



A Phylogenetic Approach to Following West Nile Virus

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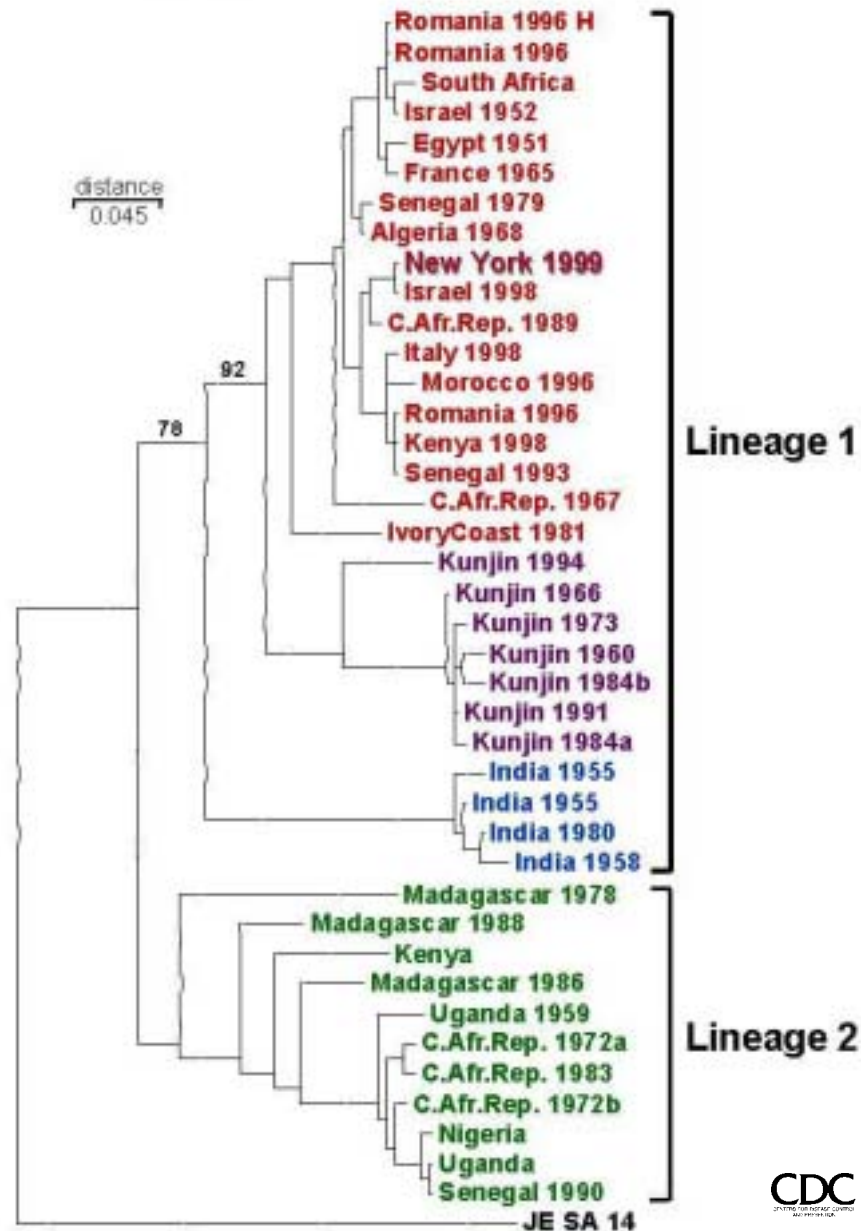
New Haven, CT 06511

