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The Role of Long Distance Movement and Genetic Adaptation on the Evolution of West Nile virus in the New World

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The Role of Long Distance Movement and Genetic Adaptation on the Evolution of West Nile virus in the New World

by

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Dedication

For the woman I called mom, Loretta Freeman and the teacher that noticed, Randa Flinn.

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Finally, I must thank two individuals for their personal support and encouragement. I have cherished the continuous loyalty of my best friend and sister, Allison Freeman as well as John Heymann, who is my biggest champion and most tenacious advocate. Together they have provided an infinite source of empowerment and inspiration.

The Role of Long Distance Movement and Genetic Adaptation on the Evolution of West Nile virus in the New World

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West Nile virus (WNV) is an emerging arbovirus that is maintained in an epizooitic cycle involving birds and mosquitoes. After a long history of circulation in the Old World, WNV was introduced into the US and identified in the New York Metropolitan Area during the summer of 1999. While the initial outbreaks were restricted to the northeastern USA, the geographic range of WNV expanded rapidly south reaching Florida and the Caribbean by 2001, and west to California by 2003. The accelerated rate and distinct pattern of expansion suggested that migratory birds played a role in the dissemination of WNV throughout the New World. In this study, the general patterns of WNV circulation were defined in the Americas revealing correlation between the movement of WNV and the migration of terrestrial birds. To our knowledge, this is the first time phylogeographic methods have been used to correlate pathogen and terrestrial bird migration in the New World. The major sources of WNV migration events were also identified to determine the optimal locations for targeted surveillance efforts (New York, Illinois and Texas). To investigate the effectiveness of monitoring WNV evolution in these locations, recent isolates from Texas were analyzed using Next Generation Sequencing and phylogeny. The results of this analysis demonstrated that WNV sequences collected in Texas could be used to identify genetic selection occurring throughout the country. However, sequencing data could not be used to predict virulence as there was no relationship between intra-host variation and phenotype. Finally, the relationship between WNV circulating in North and South America was considered. The results of this analysis confirmed that WNV circulating in South America was introduced from North America prior to 2001. Together the results of this dissertation demonstrate the power of using sequencing and phylogeny to inform public health strategies.

Table of Contents

TABLES

FIGURES

CHAPTER 1 INTRODUCTION

1. Basic Virology of WNV

1.1 WNV TAXONOMY

West Nile virus (WNV) is an arthropod-borne (arbovirus) in the family *Flaviviridae*. The *Flaviviridae* family is a diverse group containing 82 viruses in four genera: *Flavivirus* (52 species), *Hepacivirus* (14 species), *Pegivirus* (11) and *Pestivirus* (4 species).[1] WNV belongs to the genus *Flavivirus* (Figure 1.1), along with several other clinically significant arboviruses, including yellow fever virus (YFV), dengue virus (DENV), Zika virus (ZIKV) and Japanese encephalitis virus (JEV). Due to antigenic similarity and serological cross-reactivity, WNV is described as a member of the Japanese encephalitis (JE) serocomplex, along with eight additional *Flaviviruses*, including Alfuy (ALFV); Koutango (KOUV); Kokobera (KOKV); Murray Valley encephalitis (MVEV); JE; Stratford (STRV); Usutu (USUV); and St. Louis encephalitis (SLEV) viruses.[2]

1.2 WNV GENOME STRUCTURE AND REPLICATION

The WNV genome is encoded by a single-stranded positive sense RNA molecule approximately 11 kb in length. The genome contains a single open reading frame (ORF), 5'-cap, and untranslated regions (UTRs) at the 5' and 3' ends. While there is no polyadenylation at the 3' end of the WNV genome; the 3' UTR is highly ordered and contains conserved stem-loop structures.[3,4] The genome is transcribed as a single polyprotein that is post- and co-translationally cleaved into three structural proteins [Capsid (C), preMembrane (prM), Envelope (E)] and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (Figure 1.2).

Figure 1.1 Phylogeny of the *Flavivirus* genus.
Adapted from Cook, S. 2006, the phylogenetic relationships of 72 members of the *Flavivirus* genus are represented as a maximum likelihood tree using the NS5 gene. Branch support for clades of interest are depicted as quartet puzzling support values.[5] Standard virus abbreviations are used.

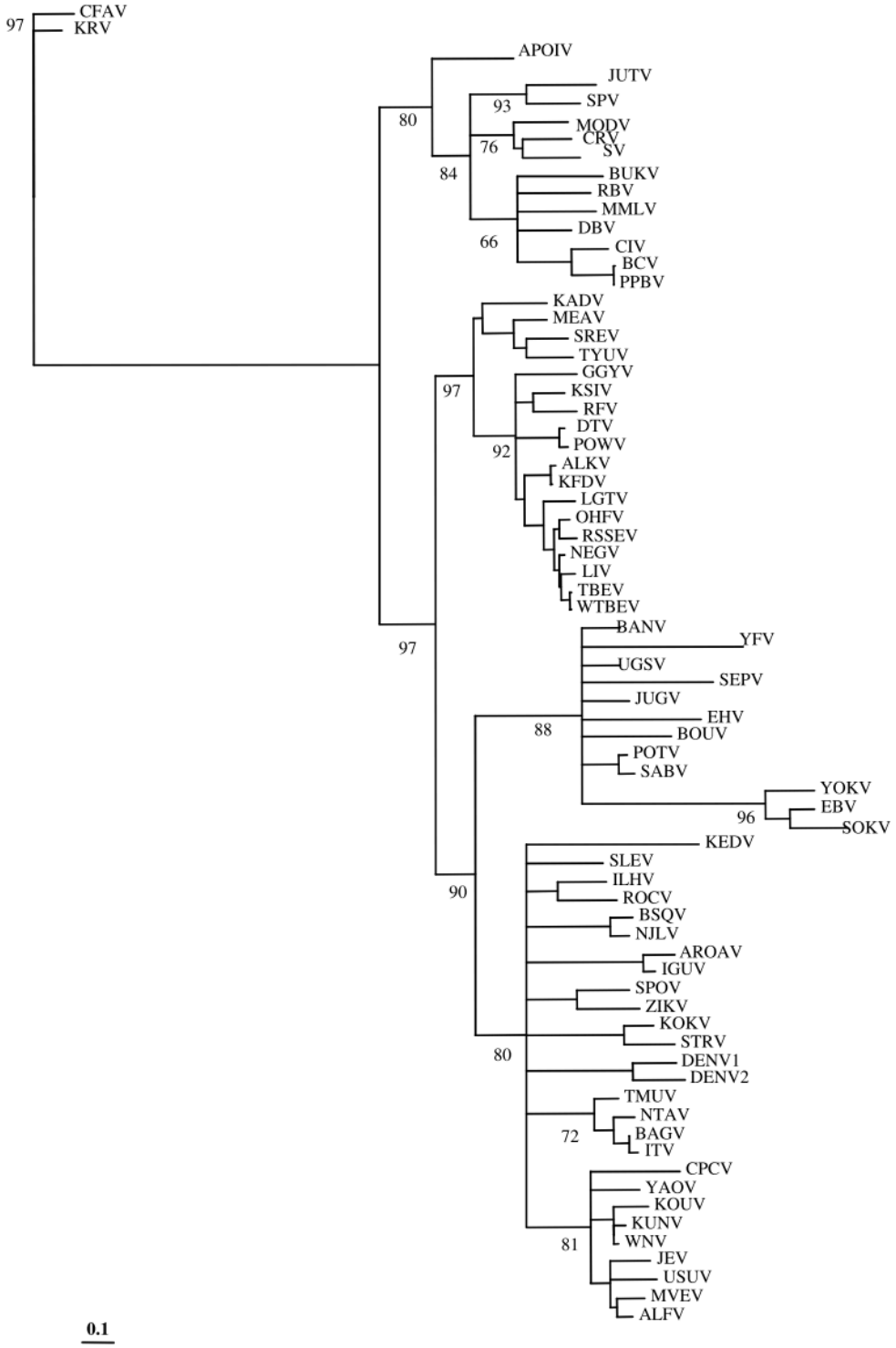
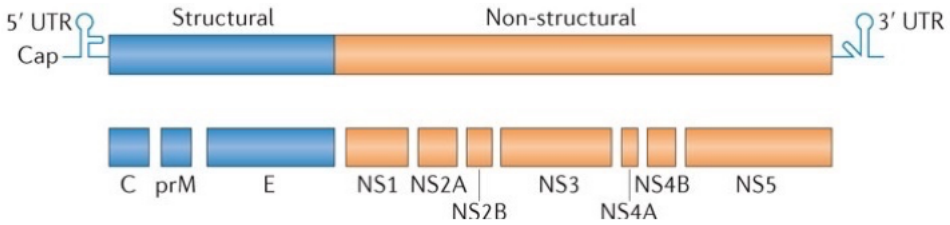


Figure 1.2 Diagram of the WNV genome.
A cartoon depiction of the WNV genome illustrating the 10 genes of WNV and the UTR regions. Structural genes are in blue and non-structural gene are in orange.



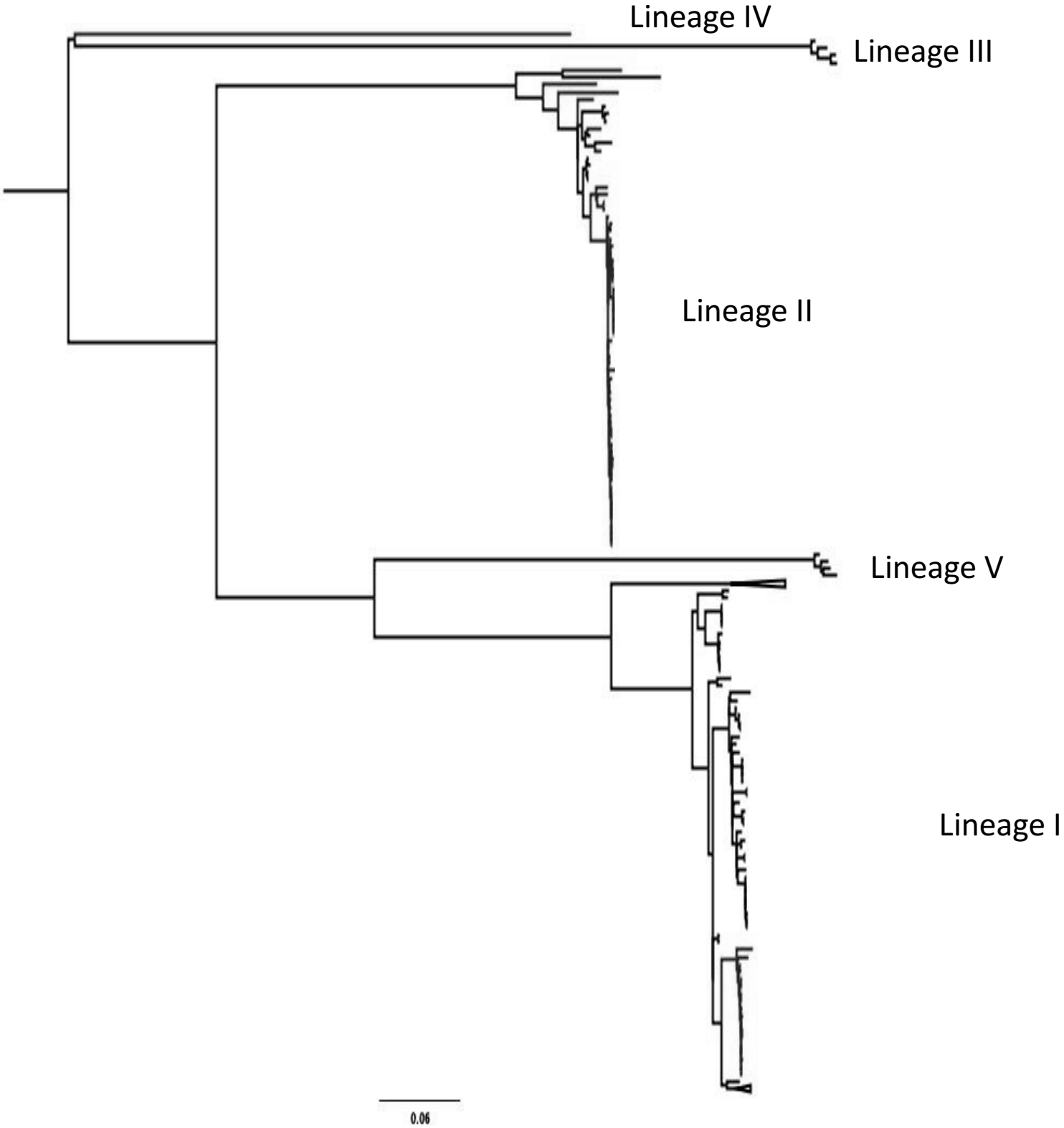
The structural proteins form the WNV virion, which is an enveloped icosahedral particle approximately 50nm in diameter.[6] Following entry into the host cell, translation of the viral genome is initiated by host machinery. Accumulation of viral nonstructural proteins allows replication of the viral RNA genome to commence. Replication of the WNV genome is driven by the RNA-dependent-RNA-polymerase (RdRp) encoded by the NS5 gene, which forms the replication complex with the other NS proteins.

The error-prone nature of RdRps ensure high mutation rates that enable rapid evolution within and between hosts.[7,8] Consequently, RNA viruses are transmitted as a population of genetically related virions that differ in nucleotide sequence, referred to as a quasispecies, mutant swarm, or population.[9] While the error-prone replication of WNV encourages high genetic diversity, arboviruses, such as WNV, evolve slower than single-host viruses, likely due to the complex nature of replication in a dual host (arthropod and vertebrate) system.[10] Evidence suggests that the diversity of WNV quasispecies increases during replication in mosquitoes,[11,12] likely due to diversifying selection pressure exerted by RNAi,[13] and contracts during replication in birds due to the effects of strong selection purifying selective pressure.[11,14] Together these factors drive WNV evolution in the environment.

1.3 Classification of WNV

At least five major phylogenetic Lineages have been described for WNV (Figure 1.3).[15] However, at this time, there are no clear guidelines for the designation of viral Lineages, and thus the classification of WNV lineages, in some cases can appear inconsistent or even arbitrary. In fact, as many as nine Lineages have been proposed based on genetic diversity and

Figure 1.3 The five Lineages of WNV.
A maximum likelihood phylogeny was generated using the full ORF of 1705 WNV sequences. Sequences collected in the USA are collapsed to assist with visualization. Lineages are indicated.



phylogenetic clustering, depending on the amount of genetic variation that is used to define a Lineage.[15]

WNV-Lineage I is made up of two clades (IA-IB). Lineage IA has the broadest geographic distribution and has been identified in Africa, Europe, the Middle East, Russia, and most recently in the Americas. It is also associated with elevated virulence in birds and humans.[16,17] Lineage IB, also known as Kunjin virus, is made up of WNV isolates from Australia and Papua New Guinea.[18]

Historically, WNV-Lineage II has largely been restricted to Africa (Madagascar, Central Africa and Southern Africa).[19] However, at least three introductions of WNV-Lineage II into Europe and Russia have resulted in significant outbreaks of human disease.[20–22] The first introduction occurred during the 19th century/early 20th century and affected the Mediterranean region resulting in virus isolation in Cyprus during 1968, and more recently central Europe and southern Russia.[22]

The second introduction of Lineage II from Africa occurred between 1914 and 1951 and was detected in Southern Russia in 2007.[23] Outbreaks stemming from the Russian introduction have since spread into Romania[24] and Italy.[25] In 2010, Lineage II spread into the Balkan states and northern Greece, resulting in large outbreaks of human disease.[26]

The final introduction of WNV-Lineage II isolates into Europe occurred between 1936 and 1981 and was detected in Hungary during an outbreak of fatal disease in birds.[21,22] Investigators suspected that these isolates were introduced by migratory birds traveling from Central Africa.[21] Between 2005 and 2007, moderate geographic spread and sporadic cases were reported among birds, sheep, horses and humans in Hungary. However, in 2008 and 2009, the

geographic range of Lineage II spread rapidly south and west throughout Hungary and Austria.[27,28]

The European and Russian introductions of Lineage II isolates from Africa continue to cause outbreaks of human disease today. While Lineage II isolates were previously thought to cause less severe disease,[29] recent European Lineage II isolates have a similar virulence phenotype to the Lineage 1A isolates of the Americas.[30]

Lineage III (also known as Rabensburg virus)[31] and Lineage IV[32–34] isolates have only been observed in mosquitoes in the in the Czech Republic and Russia, respectively. Lineage V was previously designated Lineage 1C, but it has since been designated separately because it differs from other WNV Lineages by 20-25% at the nucleotide level.[35] Lineage V contains isolates from India collected from humans and mosquitoes between 1950 and 1980.[35]

Further subdivision of Lineage IV has been proposed, including an isolate from Spain sometimes termed Lineage VI [33] and an isolate from Austria sometimes termed Lineage IX.[32] At this time, Koutango virus remains an independent virus species, but it may be reclassified as WNV Lineage VII due to its genetically similarity to WNV.[36] Finally, putative Lineage VIII contains a virus restricted to *Cx. neavei* mosquitoes that was isolated in Kedougou, southeastern Senegal, in 1992.[37]

1.4 History of West Nile Virus

1.4.1 Discovery and Early Characterization (1930s-1950s)

WNV was first isolated from in the serum of a woman (age 37) in Omogo, West Nile District, Northern Province of Uganda in December of 1937, during a routine screening for YFV.[38] She presented with a mild fever, but did not report feeling ill. Following isolation,

experimental infection of mice and Rhesus monkeys revealed a neurovirulent and neuroinvasive phenotype, suggesting that WNV was a potentially neurotropic virus.

Following the initial discovery, little was known about the clinical disease associated with WNV or the mechanisms of transmission until the 1950s. At the start of that decade, WNV was isolated from the serum of three children living north of Cairo, Egypt.[39] The study also found that more than 70% of the inhabitants in the area were seropositive for WNV. Similar to the Ugandan isolate, WNV isolates from Cairo displayed a neurovirulent phenotype in monkeys. Subsequent studies demonstrated that WNV was endemic throughout Egypt (61% seropositivity rate) and Sudan (40% seropositivity rate) with peak circulation during mid-summer.[40] The study also suggested that WNV infection in humans resulted in a self-limiting, non-fatal, childhood disease with rare encephalitic complications.

During the same time period, WNV also was isolated from *Culex* (*Cx.)* mosquitoes (*Cx. univittatus* and *Cx. antennatus*) and from passerine birds [two pigeons (*Columba livia)*, and one hooded crow (*Corvus corone sardonius*)] in the Nile Delta region of Egypt.[40–42] Neutralizing antibodies were detected in 65% of crows and 42% of sparrows.[40] This was the first indication that birds and mosquitoes were the primary reservoirs and vectors for WNV in nature. Later, the transmission cycle of WNV was confirmed experimentally by allowing naïve and WNV-infected mosquitoes *(Cx. pipiens* and *Cx. univittatus)* to feed on hooded crows, house sparrows, and buff-backed herons.[41] At the time, investigators suggested that non-migratory birds were involved in the transmission of WNV because virus circulation appeared to be restricted to the Nile Delta region.[40]

1.4.2 First Outbreaks of WNV (1950s-1980s)

The first outbreak[a] of WNV-associated disease was reported in Maayan Zvi, Israel, affecting hundreds of people between 1951 and 1952.[43] Maayan Zvi was an agricultural settlement located in the coastal plain region, 30 km south of Haifa. During the outbreak, 41% (123) of the 303 inhabitants developed a self-limited and non-fatal fibrile disease.

During the summer of 1957, additional outbreaks of WNV disease occurred in Israel in the Shomron area affecting soldiers in an army camp (297 cases), residents in and around the town of Hadera (65 cases), and elderly residents living in the Malben homes for the aged in Ein Shemer and Pardes Hanna (49 cases).[44] Among the infected soldiers and Hadera residents, one child and two adults (0.8%) developed meningoencephalitis. In addition, 12 patients (24.5%) from the Malben homes developed severe disease complicated by meningoencephalitis. This was the first report of severe neurologic disease occurring in humans infected with WNV and the first indication that the incidence of WNV disease was elevated in elderly patients.

In 1962, the first outbreak of WNV disease in Europe was reported. Eighty horses with a neurological disease were identified in the Camargue region of France.[45] Fatal outcomes were observed in 25 to 30% of the horses.[46] Several human cases of encephalitis were reported at that time. Additional humans and equine cases were reported in the Camargue region during 1964 and 1965, respectively.[47]

Large outbreaks were also reported in Africa. In 1974, an outbreak of WNV occurred in South Africa following heavy rains.[48] Post-epidemic antibody surveys indicated that approximately 55% of the population had been infected with WNV. Serological evidence also suggested that WNV may have been responsible for regular outbreaks of encephalitic illness occurring in the Kolar district of Karnataka State, India in 1977, 1979 and 1981.[49] However, co-

[a] The term outbreak refers to the sudden and unexpected occurrence of disease that significantly exceeds the expected number of cases or disease severity.

circulation with JEV prevented identification of the specific etiological agent during those outbreaks. However, in 1980 and 1981, WNV was isolated post-mortem from three children presenting with encephalitis. Patients included a 14-year old boy from the Budithitoo village in Mysore district, a six-year old boy from the Malur *taluk* in Kolar district, and a four-year old girl from the Kolar *taluk* in Kolar district.

1.4.3 Association of WNV with Large Outbreaks (1990s)

Outbreaks of WNV were also reported in Northern Africa in the early to mid-1990s. In 1994, an outbreak of WNV infection with neurological complications in humans was reported in the Timimoun region of Algeria.[50] Fifty cases (20 confirmed by case definition) of encephalitis and eight deaths were reported. Two years later, in 1996 an equine outbreak of WNV disease was reported in the Atlantic coastal plains of northwest Morocco.[51] The outbreak resulted in paralysis of 94 horses and 42 deaths.

During the same year, WNV was identified as the causative agent of a major outbreak of neuroinvasive disease in humans in Romania.[52] More than 800 patients were hospitalized with suspected infection of the central nervous system between July15 and October, 1996. Laboratory confirmation of WNV infection was obtained for 393 cases. In previous outbreaks, the incidence of neurological or fatal disease was rare; however, the case fatality rate of the Romanian 1996 outbreak was 4.3%. The outbreak spread across 15 districts in southeastern Romania, with the area surrounding the Danube River the most significantly affected. Environmental surveillance indicated that *Cx. pipiens* mosquitoes were most likely the dominant vector involved. Interestingly, the Carpathian Mountains appeared to provide a barrier against the transmission of WNV further north, and it was hypothesized that WNV was introduced into Romania by migratory birds from Africa.

During the following year, between September 7 and December 12, 1997, WNV outbreaks were reported in the Sfax and Mahdia districts of Tunisia.[47] One hundred and seventy-three patients developed meningitis or meningoencephalitis disease. All but eight recovered. An additional outbreak of WNV infection among horses was reported in Italy during 1998 (August to October). Subsequently, large outbreaks of WNV infection were reported in southern Russia in 1999 with at least 1,000 cases and 40 deaths in the Volgograd, Astrakhan, and Krasnodar regions of southern Russia.[53]

1.5 WNV IN THE NEW WORLD

1.5.1 Introduction of WNV into the New World

While historically restricted to the Old World, WNV was identified as the causative agent during an outbreak in the New York City (NYC) Metropolitan area during the summer of 1999 that resulted in fatal disease among humans, birds and horses.[54–56] The outbreak was first reported to the New York City Department of Health (NYCDOH) on August 23, 1999 by an infectious disease physician at the Flushing Hospital in Queens, NYC.[54] She reported two human cases of encephalitis.[57] Further investigation revealed a total of eight patients admitted to Flushing Hospital between August 12 and September 2.[17] The patients were later found to live within a two-mile radius.[54] By the end of 1999, the NYC outbreak resulted in 62 confirmed human cases; however, door-to-door serosurveillance studies showed that the incidence of WNV in Queens, NYC was much higher than expected (2.6%).[55,58]

Human sera and CSF fluid of the eight patients were evaluated by the CDC's Division of Vector-Borne Diseases for evidence of exposure to known North American arboviruses. As WNV was considered an "Old World" virus, it was not included in the analysis. On September 3, 1999, the sera and CSF samples tested positive by IgM capture ELISA for St. Louis encephalitis

virus (SLEV), resulting in the initial misdiagnosis of the outbreak as SLEV.[57] This result was not surprising as SLEV and WNV are antigenically similar and both are members of the JE serogroup. In response, the NYCDOH initiated vector reduction campaigns to limit mosquito populations in northern Queens and southern Bronx utilizing both mosquito larvicides and mosquito adulticides.[57]

Increased fatality was also reported among birds in the same geographic area, especially among American crows (*Corvus brachyrhynchos*) and fish crows (*Corvus ossifragus*).[59] At the Bronx Zoo/Wildlife Conservation Park, 24 exotic birds died between August 10, 1999 and September 23, 1999.[56] On September 10, 1999, tissue samples from the exotic birds, including one from a Chilean flamingo (*Phoenicopterus chilensis*), were submitted to the National Veterinary Services Laboratories, U.S. Department of Agriculture for virus isolation.[57,59] All samples tested negative for common avian pathogens and equine encephalitic viruses.[57]

On September 20, the viral isolates were sent to the CDC for identification by RT-PCR and sequencing. and on September 23, the viral isolates were shown to be related to Kunjin, a subtype of WNV.[57,59] This result was confirmed by immunofluorescence antibody testing with monoclonal antibodies (mABs) specific for WNV and related flaviviruses.[59] The complete genome sequence (Genbank Accession AF196835) was determined for the Chilean flamingo sample and designated WN-NY99.[59] Phylogenetic analysis of the E gene showed the WN-NY99 was most closely related to an isolated collected from the brain of a dead goose in Israel during 1998 (Genbank Accession AF205882).[59] To this day, the NY99 sequence has remained the prototypical sequence for WNV in the New World.

