab1      ATTGTCTAGGTTTTTTGATTCGAGCATTCCCAGATCCCGTAACAGAAAGTCT
6. MT437283                ATTGTCTAGGTTTTTTGATTCGAGCATTCCCAGATCCCGTAACAGAAAGTCT

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Figure. S5. Alignment of VSIV sequences of positions 7,288 to 7,456 (numbered from the first nucleotide in the genome) within the L gene obtained from black fly pools collected in Southern NM (sequence names starting with VSIV) in 2020 and a VSIV published sequence from an infected cow in Wyoming during the 2019 outbreak (MT437283).

Table S1: Correlation matrix based on Spearman's rho. Spearman rho for each combination of variables is shown on the bottom of the matrix and the p-value for each correlation is shown on the top of the matrix. P-values indicating significant correlation is highlighted in bold.

	NDVI	Max temperature	Flow rate	2- month lagged precipitation	1-year lagged precipitation
NDVI		<0.001*	0.48	<0.001*	0.004*
Max temperature	0.22		<0.0001*	<0.001*	<0.001*
Flow rate	0.04	0.34		0.14	<0.001*
2- month lagged precipitation	-0.20	-0.20	-0.08		<0.001*
1-year lagged precipitation	0.15	0.22	0.25	0.42	

Table S2: Model output for three negative-binomial GLMs run on normalized black fly and six predictor variables. Significant correlations are indicated in bold.

	Early time period: March to May			Middle time period: June to August			Late time period September to November		
Predictor	Parameter estimate	DF	P-value	Parameter estimate	DF	P-value	Parameter estimate	DF	P-value
Intercept	-1.3477	112	0.785	-5.1653	86	0.118	-3.6582	134	0.0036
Mean NDVI	-2.1817	112	0.051	-0.7166	86	0.479	-6.8087	134	<0.001*
Average flow rate	0.0007	112	0.366	-0.00048	86	0.208	-0.00064	134	0.0772
Maximum temperature	0.04789	112	0.446	0.1601	86	0.043	0.04996	134	0.0823
Two-month lagged precipitation	0.0615	112	<0.001*	0.01007	86	0.232	-0.01254	134	0.3148
One-year lagged precipitation	-0.0149	112	0.200	-0.00199	86	0.779	0.01682	134	0.0120

Table S3: Primer and probe sequences used in molecular analyses for black fly barcoding and VSV-IN detection.

Primer	Sequences (5' to 3')	Target Gene
CoxI-F	5'-ACAAAYCAYAARGATATYGG-3'	CoxI
CoxI-R	5'-YTARRTTWGMTCATGTTGMCAT-3'	CoxI
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	CoxI
HC02198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3	CoxI
VSIN-F	5'TGATACAGTACAATTATTTTGGGAC-3'	VSIN L gene
VSIN-R	5'-GAGACTTTCTGTTACGGGATCT-3'	VSIN L gene
VSIN-probe	FAM-ATGATGCATGATCCAGC-BHQplus	VSIN L gene