Possible Pathways of Introduction of WNV into the US

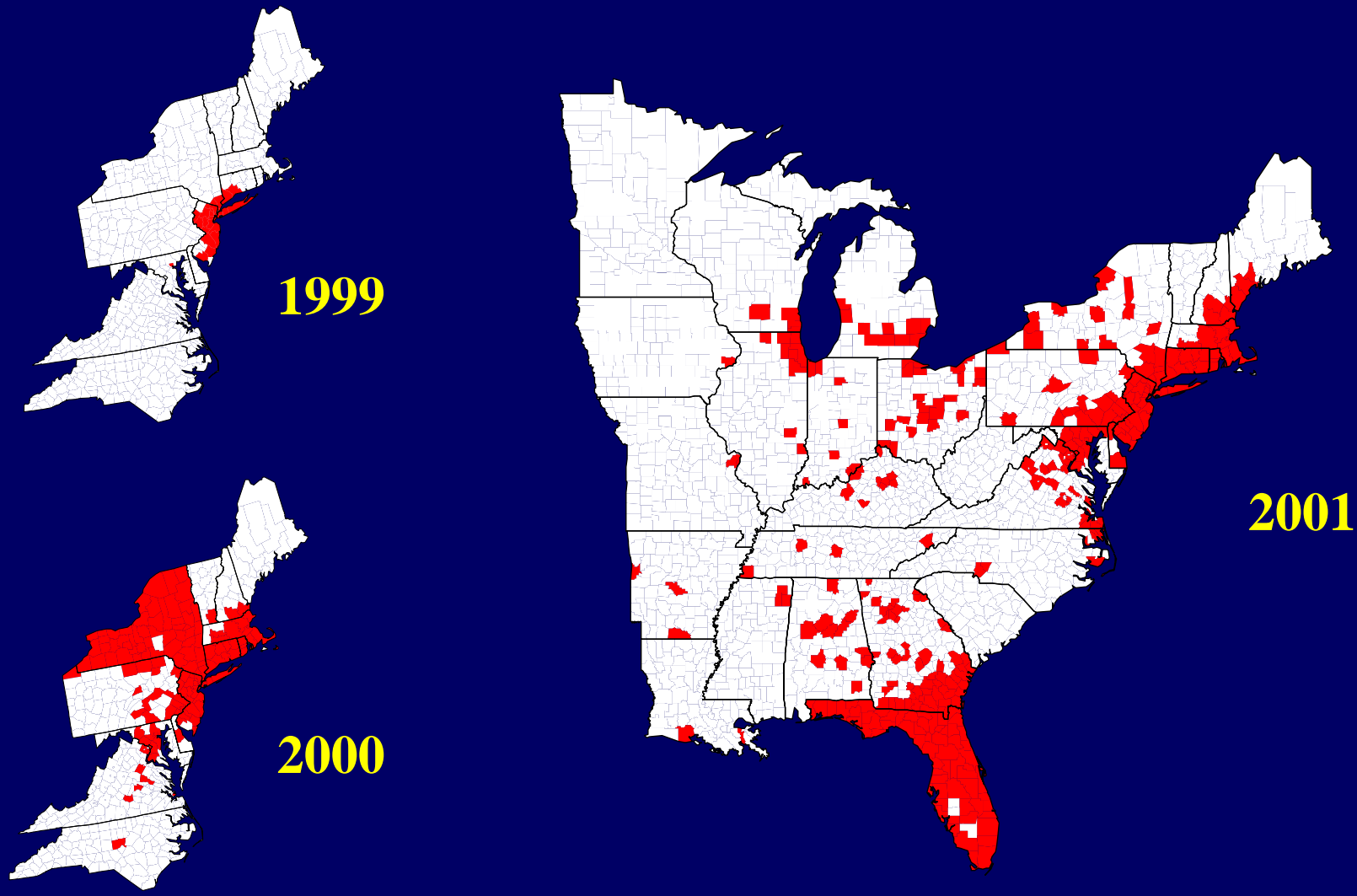
- Infected Human
- Human-transported vertebrate host
 - Legal
 - Illegal
- Human-transported mosquito vector
- Storm-transported vertebrate host (bird)
- Intentional introduction (terrorist event)