In the following years, the virus spread north into Ontario, Canada and south along the east coast reaching Florida and the Caribbean by 2001. WNV then spread west to the Rocky

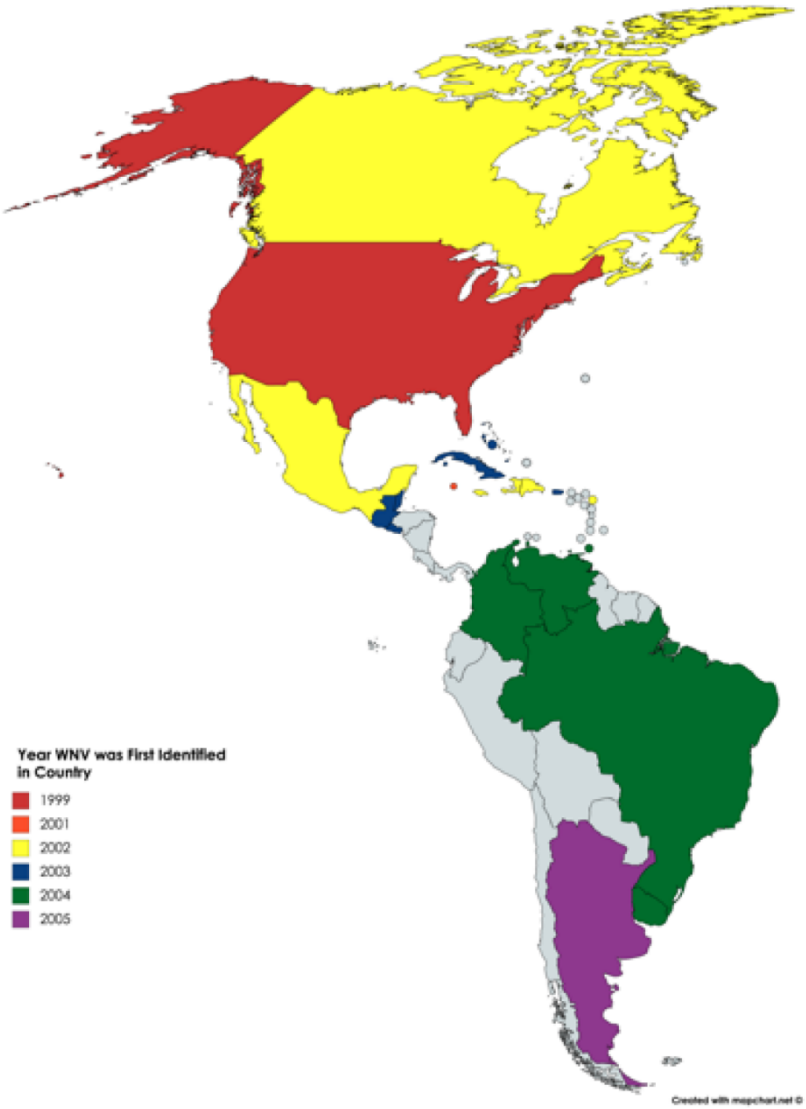
Mountains by 2002,[60] and into Jamaica,[61] Mexico, [62] Hispaniola[63] and Guadeloupe[64] (Figure 1.4). It is important to note that a single human case of WNV was identified in California (CA) in 2002, but WNV was not detected by bird or mosquito surveillance efforts until 2003.[65] WNV also was detected in Belize, Guatemala, Cuba, Puerto Rico and the Bahamas the same year. During that time, the geographic range of WNV continued to expand throughout North, Central and South America reaching Trinidad[66] and mainland South America (Colombia[67] and Venezuela[68]) in 2004 and as far south as Argentina by 2005[69] and Uruguay by 2007.[70]

1.5.2 WNV Epidemiology

A household-based seroepidemiological survey was used to estimate that approximately 70-80% of WNV cases are asymptomatic and resolve without intervention.[58] Twenty percent of infections result in mild to severe febrile illness (WNF). The clinical WNF symptoms, if present, generally arise 2-14 days following infection and include fever, headache, fatigue, myalgia and gastrointestinal complaints.[58,71] In addition, approximately 1% of infected humans develop neuroinvasive disease (WNND),[55] presenting as meningitis, encephalitis, or poliomyelitis-like acute flaccid paralysis.[72] WNF can also be associated with rash on the torso and extremities, and is more common among younger patients and patients with WNF than with WNND.[73] Evidence of persistent infection in the kidney has also been detected by WNV-positive RT-PCR results from urine.[74]

Severe outcomes with WNV infection are more common in elderly patients. Between 15-29% of cases in patients above 70 years of age are fatal and 50% of elderly patients develop significant disease with symptoms persisting for up to a year.[58,75] Furthermore, elderly patients have an increased risk of death for three years following infection.[76] Mortality is also elevated in

Figure 1.4 Map of WNV in the New World.
Locations where investigators and public health officials have reported evidence (virus isolation, RT-PCR, serology) of WNV circulation. Countries are color coded to indicate the year in which WNV was first detected.



infants and immunocompromised patients. Although rare, human-to-human transmission has also been documented in specialized circumstances, including blood transfusion,[77,78] tissue and organ transplant,[79] breast feeding[80] and intrauterine exposure.[81]

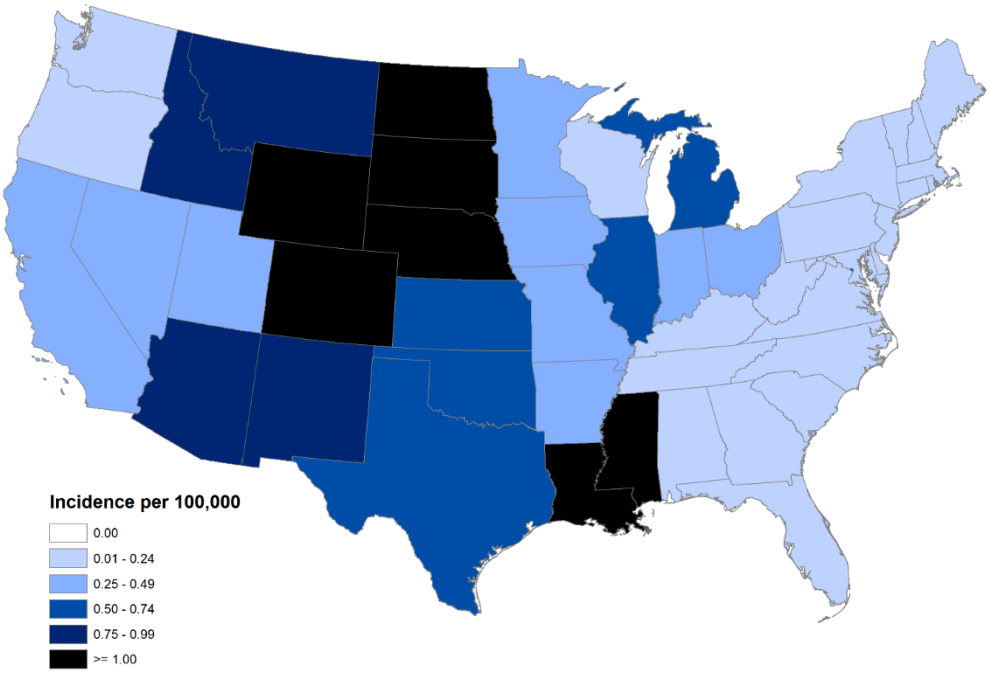
Since the introduction of WNV into the New World, significant morbidity and mortality was been reported, especially in the Central USA (Figure 1.5). Large outbreaks were reported in 2002 and 2003, with 2,946 and 2,866 cases of human WNND, respectively (Figure 1.6).[82] In the years that followed, the number of WNND cases reported to the CDC decreased, and between 2008 and 2011, less than 1,000 WNND cases reported were each year. Then, in 2012, another large outbreak of WNV occurred in the USA with 2,873 cases of WNND.[60] Approximately a third of cases were reported in Texas.[83]

As of October 9, 2017, there have been a total of 22,146 cases of WNND and 2061 WNV-related deaths reported to the CDC since WNV emerged in the USA in 1999.[60] However, current estimates suggest that over 3 million individuals in the US have been infected with WNV between 1999 and 2010.[84] Furthermore, it is estimated that WNV-related hospitalization costs were $673 million–$1.01 billion between 1999 and 2012.[85]

In comparison to the USA, outbreaks of WNV in Latin America and the Caribbean have been reported less frequently.[66] Despite ample serological evidence of WNV circulating in South America, collection of WNV isolates has been extremely limited. In fact to date, there have only been four full genome sequences published from WNV isolates collected in South America.[86,87]

1.5.3 Evolution of WNV in the USA

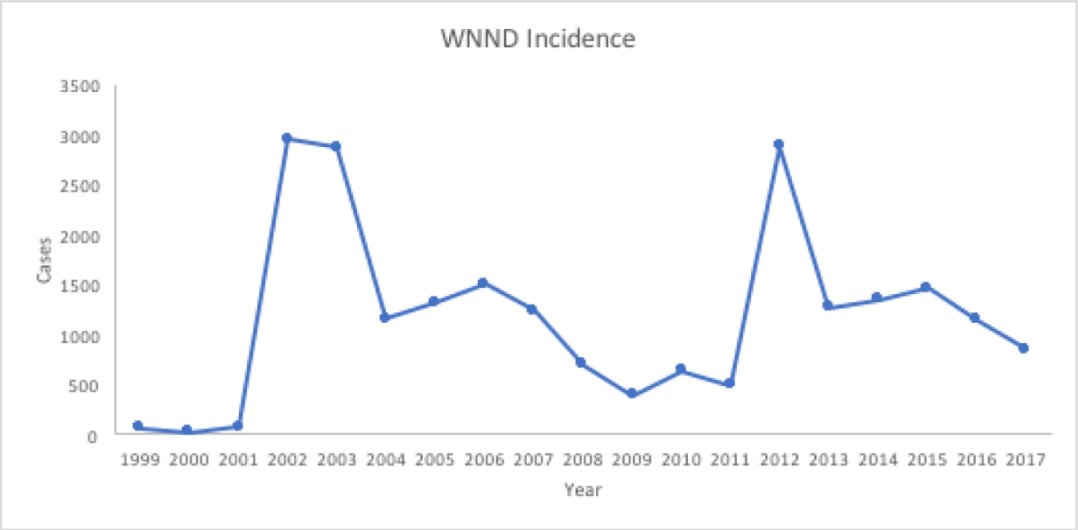
Figure 1.5 Map of the cumulative annual WNND incidence in the USA between 1999 and 2015. Map was made available by the CDC.[82]



Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention

Figure 1.6 WNND incidence in the USA over time.

There is mandatory reporting for all WNND cases in the USA. The number of WNND cases reported to the CDC are shown by year.[60]



In the USA, WNV has undergone significant genetic adaptation. The initial WNV genotype[b] was termed New York 1999, or NY99, due to being first isolated in New York in 1999. This genotype was rapidly displaced by the North America or West Nile 2002 (NA/WN02) genotype which arose in 2002.[88,89] The NA/WN02 genotype had 13 nucleotide differences, including a single amino acid substitution in the envelope protein at residue 159 where a valine was replaced by an alanine (E-V159A), from the NY99 genotype. However, there is evidence that several of the silent nucleotide changes in the NA/WN02 genotype have reverted to the NY99 sequence over time.[90] Despite being a conservative substitution, the E-V159A substitution has been associated with reduced extrinsic incubation period (up to four days) in *Cx. pipiens* mosquitoes suggesting that the NA/WN02 genotype can be transmitted faster than the NY99 genotype.[88,91]

Simultaneously, an additional geographically restricted genotype named the Southeast Coastal Texas (SECT) genotype arose in the Gulf Coast region of TX, but it is presumed to have undergone rapid extinction as it was only observed in 2002. [92,93] The SECT genotype was characterized by five amino acid substitutions: E-T76A, NS1-E94G, NS2A-V138I, NS4B-V173I and NS5-T526I. The southwest genotype (SW/WN03) arose from within the NA/WN02 genotype in 2003 and was first identified in the southwestern US in Arizona and New Mexico. It is defined by an amino acid substitutions at NS4A-A85T and often observed with an additional substitution at NS5-K314R.[94] The functions of these substitutions are not known. Finally, the MW/WN06 genotype was identified from within the SW/WN03 genotype.[90] The MW/WN06

[b] A genotype is a group of sequences that cluster together phylogenetically and share one or more conserved substitutions. In some cases, published literature has used the term genotype to describe any sequences that share a common substitution, even if they do not cluster together.[110] For simplicity sake, this dissertation will only use the term genotype to describe sequences that cluster together and share substitutions.

genotype was made up of eight human and birds isolates from North Dakota and Idaho collected between 2006 and 2007. In the US, both the NA/WN02 and SW/WN03 genotypes still remain in circulation.[90]

1.5.3.1 Regional Evolution of WNV in Texas

A) Initial Introduction

In 1964, there was a large outbreak of SLE in Harris county, TX (which includes the metropolitan Houston area) that affected 243 patients and resulted in 27 deaths.[95] Following secondary outbreaks between 1975 and 1976 (58 cases and 11 deaths),[95] the Harris County Mosquito Control Division developed a SLEV surveillance program that routinely surveyed birds and mosquitoes for evidence of SLEV infection. In anticipation of the spread of WNV, the Harris County SLEV surveillance program was expanded to include screening for WNV. In 2001, the Avian Mortality Surveillance system was developed in partnership with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch.[96]

During 2002, WNV and SLEV were both detected in Harris County.[97] Co-circulation of the two viruses resulted in 113 viral encephalitis cases in humans. WNV was confirmed in 93% of the cases. The first human case was identified on July 23, 2002, while the first dead bird samples and positive mosquito pools on June 10 and 11, respectively.

Blue Jays were the most significantly affected bird species; 95.1% (281) of 307 Vero cell cultures inoculated with dead bird brain were positive for WNV (immunofluorescent antibody technique, [IFAT]).[97] In contrast, only 4.2% (13) of American crows and 1.3% (4) of house sparrows were IFAT-positive. Survey of wild live birds revealed that 31.2% of birds and 32.6% of house sparrows were seropositive (by hemagglutination-inhibition [HI] test). While *Cx.*

pipiens and *Cx. resturans* were important for WNV transmission in the northeastern USA and IL, *Cx. quinquefasciatus* mosquitoes appeared to be the dominant vector involved in WNV transmission in Harris County.

Phylogenetic analysis of a 2,004nt fragment in the prM-E region demonstrated that WNV strains circulating in TX during 2002 belonged in two distinct genotypes, the NA/WN02 genotype and the SECT genotype.[92,98] This indicated there were at least two independent introductions of WNV into the TX. While widespread circulation of the NA/WN02 genotype was observed, the geographic range of the SECT was restricted to a few counties in Eastern TX bordering Louisiana. The presence of both the widespread NA/WN02 genotype and the geographically limited SECT genotype suggested that WNV transmission in TX was governed by the movements of both migratory birds and residential (non-migratory birds). The wide distribution of the NA/WN02 genotype could have been driven by the long distance movements of avian migration, while the restricted SECT genotype was probably maintained by transmission among residential birds.[92]

B) Maintenance and Continued Circulation of WNV

In 2003, seven WNV isolates collected in TX (Bird 1153, Bird 1171, Bird 1175, Bird 2529, Bird 1181, Mosquito v4369 and Mosquito v4380) displayed small or mixed plaque size and a temperature sensitive phenotype.[99] These isolates were also attenuated in mice for neuroinvasiveness, but not neurovirulence. While naturally attenuated WNV isolates were previously reported in Australia (Kunjin), Central Africa Republic, Cyprus, Egypt, Ethiopia, India, Madagascar and Senegal,[29] this was the first documented evidence of naturally attenuated WNV isolates circulating in the USA.[99] Isolates with these attenuated phenotypes were observed in 2003 only.

Phylogenetic analysis of partial sequences (prM-E) of WNV strains collected in Harris County between 2002 and 2006 revealed large unresolved polytomies with limited evidence of substitutions shared between WNV isolates collected over consecutive years.[100] Apart from the nucleotide substitutions associated with the NA/WN02 genotype, only one noncoding substitution was observed in isolates collected during consecutive years in Harris County, E-C2469U. The E-C2469U substitution arose in WNV isolates collected during June and July of 2005 and was also present in WNV isolates collected in 2006.[100] The limited fixation of nucleotide substitutions is consistent with strong negative selection or the lack of positive selection. While two studies noted limited evidence of positive selection in Harris County,[98,100] it is important to note that these studies relied on a 2004nt fragment in the prM and E region. Later studies utilizing the full genome sequences of WNV isolates (1999-2009) were able to identify significantly more evidence of positive selection, including the selection of the SW/WN03 genotype.[94]

C) Outbreak of 2012

In 2012, following 3-4 years of declining WNV activity, large outbreaks of WNV were reported across the USA. The outbreak prompted additional phylogenetic studies to evaluate the genetic composition of WNV isolates involved. Analysis of 42 WNV sequences collected in Harris County, TX between 1999 and 2012 revealed that the 2012 isolates clustered in four independent groups among 2003-2006 isolates, rather than with isolates collected more recently (2007-2010).[101] Interestingly, while the NA/WN02 and SW/WN03 genotypes co-circulated in TX until 2011, the isolates from 2012 were all members of the NA/WN02 genotype. There was also evidence that the 2012 isolates were phylogenetically related to WNV strains that had circulated in the northeastern USA between 2006 and 2009. Together these results suggest that

the activity of WNV in Harris County during 2012 was the result of multiple introductions from outside of TX.

Interestingly, the 2012 outbreak in Dallas County, TX was larger much than the outbreaks in Harris County or the neighboring Montgomery County, TX. To determine if genotypic differences were responsible for the differing incidence, 17 WNV isolates collected in Dallas and Montgomery County were compared and evaluated phylogenetically.[83] In this case, TX 2012 sequences from the two counties clustered together into two clades with isolates collected in NY during 2009 and CT during 2008. As seen in Harris County, WNV strains associated with the Dallas outbreak of 2012 appeared to originate in the northeastern USA. There was no evidence of genetic differences between WNV strains circulating in Dallas and Montgomery Counties, suggesting that the size of the outbreak may have been due to unidentified ecological factors. One possible explanation is that temperatures differences may have contributed to the larger outbreak in Dallas County. Indeed, during the outbreak season, Dallas County was 1-6˚F warmer than Harris County, and increased temperature is associated with a decreased extrinsic incubation time for WNV in mosquitoes.[102,103] Despite the continued circulation of WNV in TX, the ongoing patterns of local WNV evolution have not been considered in TX since 2012. That gap was addressed in Chapter 5 of this dissertation.

1.5.3.2 Additional Considerations of Regional Adaptation

The evolution of WNV has also been considered in locations outside of TX. In Illinois (IL) the evolution of WNV has been monitored from 2002 to 2007 using a combination of phylogenetic and population genetic approaches.[104–106] These studies found little evidence of geographic substructure, which is consistent with the frequent influx of new WNV isolates from outside IL. But, some examples of trans-seasonal maintenance were observed indicating that

WNV in IL arose due to both local evolution and novel introductions.[105] Similar studies in Connecticut (CT) showed limited evidence of geographic structure in WNV phylogenies, indicating again that new strains of WNV were introduced within and between years. Again, evidence of local maintenance of WNV from year-to-year was also observed. Taken together with studies of WNV evolution in TX,[92,98] it appears that WNV populations in CT and IL were maintained locally by overwintering with frequent mixing with nonlocal populations and occasional instances of region-specific adaptation.

Broader studies investigating the geographic substructure of WNV across the USA have reported similar results.[90,98,107–109] A notable exception is observed with WNV circulating in CA, where significant clustering of viral sequences is observed. [90,107–110] This pattern is consistent with few introductions of WNV into or out of CA,[110] suggesting WNV in CA is evolving in isolation, compared to WNV isolates circulating in the remaining regions of the USA, possibly due to the Rocky Mountains as a physical barrier.

1.5.4 Ecology of WNV in the USA

1.5.4.1 WNV REPLICATION IN INSECTS

WNV is maintained in an enzootic cycle involving birds and mosquitoes. In the USA, Culex (Cx.) species, including *Cx. quinquefasciatus*, *Cx. tarsalis, Cx. pipens, Cx. nigripalpus, Cx. stigmatosoma and Cx. erythrothorax* and are the most effective WNV vectors.[111–113] *Cx. pipiens* and *Cx. tarsalis* can transmit as much as $10^{6.1}$ and $10^{5.0}$ PFU, respectively, to a host during a blood meal.[114] *Aedes, Ochlerotatus* and *Culiseta* mosquito species are less susceptible to WNV infection and are only weak to moderate WNV vectors.[111,113] Additional factors such as feeding time, flight range, population density, location of breeding sites and host preference vary among mosquito species and contribute to their vector competence.

Mosquitos become infected with WNV while taking a blood meal from an infected host. Following a blood meal, the mosquito midgut epithelium becomes the primary site of virus replication.[115] Following replication in the midgut, WNV is transported through the hemolymph to the salivary glands. Viral replication in the salivary glands results in accumulation of WNV in the saliva and facilitates transmission to naïve vertebrates. Depending on the species a single mosquito bite can transmit between $10^{1.2}$ to $10^{4.3}$ PFU.[116–118] However, mosquitoes often probe the host, biting multiple times to locate a dermal blood vessel in the skin to feed. The repetitive nature of probing can result in a single mosquito administering as much as $10^{3.4}$ and $10^{6.1}$ PFU during a single blood meal.[114]

1.5.4.2 WNV REPLICATION IN BIRD SPECIES

Interestingly, WNV was not associated with fatal disease in birds until September 1998 when an outbreak of fatal WNV disease was reported among geese in Eilat, Israel.[119] An increase in virulence among corvid birds (order: *Passeriformes*) was detected in association with a single amino acid substitution (NS3-249) that is conserved among WNV isolates in Lineage IA, including the New World strains.[16] Following the introduction of the bird-virulent Lineage IA strains into the USA, many American bird populations were significantly impacted. For instance, American crow (*Corvus brachyrhynchos*) populations experienced large-scale declines of 45%.[120]

To date, more than 300 species of birds have been identified with evidence of WNV infection in the USA.[121] However, disease manifestations, including pathology, mortality rate, viremia level and duration of viremia, vary greatly among species.[122] Of these birds, species within the order Passeriformes are the most susceptible to WNV infection and have the highest reservoir competence, especially Blue Jays (*Cyanocitta cristata*), Common Grackles (*Quiscalus*

quiscula), House Finch (*Carpodacus mexicanus*), American Crow (*Corvus brachyrhynchos*, and House Sparrow (*Passer domesticus*).[123] These species develop WNV viremias above 10 $\log_{10}$PFU/ml. In contrast, other species, such as the Monk Parakeets, Japanese Quails and Ring-necked Pheasants (*Phasianus colchicus*), never develop viremias above 3 $\log_{10}$PFU/mL.[123]

1.5.4.3 Nontraditional Transmission of WNV among animals

While the dominant route of WNV transmission between birds occurs through the bite of an infected mosquito, several additional routes of infection have been observed. For instance, birds with high viremia shed significant amounts of WNV in oral and cloacal secretions, which may facilitate virus transmission between birds.[123,124] This may contribute to the transmission among infected and naive birds during co-housing experiment.[123] In addition, the consumption of WNV-infected prey has also led to transmission to raptors.[123–125]

Spill-over into susceptible non-avian animal populations have also been observed, most notably with humans and horses. While these spill-over events can result in fatal disease, infection of non-avian vertebrate species generally leads to dead-end infections because the viremia in these species is too low to allow transmission to feeding mosquitoes. Viremia of at least 10^5 PFU/ml is required for a naïve Culex pipiens mosquito to develop infection.[111,126] Some examples of species that develop dead-end infection are horses[45] pigs,[127] big brown rats (*Eptesicus fuscus*), and Mexican free-tailed bats (*Tadarida brasiliensis*),[128] green iguanas (*Iguana iguana*), red-ear sliders (*Trachymes scripta elegans*), garter snakes (*Thamnophis sirtalis sirtalis*), bull frogs (*Rana catesbeiana)*,[129]and companion animals, such as dogs and cats,[130] However, dogs receiving glucocorticoids (an anti-inflammatory medication) develop elevated viremia, which is consistent with higher susceptibility seen in immunosuppressed humans.[131]

Although rare, there are also several instances of transmission between non-avian species as been observed in a laboratory setting. Interestingly, despite low viremia, WNV transmission has been observed between mosquitoes and cottontail rabbits (*Sylvilagus floridanus*).[132] In addition, American alligators (*Alligator mississippiensis*) can develop prolonged (14 dpi) high titer (peak 10^5-10^6 PFU/ml) viremia following experimental infections using multiple routes of infection, including needle injection, consumption of WNV-infected mice and contact with other infected alligators.[133] This evidence suggests that American alligators may be an important reservoir for WNV in nature.

1.5.4.4 The Potential ***Role of Birds in WNV Migration***

Due to the highly mobile nature of birds, it is likely that avian behavior has significantly contributed to the movement of WNV throughout the world. Particularly in the USA, the rapid geographic expansion of WNV has been noted by many investigators.[134–139] In fact, WNV spread at a rate of ~1000km/year from 1999 until 2004, which is much faster than could be expected by simple contiguous diffusion.[135,136,139,140] Furthermore, the pattern of diffusion has been characterized as heterogeneous, suggesting that perhaps the contiguous diffusion of WNV was punctuated by long-distance translocation events. This lead some investigators to propose that the rapid expansion of WNV was driven by the annual movements of WNV-infected migratory birds.[134–139]

In the Americas, it is estimated that 5 billion birds from 338 species participate annually in long-distance migration.[141,142] Traditionally, it was thought that migratory birds in the Americas travel along four major flyways: the Atlantic, the Mississippi, the Central and the Pacific. These flyways are defined by the Appalachian Mountains (that separate the Atlantic and

Mississippi Flyways), the Mississippi river (that separates the Mississippi and Central Flyways) and the Rocky Mountains (that separate the Pacific and Central Flyways).[143]

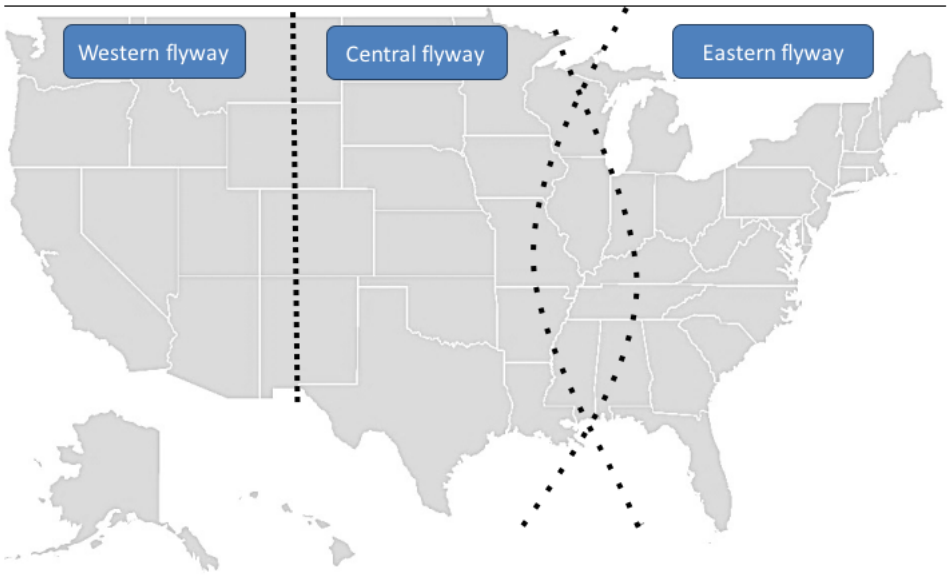
Several studies have attempted to correlate the circulation of WNV with avian migratory patterns. Serological studies testing neutralizing antibody titers in the sera of wild birds have found evidence of WNV infection in birds migrating southwards during the Fall, but not in birds flying north during the Spring in the Atlantic, Mississippi and Pacific Flyways.[134,137] Furthermore, there is some limited phylogenetic evidence that suggests WNV isolates cluster by flyway; however, this study did not address the direction of WNV movement within or between flyways.[108]

It should be noted our understanding of avian flyways is based largely on the movements of waterfowl (ducks, geese, swans, etc.). As described above, the most important vertebrate hosts for WNV are passerine birds that are a type of terrestrial bird. These birds follow their own unique flyways that are distinct from those of waterfowl. Migratory patterns of terrestrial birds are often more irregular than waterfowl, as they prefer looped routes,[144–149] which are typically longer, but allow the birds to take advantage of food avalibility[150] and atmospheric conditions.[151] Little was known about the general flyways of terrestrial birds until recently, when studies revealed the distinct Western Flyway and the two overlapping Eastern and Central Flyways (Figure 1.7).[151] Currently, no studies have ever considered the effect of terrestrial bird migrations on the movement of any infectious disease in the USA.

1.6 Phylogenetic Methods

Phylogeny is a powerful tool used to define the relationship among viral isolates using genetic sequences. While the field is constantly changing, the two most common approaches are the maximum likelihood and Bayesian methods. Most of the analyses in this dissertation rely on

Figure 1.7 Terrestrial Bird Flyways.
Recently, three flyways have been identified for terrestrial birds.[151] The distinct Western Flyway and the overlapping Central and Eastern Flyways.



Bayesian methods. While both methods utilize sequence alignments, maximum likelihood analyses are relatively simplistic with few assumptions while Bayesian methods make assumptions based on "prior knowledge", or more simply priors. Priors provide information about the nucleotide substitution model, date and locations of the sample collection, the distribution of possible mutation rates, changes in the effective size of the population, etc.

To generate a Bayesian phylogeny, a random tree is generated. Then using the distributions specified in the pre-selected priors, the random tree is optimized through a series of branch switches using a Markov chain Monte Carlo (MCMC) method. The number of optimizations is selected by the user and usually runs for 10 million to 250 million steps. Given that each phylogeny starts with a random tree, multiple independent runs must be combined and compared to ensure that all trees converge onto the same topology with sufficient statistical support (Effective Sample Size (ESS) >200).

1.7 The Aims of This Dissertation

The rapid genetic adaptation and geographic expansion of WNV in the New World provides a unique opportunity to study successful spread and adaptation of an emerging viral disease in a large population of naïve hosts. The overall objective of this dissertation is to identify the genetic and evolutionary mechanism(s) that have facilitated the successful invasion and continued circulation of WNV in the New World. In doing so, the relationship between WNV adaptation, circulation and host migration was proposed to be examined with the following hypothesis and Specific Aims:

Evolution of WNV in the New World has been enhanced by long distance travel and concurrent genetic adaptation.

Specific Aim 1: Evaluation of WNV circulation within and between flyways.

Specific Aim 2: Evaluation of the ongoing evolution of WNV in Texas, with a focus on the 2014 outbreak in Harris County.

Specific Aim 3: Evaluation of WNV isolates collected in Colombia.

1.6.1 Specific Aim 1: Evaluation of WNV circulation within and between flyways.

1.6.1.1 Hypothesis:

The circulation of WNV in the USA is influenced by the movement of migratory birds.

1.6.1.2 Rationale:

Avian migration has been implicated in the spread of infectious diseases around the world. In the case of avian influenza, phylogenetic studies have provided powerful insight into the relationship between host and virus migration that in turn has supported the development of surveillance and early warning programs. Studies involving WNV have been much more limited. Serological evidence has suggested that the movement of WNV within North American flyways was unidirectional and southward,[134,137] and phylogenetic studies have demonstrated that avian flyways contribute to the clustering pattern of WNV in the USA.[108] However, no studies have identified the major sources (origin or departure location) or sinks (destination or arrival location) of WNV movement, and studies to date have not evaluated the magnitude of virus movement within or between flyways. Furthermore, all studies investigating the role of bird migration on WNV circulation have relied on the patterns of waterfowl migration, even though waterfowl are not significant contributors to WNV transmission. In this study, the movement of WNV was evaluated with regard to terrestrial bird flyways. Specifically, viral migration was defined in the Eastern and Central Flyways.