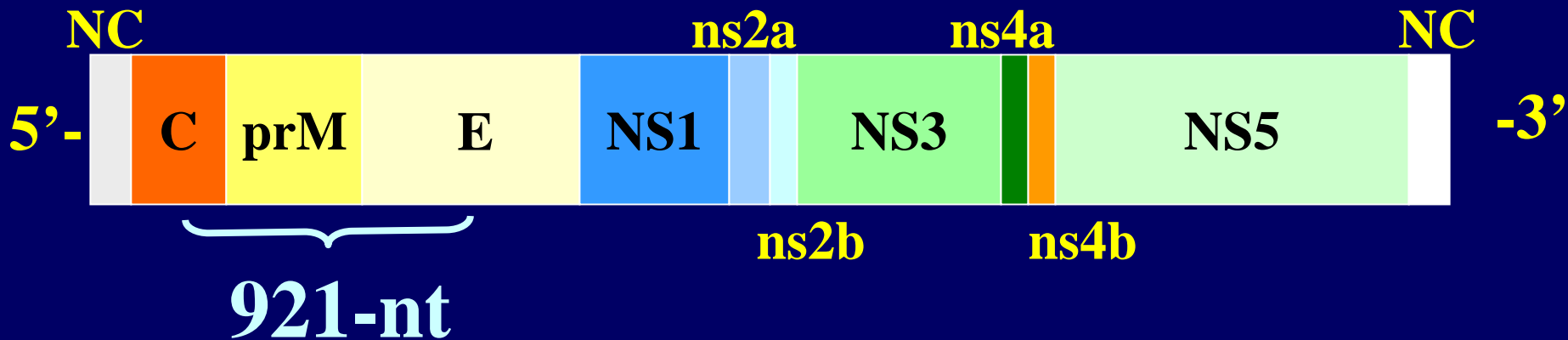
Phylogenetic Tree Based on Envelope Glycoprotein Sequence Data



DISTRIBUTION OF WEST NILE VIRUS IN NORTH AMERICA



Genomic Structure of Flaviviruses



- NC noncoding (at ends)
- C, M, E structural proteins (3)
- prM precursor protein for M
- NS nonstructural proteins (7)

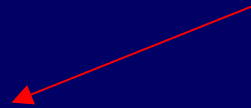
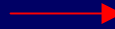
Information from L.R. Peterson and J.T. Roehrig (2001)

Primers Used to Amplify the 921-nucleotide Sequence of West Nile Virus from Positions 205-1125

WN-233F (5'-GACTGAAGAGGGCAATGTTGAGC-3')

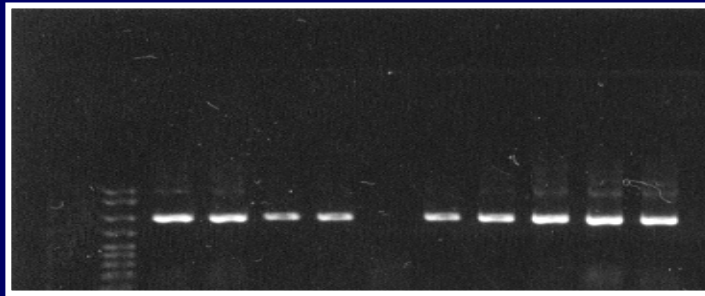
WN-1189R (5'GCAATAACTGCGGACYTCTGC-3')