1.6.1.3 Approach:

A) Selection of Sequences

To address the hypothesis of this Aim, a significant limitation had to be addressed, namely the limited number of available WNV sequences. In 2013, when this study began, only 454 full genome sequences of WNV were available from 24 states (Figure 1.8A).[152] Now, thanks

to the advent of Next Generation Sequencing (NGS), that number has almost doubled and now more than 905 unique WNV sequences are available (Figure 1.8B), including 142 WNV isolates sequenced in this study. The collection information and intra-host variation is of these isolates is discussed in Chapter 3.

B) Analysis of WNV Migration within the Eastern and Central Flyway

Migration along the Eastern Flyway was evaluated using sequences collected from NY, Virginia (VA) and Georgia (GA); and the Central Flyway was evaluated with sequences from CO, North Dakota (ND), South Dakota (SD) and TX. As IL is situated in a region of overlap between the two flyways, it was used in both models. Sequences were analyzed with Bayesian phylogenetic methods and an asymmetrical discrete trait model. Ancestral state reconstruction was used to determine the location of each common ancestor and Markov Jumps were counted to determine the minimum number of migration events between each location.

C) Analysis of WNV Migration Between Flyways

The phylogeographic relationships of WNV sequences from all locations (NY, VA, GA, TX, IL, ND, SD, and CO) was defined as described above. The major sources and sinks of WNV migration was defined to identify the most efficient locations to focus surveillance efforts. Also, the patterns of WNV movement was evaluated with respect to terrestrial bird flyways to determine the role of avian migration in virus dissemination.

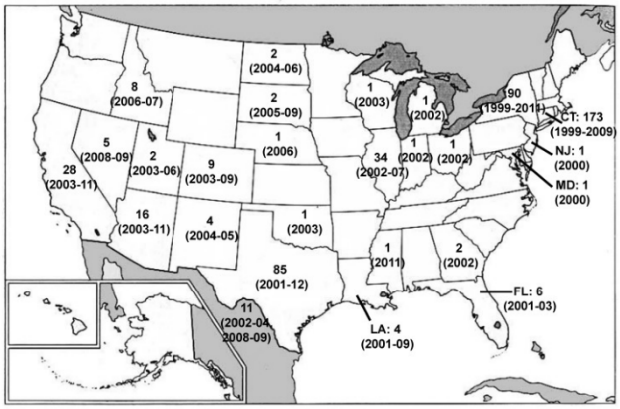
1.6.2 Specific Aim 2: Evaluation of the ongoing evolution of WNV in Texas, with a focus on the 2014 outbreak in Harris County.

1.6.2.1 Hypothesis

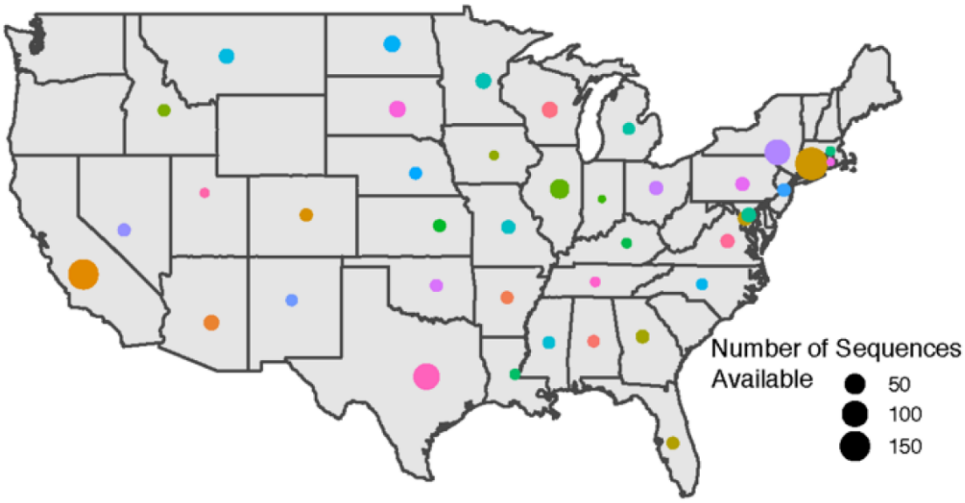
Harris County, Texas can serve as a national model for WNV evolution.

Figure 1.8 Summary of the number of WNV sequences available in the USA available in 2013 (A) and in 2017 (B). Only sequences containing the full ORF were included. Duplicate sequences, sequences with degenerate nucleotides, and sequences without date or location information were excluded.

A) Distribution of WNV sequences as reported by Mann et al. 2013



B Distribution of WNV sequences available on Genbank in on January 2017



1.6.2.2 Rationale

Due to the availability of genomic sequence from multiple years, the evolution of WNV was evaluated in Harris County, TX between 2012 and 2016. Special attention was given to WNV isolates from the recent 2014 outbreak because it was the largest outbreak of WNV in Harris County to date.

1.6.2.3 Approach

A) Analysis of Consensus Sequences

Several studies have asserted that WNV evolution in the USA has reached homeostasis.[83,90,98,108,109] The degree to which stochastic variation or selection influences WNV evolution was determined by comparing the amino acid sequences of the Harris County 2014 isolates to the prototype NY99-flamingo 382-99 strain. Phylogenetic analyses were used to define the evolutionary relationships among WNV isolates collected in TX. Both maximum likelihood and Bayesian methods were utilized.

B) Analysis of Diversity and Virulence

Intra-host variation was evaluated using a robust bioinformatics pipeline. Two measures of diversity were used, Shannon's entropy and single nucleotide variant identification.

1.6.3 Specific Aim 3: Evaluation of WNV isolates collected in Colombia.

1.6.3.1 Hypothesis

WNV in South America is descended from WNV strains originating in North America.

1.6.3.2 Rationale

In North America, the NY99 genotype was completely displaced by the dominate NA/WN02 and SW/WN03 genotypes in 2002. However, studies of isolates from Colombia[87] and

Argentina[69] collected in 2006 and 2008, respectively, revealed that the NY99 genotype continues to circulate in South America. Genbank contains only four South American isolates that span the entire open reading frame: two isolates from Colombia collected in 2008, and two isolates from Argentina collected in 2006.

1.6.3.3 Approach

In this study, four Colombian isolates from 2008 were sequenced using NGS to evaluate the intra-host diversity of WNV isolates circulating in South America. The evolutionary patterns of WNV in South America were considered using phylogenetic methods to determine the relationship between WNV in the Old World, North America and South America.

CHAPTER 2 MATERIALS AND METHODS

2.1 Generation of Alignments

All unique sequences of natural and [c]laboratory strains of WNV were identified using the Virus Variation Resource[153] and obtained from Genbank through R with the following code:

```
#acc is a string of Genbank Accession numbers identified in Virus Variation Resource
# acc<-c("xxxx","yyyy".....)
WNV<-read.GenBank(acc)
write.dna(WNV, file ="alignment_name ", format = "fasta")
```

All sequences were manually aligned in BioEdit v7.1.3 or MEGA7 and noncoding regions were removed, i.e., the open reading frame was used for analyses. The sequence inclusion criteria varied between experiments to allow the hypothesis of each Aim to be addressed. Unless otherwise stated, viral sequences meeting the following criteria were included in this study: (a) the nucleotide sequence spanned the complete open reading frame, (b) the sequence was derived from natural isolates and not laboratory strains, (c) the sequence was unique, i.e. the sequences were not identical to any other sequence in the alignment, and (d) no degenerate nucleotides were in the sequences.

2.2 Selection of Virus for Isolation

Additional WNV isolates were obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch. These virus isolates were originally collected in Colombia and from four states in the USA: VA, GA, TX and CO. Details concerning the original source, collection method, passage history, etc. for each of the isolates are explicitly detailed in appropriate chapter(s).

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[c] Laboratory strains are those that were derived from an infectious clone or have been used in experiments, for example infectious clone derived viruses, viruses that have undergone extensive passage for the purpose of attenuation or adaptation to a new host, or viruses that were isolated from animals following experimental infection when the sequence of the parental strain was already known.

2.3 Isolation of Viral RNA and Next Generation Sequencing

Viral RNA was extracted from the supernatant of infected Vero cells with a QIAamp Viral RNA Mini Kit (Qiagen) per the manufacturer's instructions. Libraries were generated with a TruSeq RNA v2 kit (Illumina) and samples were sequenced by the University of Texas Medical Branch Next Generation Sequencing Core on an Illumina 1500 Seq platform. Adaptor sequences and poor quality reads (Q score < 20) were removed with Trimmomatic.[154] Reads were aligned with bowtie2[155] under the very sensitive local parameter against the prototypical strain of WNV (NY99 flamingo- 382-99, Accession: AF196835). Consensus sequences were generated using the mpileup function in Samtools.[156] The author of this dissertation developed the NGS pipeline used in this dissertation. Briefly, bam files were realigned to the consensus sequence with bowtie, and then sorted, indexed, processed to remove PCR duplicates and down-sampled to the mean depth of 1000 or 3000 using functions within Picardtools[157] and Samtools[156] (Figure 2.1 Lines 1-20).

Single nucleotide variants (SNVs) were identified using V-phaser2 while applying false detection rate and the strand bias filters (Figure 2.1 lines 21-26).[158,159] Nucleotide counts were identified with the bam2R function of the deepSNV library in R. Shannon's entropy was calculated as below, where *f(a)* is the frequency of the As at a single position, *f(g)* is the frequency of Gs, *f(u)* is the frequency of u, *f(c)* is the frequency of Cs *f(-)* is the frequency of gaps, and *n* is the population size, in this case 1:

Shannon's Entropy=

$$\frac{f(a)ln(f(a))+f(g)ln(f(g))+f(u)ln(f(u))+f(c)ln(f(c))+f(-)ln(f(-))}{n}$$

Figure 2.1 Summary of NGS Pipeline Codes and Programs used to Process NGS Data

NGS processing codes were written in bash and relied on the following programs: Bowtie, Picard tools, Samtools.

NGS Data Preprocessing

```
FOR I IN *.BAM DO
    JAVA -JAR -XMX6G SAMTOFASTQ.JAR INPUT=I FASTQ=${I}A
SECOND_END_FASTQ=${I}B;
    BOWTIE2-BUILD-S -F ${I}.FASTA ${I}REF;
    BOWTIE2-ALIGN-S --VERY-SENSITIVE-LOCAL -X ${I}REF -1 ${I}A -2 ${I}B –S
     ${I}ALIGNMENT
          2>${I}BOWTIE.REPORT;
    SAMTOOLS VIEW –SB ${I}ALIGNMENT > ${I}VSL;
     JAVA –XMX8G –JAR SORTSAM.JAR INPUT=${I}VSL
OUTPUT=${I}.COORDINATE.BAM SORT_ORDER=${I}COORDINATE;
     JAVA –XMX8G –JAR MARKDUPLICATES.JAR I=${I}.COORDINATE.BAM
OUTPUT=${I}_RE.BAM REMOVE_DUPLICATES=TRUE
OPTICAL_DUPLICATE_PIXEL_DISTANCE=0
METRICS_FILE=${I}.RE.OUTPUT;
    SAMTOOLS DEPTH ${I}_RE.BAM > ${I}DEPTH.TXT;
    SAY "TASK COMPLETE";
DONE
```

Down-sampling procedure

```
#where I= the input file name, O= the output file name, p= #the mean depth of
#coverage/the # desired depth of coverage
Java -jar -Xmx4g /Volumes/seagate/ngs/picardtools/DownsampleSam.jar I= O= p=
```

Variant Detection with Vphaser

```
for a in *; do
        mkdir ${a}_ds1000;
       /Volumes/seagate/programs/NGS/V-phaser/VPhaser-2-
       02112013/bin/variant_caller  -i ${a}_ds1000.bam -o ${a}_ds1000;
        say "done with ${a}";
  done
```

2.4 PHYLOGENY

2.4.1 Maximum Likelihood Phylogeny

Maximum likelihood trees were generated with RAxML-HPC Black Box on the Cyberinfrastructure for Phylogenetic Research (CIPRES) V.3.3 with automatic halting determined by bootstrapping.[160] The frequency of invariable sites was estimated prior to generation of the phylogeny to protect against long branch attraction (LBA).

2.4.1.1 INVESTIGATION OF TEMPORAL STRUCTURE

Temporal structure is the relationship between time and genetic distance. Verifying temporal structure was important because it demonstrated that it was possible to create an accurate time-scaled Bayesian phylogeny. To evaluate temporal structure, a time-naïve phylogeny, in this case a maximum likelihood phylogeny, was generated to allow the genetic distance to be determined between all isolates on the phylogeny. The root-to-tip distance, which is a phylogenetic measure of genetic distance, was determined for each isolate of the maximum likelihood phylogenies in TempEst (formerly Path-o-gen).[161] The correlation between root-to-tip distance and collection date was evaluated using the Pearson correlation method in R.

2.4.1.2 INVESTIGATION OF SELECTION PRESSURE

Codons undergoing positive selection pressure were identified by Fixed Effects Likelihood (FEL)[162] and Mixed Effects Model of Evolution (MEME)[163] methods implemented by the HYPHY program through datamonkey.org.[164,165] Two types of positive selection pressure were considered: (1) pervasive positive selection that indicates directional selection and (2) episodic selection that may indicate diversifying selection or changes in the selection pressure acting on a pathogen (e.g. hosts acquiring adaptive immunity, host switching, etc.). While FEL

and MEME are both capable of detecting pervasive positive selection pressure, only MEME can identify episodic selection pressure. Therefore, sites that were identified by both the FEL and MEME methods are likely undergoing pervasive selection pressure and sites that are only identified by the MEME method are likely undergoing episodic selection pressure.

2.4.2 Bayesian Phylogeny

2.4.2.1 Model Selection

The most appropriate nucleotide substitution model[d] was determined by comparing all 203 models available in JModelTest2 with Alkaline and Bayesian information criteria.[166] Path-sampling and stepping-stone approaches were used to compare uncorrelated clock models (exponential and lognormal) and tree priors (Bayesian skyline, Bayesian Skygrid and Bayesian Skyride). Marginal Likelihood Estimation (MLE) files were generated using 100 path-steps and a MCMC chain length of one million. During the course of this dissertation, Beast v1.8.4 was released providing one additional clock model, the uncorrelated gamma clock. This model was considered in Aim 3.

2.4.2.2 Generation of Phylogenies

Phylogeographic relationships were inferred using a Bayesian MCMC approach. Xml files were generated in BEAUti v1.8.3 or 1.8.4 and run with BEAST v1.8.3 or 1.8.4[167] on CIPRES.[160] The General-Time Reversible model was used to infer nucleotide substitution frequencies with a gamma rate distribution and invariable sites (GTR +I+Γ). The mutation rate was inferred with uncorrelated lognormal (or gamma) clock model and changes in population size were modeled with a Bayesian Skyline tree prior. The UCLD mean was restricted between 1

[d] Nucleotide substitution models describe the relative substitutions between bases, (A>T, A>C, A>G, T>A, etc).

x 10^{-4} and 9 x 10^{-4} substitutions per site per year, which is consistent with previously reported rates for WNV evolution.[90,168]

Trees were run with an MCMC chain length of 100 or 50 million and were sampled every 5,000 steps. Log files were reviewed in Tracer to determine burn-in, which ranged from 5-10%. Multiple independent MCMC chains were run until ESS values exceeded 200. Log and tree files were combined in LogCombiner v.1.8, and a maximum clade credibility tree was generated in TreeAnnotator. Locations were inferred using ancestral state reconstruction with an asymmetrical discrete trait substitution model.[169]

2.4.2.3 Analysis of Migration

After the Xml files were generated in BEAUti, they were manually edited to allow all Markov Jumps between 2001 and 2009 to be counted,[170] which described the relative magnitude of migration between locations. Unfortunately, as expected for a zoonotic emerging disease, both the annual WNND incidence, and sample collection efforts varied dramatically among the states over time, adding significant complexity to the model.

To mitigate the effects of inconsistent sampling and to confirm the observed results, a stricter inclusion criterion was applied to confirm the results obtained using the full data set. This analysis ensured that the dataset was representative of the WNV activity of each region in a particular year. In this approach, the sequences were randomly down-sampled such that the number of sequences correlated [Pearson method ($p < 0.05$)] with the incidence of WNND reported to the CDC in a particular year, as this is the most accurate record of relative WNV activity.

Incidence was calculated using the number of WNND cases reported to the CDC from each state during each year and divided by the estimated population of each state. The population estimates were obtained from the Time Series of Intercensal State Population Estimates: obtained from the Population Division of The U.S. Census Bureau.[171] States with insufficient sequences to represent the WNND incidence were excluded. Down-sampling was done in at least duplicate to ensure that reduction in sample size and diversity did not remove important relationships.

2.5 *IN VITRO* STUDIES AND TEMPERATURE SENSITIVITY ASSAYS

2.5.1 Cell Culture

All in vitro studies and virus passage was undertaken using Vero cells (African green monkey kidney), which were grown in T-150 flasks in growth medium at 37˚C with 5% CO2. Growth medium was prepared using commercially available minimum essential media (MEM) supplemented with Earle's salts and L-glutamine (Gibco, Carlsbad, CA). The medium was supplemented with 1% penicillin-streptomycin solution (5,000 units penicillin and 5,000 µg/mL streptomycin in 0.85% saline) (Gibco), 1% non-essential amino acids (10nM) (Gibco) and 1% (200nM) L-glutamine (Gibco). Growth medium contained 8% Fetal Bovine Serum (FBS) (Gibco or Invitrogen), while maintenance medium only contained 2% FBS.

For passage, cells were washed with 5-10mls of PBS, and the monolayers were dissociated from the flask with 5mls of trypsin and diluted in 25mls of fresh growth media. Passage occurred twice a week.

2.5.2 Virus Isolation

Dead birds in Harris County, TX were collected by the Mosquito Control Division of Harris County Public Health and Environmental Services (HCPHES). Screening for WNV

infection and virus isolation was undertaken by the World Reference Center for Emerging Viruses and Arboviruses at the University of Texas Medical Branch. Briefly, brain homogenates from dead birds were passaged once or twice in Vero cells, and virus was collected from the supernatant of infected Vero cells at three days-post infection.

2.5.3 Infections and Plaque Assays

Vero cell monolayers were used for virus infection when they were 90-100% confluent. Old medium was removed and monolayers were washed with 5 ml PBS and inoculated with a small volume (100ul/ T-25 flask) of virus. After incubation for 30 minutes at 37˚C, monolayers washed with PBS, and 10mls of fresh maintenance medium was added. Infections were allowed to continue until significant CPE was apparent, typically 3 days post-infection.

Plaque assays were used to quantify virus. Briefly, virus stocks were serially diluted 10-fold from 1 in 10 to 1 in one million in maintenance media to generate virus inoculum. Six-well plates containing Vero cells were washed with 1 ml PBS, and each well inoculated with100ul of one of the virus dilutions in a serial manner with one virus per six-well dish. Following 30-minute absorption time at 37˚C, 3 mls of agar media was applied. To prepare the agar media solution, 2% tissue culture grade agar in water was heated until boiling and cooled to 42˚C in a water bath. An equal volume of the melted 2% gar solution was then mixed with pre-warmed (42˚C) 2X maintenance medium containing 4% FBS immediately prior to use. Agra overlay was left at room temperature to solidify. Plates were incubated at 37˚C with 5% CO_2 unless otherwise stated. On 2 days post-infection (dpi), 4% neutral red solution was added to monolayers and plaques were counted three dpi.

2.6 Animal Studies

All animal procedures were approved by the University of Texas Medical Branch (UTMB) Institutional Animal Care and Use Committee and studies were carried out in strict compliance with the recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council).

Neuroinvasive virulence studies were performed in three to four-week-old female Swiss Webster mice. The 50% lethal dose (LD_{50})[e] was determined using groups of five mice infected with 100ul at doses from 0.1 to 1000 PFU/mouse (0.1, 1, 10, 100, or 1000 PFU/mouse). Virus was administered by the intraperitoneal route. Mice were monitored daily for clinical signs and mortality. Animals were humanely euthanized as significant disease became apparent. Survival Curves were calculated in Prism 7.

[e] LD50 is the dose at which 50% of infected mice succumb to infection.

CHAPTER 3 SAMPLE COLLECTION AND SEQUENCING

3.1 INTRODUCTION

The goal of this dissertation research was to evaluate the evolutionary patterns of WNV in the New World. The first objective was to define the patterns of WNV circulation and to identify the major sources and sinks of WNV movement events. Preliminary studies identified significant variation in the number of sequences available by geographic location throughout the USA, with a few locations accounting for most of the available WNV sequences (Figure 1.8A). In fact, 58% of the 454 WNV sequences available at the beginning of this study were collected from southern New York (NY) and Connecticut (CT), two relatively small regions in the northeastern USA that share a common border.

Such variation in the number and distribution of WNV sequence information would have introduced significant bias into the analysis. To address this concern, additional WNV isolates present in the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) were reviewed to identify locations where multiple isolates had been collected over consecutive years. WNV strains from Virginia (VA), Georgia (GA) and Colorado (CO) met this requirement; and isolates from these locations were sequenced using Next Generation Sequencing (NGS) technology. While the ultimate goal of sequencing these isolates was to provide a sufficient dataset for phylogenetic analyses (Chapter 4), NGS provides an opportunity to investigate the quasispecies diversity of each isolate. The purpose of this chapter is to provide a descriptive quasispecies analysis of each WNV isolate sequenced in preparation of Chapter 4. The observations of this Chapter provide interesting preliminary data that could prompt future studies into evolutionary quasispecies dynamics.

3.2 RESULTS

3.2.1 Isolate Collection

The collection of WNV isolates available in the WRCEVA was reviewed to identify states where there were at least 20 isolates available across multiple consecutive years. States with at least 20 sequences already available on Genbank were excluded. In this way, three states were identified that had at least 20 distinct samples available (GA, VA and CO) (Table 3.1). In total, 91 low-passage WNV isolates were obtained from the WRCEVA (Appendix I) and sequenced directly without amplification or additional cell culture passage to avoid introduction of selection pressures on the virus population.

Between 2000 and 2010, 40 WNV isolates were collected in Norfolk County, VA (Table 3.1). Six of the isolates were collected from birds (four were collected in 2000 and two were collected in 2002) (Table 3.1). The remaining 34 isolates were collected from mosquito pools. All mosquito isolates were collected between 2001 and 2010.

Twenty WNV isolates were collected from eight counties in GA (four from Chatham, five from Dekalb, four from Fulton, three from Lowndes, and one each from Gwinnett, Henry, Wuscogee and Wane) between 2001 and 2009. No isolates were available from either 2003 or 2006. Of the 20 GA isolates, 10 isolates were collected from birds (2001-2007) and 10 were collected from mosquitoes (2004-2009) (Table 3.1).

Finally, 31 WNV isolates (nine from birds and 22 from mosquitoes) were collected in CO between 2003 and 2008 (Table 3.1). No isolates were available from 2005. Seventeen of the 31 isolates were collected in Larimer County (six from Fort Collins, one from Lovelace, one from Scarborough, six from Wellington, and three were unknown), 13 were collected in Weld County, and one was collected from an unknown location in Colorado.

Table 3.1 Summary of the viral isolates.

The number of isolates collected in birds and mosquitoes by year from CO, GA and VA. Dots indicate instances where no viral isolates were available.

	CO			GA			VA		
year	Total	bird	mosquito	overall	bird	mosquito	overall	bird	mosquito
2000	.	.	.	.	.	.	4	.	4
2001	.	.	.	3	3	.	1	1	.
2002	.	.	.	3	3	.	6	2	4
2003	8	6	2	.	.	.	3	.	3
2004	4	2	2	2	1	1	4	.	4
2005	.	.	.	2	1	1	3	.	3
2006	7	.	7	.	.	.	4	.	4
2007	6	.	6	5	2	3	2	.	2
2008	6	.	6	2		2	4	.	4
2009	.	.	.	3		3	5	.	5
2010	.	.	.	.	.	.	4	.	4
Total	31	8	23	20	10	10	40	3	37

3.2.2 Analysis of Consensus Sequences

Eleven isolates (CO07E-TWN2998, GA01-TWN3014, GA01-TWN2971, GA01-TWN2984, VA00A-TWN2672, VA00B-TWN2733, VA00C-TWN2731, VA00D-TWN2714, VA01A-TWN2693, VA02C-TWN2694, VA02D-TWN2717) did not possess the E-V159A substitution, indicating they belonged to the NY99 genotype (Figure 3.1). Despite being displaced by the NA/WN02 genotype in 2002, one isolate, CO07E-TWN2998 that was isolated in 2007, belonged to the NY99 genotype suggesting that the NY99 genotype may have persisted in the USA at low levels or that the E-159 position in this isolate may have reverted to the NY99 polymorphism.

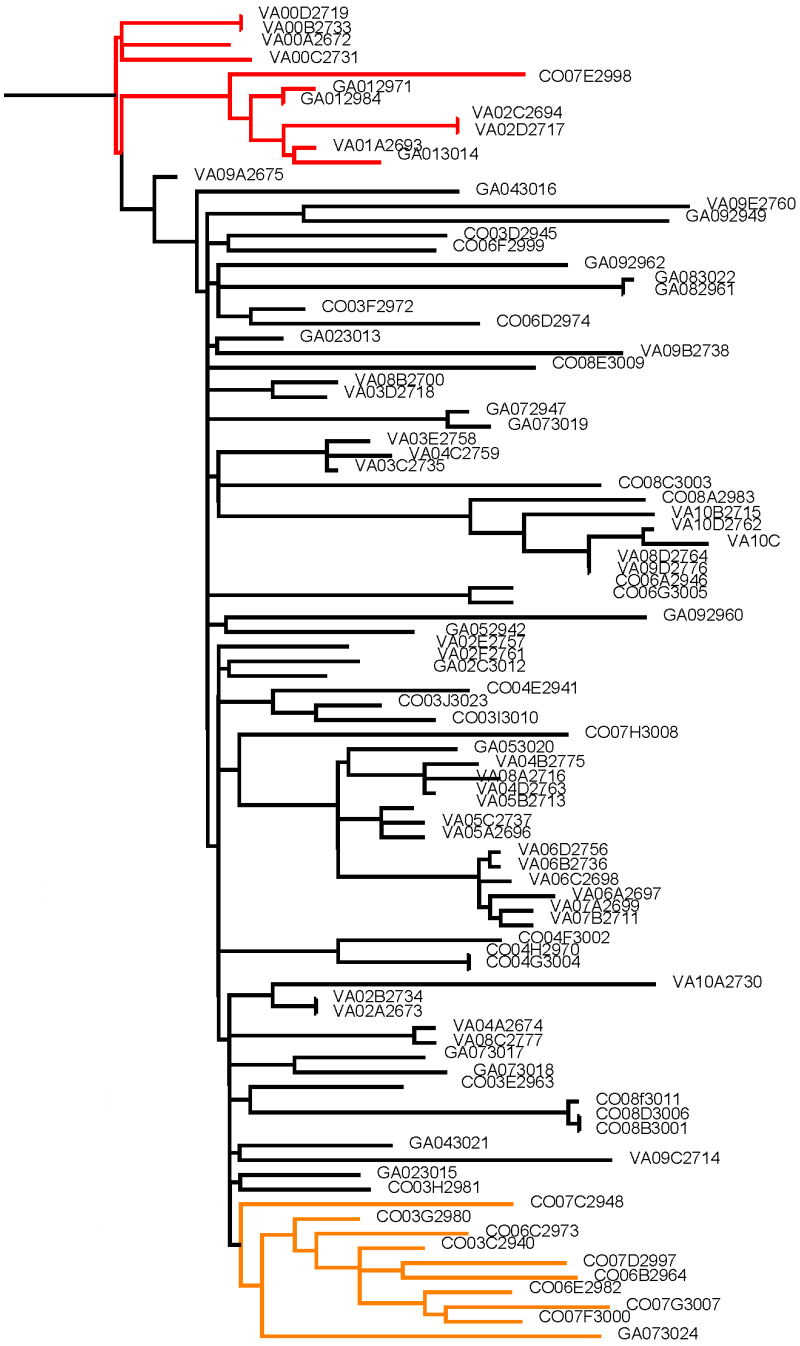
Ten isolates (CO03C-TWN2940, CO03G-TWN2980, CO06B-TWN2964, CO06C-TWN2973, CO06E-TWN2982, CO07C-2948, CO07D-2997, CO07F-3000, CO07G-TWN3007, GA07-TWN3024) contained the NSA-A85T substitution indicating that they were part of the SW/WN03 genotype. All other isolates were part of the NA/WN02 genotype. Interestingly, none of the isolates from VA and only one isolate from GA belonged to the SW/WN03 genotype. Based on a limited dataset, this may suggest that the SW/WN03 genotype may be less fit in the eastern USA or that there has been limited gene flow from the western and central USA where the SW/WN03 genotype circulates into the eastern USA.