Protocol Used to Develop a Phylogeny of West Nile Virus Isolates

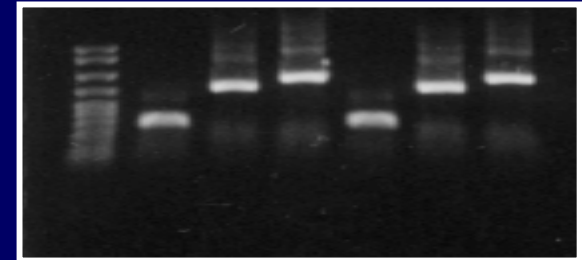


Protocol Used to Develop a Phylogeny of West Nile Virus Isolates

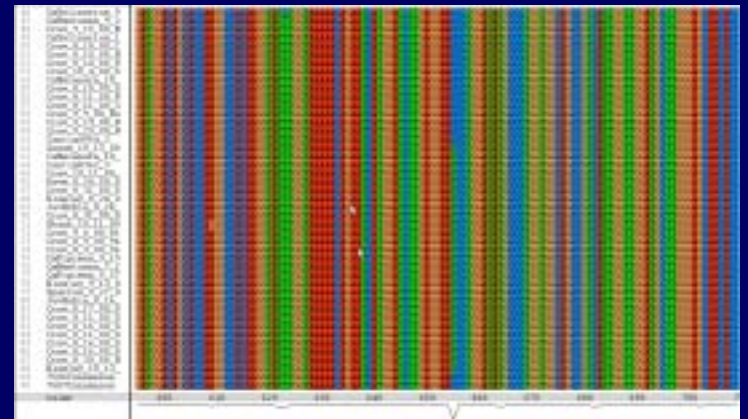