3.2.3 Identification of single nucleotide variants (SNVs) including nucleotide substitutions (NSubs) and length polymorphisms (LP)

3.2.3.1 Location

SNVs were distributed through the WNV genome with no clear regions with enriched diversity in isolates from any location.

Figure 3.1 Maximum likelihood phylogeny of isolates from CO, VA and GA.
A maximum likelihood phylogeny was generated using all WNV sequences generated for Chapter 4. Branches in red belong to the NY99 genotype and branches in orange belong to the SW/WN03 genotype. All other branches belong to the NA/WN02 genotype. The first two letters of each sequence indicate the collection location, the second two numbers indicate the year of collection and the final four numbers represents a unique identification number (TWN number).



A. Isolates from Colorado

Eight hundred and sixty-nine single nucleotide variants (SNVs) were identified in the CO isolates (mean= 28.03 SNVs/isolate), of which 247 (mean=30.88 SNVs/isolate) SNVs were identified in bird isolates and 622 (mean= 27.04 SNVs/isolate) were identified in mosquito isolates (Table 3.2, Figure 3.2, Appendix II). The most diverse isolate (TWN3008) had 94 SNVs while the least diverse isolate had four SNVs (TWN2997 and TWN3007) (Appendix I). The mean frequency of the SNVs identified in CO isolates was 3.99% (Figure 3.3), of which 15.65% (136) were length polymorphisms (LPs) and 84.35% (733) were nucleotide substitutions (NSubs).

B. Isolates from Georgia

A total of 363 SNVs were identified in the WNV isolates from GA (mean= 18.15 SNVs/isolate) (Table 3.2). The most diverse isolate (TWN2984) had 52 SNVs, while the least diverse isolates (TWN2962) had six SNVs (Appendix I). Two hundred and thirty-six SNVs were identified in bird isolates (mean = 23.6 SNVs/isolate) and 127 SNVs (mean= 12.1 SNVs/isolate) were identified in mosquito isolates (Table 3.2). SNVs identified in GA isolates occurred at a mean frequency of 2.51% (Figure 3.4). LPs accounted for 22.87% (83) of the SNVS and 77.13% (280) were NSubs.

C. Isolates from Virginia

A total of 819 SNVs were detected in the VA isolates (Table 3.2). The most diverse isolate had 76 SNVs (TWN 2734) and the least diverse isolates had five SNVs (TWN2713) (Appendix I). Three hundred and seven SNVs (mean 51.17 SNVs/isolate) occurred in bird isolates and 512 (mean 15.06 SNVs/isolate) occurred in the mosquito isolates (Table 3.2). The

Number of SNVs identified in each isolate given the location and host.

The total number of isolates, total number of SNVs and the mean number of SNVs are summarized by location and host.

Location	Species	Number of Isolates	Number of SNVs	Mean SNVs/Isolate
CO	Avian	8	247	30.88
CO	Mosquito	23	622	27.04
GA	Avian	10	236	23.60
GA	Mosquito	10	127	12.70
VA	Avian	6	307	51.17
VA	Mosquito	34	512	15.06
CO	Total	31	869	28.03
GA	Total	20	363	18.15
VA	Total	40	819	20.48
Total	Total	91	2051	22.54

Figure 3.2 Comparison between the number of SNVs by location.
SNVs were identified with Vphaser2. The number of SNVs per isolate are summarized as box plots. No statistical differences were observed between the mean number of SNVs from isolates collected in CO, GA or VA.

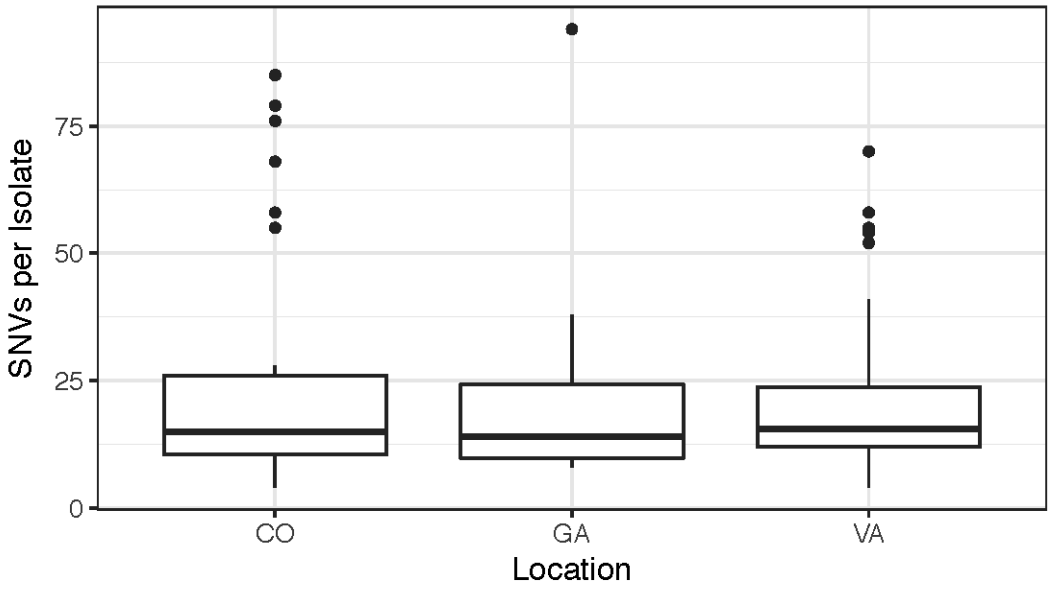
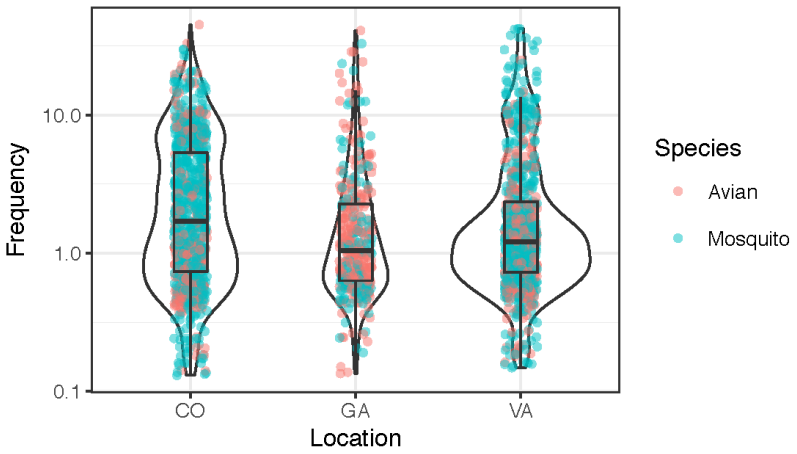


Figure 3.3 Comparison of SNVs frequency by location.
The frequency of each SNV is displayed as a dot plot. Red dots indicate SNVs that were identified in avian isolates while blue dots indicate SNVs that were identified in mosquito isolates. The density and distribution of dots are summarized by violin plots and box plots.



mean frequency of SNVs was 3.07% (Figure 3.3). Twenty-three point zero-eight percent (189) of the SNVs were LPs, including insertions and deletions while 76.92% (630) were NSubs days post-infection.

3.2.3.2 Comparison Between Species and SNV Type- Length polymorphisms (LP) vs nucleotide substitutions (NSubs)

Of the 91 isolates, 66 were collected from mosquitoes and 25 were collected from birds (Table 3.1). In total, 2051 total SNVs were identified (Appendix I and II). Twelve hundred and sixty-one SNVs identified were among the mosquito isolates (mean= 18.82 SNVs per isolate) and 790 SNVs were identified among the bird isolates (mean= 32.91 SNVs per isolate). The number of SNVs identified per bird isolate was significantly greater than the number of SNVs identified per mosquito isolate (Kruskal-Wallis p= 0.001) (Figure 3.4).

Only 408 (19.89%) of the SNVs were LP, of which 302 occurred in mosquitoes and 106 occurred in birds. NSubs accounted for 80.11% (1643) of the total SNVs. Nine hundred and fifty-nine NSubs occurred in mosquito isolates and 684 occurred in bird isolates. There were significantly more NSubs than LPs in each isolate (Kruskal-Wallis $p=3.11 \times 10^{-15}$).
LPs occurred at similar frequencies in both bird and mosquito isolates (2.25% and 2.10%, respectively) (Figure 3.5). However, while more NSubs were identified in bird isolates, NSubs occur at higher frequencies in mosquito isolates (mean = 4.44%) than in bird isolates (mean=2.64%). Two-way ANOVA comparison of the mean SNV frequencies revealed statistically significant differences related to the type of SNV (NSubs vs LPs) ($p=1.44 \times 10^{-9}$) and host (birds vs mosquitoes) ($p= 5.09 \times 10^{-7}$) (Table 3.3). However, there was evidence of a combined effect between host and SNV type (p=0.01). The most frequent LP in mosquito isolates occurred at 13.23% and in bird isolates at 11.93% (Figures 3.5). In contrast, the most

Figure 3.4 Comparison of diversity between species.
The number of SNVs identified in each isolate are summarized below as a dot plot. The color of the dots indicates the year the virus isolate was collected. The density and distribution of the dots are summarized by violin and box plots.

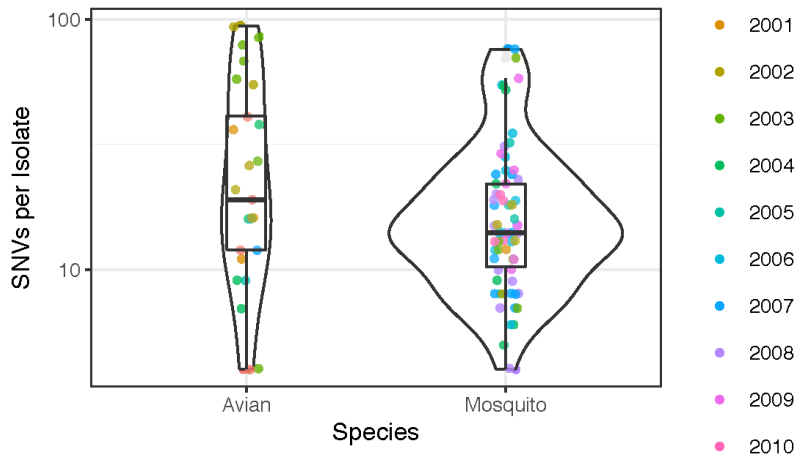


Figure 3.5 Frequency of LPs and NSubs across the WNV genome.
The frequency of each SNV identified by Vphaser2 was summarized as scatter plots. Separate plots were used to illustrate the frequency of length polymorphisms (LP) and nucleotide substitutions (NSubs) present in birds and mosquito samples. Color indicates genome position.

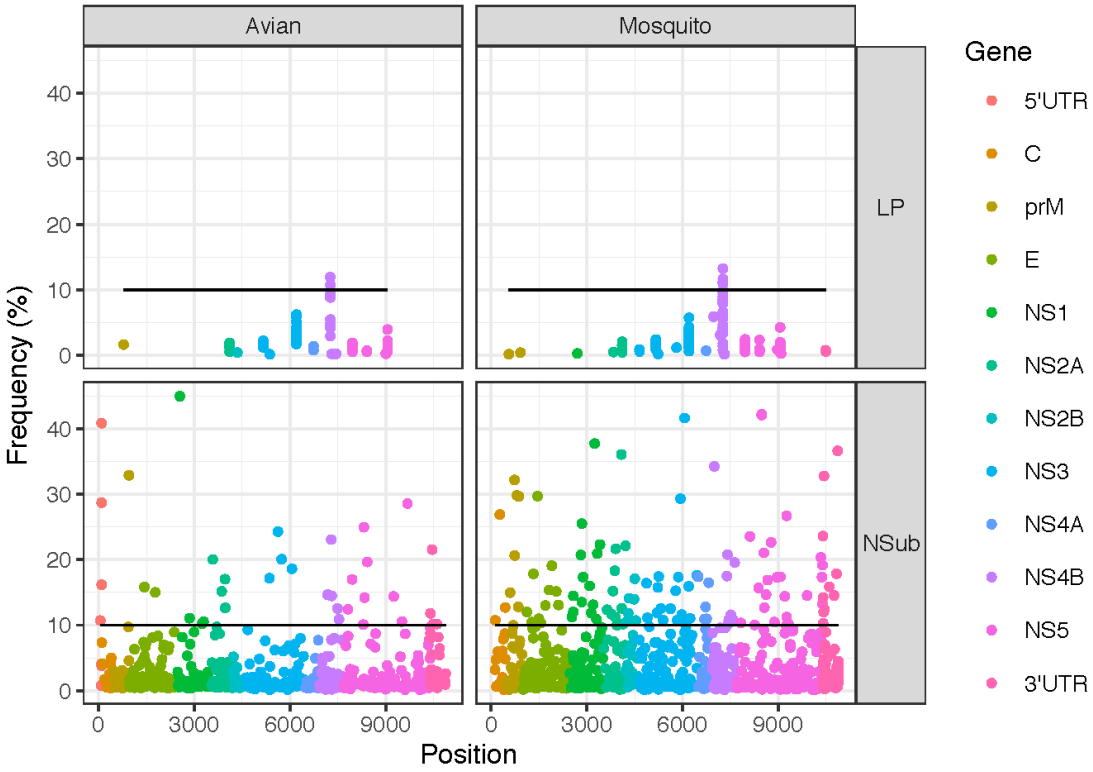


Table 3.3 Two-way ANOVA between SNV frequency, host type and SNV type.

A two-way ANOVA was performed to test the difference between the frequency of LPs and NSubs identified in bird and mosquito isolates. P-value (P), F- statistic (F) and degrees of freedom (DF) are provided for each factor. The sum of the squares (Sum Sq) and the mean of the squares (Mean Sq) of the deviations of all the observations are also provided.

Two-Way ANOVA- SNV Frequency					
SNV Frequency	Df	Sum Sq	Mean Sq	F	P
Species	1	628	628.33	25.3969	5.09E-07
Type	1	914	914.38	36.9593	1.44E-09
Species:Type	1	170	169.92	6.8684	0.00884
Residuals	2008	49678	24.74		

frequent NSubs in bird isolates occurred at 44.95% and mosquitoes occured at 42.2% (Figures 3.5).

3.2.3.3 VARIANTS THAT OCCUR IN MULTIPLE ISOLATES

LPs were identified at 35 positions. Twenty-four positions were identified with a LP in one isolate only and three LPs [NS4A-272, NS4B-396, NS4B-404 (nucleotide position in gene)] were identified in two isolates. Four isolates had LPs at nucleotide position NS3-39, 65 isolates had LPs at NS2A-584, four isolates had LPs at NS3-39, 41 isolates had LPs at NS3-555, 52 isolates had LPs at NS3-1592, 43 Isolates had LPs at NS4B-352, 83 isolates had LPs at NS5-280, 11 isolates had LPs at NS5-733, and 67 had LPs at NS5-1383. Interestingly, only 3 occurred in the structural genes (in the prM coding region) and only two were identified in non-coding regions, 3'UTR-94 and 3'UTR-97 (Figure 3.5).

NSubs were identified at 1,137 positions. Eight hundred and ninety-two positions were identified with NSubs in one isolate only suggesting the arose from stochastic variation and not selection pressure. One hundred and sixty-one NSubs were identified in two isolates, 42 NSubs reoccurred in three isolates, seven NSubs reoccurred in five isolates, three NSubs reoccurred in six isolates, two NSubs reoccurred in seven isolates, three NSubs reoccurred in eight isolates, one NSub reoccurred in nine isolates, two NSubs reoccurred in 12 isolates, one NSub reoccurred in 28 isolates, and one NSub reoccurred in 36 isolates. NSubs were identified in both structural and nonstructural genes as well as both noncoding regions. The most frequent NSub was identified in 36 isolates at position NS3-555 (Figure 3.5).

3.2.3.4 Relationship Between Time and Diversity

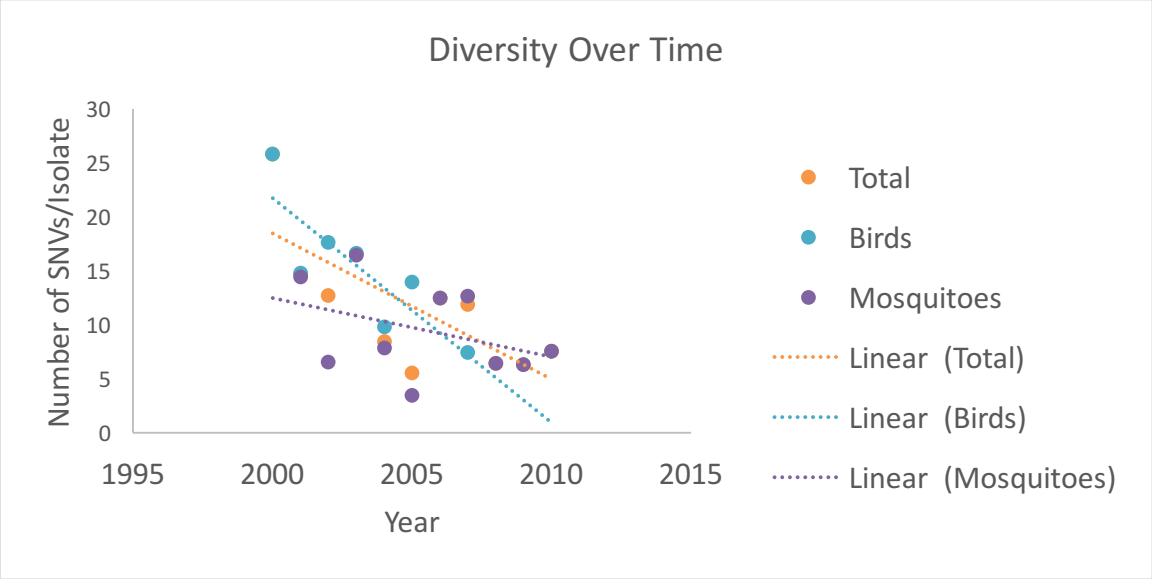
There was a statistically significant negative correlation between year and the number of SNVs/isolate (Pearson: r=-0.75, 95% CI= p=0.01), indicating that isolates collected earlier in time had a greater number of SNVs than the more recent isolates (Figure 3.6). However, it was possible that this result could be biased due to the inconsistent number of bird and mosquito isolates made over time. To address that concern, bird and mosquito isolates were analyzed separately. Among avian isolates alone (n=21), there was a statistically significant negative correlation between year and the number of SNVs/isolate (r=-0.84, 95% CI=-0.98 to -0.24, p=0.02). However, while mosquito isolates (n=70) also demonstrated a negative correlation between year and the number of SNVs/isolate (r= -0.39), it was not statistically significant (95% CI= -0.82 to 0.32, p=0.27).

3.4 Discussion

WNV geospatial evolution has been characterized previously using consensus sequences; however, this analysis has been limited by inconsistent sample collection.[152] Likewise WNV quasispecies dynamics to date have been investigated using either high passage laboratory strains or infectious clone-derived viruses.[14,172,173] However, little is known about the evolution of WNV quasispecies in nature.

To address this gap, 91 low-passage (one to two passages in Vero cells for virus isolation) WNV isolates were obtained from three US locations (VA, GA, CO) between 2000 and 2010 (Table 3.1) and sequenced directly (i.e., no additional passaging) with NGS technology. SNVs were identified using a robust bioinformatics pipeline and well-characterized variant caller program, V-Phaser 2[158,159], which has the capacity to identify low frequency variants including both NSubs and LPs.

Figure 3.6 Diversity over Time.
The mean number of SNVs in each isolate was determined and plotted overtime. Bird isolates are in blue, mosquito isolates are in purple and all isolates are in orange. Linear regression (Pearson's method) was applied to determine the correlation between diversity and time.



The results of this analysis revealed a total of 2051 SNVs distributed across the WNV genome (Figure 3.5, Appendix II). There was no statistical difference in the number and frequency of SNVs per isolate among the isolates collected from all three locations (Figures 3.2 and 3.3). NSubs were more common than LPs in each of the isolates (Figure 3.5). This was not surprising as LPs are associated with frameshifts, which are usually lethal for the virus. While it is possible that the LPs are a result of sequencing errors,[174] additional studies are needed to verify the presence of LPs experimentally.

In this study, no LPs were identified above a frequency of 15% (Figures 3.5), suggesting that RNA genomes with LPs are maintained at low frequency, even following two passages in cell culture where opportunities for superinfection are potentially high and selection pressures are relatively weak. Furthermore, while NSubs are distributed across the genome, only three LPs were observed in the structural genes, and all occurred in the prM (Figure 3.5). This suggests that truncation of nonstructural genes may be tolerated whereas it is not tolerated in structural genes, probably due to the requirement to maintain conformation of virion proteins to infect cells.[175,176] Furthermore, many of the LPs appeared to cluster at specific positions (Figure 3.5). It is possible that the RNA sequence or secondary structure at these sites may induce stalling of the polymerase, which could promote insertions at these locations. It is also possible that the LPs arose in tissue culture during blind (possibly high MOI) passage for isolation.

Additionally, the patterns of WNV quasispecies were compared by host (Figures 3.4 and 3.5). Previous studies have suggested expansion of WNV quasispecies is driven by RNAi during replication in mosquitoes and is reduced during replication in birds.[11,13] However, in this study, more SNVs were identified in bird isolates than those from mosquitoes. It is possible that this difference may be attributed to differences in the method of virus isolation. Previous studies have

focused on virus isolated from bird sera,[11,13] while avian samples used in this study were derived from brain homogenate. This suggests that quasispecies diversity may be tissue specific and that quasispecies diversity may increase in bird brains despite being restricted in the serum. Additional experimental studies are needed to investigate this hypothesis further.

When accounting for variant type (LP vs NSubs), a statistically significant difference was observed between mean frequency of SNVs identified in bird and mosquito isolates (Figure 3.5 and Table 3.3). However, this may be the result of the combined effect of variant type and host. While LPs occurred at similar frequencies in birds and mosquitoes, NSubs appeared to occur at higher frequencies in mosquitoes. It is possible that the stochastic nature and diversifying selection of the iRNA pathway in mosquitoes allows existing and new NSubs to amplify while strong purifying selection in birds restricts NSubs to lower frequencies.

Finally, there appeared to be a negative correlation between date of isolation and diversity among bird isolates (Figure 3.6). This suggests that WNV was able to diversify more during replication in birds during early outbreaks when the bird population was largely naïve while diversification was more restricted when the bird population contained those with immunity to WNV. Additional studies are needed to test this observation, however, if confirmed, these results could provide important information about quasispecies diversification in nature and the general mechanisms that allow pathogens to emerge in novel locations.

Taken together, the results in this Chapter suggest that host, and not geographic location (at least for VA, GA and CO), influences quasispecies diversity; however, additional studies are needed to further investigate the relationship between host and quasispecies size, especially as it relates to tissue compartmentalization and evolution over time. This study also suggests that LPs are maintained in natural WNV quasispecies at low (<15%) frequency, despite potential fitness

losses associated with defective genomes. While this study provides preliminary data for several interesting experimental studies, it is also important to note that limitations exist basis on the number of WNV sequences collected from three geographic locations. Continued sequencing of WNV isolates collected from additional locations may support or challenge these observations.

CHAPTER 4 PATTERNS OF WNV CIRCULATION IN THE USA

4.1 INTRODUCTION

Following the introduction of WNV into NYC, the geographic range expanded quickly, reaching the west coast in 2003. Since that time, the movement of WNV between locations appears to have continued because phylogeographic studies have reported minimal geographic structure,[f] consistent with frequent mixing of WNV strains from distant locations. The most notable exception being CA, where several genetic studies have shown significant isolation of WNV.[177] This suggests limited movement of WNV into CA and no movement out of the state.

While there is limited evidence of geographic clustering by location, one study recently reported that WNV clustered by avian flyway.[108] As birds are the primary reservoir of WNV, this was not surprising; however, it is significant because avian migration has been implicated in the movement of influenza A,[178] Lyme disease,[179] other pathogenic organisms[140] and even invasive invertebrate organisms.[180] In particular, characterization of the relationship between avian influenza virus movement and waterfowl migration has significantly enhanced surveillance and early warning programs.[140,181] However, in the Americas, studies of virus movement associated with avian hosts have mainly concentrated on the migration of waterfowl to the exclusion of terrestrial birds. This is largely because the migratory patterns of waterfowl have been thoroughly characterized with banding studies, which were possible because of the large size of waterfowl and the support among hunting communities. However, it is important to note that passerines, the primary reservoir for WNV, are terrestrial birds and not waterfowl.

In the Americas, waterfowl travel along narrow corridors called flyways that were defined in the 1950s: the Atlantic, Mississippi, Central and Pacific. Unlike waterfowl, terrestrial

[f] Geographic or spatial structure occurs when sequences collected from similar locations cluster together. An example of geographic structure is that all WNV sequences from North America cluster together.

birds fly along more irregular patterns that are influenced by atmospheric conditions[151] and vegetation[150] and often follow looped or elliptical migratory patterns, favoring easterly routes during fall migration and westerly routes during the spring. Until recently, the general flyways of terrestrial birds have been poorly understood, in part because terrestrial birds are too small for use in banding studies and not often pursued as game birds, limiting collaboration with hunting communities. However, in 2014, La Sorte et al. provided the first description of terrestrial bird flyways in North America (Figure 1.7).[151] They defined a single distinct flyway, the Pacific flyway and two overlapping flyways; the Central and Eastern Flyways.

As with other avian pathogens, all previous attempts to correlate the movement of WNV with avian migration have relied exclusively on waterfowl migration patterns. Serological studies have been used to determine the direction of WNV movement within the Atlantic, Mississippi and Pacific Flyways and demonstrated evidence (ELISA, PRNT) of WNV in birds migrating southward, whereas they found limited evidence in birds during northward migration.[134,137] Phylogenetic studies have also found some evidence of geographic clustering in the Atlantic and Pacific Flyways.[108]

In this chapter, phylogeographic approaches were used to investigate the phylogenetic relationships between WNV isolates in the U.S. to identify the major sources of WNV circulation. Furthermore, the pattern of WNV movement was also evaluated with respect to the flyways of terrestrial birds.

4.2 Results

4.2.1 Sequence Collection

All previously published sequences of natural WNV isolates collected in the U.S. were obtained from Genbank on January 1, 2016 (Appendix III). The number of WNV sequences varied significantly over time and among locations, which presented significant statistical challenges. In particular, while Genbank has over 900 WNV open reading frames, the vast majority come from a few states where labs were actively undertaking surveillance and research on WNV, e.g. UTMB in TX. The ability to compare multiple isolates over multiple years was critical to the analysis. Only a few states had sufficient number of WNV sequences to allow analysis of multiple consecutive years: NY, CT, IL, ND, SD, TX and CA. To mitigate the influence of sampling bias additional WNV isolates were obtained from the WRCEVA for three states: VA (n=39), GA (n=20) and CO (n=31) to support the analysis. Given that previous studies have demonstrated significant isolation of WNV in CA, it was not included in the analysis. Similarly, due to the close proximity of NY and CT, one location, NY, was chosen to represent WNV in the Northeast as CT is a small state by size. Finally, to ensure that each location was represented across a similar time frame, only isolates collected between 2001 and 2009 were included in this study. The states and availability of isolates by year is shown in Table 4.1.