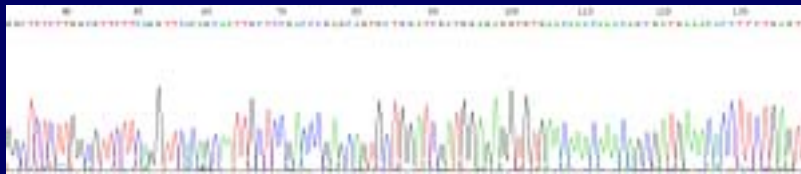
A 921-nucleotide sequence using WNV primers 233F-1189R



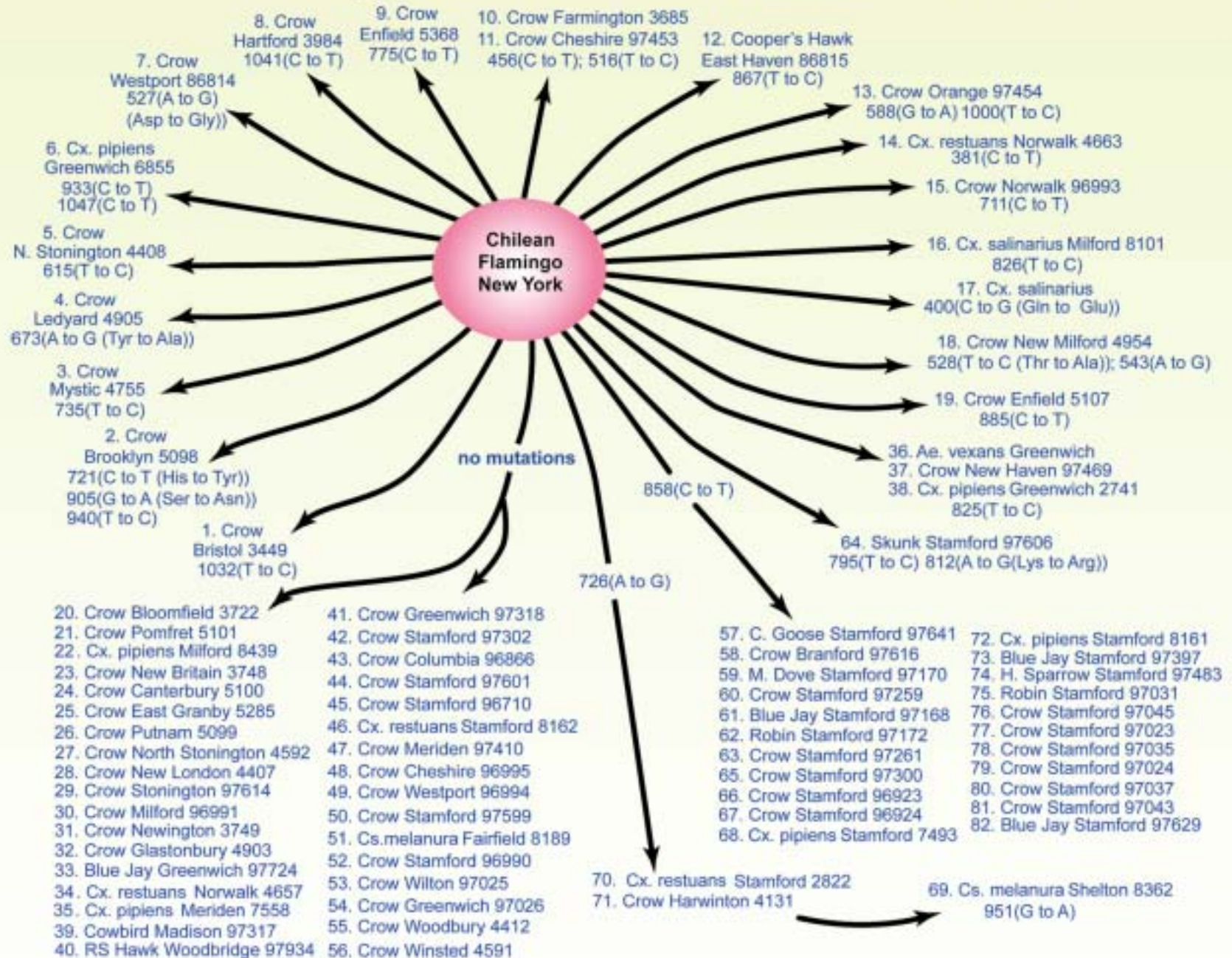
Three different-sized sequences for each of 2 isolates, using primers 233F-540R, 451F-1189R, and 233F-1189R

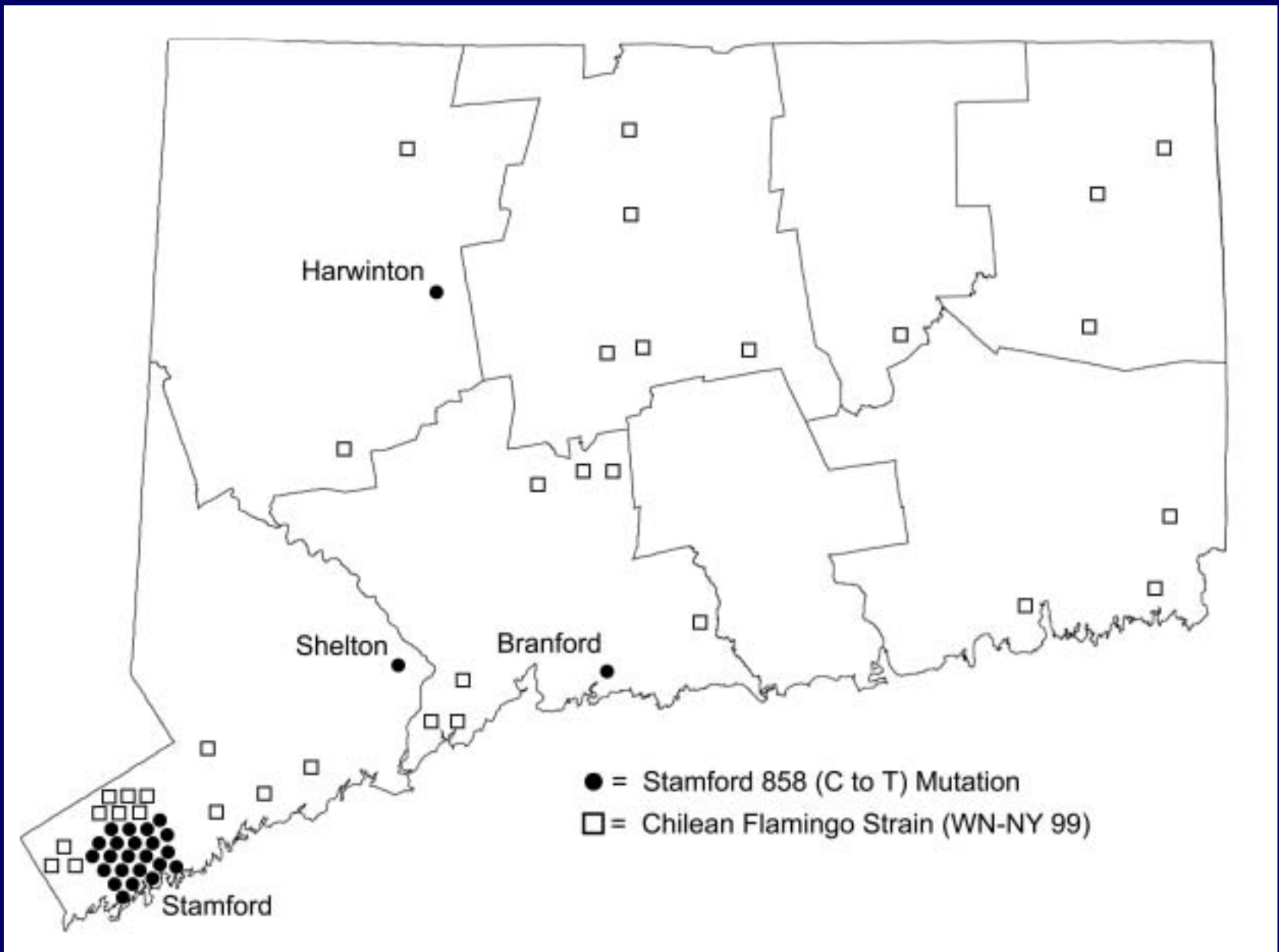


Protocol Used to Develop a Phylogeny of West Nile Virus Isolates



Mutational Changes in WN-NY99 in 1999/2000 CT Isolates





Geographically Based West Nile Virus Mutation

Significant Findings

- 30 of the 921 nucleotides showed variability
 - 3 occurred in the nucleocapsid coding region
 - 11 occurred in the premembrane coding region
 - 12 occurred in the membrane coding region
 - 4 occurred in envelope coding region
- 6 nucleotide positions showed identical changes in 2 or more isolates
- 34 isolates had sequences identical to WN-NY99
- 37 isolates had a single nucleotide change
- 8 isolates had two nucleotide changes
- 3 isolates had three nucleotide changes
- Amino acid changes occurred at 7 loci in 6 isolates

Comparison of the 921-nt Sequence of WN-NY99 to WN Virus From Brain and Heart Tissues From Same Avian Host