4.2.2 Model Selection

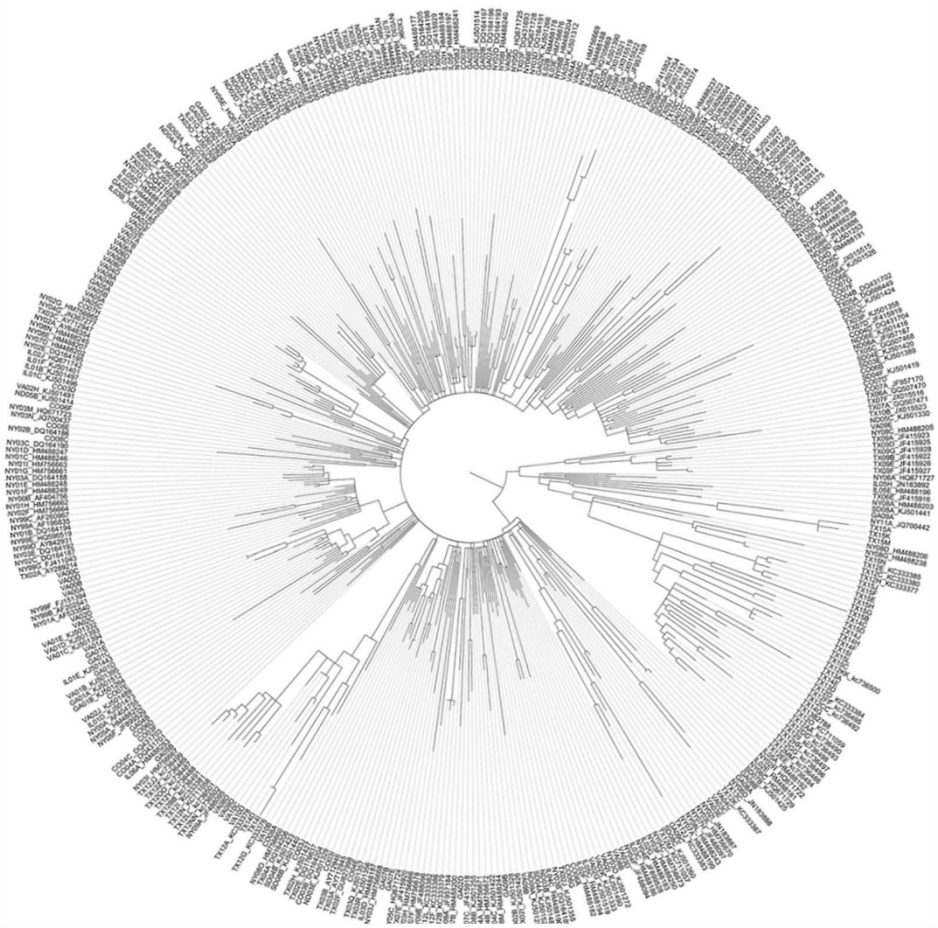
Two hundred and three nucleotide substitution models were compared using Bayesian and Alkaline Information Criteria in JModelTest2. The GTR+G+I model was found to be the most appropriate. A maximum likelihood tree using sequences of WNV strains from NY, VA, GA, IL, ND, SD, TX, and CO (n=405) (Figure 4.1) was used to assess temporal signature by determining the correlation coefficient between the root-to-tip distance and the date of isolation

Table 4.1. Summary of the years with available WNV sequences available.

The number of WNV sequences available are summarized in the table below. Xs indicate the years with sequences available.

Location	Years																# of Isolates
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	
NY	x		x	x	x	x	x		x	x			x				95
VA		x	x	x	x	x	x	x	x	x	x	x					52
GA			x	x		x	x		x	x	x						31
IL				x	x	x	x	x	x								41
TX				x	x		x	x	x		x	x	x	x	x	x	113
CO				x	x	x		x	x	x	x						50
ND				x	x	x	x	x		x	x						23
SD					x	x	x	x	x	x	x						22

Figure 4.1 Maximum likelihood phylogeny.
A maximum likelihood phylogeny was generated with all sequences from NY, VA, GA, IL, ND, SD, TX, and CO (n=405). Sequence name include the two-letter state abbreviation to indicate the origin of isolation, followed by the year of isolation. Multiple isolates collected from the same state within the same year are differentiated by letter. When possible, accession number is provided.



in Temp-Est (formerly known as Path-O-gen). A statistically significant positive correlation ($r=0.93$, 95% HPD= 0.92 - 0.94, $p<2.2 \times 10^{-16}$) was identified (Figure 4.2). The mutation rate was estimated to be 4.05×10^{-4} substitutions/per site/year (s/s/y) and the most recent common ancestor (MRCA) was in the year 1997. Together these results indicated that there was a strong temporal signal in the dataset. Finally, Bayesian tree priors (skride, skygrid and skline) and uncorrelated clock models (lognormal and exponential) were evaluated using path-sampling and stepping stone approaches. The uncorrelated lognormal clock model with the Bayesian skyline tree prior was found to be the most appropriate.

4.2.3 Phylogeographic analysis of the USA as a whole

Analysis of all WNV sequences collected from NY, VA, GA, IL, ND, SD, TX and CO between 2001 and 2009 provided estimates of the introduction date (MRCA) and mean evolution rate that were consistent with the estimates of the root-to-tip distance analysis (Figure 4.3, Table 4.2). The MRCA was estimated as 1997 while the average evolution rate was 3.92×10^{-4} s/s/y.

Markov Jumps between reconstructed ancestral states were used to estimate the magnitude of relative migration out of, or into, each of the eight regions (Table 4.3). Frequent migration (>2 Markov jumps) was detected from IL to CO (8.23 Markov jumps), IL to GA (8.11 Markov jumps), IL to ND (10.27 Markov jumps), Il to NY (29.46 Markov jumps), IL to SD (6.67 Markov jumps), IL to TX (22.49 migration events, IL to VA (11.25 Markov jumps), NY to CO (4.45 Markov jumps), NY to GA (7.17 Markov jumps), NY to SD (2.23 Markov jumps), NY to TX (4.93 Markov jumps), NY to VA (4.34 migration events), TX to CO (9.77 Markov jumps), TX to ND (5.19 Markov jumps), TX to SD (7.54 Markov jumps), and VA to GA (3.62 Markov jumps).

Figure 4.2 Analysis of temporal structure.
Root-to-tip distances were determined for each isolate using the maximum likelihood tree presented in supplemental Figure 1 and plotted against the year of isolation. The correlation between the root to tip distance and year of isolation was determined with linear regression shown blue. 95% Confidence interval is shown in grey. The equation of the linear regression line was used to estimate the year of the most recent common ancestor (MRCA) and the mutation rate (m): y= mx+MRCA

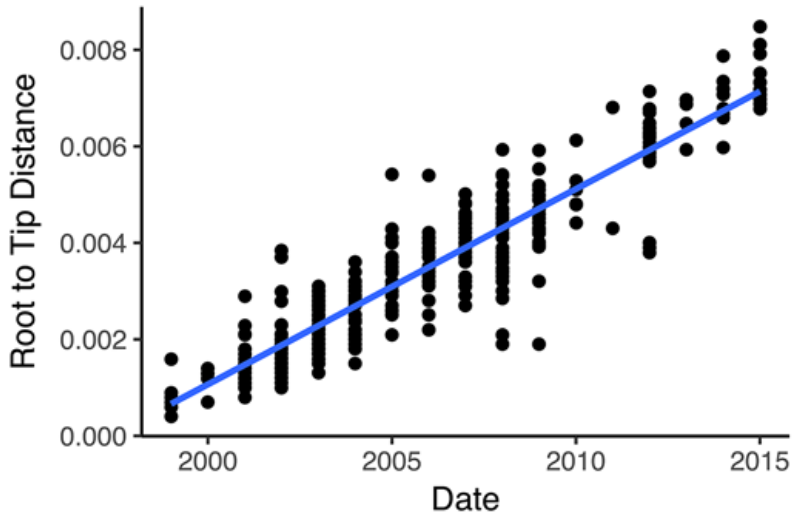


Figure 4.3 Bayesian phylogeny of Eastern and Central Flyways combined.
Bayesian phylogeny of WNV isolates collected in representative regions along the Eastern and Central Flyways between 2001 and 2009. Maximum clade credibility tree obtained using a Bayesian approach. The location of each isolate and the inferred location of each ancestor are depicted in the colors as described.

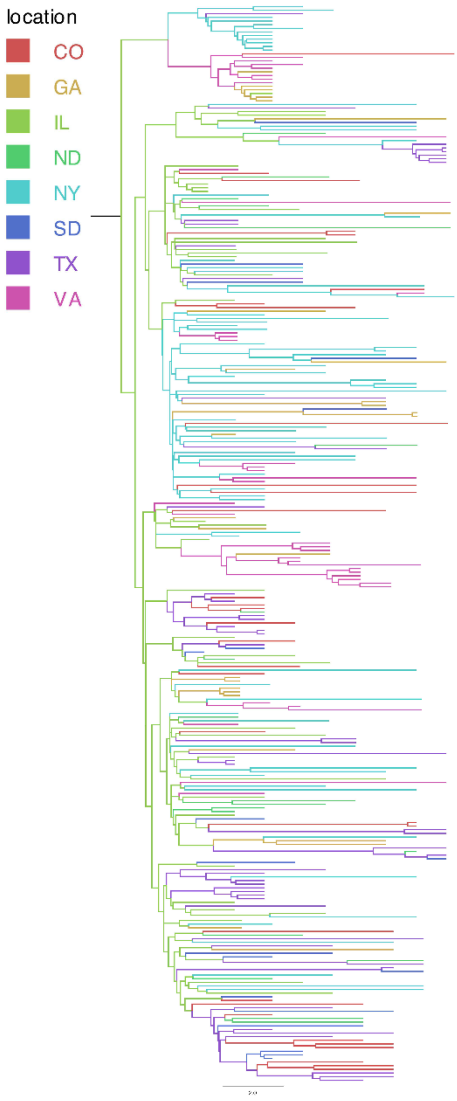


Table 4.2 Statistical support for the Eastern and Central Flyways combined.

Statistical support for the MCC tree, the estimated date of the most recent common ancestor (MRCA) and the mutation rate (ucld.mean).

	Mean	ESS[g]	95% HPD Interval
posterior	-49722.50	1370	-49803.21, -49640.55
prior	-3987.09	1110	-4051.92, -3916.56
likelihood	-45735.41	2179	-45780.00, -45691.43
MRCA	11.92	3119	10.82, 13.08
ucld mean	3.92E-4	1604	3.55e-4, 4.49e-4

[g] Effective sample size (ESS) is a measure of statistical support used in Bayesian phylogeny. Values above 200 are deemed acceptable.

Overall, three major sources of WNV circulation, NY, IL and TX, appeared to be the origin of 88.5% of the total migration events observed (Figure 4.4, Table 4.3). South and westward movement was detected along east coast, while only northward movement was observed along the central USA. A notable exception was observed in IL, which demonstrated evidence of WNV movement in all directions.

4.2.4 Phylogeographic analysis of the Eastern Flyway alone

Next, the movement of WNV within the Eastern and Central Flyways were considered separately. As IL is positioned in a region of overlap between the Eastern and Central Flyways, it was included in the models of both flyways. Analysis of WNV sequences collected along the Eastern Flyway (NY, VA, GA and IL) estimated the MRCA existed in 1997 and that the evolution rate was 3.73×10^{-4} s/s/y (Figure 4.5A and Table 4.4). Frequent movement was detected from IL to NY (29.59 Markov jumps), IL to VA (10.36 Markov jumps), IL to GA (8.94 Markov jumps), NY to GA (7.49 Markov jumps), NY to VA (5.72 Markov jumps), VA to GA (3.58 Markov jumps) and NY to IL (2.02 Markov jumps). In summary, movement was predominately detected heading south along the east coast (NY to VA, NY to GA, NY to IL, IL to GA, IL to VA and VA to GA) with the only evidence of northward movement occurring through IL [GA to IL (1.24 Markov jumps) and IL to NY (29.59 Markov jumps)]. The pattern of WNV circulation appeared to follow the elliptical pattern similar to the migration of terrestrial birds.

4.2.5 Phylogeographic analysis of the Central Flyway alone

In the Central Flyway, the MRCA was estimated to have occurred in 1998 (95% HPD 1999.29-1995.3) (Figure 4.5B and Table 4.4). The evolution rate was 4.07×10^{-4} (95% HPD

Table 4.3 Source sink analysis

Table summarizes the mean number of Markov Jumps detected between each source (origin) and sink (destination) location, or the minimum number of migration events observed from each source to each sink.

Source	Sink	Migration events (Markov Jumps)	ESS	95% HPD Interval
CO	GA	0.164	15683	[0, 1]
CO	IL	0.111	33920	[0, 1]
CO	ND	1.368	8603	[0, 3]
CO	NY	0.261	16508	[0, 1]
CO	SD	0.486	8222	[0, 2]
CO	TX	0.337	5909	[0, 2]
CO	VA	0.165	24157	[0, 1]
GA	CO	0.314	13738	[0, 2]
GA	IL	1.31	31284	[1, 3]
GA	ND	0.122	26193	[0, 1]
GA	NY	0.821	6736	[0, 3]
GA	SD	0.719	8787	[0, 2]
GA	TX	0.313	11570	[0, 2]
GA	VA	0.334	10761	[0, 2]
IL	CO	8.376	3311	[1, 14]
IL	GA	8.226	3046	[3, 13]
IL	ND	10.43	15423	[6, 14]
IL	NY	29.965	1355	[20, 40]
IL	SD	6.691	3635	[1, 11]
IL	TX	22.872	4545	[14, 30]
IL	VA	11.449	1631	[6, 16]
ND	CO	0.618	8832	[0, 3]

ND	GA	0.147	23908	[0, 1]
ND	IL	0.144	25515	[0, 1]
ND	NY	0.288	12855	[0, 2]
ND	SD	0.183	20239	[0, 1]
ND	TX	0.521	9813	[0, 2]
ND	VA	0.255	15630	[0, 1]
NY	CO	4.362	2143	[0, 8]
NY	GA	7.039	2170	[2, 11]
NY	IL	1.012	3023	[0, 4]
NY	ND	0.448	7434	[0, 2]
NY	SD	2.177	2740	[0, 5]
NY	TX	4.564	2149	[0, 9]
NY	VA	4.24	1171	[0, 8]
SD	CO	1.328	3964	[0, 5]
SD	GA	0.567	10905	[0, 2]
SD	IL	0.143	26368	[0, 1]
SD	ND	0.301	8901	[0, 2]
SD	NY	0.236	19732	[0, 1]
SD	TX	0.93	3517	[0, 4]
SD	VA	0.116	32792	[0, 1]
TX	CO	9.775	6039	[3, 16]
TX	GA	0.669	6777	[0, 3]
TX	IL	0.441	10249	[0, 2]
TX	ND	5.177	7617	[2, 9]
TX	NY	1.606	9410	[0, 4]
TX	SD	7.557	5113	[2, 12]
TX	VA	0.296	13706	[0, 2]
VA	CO	1.16	9807	[0, 3]
VA	GA	3.616	14054	[2, 6]
VA	IL	0.261	14878	[0, 1]
VA	ND	0.194	20523	[0, 1]
VA	NY	0.96	4048	[0, 3]
VA	SD	0.176	23197	[0, 1]
VA	TX	0.247	15068	[0, 1]

Figure 4.4 Summary of source sink analysis
The minimum number of migration events detected from the (A) Eastern Flyway (B) IL (C) Central Flyway. Only events that occur at least twice are depicted in the figure. Migration in the northward is indicated in red, Southward in teal and lateral migration in purple. Dotted arrows indicate migration that could not be confirmed by incident controlled down sampling due to an insufficient number of sequences.

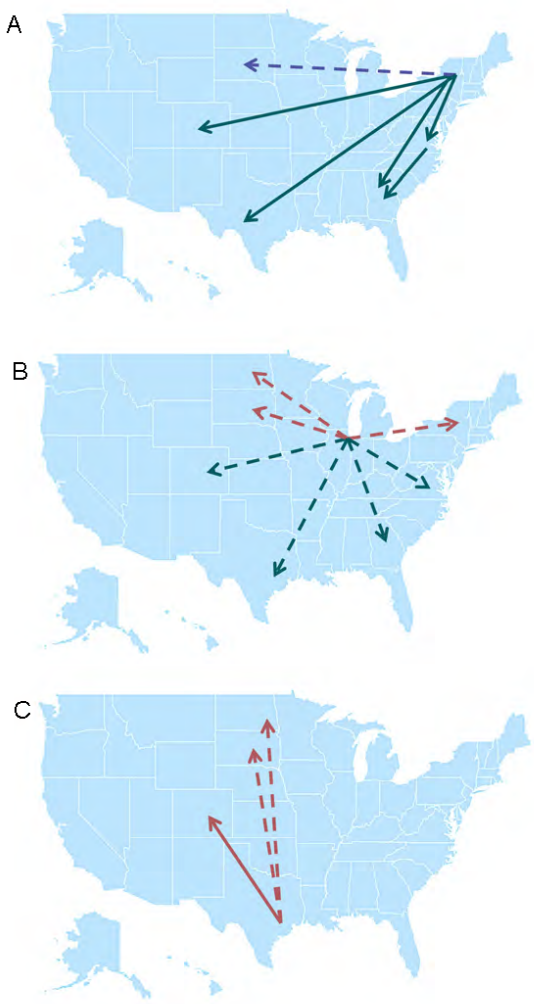


Figure 4.5 Bayesian phylogeny of Eastern or Central Flyways alone.
Bayesian phylogeny depicting the intra-flyways relationships of WNV isolates collected within the (A) Eastern or (B) Central Flyway between 2001 and 2009. The locations of each inferred ancestors are depicted in color.

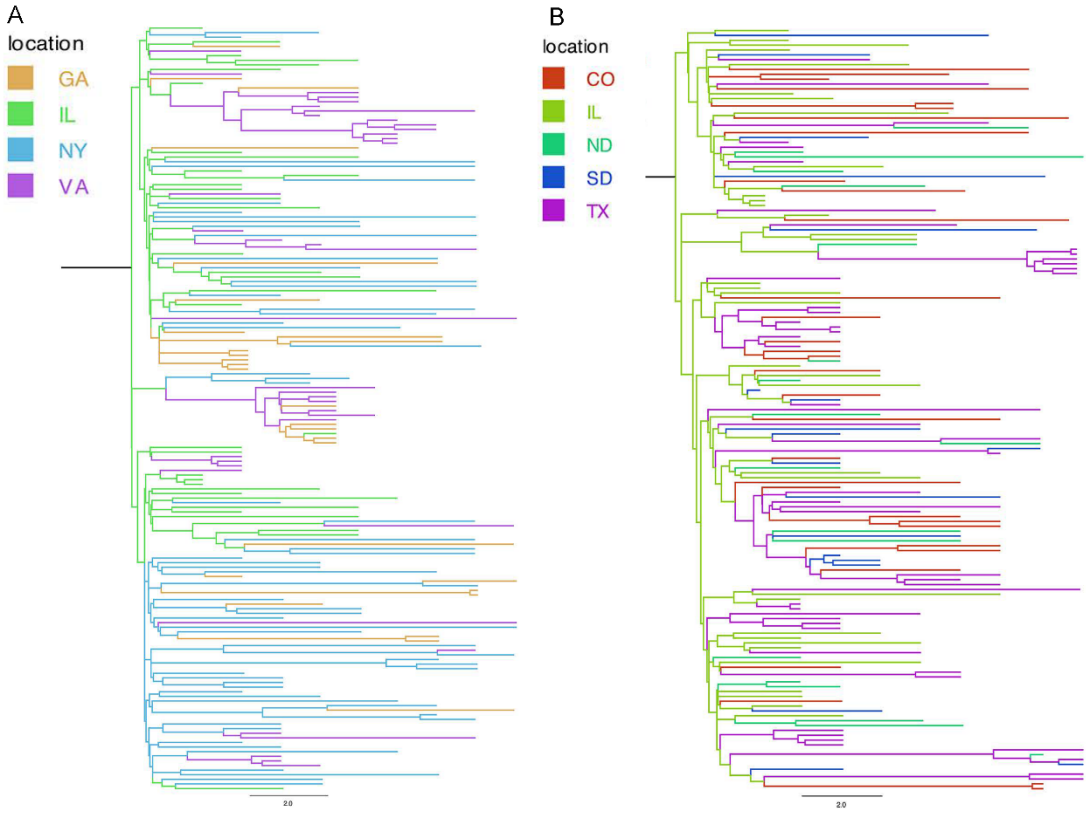


Table 4.4 Statistical support for the Eastern and Central Flyways separately.

Statistical support, estimated date of the most recent common ancestor (MRCA) and estimated evolution rate (ucld.mean) are provided.

	Eastern Flyway		
	Mean	ESS	95% HPD Interval
posterior	-35156.62	1057	-35235.25, -35074.35
prior	-3062.02	931	-3133.04, -2982.59
likelihood	-32094.59	2249	-32128.02, -32062.46
MRCA	11.59	19338	10.5334, 12.7521
ucld mean	3.73E-04	6801	3.32E-4, 4.14E-4
	Central Flyway		
	Mean	ESS	95% HPD Interval
posterior	-34949.296	802	-35003.2154, -34893.1167
prior	-3085.637	699	-3131.0506, -3038.286
likelihood	-31863.658	3986	-31892.3326, -31834.7097
MRCA	11.68	407	9.7063, 13.7041
ucld mean	4.07E-04	603	3.5387E-4, 4.6119E-4

3.54 x 10^{-4} - 4.61 x 10^{-4}) s/s/y. The results of the Markov Jump analysis revealed strong evidence of northward, not southward, movement in the Central Flyway (Figure 4.6). Northward movement was detected from IL to SD (11.39 Markov jumps), IL to ND (11.61 Markov jumps), TX to CO (7.25 Markov jumps), TX to SD (5.77 Markov jumps) and TX to ND (4.56 Markov jumps). Interestingly, the only significant evidence for southward movement was observed from IL to TX (28.87 Markov jumps) and from IL to CO (16.36 Markov jumps). Again, like the Eastern Flyway WNV movement appears to follow the elliptical patterns of terrestrial birds with southward movement occurring further east and northward movement occurring further west.

4.2.6 Incidence-controlled phylogeny

Unfortunately, both the annual WNND incidence and sample collection efforts varied dramatically among the states over time, adding significant complexity to the model. To mitigate the effects of inconsistent sampling, a stricter inclusion criterion was applied to ensure that the dataset was representative of WNV activity in each region in a particular year. In this approach, the sequences were randomly down-sampled using the sample command in R, such that the number of sequences was proportional to the incidence of WNND reported to the CDC (Figure 4.7, Table 4.5). IL, ND and SD were not included in the down-sampled datasets as there were insufficient sequences to represent the WNND incidence in these states. To ensure that reduction is sample size and diversity did not remove important relationships, the down-sampling was done twice independently.

According to the two incidence-controlled datasets, the MRCA was 1997 in both down-sampling exercises (95% HPD 1996.00-1998.52 and 95% HPD 1995.7-1998.25, respectively) and the overall mutation rate was estimated to be 4.02 x 10^{-4} s/s/y and 3.83 x 10^{-4} s/s/y,

Figure 4.6 Summary of Markov jumps within the Eastern and Central Flyways.
The minimum number of Markov jumps detected between each location within the Eastern (A) and Central (B) Flyways are depicted as box plots. Markov jumps occurring at least twice are summarized in figures (C) and (D) respectively. Southward movement was depicted in teal and northward movement was in red. Dotted arrows represent relationships that could not be confirmed by incident-controlled datasets because too few sequences were available from one or more of the locations involved.

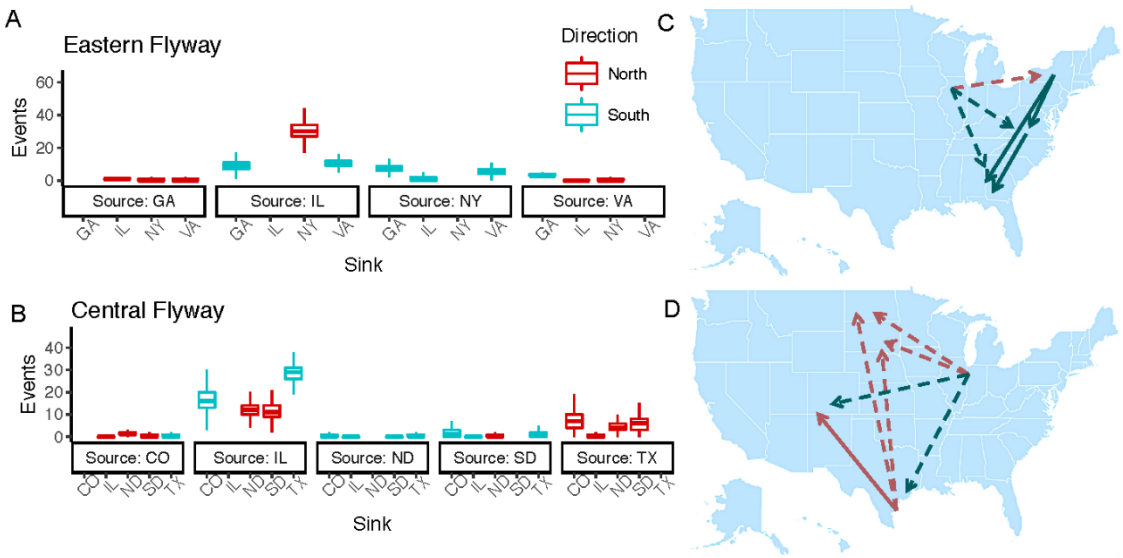
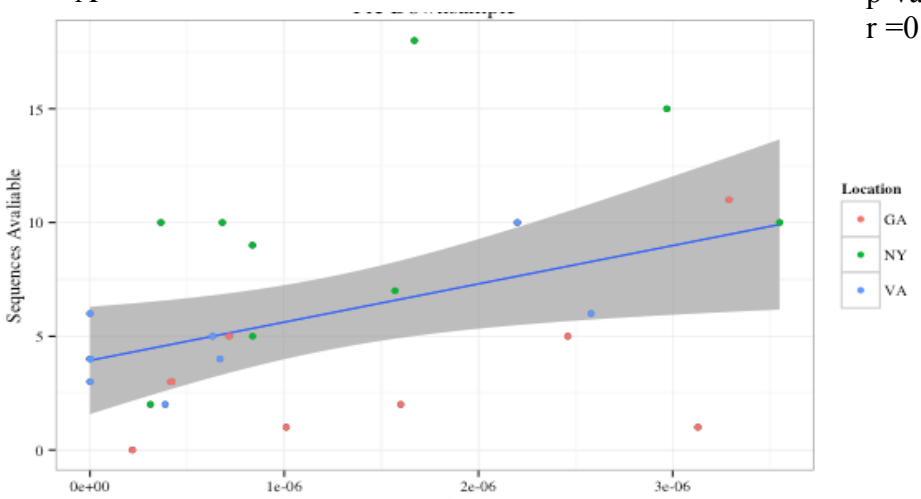


Figure 4.7 Incidence-controlled down-sampling strategy.
The correlation between the number of WNV sequences available and WNND incidence was evaluated using linear regression and Pearson's correlation method before (A) and after (B)down-sampling. Linear regression line is indicated in blue and the 95% CI is represented in grey.

A

p-value = 0.02379
r =0.4337719



B

p-value = 8.232e-05
r =0.6844588

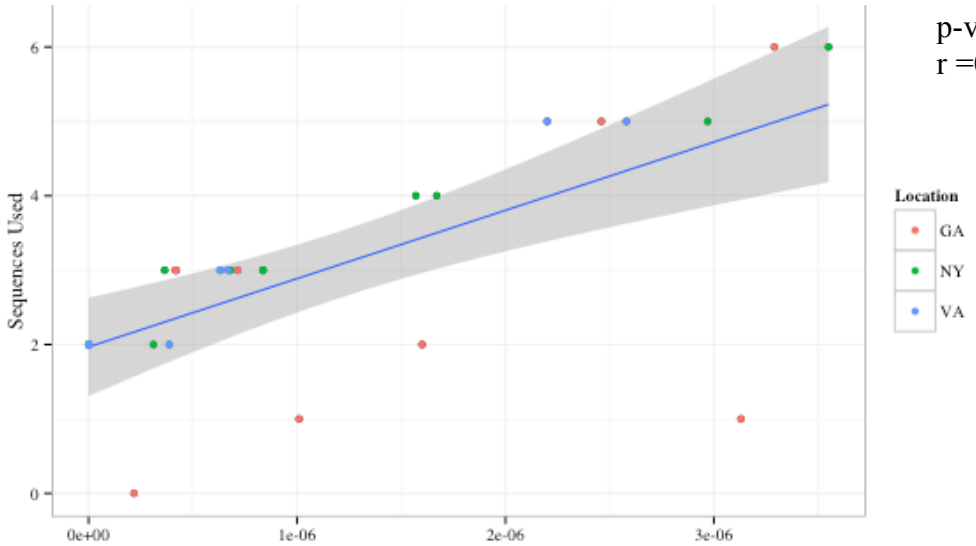


Table 4.5 Incidence-controlled down-sampling strategy.

The number of WNV sequences available and the number of sequences used in the down-sampled dataset are summarized below.