RT-PCR Sequences That Differ From WN-NY99

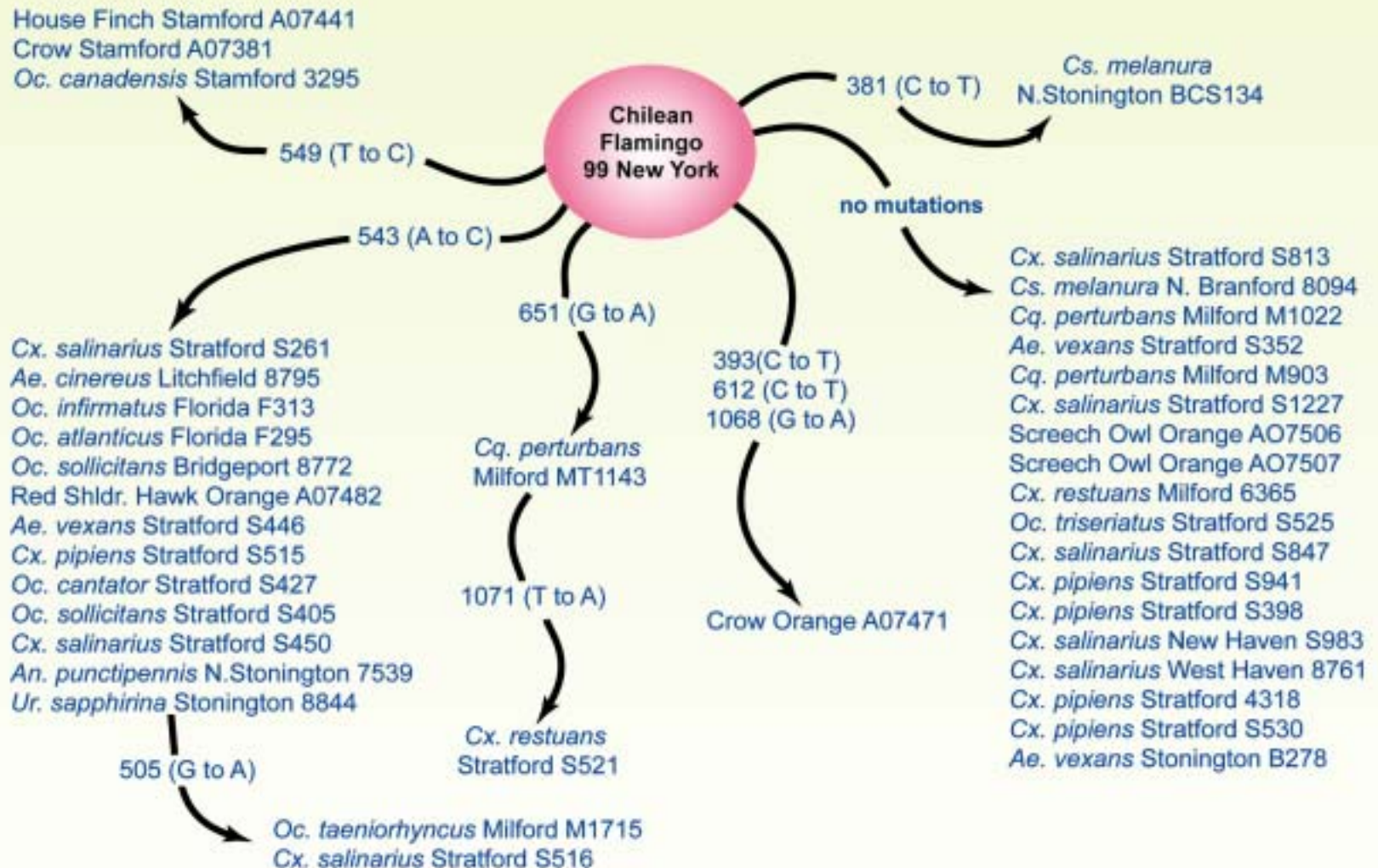
<u>Host</u>	<u>Brain</u>	<u>Heart</u>
Crow	456, C to T; 516, T to C	456, C to T; 516, T to C
Crow	588, G to A; 1000, T to C	588, G to A; 1000, T to C
Crow	None	None
Blue Jay	None	None
Crow	825, T to C	825, T to C
House Sparrow	858, C to T	858, C to T

Comparison of the 921-nt Sequence of WN Virus in Different Passages in Vero Cell Culture

RT-PCR Sequences That Differ From WN-NY99

Host	(Passage Number)	(Passage Number)
Crow	1032 T to C(1)	1032 T to C(3)
Crow	721 C to T; 905 G to A; 940 T to C(1)	721 C to T; 905 G to A; 940 T to C(3)
Crow	673 A to G(1)	673 A to G(3)
<i>Cx. pipiens</i>	933 C to T; 1047 C to T(0)	933 C to T; 1047 C to T (2)
Crow	528 T to C; 543 A to G(0)	528T to C; 543 A to G(3)
<i>Cx. restuans</i>	None (0)	None (2)
Crow	726 A to G; 858 C to T(0)	726 A to G; 858 C to T(3)

Mutational Changes in WN-NY99 Observed in Virus Isolates from CT and Florida in 2001



Experiment Station Staff Participating in This Study

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Michael Vasil

Participants in This Study From Other Agencies

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