Location	Year	WNV Incidence	Sequences Available	Sequences Used
GA	2001	7.16E-07	5	3
	2002	3.29E-06	11	6
	2003	3.13E-06	1	1
	2004	1.60E-06	2	2
	2005	1.01E-06	1	1
	2006	2.18E-07	0	0
	2007	2.46E-06	5	5
	2008	4.21E-07	3	3
	2009	4.16E-07	3	3
NY	2001	6.81E-07	10	3
	2002	3.55E-06	10	6
	2003	2.97E-06	15	5
	2004	3.65E-07	10	3
	2005	1.57E-06	7	4
	2006	8.37E-07	5	3
	2007	8.36E-07	9	3
	2008	1.67E-06	18	4
	2009	3.11E-07	2	2
VA	2001	0.00E+00	6	2
	2002	2.20E-06	10	5
	2003	2.58E-06	6	5
	2004	6.69E-07	4	3
	2005	0.00E+00	3	2
	2006	0.00E+00	4	2
	2007	3.87E-07	2	2
	2008	0.00E+00	4	2
	2009	6.31E-07	5	3

respectively (Figure 4.8, Table 4.6A and B). As with the full dataset, the Markov analysis demonstrated that NY and TX were strong sources of WNV circulation. Significant movement (mean >2 Markov jumps)was detected from TX to CO (20.42 and 20.44 Markov jumps), TX to NY (12.36 and 11.77 Markov jumps), TX to GA (8.28 and 9.55 Markov jumps), TX to VA (7.14 and 7.732 Markov jumps), NY to GA (6.1 and 5.38 Markov jumps), NY to VA (4.95 and 3.65 Markov jumps), NY to CO (4.04 and 2.66 Markov jumps), NY to TX (2.66 and 2.73 Markov jumps), VA to GA (1.55 and 3.62 Markov jumps)and in dataset 2 only VA to CO (1.31 Markov jumps).

Together, the Markov Jump analysis of the incidence-controlled dataset and the full dataset illustrated an interesting pattern of WNV circulation. All southward movement originated in the eastern US (NY and VA) and most of the northward movement originated in the Central US (TX).

4.2.7 Incidence-controlled Eastern Flyway phylogeny

In the Eastern Flyway, down-sampling was undertaken twice, independently. Unfortunately, all sequences from IL had to be removed from the down-sampled datasets because too few sequences were collected in the years 2007-2009. The results of the incidence-controlled analysis in the Eastern Flyway were consistent with the trends observed in the full dataset. The topology of each maximum clade credibility tree is summarized in Figure 4.9A-B. The mutation rate for each of the two trees was found to be very similar, 3.37×10^{-4} -3.49×10^{-4} s/s/y. The two datasets also reported similar MRCA estimates (1996 and 1997) (Table 4.6C and D). Markov jump analysis confirmed strong evidence of southward movement in the incidence-controlled sampled datasets (Figure 4.10). NY was identified as a major source of WNV

Figure 4.8 Incidence-controlled phylogeny of Eastern and Central Flyway together. Sequences were down-sampled such that the number of sequences was proportional to the WNND incidence in each location during each year between 2001-2009. Down-sampling was undertaken (a and b) twice to ensure that the reduction in sequences did not result in a significant loss in diversity. Illinois, North Dakota and South Dakota were not included the incidence-control analysis because too few sequences were available from either location to support down-sampling. Bayesian approaches was used to generate maximum clade credibility trees. The locations of each isolate and the inferred ancestors are represented in color. (Colorado= Red, Georgia= Light Green, New York= Dark Green, Texas= Blue, Virginia= Purple)

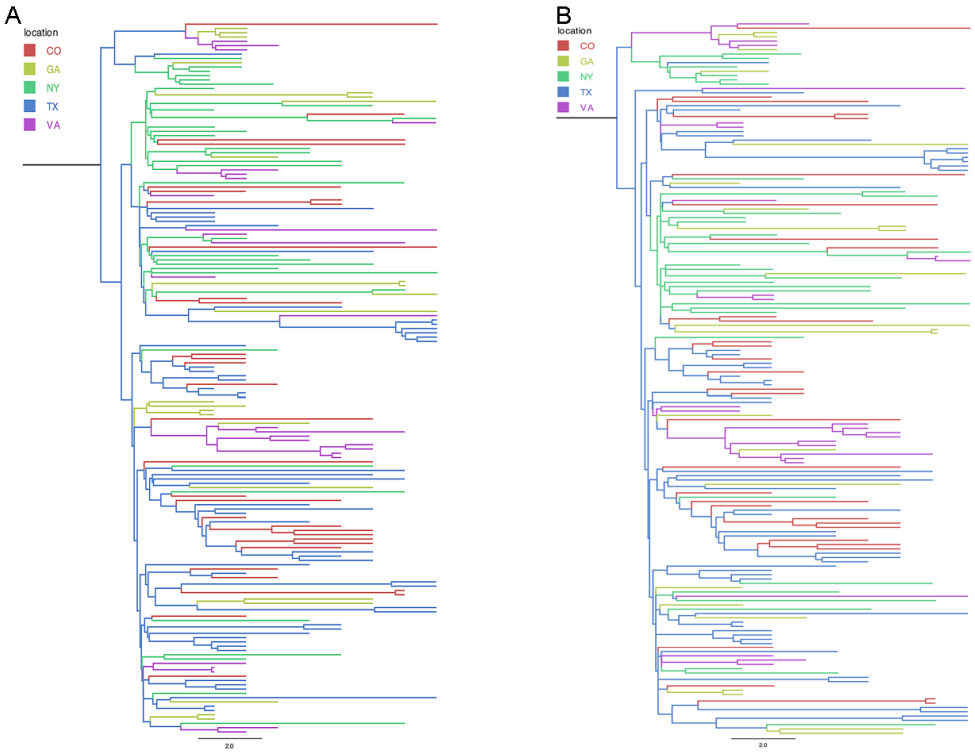


Table 4.6 Statistical support for the incidence-controlled phylogenies.

Statistical support for the incidence-controlled phylogenies. Summary of the statistical support of the (a) Combined Eastern and Central Flyway, (b) Eastern Flyway and (c) Central Flyway. Posterior, prior and likelihood values are provided as well as the estimated most recent common ancestor (TMRCA) and mutation rate
(ucld.mean).

	Eastern and Central Flyways Combined					
	Dataset 1			Dataset2		
	Mean	ESS	95% HPD Interval	Mean	ESS	95% HPD Interval
posterior	-36338.16	1520	-36394.41, -36280.28	-36799.24	1252	-36853.60, -36741.26
prior	-3141.85	1393	-3189.48, -3090.67	-3153.35	979	-3201.05, -3104.67
likelihood	-33196.35	61588	-33228.01, -33164.71	-33645.90	1391	-33678.63, -33614.56
tmrca	11.66	5621	10.47, 12.99	11.98	5231	10.73, 13.28
ucld.mean	4.02E-04	2754	3.51E-4, 4.52E-4	3.82E-04	1514	3.30E-4, 4.36E-4

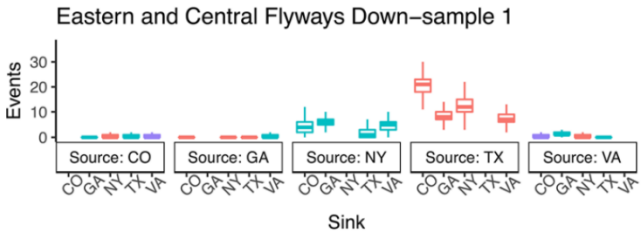
	Eastern Flyway					
	Dataset 1			Dataset2		
	Mean	ESS	95% HPD Interval	Mean	ESS	95% HPD Interval
posterior	-26208.90	2284	-26254.35, -26160.36	-26631.30	1284	-26683.99, -26575.34
prior	-2638.75	1899	-2680.76, -2594.12	-2633.50	1102	-2682.43, -2581.46
likelihood	-23570.16	6910	-23592.94, -23548.45	-23997.80	8410	-24020.50, -23975.75
tmrca	12.10	22276	10.7989, 13.5072	12.18	18599	10.88, 13.56
ucld.mean	3.59E-04	14145	3.0565E-4, 4.1408E-4	3.56E-04	3219	2.92E-4, 4.19E-4

Central Flyway			
	Mean	ESS	95% HPD Interval
posterior	-27497.43	1728	-27535.01, -27458.68
prior	-2670.41	1898	-2701.94, -2638.47
likelihood	-24827.02	4016	-24848.86, -24807.12
TMRCA	10.65	316	8.9734, 12.62
ucld.mean	4.588E-4	1227	3.7869E-4, 5.4457E-4

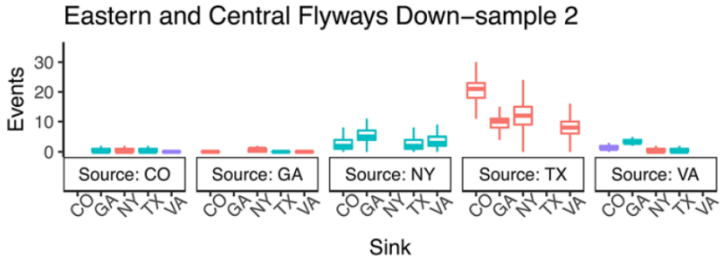
Figure 4.9 Incidence-Controlled investigation of phylogenic relationships within the Eastern and Central Flyways.
(A and B) Sequences isolated from within the Eastern Flyway were down-sampled in duplicated such that the number of sequences was proportional to the WNND incidence in each location during each year between 2001-2009. (C) Sequences isolated from the Central Flyway did not require down-sampling because there was already a positive correlation between WNND incidence and the number of sequences available. Bayesian approach was utilized to infer phylogenetic relationships and trees were summarized as maximum clade credibility trees. The locations of each isolate and the inferred ancestors are represented in color. (New York=Green, Georgia= Red, Virginia= Dark Blue, Texas= Light Blue, Colorado= Red)

Figure 4.10 Incidence-controlled analysis of virus movement within flyways. Northward movement is depicted in red and southward movement in teal. Dotted arrows indicated relationships that could not be confirmed in incident controlled datasets due to insufficient number of sequences available.

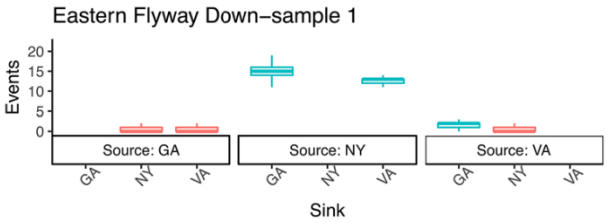
A



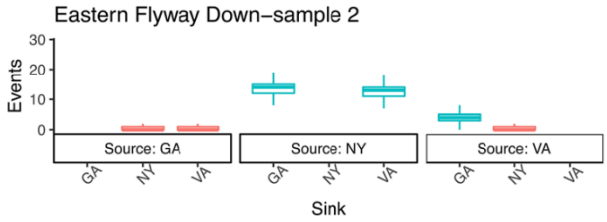
B



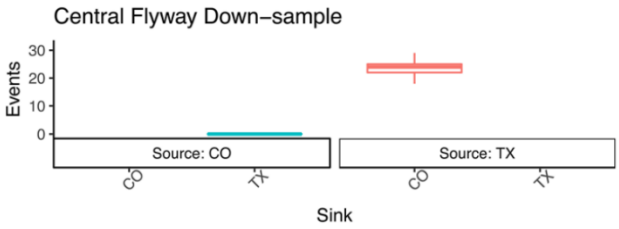
C



D



E



movement along the east coast. Both datasets detected similar amounts of movement from NY to VA (13.59 and 12.87 Markov jumps). Similar trends were observed from NY to GA (14.1 and 13.28 Markov jumps). A small amount of movement was detected from VA to GA (1.98 and 4.34 Markov jumps). Little to no movement was detected from VA to NY, GA to NY or GA to VA.

4.2.8 Incidence-controlled Central Flyway phylogeny

In the Central flyway, despite consistently high incidence of WNND in IL, ND and SD, there was poor correlation between the number of sequences available from each location and the WNND incidence. To control for biased sample collection, only TX and CO were used to define the direction of WNV movement in the Central Flyway. In TX and CO, there was a strong correlation between the number of sequences collected each year and WNND incidence between 2004 and 2009, suggesting that down sampling was not necessary; although in 2003 the number of sequences collected in CO was much less than necessary to adequately represent the incidence of WNND for that year. Nevertheless, all available sequences from CO during 2003 were utilized to ensure that the CO outbreak of 2003 was represented as well.

The topology of each maximum clade credibility tree is summarized in Figure 4.9C. Like the Eastern Flyway, the mutation rate was estimated to be 4.49 x 10^{-4} s/s/y with the MRCA 1998 (95% HPD 1996-2000) (Table 4.6). Ample movement was detected from TX to CO (23.25 Markov jumps), but little to no movement was observed from CO to TX (0.31 Markov jumps) (Figure 4.10E).

4.3 Discussion

The introduction and subsequent spread of WNV into the Americas underscores the

invasive potential of emerging pathogens in the New World, which has been recently exemplified by Zika virus, another mosquito-borne flavivirus. Dramatic variation in the location, timing, and intensity of WNV strain collection and sequencing has left the field with a limited understanding of virus circulation patterns and no reliable way of predicting the flow of WNV outbreaks. This gap in knowledge is addressed here by characterizing the movement of WNV with regards to the migratory patterns of its natural hosts, terrestrial birds. To this end, 405 viral sequences were compiled for analysis, including 379 previously reported sequences from NY, VA, GA, IL, ND, SD, TX and CO plus 90 novel sequences from VA, GA, CO and TX.

Phylogeographic analysis revealed that three geographic locations, NY, IL and TX, accounted for 88.5% of the total WNV Markov jumps inferred. As NY was the original introduction point for WNV into the U.S., its role as a major source of WNV movement was expected. However, 74.2% of the observed Markov jumps originated in IL and TX only. Interestingly, ND and SD, which are two of the states with the highest annual incidence of WNV, appear to be strong sinks for WNV moving out of both IL and TX.

Furthermore, the contribution of both IL and TX to WNV circulation is not surprising, as both locations are situated at important convergence points between the Eastern and Central Flyways. In the case of TX, birds from both flyways may avoid long distance flights across the Gulf of Mexico by traveling along the circa-gulf route that follows the Gulf Coast through TX into Mexico (Figure 4.11). In the case of IL, seasonal shifts in terrestrial bird migration routes ensure that IL supports birds from the Eastern Flyway during spring migration, and birds from the Central Flyway during fall migration.

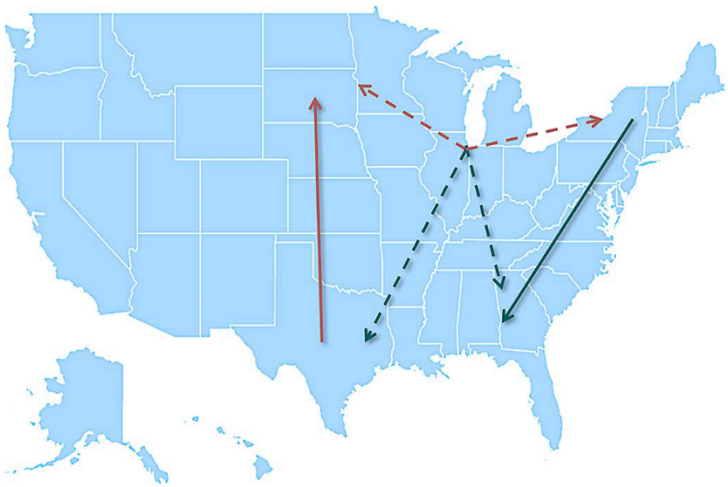
Figure 4.11 Circa-Gulf route.
As birds from the eastern and central USA migrate south they encounter the Gulf of Mexico. Instead of flying directly across the Gulf of Mexico, some birds prefer to fly along the coast on the circa-Gulf route. Map was created with ArcGIS.

While it is recognized that other geographic sources may exist, there were insufficient viral sequences available from other states to undertake an analysis. Thus, based on the information currently available, NY, IL and TX are the optimal sites to efficiently monitor ongoing WNV evolution and target insecticide campaigns. For instance, insecticide campaigns are currently focused on urban areas; however, information provided in this study suggests that WNV transmission among resident and migratory birds could be reduced by spraying in rural stop-over sites located in major source locations (NY, IL and TX).

In addition, the overall pattern of WNV circulation in the USA was defined (Figure 4.12). The results of the analysis demonstrated elliptical virus movement patterns in the Eastern and Central Flyways that are bridged by IL, a region shared between the two flyways. This specific pattern correlates with the elliptical migration patterns of terrestrial birds. To my knowledge, this is the first time phylogeographic methods have been used to correlate pathogen and terrestrial bird migration patterns in the Americas.

Unfortunately, due to computational challenges and the limited number of WNV sequences available, only eight locations could be used in this study. However, it is possible, if not likely, that additional locations exist that are also important sources of WNV circulation, but could not be identified here due to the unavoidable limitations describe above. As new WNV sequences become available, similar phylogeographic methods can be used to develop a more detailed understanding of WNV circulation in the USA. For example, on the east coast, WNV circulation occurs southward direction, so surveillance efforts in the northeast are likely to be

Figure 4.12 Model summarizing the general patterns of WNV movement in the US. Northward movement is depicted in red and southward movement in teal. Dotted arrows indicated relationships that could not be confirmed in incident controlled datasets due to insufficient number of sequences available.



more informative than surveillance in the southeast. Conversely, WNV in the central USA travels north, so surveillance in the southcentral USA is more likely to be informative than the surveillance in the northcentral USA. Finally, surveillance efforts in the region of overlap between the Eastern and Central Flyways are the most likely locations to give rise the important surveillance information because WNV in this area travels in all directions.

Taken together, the results in this chapter illuminate the value of multidisciplinary approaches to surveillance of infectious diseases, especially in the case of zoonotic diseases. Animal migration is shaped by a delicate balance of ecological factors and anthropomorphic barriers. Natural and man-made events, such as climate change, atmospheric fluctuations, habitat destruction, etc., can drastically alter host behavior that, in turn, affects the circulation patterns of infectious agents such as WNV. In this study, the patterns of WNV circulation and key areas for surveillance were defined and correlated to the migratory patterns of their primary reservoir, terrestrial birds. While this information does not allow investigators to predict the size of annual WNV outbreaks, these advancements support the construction of targeted surveillance and vector mitigation strategies, predict the annual flow of WNV strains, and allow public health officials to anticipate changes in WNV circulation due to altered bird migration.

CHAPTER 5 EVOLUTION OF WEST NILE VIRUS IN TEXAS WITH A FOCUS ON THE HARRIS COUNTY 2014 OUTBREAK

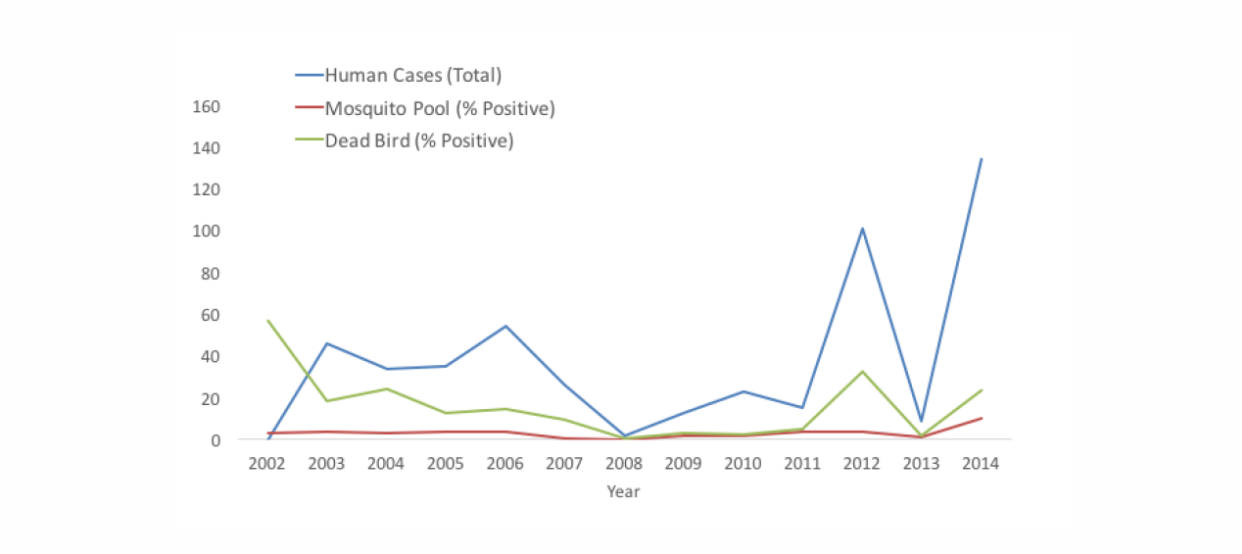
5.1 INTRODUCTION

The successful introduction of WNV into the USA has provided an exciting opportunity to study the evolution of an emerging pathogen in a large population of naïve hosts. However, given the size and varied topology, comprehensive monitoring of WNV throughout the USA has proven to be an expensive and time-consuming process. In fact, only 36 full ORF sequences of WNV have been published since 2013. Fortunately, in Chapter 4, three locations (TX, IL, and NY) were identified as major sources of WNV circulation in the USA. With so few recent sequences available, one location, TX, was chosen as a surrogate to potentially model the national evolution of WNV.

Within TX, the evolution of WNV has been most thoroughly characterized in Harris County, which is home to more than 4.5 million residents. It is also situated along the avian circa-Gulf route, which is a major point of convergence for multiple avian migration flyways. These features make Harris County an especially important location for WNV surveillance within TX.

Furthermore, this chapter will focus on the genomic and phenotypic characteristics of WNV isolates collected during 2014, which was the largest outbreak of WNND reported in the county to date. During the 2014 outbreak, 134 people were diagnosed with symptomatic infection.[182] Concurrently, this was associated with 1285 WNV-positive mosquito pools, which was more than 20% higher than had been observed in any year previously (Figure 5.1). Interestingly, numbers of WNV positive birds were higher in 2002 and 2012 (Figure 5.1); however, this may be due to changes in surveillance practices, which can be influenced by public involvement, community concerns, and political or financial support.

Figure 5.1 Summary of WNV Surveillance in Harris County Between 2002 and 2014.
WNV was first detected in June 2002; The number of human WN disease cases in Harris County are displayed in blue. The percent of WNV-positive dead birds and mosquito pools were indicated in red and green, respectively. The number of human cases was determined from CDC ArboNET (https://diseasemaps.usgs.gov/mapviewer/). Mosquito and bird surveillance was provided by Martin Reyna Nava of the Harris County Public Health & Environmental Services Department.



5.2 RESULTS

5.2.1 Genetic Analysis

Ten virus isolates were obtained from the brains of dead birds collected in Harris County between June 17, 2014 and October 27, 2014 (Figure 5.2). The isolates clustered from three geographic areas within Harris County: Northeast, Northwest and Southern. Four of the isolates were collected from blue jays, three from northern mockingbirds, and one each from a great-tailed grackle, house sparrow, and scissor-tailed flycatcher (Figure 5.2). Viral RNA was extracted from each isolate and sequenced using NGS technologies. The amino acid sequences of the ten Harris County isolates were compared to the prototype NY99-flamingo 382-99 strain (Tables 5.1 and 5.2).

Twenty-nine amino acid differences were identified (Table 5.1). All isolates contained the E-V159A substitution of the NA/WN02 genotype,[88,89] but not the NS4A-A85T or NS5-K314R substitutions characteristic of the SW/WN03 genotype.[94] Fifteen of the amino acid substitutions occurred in only one isolate while 14 occurred in multiple isolates. Interestingly, eight out of ten isolates had a substitution at position NS2A-R188K and six at position NS4B-I240M.

5.2.2 Phylogeny

A maximum likelihood phylogeny was generated of all WNV isolates collected in the Americas (Figure 5.3). The 2014 Harris County isolates clustered within the NA/WN02 genotype along with isolates collected from Harris County in 2012, 2013, 2015 and 2016. Isolates containing the NS2A-R188K substitution clustered together in a single clade (bootstrap 99) of WNV isolates collected in TX, NY, CT, Mississippi (MS), New Mexico (NM), Wisconsin

Figure 5.2 Sample information for WNV isolates collected in Harris County during 2014. The map summarizes the location from which WNV-positive bird isolates were collected in Harris County. The species of bird and the date of collection are also provided.

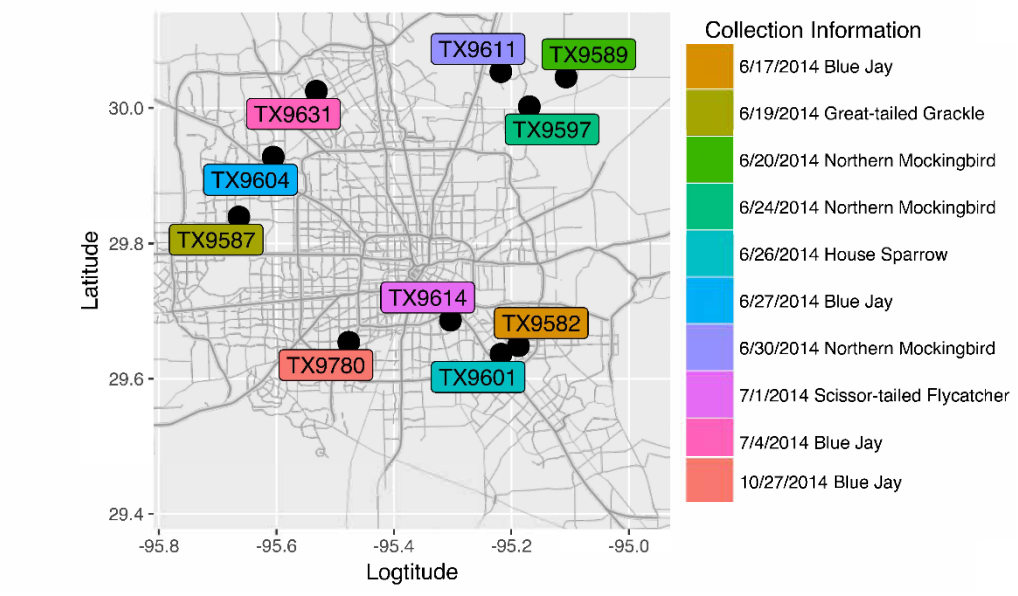


Table 5.1 Summary of amino acid substitutions.

All amino acid substitutions are summarized relative to the NY99 prototypical strain of WNV.

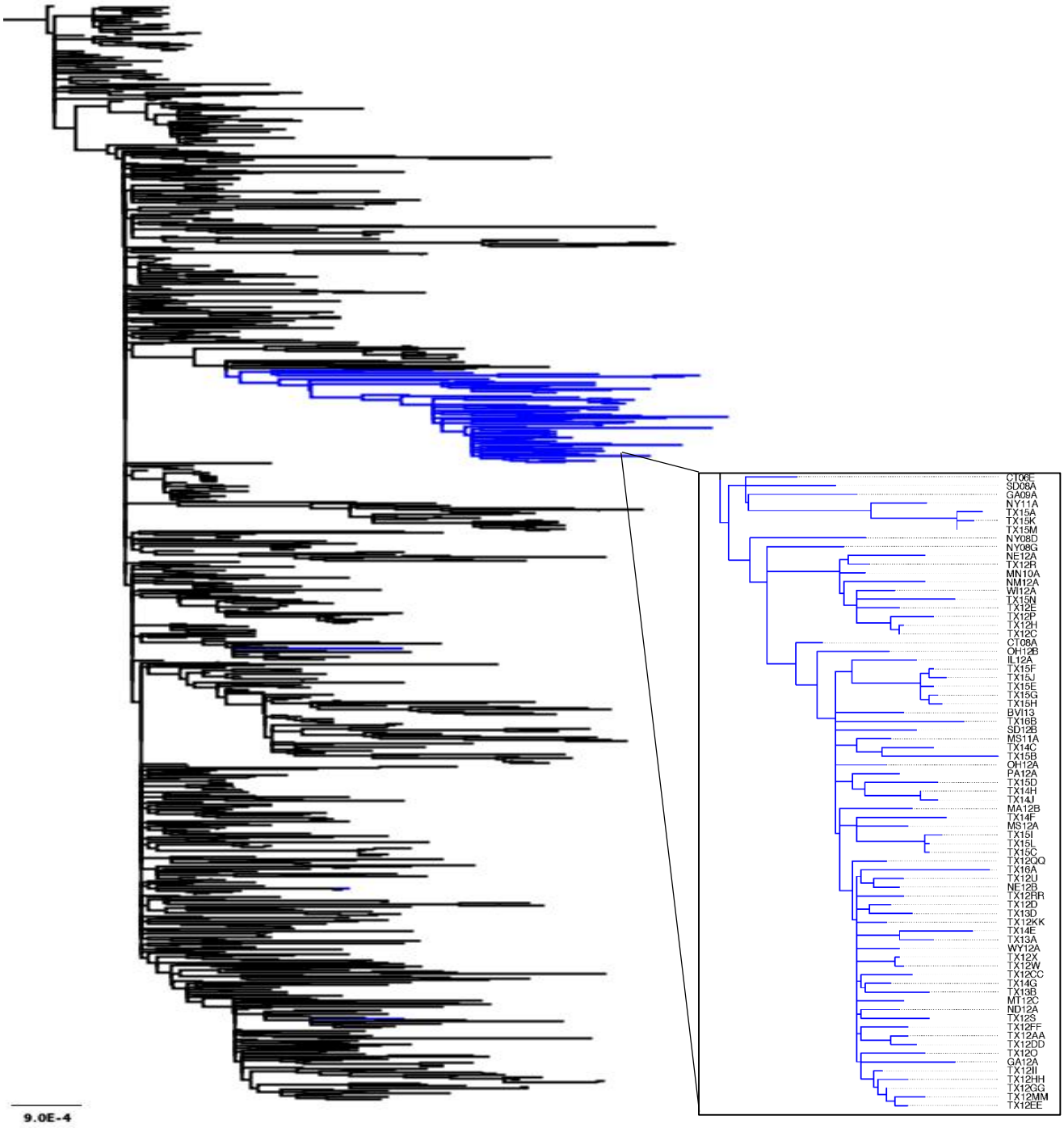
		NY99	TX 9582 (D0130)	TX 9589 (D0137)	TX 9587 (D0135)	TX 9631 (D0179)	TX 9614 (D0162)	TX 9780 (D0329)	TX 9601 (D0149)	TX 9604 (D0152)	TX 9597 (D0145)	TX 9611 (D0159)
C	10	K	.	.	.	.	.	.	.	R	.	.
	144	M	.	.	.	.	.	.	.	.	.	I
prM	157	V	.	.	.	.	A	A	.	.	.	.
	159	V	A	A	A	A	A	A	A	A	A	A
E	361	F	.	Y	.	.	.	.	.	.	.	.
NS1	108	T	.	.	.	M	.	.	.	.	.	.
	52	T	.	.	.	.	I	I	.	.	.	.
	58	V	.	I	.	I	.	.	.	.	.	.
	95	L	F	.	.	.	F	F	F	.	.	F
	188	R	K	K	K	K	.	.	K	K	K	K
NS2A	200	A	.	.	.	S	.	.	.	.	.	.
	26	I	.	.	.	.	.	.	.	V	.	V
NS2B	14	A	T	.	.	.	.	.	T	.	.	.
	162	I	.	.	M	.	.	.	.	.	.	.
	334	S	.	.	.	.	T	T	.	.	.	.
	356	T	.	.	I	.	.	.	.	.	.	.
NS3	436	T	.	.	.	.	.	.	.	.	.	P
	14	S	.	.	.	.	I	I	.	.	.	.
	15	S	.	.	N	.	.	.	.	.	.	.
	18	G	E	.	.	.	.	.	E	.	.	.
	163	E	.	.	.	.	D	D	.	.	.	.
NS4B	240	I	M	M	M	.	.	.	M	.	M	M
	91	M	.	.	.	.	.	I	.	.	.	.
	195	M	I	.	.	.	.	.	I	.	.	.
	673	K	.	R	.	.	.	.	.	.	.	.
	745	I	.	.	.	.	.	V	.	.	.	.
	814	M	.	.	L	.	.	.	.	.	.	.
	837	K	.	.	.	R	.	.	.	.	.	.
NS5	889	S	.	.	.	R	.	.	.	.	.	.

Table 5.2 Summary of nucleotide substitutions in the UTRs.

All nucleotide substitutions within the 5’ and 3’ UTRS are summarized relative to the NY99 prototypical strain of WNV.

		NY99	TX 9582 (D0130)	TX 9589 (D0137)	TX 9587 (D0135)	TX 9631 (D0179)	TX 9614 (D0162)	TX 9780 (D0329)	TX 9601 (D0149)	TX 9604 (D0152)	TX 9597 (D0145)	TX 9611 (D0159)
	19	g	a	a	a	a	.	.	a	a	a	a
	46	g	.	.	.	a	.	.	.	.	.	.
5'UTR	56	a	.	.	.	.	.	.	.	g	.	.
	10408	c	.	.	.	.	t	t	.	.	t	.
	10425	t	c	.	.	.	.	.	c	.	.	.
	10426	a	.	.	g	.	.	.	.	.	.	.
	10427	t	.	.	c	.	.	.	.	.	.	.
	10429	a	.	.	.	.	.	.	.	.	.	g
	10459	t	c	.	c	.	.	.	c	.	c	.
	10469	t	.	.	.	.	.	.	.	.	.	-
	10470	g	.	.	.	.	.	.	.	t	.	-
	10471	t	.	.	.	.	.	.	.	-	.	.
	10472	t	.	.	.	.	.	.	.	-	.	.
	10492	a	.	.	.	.	.	-	.	.	.	.
	10493	t	.	.	.	.	.	.	.	a	.	.
	10516	g	.	.	.	.	a	a	.	.	.	.
	10520	t	.	.	.	.	.	.	.	.	c	.
	10591	c	.	.	t	.	.	.	.	.	.	.
	10632	g	.	.	.	.	t	t	.	.	.	.
	10688	t	.	.	.	.	c	c	.	.	.	.
	10851	a	g	g	g	g	g	g	g	g	g	g
	11027	t	-	.	.	.	a	.	.	.	.	.
	11209	t	-	-	.	-	-	-	-	a	-	-
3'UTR	112030	-	-	-	g	-	-	-	-	-	-	-

Figure 5.3 Phylogenetic analysis of WNV in the USA.
All WNV isolates collected in the USA between 1999 and 2016 we analyzed using maximum likelihood methods. Branches in blue indicate sequences that contain the NS2A-R188K substitution.



(WI), Ohio (OH), Illinois (IL), Ohio (OH), Pennsylvania (PA), Massachusetts (MA) and the British Virgin Islands (BVI) between 2008 and 2016. Interestingly, the NS2A-R188K substitution was also present in WNV isolates collected in Africa, Europe and Russia as early as 1958 (Figure 5.4).

A Bayesian phylogenetic approach was used to investigate the genetic relationships among all TX isolates collected between 2002 (the first year of WNV in TX) and 2016 (Figure 5.5). The inferred mutation rate was 4.88 x 10^{-4} [95% CI 4.11x10^{-4} – 5.60 x 10^{-4} s/s/y] and the MRCA occurred in 1998 (95% CI 1996-2001). All isolates collected during the 2014 Harris County outbreak clustered closely with isolates collected between 2012 and 2016 (Figure 5.5). Isolate TX9631 clustered with isolate TX9364 (posterior= 1) and shared a common ancestor in 2011 (95% CI 2010-2013). Isolate TX9589 clustered with TX9388 (posterior =0.97) and shared a common ancestor that occurred in 2010 (95% CI 2009-2012). Isolates TX9614 and TX9780, which lacked the NS2A-R188K and NS4B-I240M substitutions, clustered closely with two isolates collected during 2012 (posterior =1), TX8546 and TX AR12-7025 (posterior =1) and shared a common ancestor in 2009 (95% CI 2008-2011). The 2014 isolates TX9604, TX9582, TX9601, TX9611, TX9597 and TX9587 clustered together with isolates from 2012 (posterior =1). Isolate TX9587 diverged from the cluster during 2008 (95% CI= 2008-2010) (posterior =1). Isolates TX9582 and TX9601 (posterior = 1) shared a recent common ancestor within a year of their collection, while isolates TX9597, TX9611, TX9604 diverged in 2009 (95% CI 2008-2010, posterior=0.49). TX9597 diverged from TX9611 and TX9587 in 2009 (95% CI= 2008-2010, posterior=0.79). Finally, isolate TX9587 (posterior =1) diverged in 2008 (95% CI 2008-2010).

5.2.3 Intra-host diversity

Figure 5.4 Worldwide distribution of WNV.
A maximum likelihood phylogeny was generated to display the distributions of sequences with the NS2A-R188K substitution. Sequences containing the NS2A-R188K substitution are indicated in blue. When available the country and year of isolation is provided in the following format: Accession number_Country|Date.

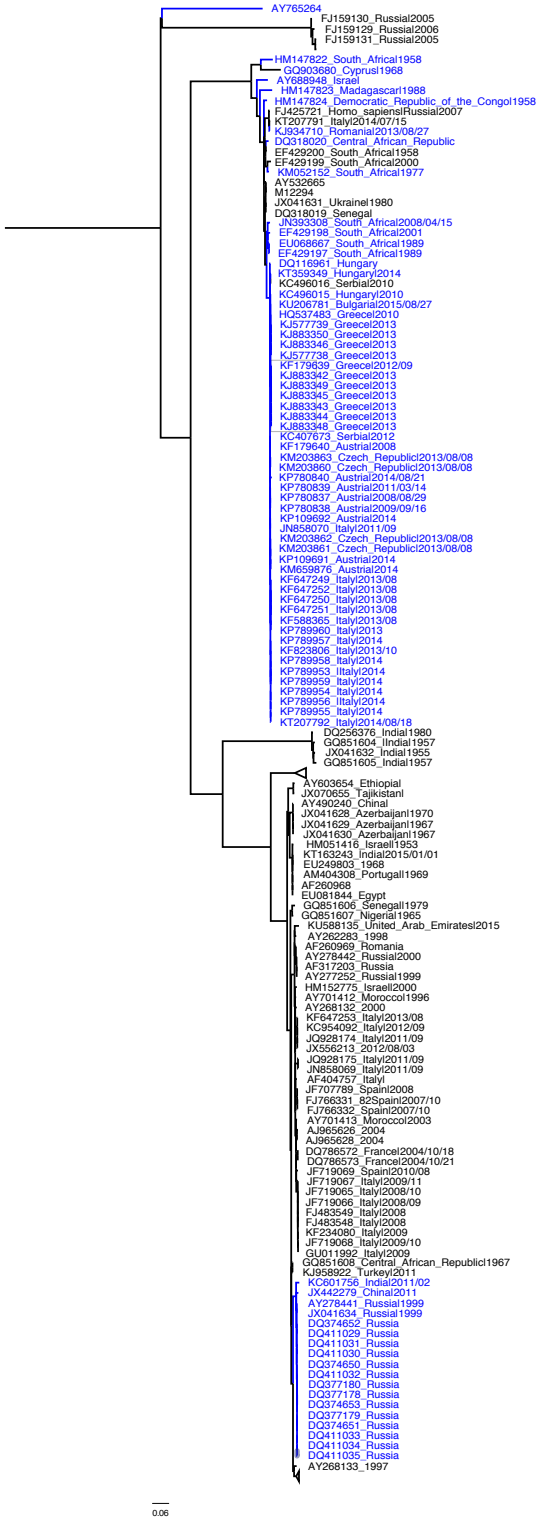
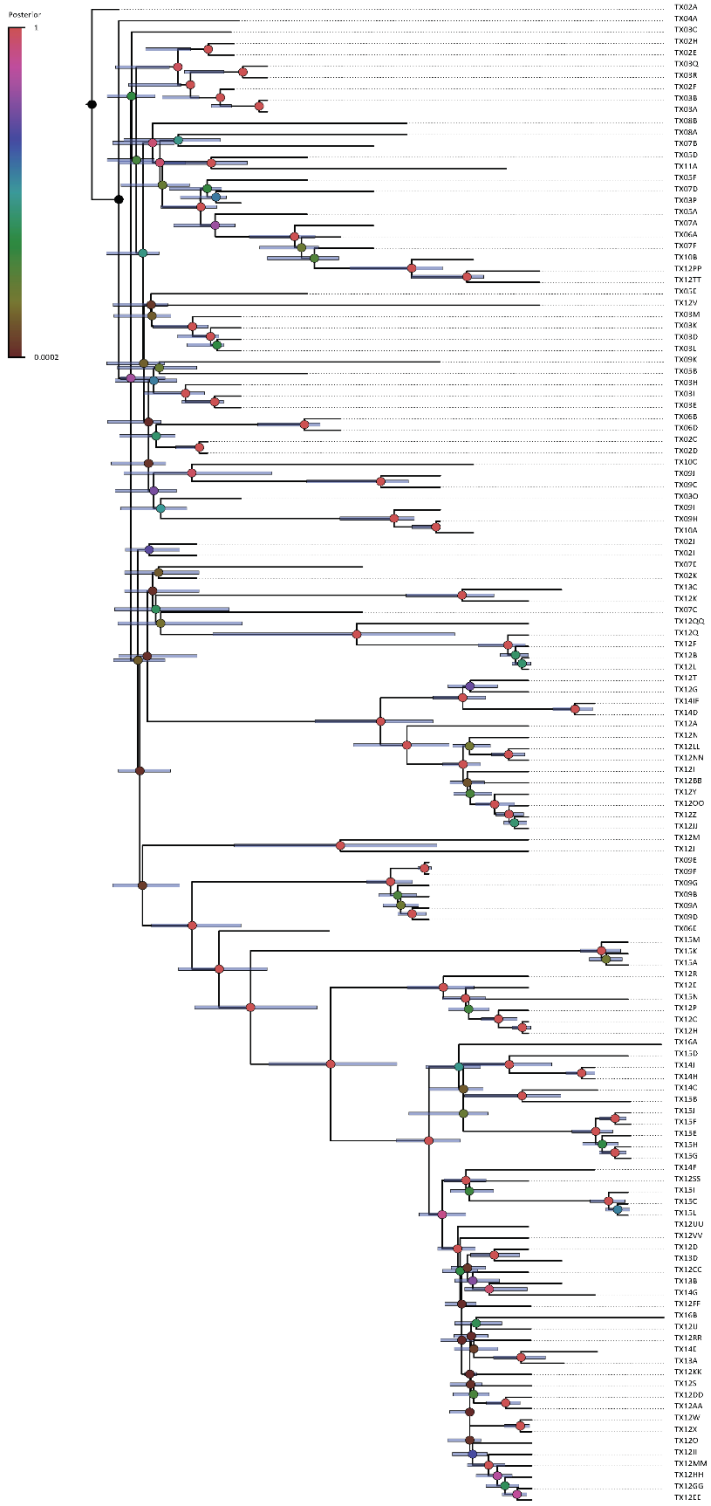


Figure 5.5 Phylogenetic analysis of WNV in Texas.
Bayesian phylogenetic approaches were used to generate an MCC tree. The posterior support for each node is indicated by colored circle and the height (95% HPD) of each node is indicated by purple bar.



Viral RNA was submitted to the NGS Core at the University of Texas Medical Branch. Datasets were down-sampled to 3000 mean coverage prior to the characterization of intra-host variation. Analysis of quasispecies population revealed a wide range of diversity among the isolates. Total nucleotide variation was modeled using Shannon's entropy while statistically significant single nucleotide variants (SNVs) were identified with Vphaser-2.

The mean Shannon's entropy for each isolate was between 1.44×10^{-3} and 2.96×10^{-3} (Table 5.3). Most positions contained low levels of Shannon's entropy with peaks below 0.01 (Figure 5.5 and 5.6). While substantial overlap was observed among the density profiles of all isolates; the peak entropy of isolate TX9611 was shifted to the right indicating the presence of more sites with higher levels of entropy (Figure 5.6). It is possible that elevated entropy observed in isolate TX9611 was the result of coinfection by one or more WNV isolates; however additional studies would be required to confirm this. Isolate TX9597 possessed the highest mean entropy (Table 5.3), contained several small peaks between 0.01 and 0.1, and one small peak greater than 0.1 ((Figure 5.6 and 5.7). These small peaks corresponded to 55 sites with elevated entropy (>0.1) in isolate TX9597 (Figure 5.8). Conversely, only 27 positions with elevated entropy (>0.1) were identified in the remaining nine isolates (Figure 5.8).

Analysis of statistically significant variation with Vphaser2 revealed 618 SNVs at 487 sites in the ten isolates (Appendix IV). Nsubs were identified at 488 sites and LPs were identified at 130 sites. Seven sites contained both NSubs and LPs: NS1-248, NS1-367, NS3-594, NS3-1670, NS4B-396, NS4B-476, 3'UTR-10487. SNVs occurring at high frequency (>10%) were identified at eight nucleotide positions: 5'UTR-79, 5'UTR-93, E-606, NS2A-459, NS2B-384, NS3-1458, NS3-1125, and NS5-1719 (Figure 5.9).

Table 5.3 Phenotypic Summary of Harris County Isolates.

Isolates were divided into four groups based on quasispecies phenotype. The number of SNVs, mean SNV frequency, mean Shannon's Entropy and mouse neuroinvasiveness expressed as

Isolate	Mouse neuroinvasiveness (ip pfu/LD_{50})	Number of SNVs	Mean SNV Frequency	Mean Shannon's Entropy
Few SNVs occurring at high frequency				
TX 9611 (D0159)	0.1	11	4.39	1.44E-03
Moderate number of SNVs				
TX 9604 (D0152)	0.50	38	0.55	1.87E-03
TX 9780 (D0329)		31	0.65	1.92E-03
TX 9589 (D0137)		83	0.83	1.79E-03
TX 9601 (D0149)	1.30	43	0.88	1.87E-03
Moderate to many SNVs occurring at low frequency				
TX 9614 (D0162)		39	0.16	1.56E-03
TX 9631 (D0179)		108	0.27	1.89E-03
TX 9587 (D0135)	1	124	0.20	2.06E-03
Many SNVs, high frequency, high entropy				
TX 9582 (D0130)		41	2.83	1.91E-03
TX 9597 (D0145)	0.3	100	2.60	2.96E-03

pfu/LD50 after intraperitoneal inoculation are summarized for each isolate.

Figure 5.6 Entropy density plot.
The density of entropy values for each isolate were plotted to illustrate the range of entropy that is most abundant. Traces for each isolate are indicated by color.

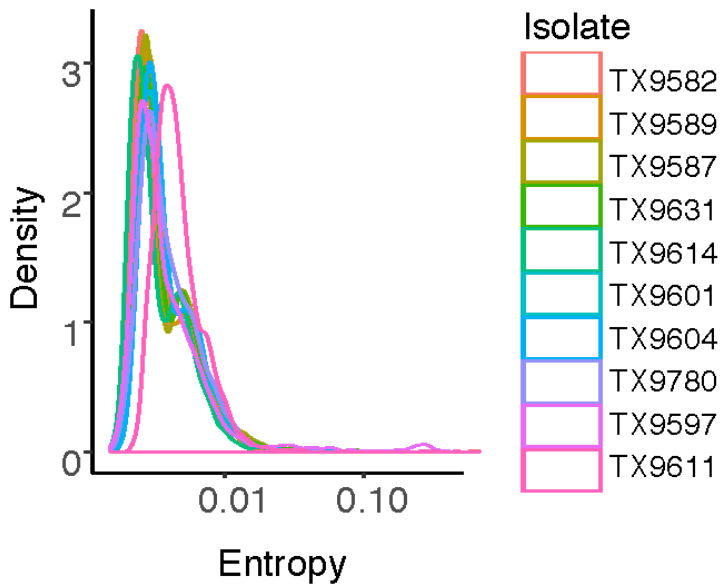


Figure 5.7 The entropy plot.
Diamond shapes indicate the mean entropy of each isolate. Violin plots were used to summarize the density and distribution of entropy in each isolate. The width of the violin plot is greatest in the range where entropy is the most frequent.

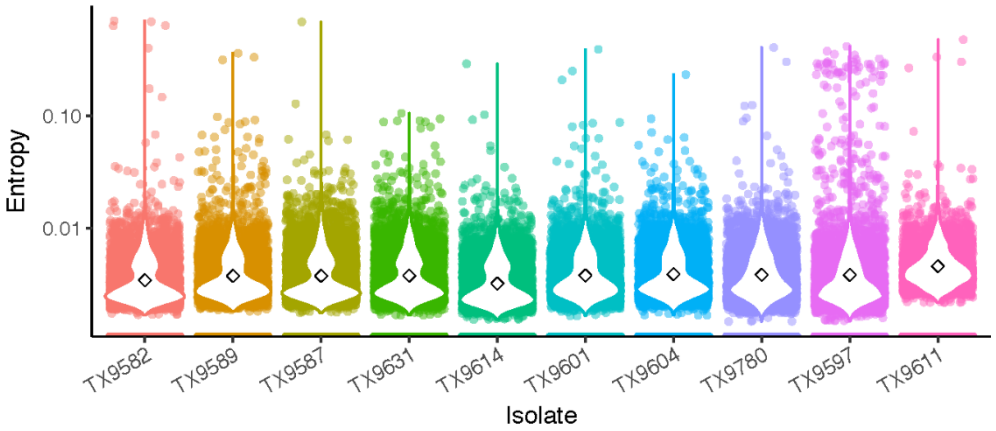


Figure 5.8 Distribution of entropy across the WNV genome. The entropy at each position is shown across the WNV genome. Color is used to indicate gene.

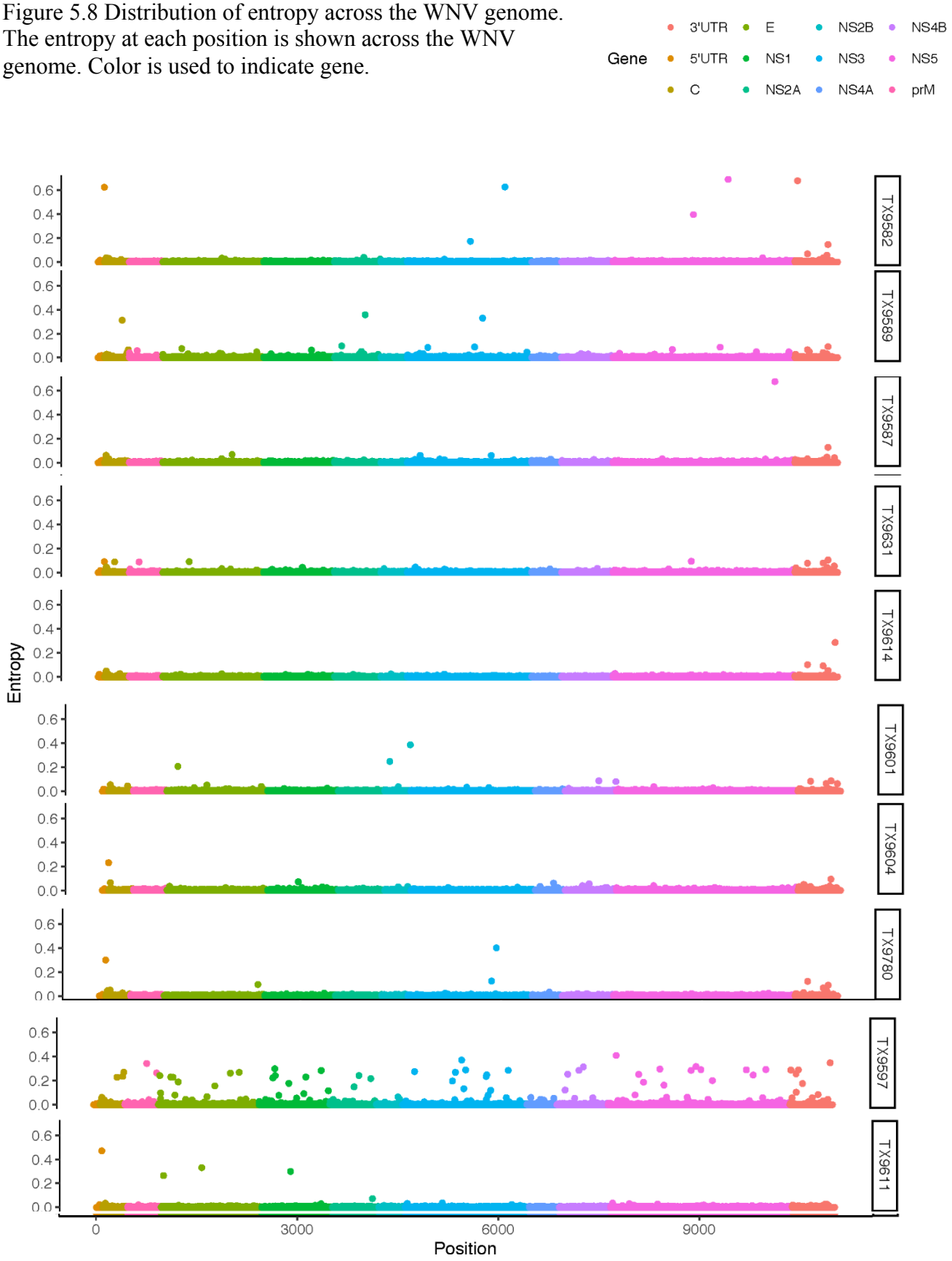
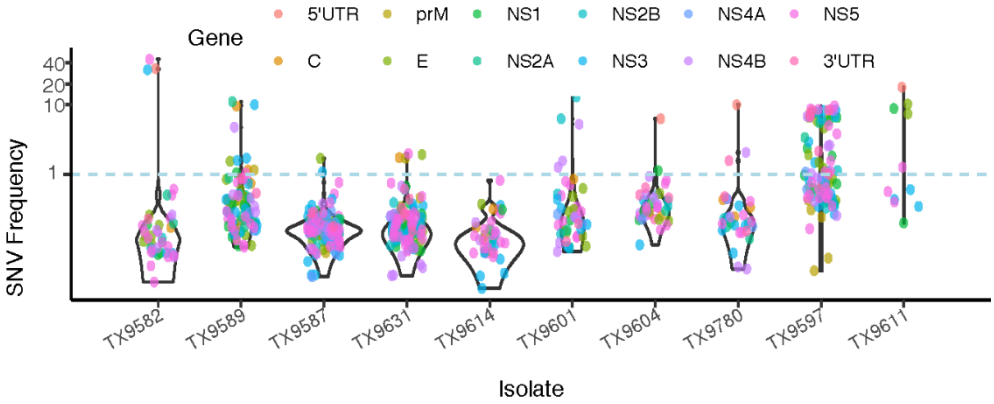


Figure 5.9 Frequency of SNVs by isolate.
SNV were identified with Vphaser2 and were summarized on a log scale. Each SNV was represented by a single point. The gene of each SNV was indicated by color. A hashed blue line was used to distinguish SNVs occurring above and below 1%.



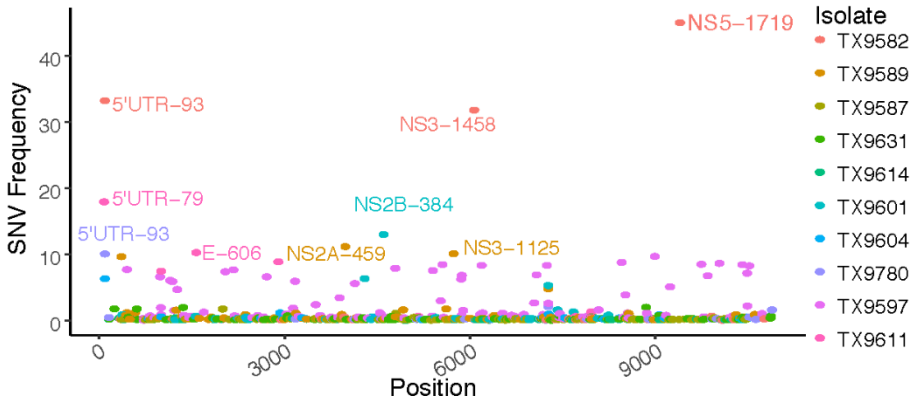
The number of SNVs identified in each isolate varied between 11 (TX9611) and 124 (TX9587) (Figure 5.10, Appendix IV). Interestingly, while TX9587 had the greatest number of SNVs, all SNVs occurred at low frequency (0.03 and 1.69) (Figure 5.10). Similarly, all 108 SNVs identified in isolate TX9631 occurred at low frequency (0.04-2.0). In contrast, 100 SNVs were identified in isolate TX9597 of which 26 occurred at frequencies between 5 and 10%. Isolate TX9589 contained 83 SNVs that ranged from 0.09% to 11.14%. Two SNVs occurred above 10%, NS2A-459 and NS3-1125.

The remaining six isolates contained less than half the number of SNVs per isolate as those reported above. Isolate TX9601 contained 43 SNVs that ranged from 0.08%-13.0%. One SNV in isolate TX9601 occurred above 10%: NS2B-C384U. Isolate TX9582 contained 41 SNVs and three occurred at more than 30%: 5'UTR-C93U, NS3-C1458U and NS5-U1719C. Isolates TX9604 and TX9780 contained 38 (0.1-6.29%) and 31 (0.04-10.05) SNVs, respectively, and both contained one SNV greater than 10% in the 5'UTR at position U93T. Thirty-nine SNVs were identified in isolate TX9614; however, all occurred at very low frequencies (0.02-0.82%). TX9611 contained only 11 SNVs (0.2-17.9%). Two of the SNVs were above 10%: 5'UTR-U79T at 17.9% and E-C606U at 10.24%

Four hundred and twenty-six (70.0%) of the SNVs occurred in one isolate only suggesting that they arose from stochastic variation and were not associated with selection. Forty-seven SNVs (7.6%) occurred in two isolates, eight (1.2%) occurred in three isolates, two (0.3%) occurred five isolates, one (0.2%) occurred in six isolates, two (0.3%) occurred in seven isolates and two (0.3%) occurred in 8 isolates.

Interestingly, low frequency SNVs were also identified at several positions that are conserved within WNV genotypes. SNVs were identified at position NS4A-85, which defines

Figure 5.10 Distribution of SNVs across the WNV Genome.
The SNVs identified in each isolate were plotted by genome position and frequency. The isolate of each SNV was indicated by color. The gene and nucleotide position of SNVs that occurred at greater that 10% were provided.



the SW/WN03 genotype[94], in TX9780 and TX9597 at 0.45% and 0.61%, respectively. Additional SNVs were identified NS2A-R188K TX9587 at 0.14% and at position NS4B-240 in isolate TX9631 at 0.19%.

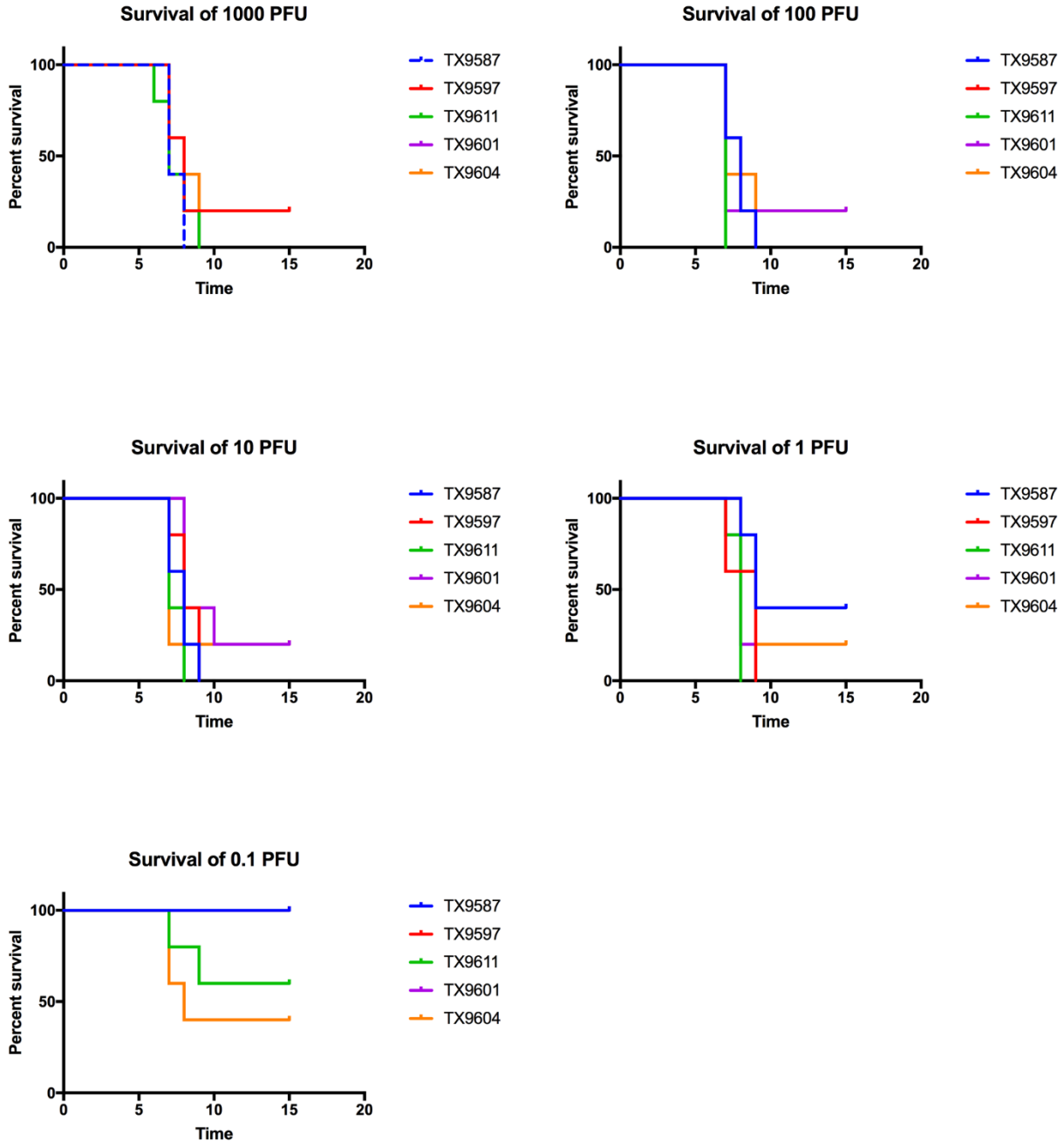
5.2.4 Phenotypic Studies

Five of the ten Harris County 2014 isolates with either elevated or reduced quasispecies diversity were selected for phenotypic analysis. No significant differences in infectivity titer at 37°C and 41°C were observed, indicating that no isolate displayed a temperature sensitive phenotype. Similarly, there were no differences in plaque morphology and mouse neuroinvasive virulence studies showed all isolates were highly virulent with LD_{50} values of <10 PFU (Table 5.3). Furthermore, there were no significant differences between the median survival time at doses 1000, 100, 10 or 1 PFU (Figure 5.11). In the 0.1 PFU dose group, a statistically significant difference was observed for TX9604 (Log-rank Mantel-cox test p=0.03), which had a median survival time of 8 dpi. The median survival times for all other isolates at that dose were undefined.

5.3 Discussion

Significant outbreaks of West Nile disease have been observed in the USA nearly every summer since the pathogen was first identified in NY in 1999. More than 2000 WNV-associated deaths have been reported to the CDC,[60] and it is estimated that more than 3 million human infections occurred in the USA between 1999 and 2010.[84] While the early WNV outbreaks were restricted to the Northeast, the central USA rapidly emerged as the region most strongly affected by WNV.[60] In chapter 4, phylogenetic approaches implicated the movement of migratory

Figure 5.11 Survival curves.
Three to four week-old female swiss webster mice were infected intraperitoneally with WNV and monitored daily. The only statistically significant differences in median surival time was observed in the 0.1 PFU dose group.



terrestrial birds in the circulation of WNV in the USA and TX was identified as a primary source of WNV circulation in North America.

As one of the most southern regions of the USA, TX is known for long summers and mild winters, fostering year-round mosquito activity and WNV transmission.[183] Furthermore, Harris County, which is the largest and most densely populated region in TX, is situated within an important point of convergence for migratory birds traveling between the USA and Mexico called the circa-Gulf route. The large human population, annual influx of migratory birds, and year-round mosquito activity makes Harris County an important location for WNV surveillance.

During the summer of 2014, the Mosquito Control Division of Harris County Mosquito Control Division reported a >20% increase in the number of WNV positive mosquito pools. This report was quickly followed by the largest outbreak of human WNV neurological disease ever reported in Harris County. In this study, ten WNV isolates made in Harris County during the 2014 outbreak were used to characterize the genetic and phenotypic properties of WNV and infer broader characteristics relating to evolutionary shifts, quasispecies diversity and mutation robustness.

Despite the co-circulation of the NA/WN02 and SW/WN03 genotypes in TX between 2003 and 2011, the NA/WN02 genotype arose as the dominate TX genotype during the 2012 outbreak.[101] Similar to Harris County isolates collected between 2012 and 2016, the WNV isolates collected during 2014 clustered within the NA/WN02 genotype. Interestingly, the most recent common ancestor of the Harris County isolates collected between 2012 and 2016 occurred between 2008-2011, a period when the SW/WN03 and NA/ WN02 genotypes co-circulated, suggestive of selection pressure in recent years.

Two dominant amino acid substitutions, NS2A-R188K and NS4B-I240M, were identified among the 2014 Harris county isolates. NS2A-R188K was identified in 8 of 10 (80%) of the 2014 isolates as well as isolates collected in Harris County during 2012 (52%) and 2013 (80%), 2015 (79%) and 2016 (100%). The two remaining isolates from the 2014 outbreak (TX9614 and TX9780) that did not possess the NS2A-R188K substitution were phylogenetically related, shared six additional amino acid substitutions in the open reading frame, and were both collected in the southern part of Harris County. Interestingly, a distinct clade was apparent containing WNV isolates with the NS2A-R188K substitution collected in NY and CT during 2008, Minnesota in 2010, an isolate from Mississippi in 2011, isolates collected from multiple locations during 2012, and an isolate collected from the British Virgin Islands during 2013. Selection of the NS2A-R188K substitution and the phylogenetic clustering of WNV isolates collected between 2008 and 2016 suggests a new genotypic designation, which is proposed to be termed Northeast 2008 or NE/WN08. However, it should be noted that the NS2A-R188K substitution also appeared to arise independently in CO in 2006, CT between 2006-2008 and NM during 2010, but these isolates do not cluster within the NE/WN08 genotype.

Phenotypic studies have shown that the NS2A-R188K substitution is also present in WNV isolates with increased peak infectivity titers in house sparrows,[177] but the significance of this observation is unknown. Nonetheless, selection of this amino acid substitution suggests that the NA/WN02 genotype is undergoing adaptation in response to strong selection pressure. It is likely that fixation of the NS2A-R188K substitution provided a fitness advantage that facilitated the displacement of the SW/WN03 genotype in TX by an unknown mechanism but probably involves ecological factors.

Furthermore, the NS2A-R188K substitution was also present in WNV isolates collected in Africa, Europe and Russia; albeit in the 1950s before WNV came to the New World. The global selection of the NS2A-R188K substitution is highly indicative of convergent evolution, suggesting that the NS2A-R188K substitution confers a significant fitness advantage for WNV world-wide. Thus, it is speculated that WNV in the New and Old World, despite significant geographic isolation, is adapting to similar ecological pressures.

In addition to consensus level analysis, deep sequencing was used to characterize the quasispecies population of Harris County 2014 WNV isolates. Due to their high mutation rates, RNA viruses exist as a population of genetically unique virions often referred to as a quasispecies, mutant spectrum, mutant swarm or mutant cloud. The quasispecies diversity has been shown to be important for viral fitness, phenotypic stability, and attenuation as multiple studies have demonstrated that significant enhancement or depletion of quasispecies diversity can drive the virus population towards extinction or attenuation.[184] In experimental studies, increasing nucleotide error rate, through exposure to mutagens or alteration of polymerase fidelity, can result in the virus population surpassing the error-threshold for viability within a host.[185] Conversely, reduction of diversity can attenuate isolates by rendering the quasispecies vulnerable to population bottlenecks and diminishing its ability to adapt to selection pressures, such as those imposed by immune responses, novel environments or tissue compartments, and new host species.[186,187] However, little is known about the precise range and specific patterns in which the mutant spectrum can exist in nature as these studies have relied on artificial methods to manipulate quasispecies diversity. The WNV isolates collected during studies, such as the 2014 outbreak in Harris County, can be used to address these questions.

Investigation of the quasispecies dynamics of the Harris County 2014 isolates revealed significant variation in Shannon's entropy and the number of SNVs found in each isolate. The mutant spectrum demonstrated in the Harris County 2014 isolates suggests that natural WNV isolates have evolved to tolerate a broad range of quasispecies diversity. Four general patterns of quasispecies diversity emerged among the ten isolates used in this study. There was no association between pattern and either bird species, or geographic location of collection or date of isolation; however, there were too few samples to test these associations robustly.

The first pattern was exemplified by isolate TX9611, which contained few SNVs; but, just under 50% occurred at high frequencies. The second pattern of quasispecies diversity where a moderate number of SNVs were identified in each isolate, and these occurred at moderately high frequencies. The third pattern was a moderate to high number of SNVs that all occurred at low mean frequency. Finally, two isolates, TX9582 and TX9597, were identified with a high number of SNVs that were also occurring at high frequencies.

Despite the various quasispecies patterns observed among the WNV isolates collecting during the 2014 outbreak, all the isolates display a non-temperature sensitive, mouse virulent phenotype. The results of this analysis demonstrated that WNV in nature can tolerate broad range of quasispecies diversity without diminished fitness or attenuation, as measured in a mouse model system. However, no studies were undertaken on the avian virulence or mosquito competence phenotypes.

The goal of this study was to characterize representative isolates from the largest outbreak to date of WNV neurological disease in Harris County, TX. Analysis of viral isolates revealed that the 2014 WNV outbreak was genotypically and phenotypically similar to the outbreaks of previous years, suggesting that unknown ecological factors, such as climate or

vector density, were responsible for the size of the 2014 outbreak. Investigation of the 2014 outbreak also revealed the presence of a novel WNV genotype, NE/WN08, characterized by the NS2A-R188K amino acid substitution. This genotype was associated with the displacement of the SW/WN03 genotype in TX and can also be observed in at least additional 11 locations throughout the USA, the BVI, Europe, Russia and Africa. This study reinforces the importance of continued WNV surveillance in Harris County as WNV evolves in North America and amino acid substitutions become fixed in the viral populations.

CHAPTER 6 DEMOGRAPHIC HISTORY AND GENOMIC VARIATION OF WEST NILE VIRUS IN COLOMBIAN AND ARGENTINA

6.1 INTRODUCTION

While the evolution of WNV in North America has been thoroughly characterized, less is known about WNV in South America. While there is ample serological evidence of WNV circulation (Figure 1.4), isolation of WNV has been extremely rare and relatively few outbreaks have been reported in South America.[188] To date, only four full open reading frame (ORF) sequences from South America are available, two from Colombia (four WNV isolates were collected, but three had identical consensus sequences)[87] and two from Argentina.[86] The apparent lack of phenotypic differences between the Colombian isolates together with the divergent epidemiological patterns warrant further investigation.

Studies using phylogenetic methods demonstrated that WNV circulating in Colombia in 2008 and Argentina in 2006 belonged to the NY99 genotype, which was displaced by the NA/WN02 genotype in USA during 2002.[87] The Argentinian study suggested that multiple introduction events occurred with at least one originating in the Old World.[86] Finally, one additional study using partial genome sequences of the WNVs isolated in Colombia reported similar results and showed the Colombian sequences clustered with the extinct SECT genotype, which was a short-lived, geographically restricted genotype from 2002.[189] However, these studies did not utilize consistent models and in some cases used parameters that were not appropriate for the WNV dataset (Table 6.1). Furthermore, additional work is needed to estimate the timeframe in which WNV was introduced into South America and to compare genotypic variation among WNV strains circulating in the two continents.

Table 6.1 Summary of parameters used to infer phylogenetic models for WNV in South America.

Three studies have investigating the phylogenetic relationships between North and South American WNV. The parameters selected for each of the models are summarized below.

Citation	Jorge E. Osorio et al 2012[87]	Cintia M. Fabbri et al 2014[86]	Richard Hoyos López et al 2015[189]
Genome Length	Full genome (ORF)	Full ORF	NS5
Nucleotide substitution	TN93	TIM+G+I	GTR + G
Clock Model	Strict molecular clock	Not specified	Random local clock
Tree Prior	Not specified	Not specified	Constant

It is important to note that WNV isolates from Argentina and Colombia are also distinct from those in Mexico. Studies have shown WNV was introduced into Mexico by at least two routes. WNV spread from the Southern USA into Northern Mexico and was also introduced directly into Tabasco, Mexico likely by WNV-infected migratory birds.[190]

In this study, the patterns of WNV evolution in South America were clarified using phylogeny and NGS using four isolates collected from healthy flamingos in Medellin, Colombia in 2008.[87] In this case, a rigorous model selection process was undertaken to ensure that appropriate parameters were selected.

6.2 RESULTS

6.2.1 Consensus Sequence Analysis

The four isolates were provided by the World Reference Center for Emerging Viruses and Arboviruses. Passage history of the isolates include 3 passages in C6/36 mosquito cells and 1 passage in Vero cells) Isolate COL524/08 was identical to the previously reported sequence.[87] Consistent with the previous study, the consensus sequences of Colombian WNV isolates COL739/08, COL928/08 and COL9835/08 were identical; however, they differed from the reported sequences by a single amino acid substitution at position NS5-M815T.[87] Disagreement at this position was not surprising as NGS analysis revealed significant entropy at this site and the surrounding residues. None of the four Colombian viruses contained the E-V159A substitution of the NA/WN02 genotype suggesting they were derived from the NY99 genotype. The four Colombian isolates shared two amino acid substitutions from NY99 at NS3-I188V and NS4A-A85T (Table 6.2).

Table 6.2 Summary of the amino acid differences among WNV isolates collected in South America.

The amino acid sequences of WNV isolates collected in South America were aligned and compared to the prototypical stain of WNV (NY99-Flamingo).

		Previously Reported					Sequenced in this study			
Accession		AF196835	GQ379160	GQ379161	JN716371	JN716372	*	*	*	*
Protein	Position	NY99A	ArEq001	ArEq003	COL524/08	COL9835/08	COL524/08	COL739/08	COL928/08	COL9835/08
C	113	V		I						
prM	14	V	I							
E	159	V								
	328	Q			H		H			
NS1	171	V	A							
	281	P			S		S			
NS2A	4	D	E							
	98	R	G							
	119	H		Y						
NS3	162	I			V	V	V	V	V	V
NS4A	85	A			T	T	T	T	T	T
	88	G		A						
NS4B	23	V		A						
	33	L	F							
	116	T	A							
	241	T			A		A			
NS5	431	P	Q							
	577	H		Y						
	791	N	D							
	814	M							T	T

6.2.2 Next Generation Sequencing

Not only were isolates COL739/08, COL928/08 and COL9835/08 identical at the consensus level, their intra-host diversity profiles were also very similar (Figure 6.1A). Overall, isolate COL524/08 was the least diverse, while isolates COL739/08, COL928/08 and COL9835/08 were more diverse. By Shannon's entropy, prM, NS4B and NS5 genes had the highest average entropy, while the most conserved genes were NS2A and NS2B (Table 6.3). The Shannon's entropy of all four isolates was concentrated below 0.001 with few sites identified with higher diversity (Figure 6.1A). Additional clusters of elevated Shannon's Entropy and high frequency SNVs were identified at genomic positions 7643-7664 and 10119-10123 in the NS4B and NS5 genes, respectively (Figure 6.2A).

Analysis of SNVs revealed similar patterns (Figure 6.1B). SNVs were identified at 169 unique sites (Appendix V). Of all the SNVs, 78.26% occurred in only one isolate while 21.74% occurred in multiple isolates. The mean SNV frequency for each isolate was 1.6 to 2.0% (Table 6.4). In all isolates, the greatest density of SNVs occurred at a frequency of less than 1% (Figure 6.1B). While all SNVs in isolate COL739/08 occurred at less than 10%, isolates COL739/08, COL928/08 and COL9835/08 contained high frequency SNVs, as high as 48.55, 41.9 and 29.24%, respectively (Figure 6.2B). One high frequency SNV was detected at position prM-104 in two isolates, COL739/08 and COL928/08, at frequencies greater than 40% (Figure 6.2B).

There was a statistically significant positive correlation between the average number of SNVs in each gene and the gene length [Kendall's rank correlation, p-value <0.05, tau= 0.81 (Figure 6.3)]. However, the NS4B gene was above the 95% confidence interval of the linear regression line, while the E and NS1 genes fell below, suggesting that diversity in the NS4B gene was elevated and diversity in E and NS1 was reduced.

Table 6.3 The mean Shannon entropy of each gene is compared between the four Colombian isolates.

The nucleotide counts at each position were determine using the Bam2R function of the DeepSNV library in R. The mean Shannon's entropy for each gene is summarized. The mean entropy of the 5'UTR and 3'UTR are not provided because low coverage at the ends of the genome prevent accurate estimation.

	Mean Entropy				
Region	COL08C	COL08D	COL08E	COL08F	Average Entropy
5'UTR					
C	0.002315	0.003621	0.002899	0.002827	0.002916
prM	0.002139	0.004154	0.004532	0.004213	0.003759
E	0.002499	0.002601	0.002602	0.002681	0.002595
NS1	0.0022	0.002889	0.002729	0.002764	0.002645
NS2A	0.001911	0.003005	0.002556	0.002415	0.002472
NS2B	0.001738	0.002517	0.002409	0.002198	0.002216
NS3	0.002158	0.002956	0.002653	0.002825	0.002648
NS4A	0.002137	0.002922	0.002567	0.002773	0.0026
NS4B	0.002529	0.004442	0.003499	0.004139	0.003652
NS5	0.002069	0.00347	0.003354	0.003303	0.003049
3'UTR					
Total	0.002146	0.003283	0.00302	0.003156	

Figure 6.1 Analysis of WNV Intra-host Diversity.
The amount of intra-host diversity was quantified in each isolate using (A) Shannon's entropy and (B) Variant detection with V-phaser2. The non-zero entropy and SNV frequency were summarized as violin plots to indicate both the range and distribution. The width of the violin correlates with density. Graphs are color coded to indicate gene and shape is used to indicate the isolate.

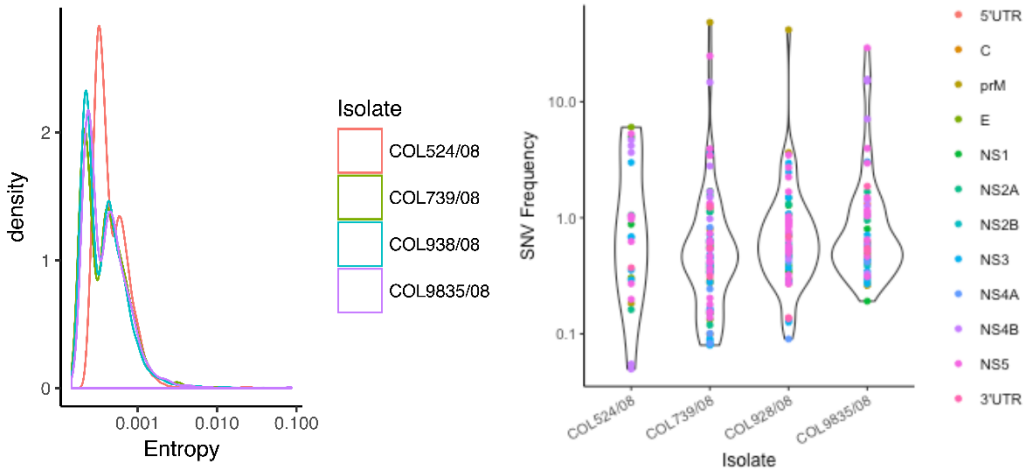


Figure 6.2. Intra-host diversity across the genome.
(A) Shannon's Entropy and (B) SNV frequency are summarized as dot plots. Dot color and shape correspond to the SNV gene location and isolate, respectively. Gene and nucleotide position are reported for all SNVs that occur at frequencies greater than 5%.

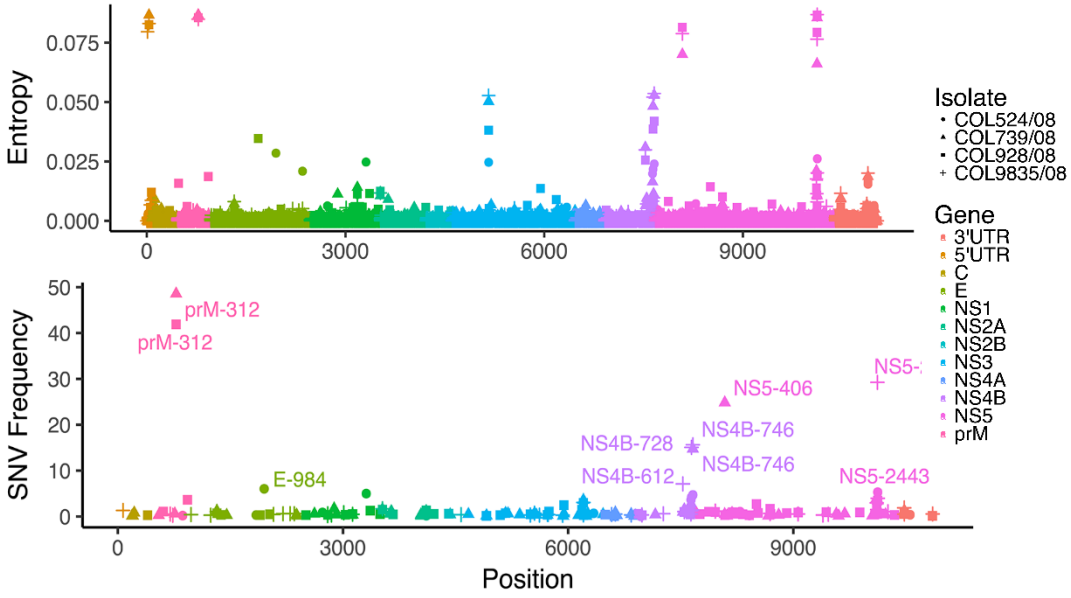
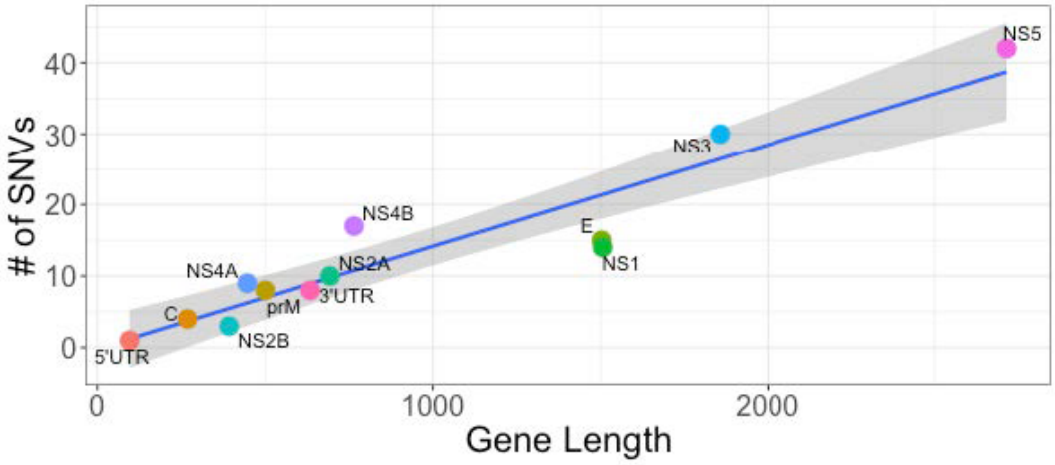


Table 6.4 Summary of NGS results.

The total number of SNVs identified in Vphaser is summarized by isolate along with the mean, standard deviation (StDev) and range of SNV frequencies.

Isolate	Total Number of SNVs	Frequency		
		Mean	StDev	Range
COL524/08	25	1.64	1.94	0.5-6.03
COL739/08	83	1.70	6.08	0.08-48.55
COL928/08	60	1.54	5.36	0.09-41.9
COL9835/08	53	2.04	4.84	0.19-29.24

Figure 6.3 Summary of the relationship between gene length and diversity.
Linear Correlation was used to determine the relationship between the proportion of sites with diversity and gene length. The 95% CI is indicated in grey.



6.2.3 Phylogeny

Maximum likelihood methods were used to generate a phylogeny of all previously reported sequences that spanned the full ORF (n=1705) (Figure 6.4). As expected, the Colombian and Argentinian isolates clustered in Lineage 1A, clade 4, among the North American isolates. A subsequent tree was generated consisting of 1377 sequences that belonged within Lineage 1A, cluster 4 (Figure 6.5).

As expected, Argentinian isolates, ArEq001 (equine isolate from 2006, Accession GQ379161), and the sequences from the Colombian isolates clustered within the NY99 genotype. However, while isolate ArEq003 (equine isolate from 2006, Accession GQ379160) clustered with an Israeli isolate from 1998, IS-98 STD (Accession: AF481864) in previous studies,[86] here it clustered with the other South American isolates.

Analysis of temporal structure revealed that there was a statistically significant positive correlation between root-to-tip distance and date of isolation suggesting that the mutation rate was similar among all branches in the tree. While several isolates displayed elevated or reduced root-to-tip distance compared to the regression line, ArEq003 had the greatest residual, indicating that it deviated most significantly (Figure 6.6).

Bayesian methods were used to interrogate the phylogenetic relationships further. A comprehensive model selection approach was applied to ensure appropriate assumptions were utilized. Previous studies either did not describe model selection or only compared nucleotide substitution models.[86,87,189] In this study, the most appropriate nucleotide substitution model was determined by comparing all 203 models available in JModelTest2 with Alkaline and Bayesian information criteria,[166] which revealed the most appropriate models included a GTR +G + I nucleotide substitution, an uncorrelated gamma clock, and a Bayesian skyline tree prior.

Figure 6.4 Maximum likelihood phylogeny of 1705 WNV sequences available of Genbank. Maximum likelihood phylogeny of All WNV sequences (1705 sequences) Red taxa represent sequences isolated in Colombia and blue taxa represent WNV taxa represented in Argentina.

Figure 6.5 Phylogenetic analysis of New World isolates.
Maximum likelihood phylogeny generated using all isolates belonging to Lineage 1 cluster 4 (n=1377). Old World isolates are indicated in purple, isolates from the NY99 genotype are red, South American isolates are in green, the NA/WN02 genotype is in blue and the SW/WN03 genotype is in orange.

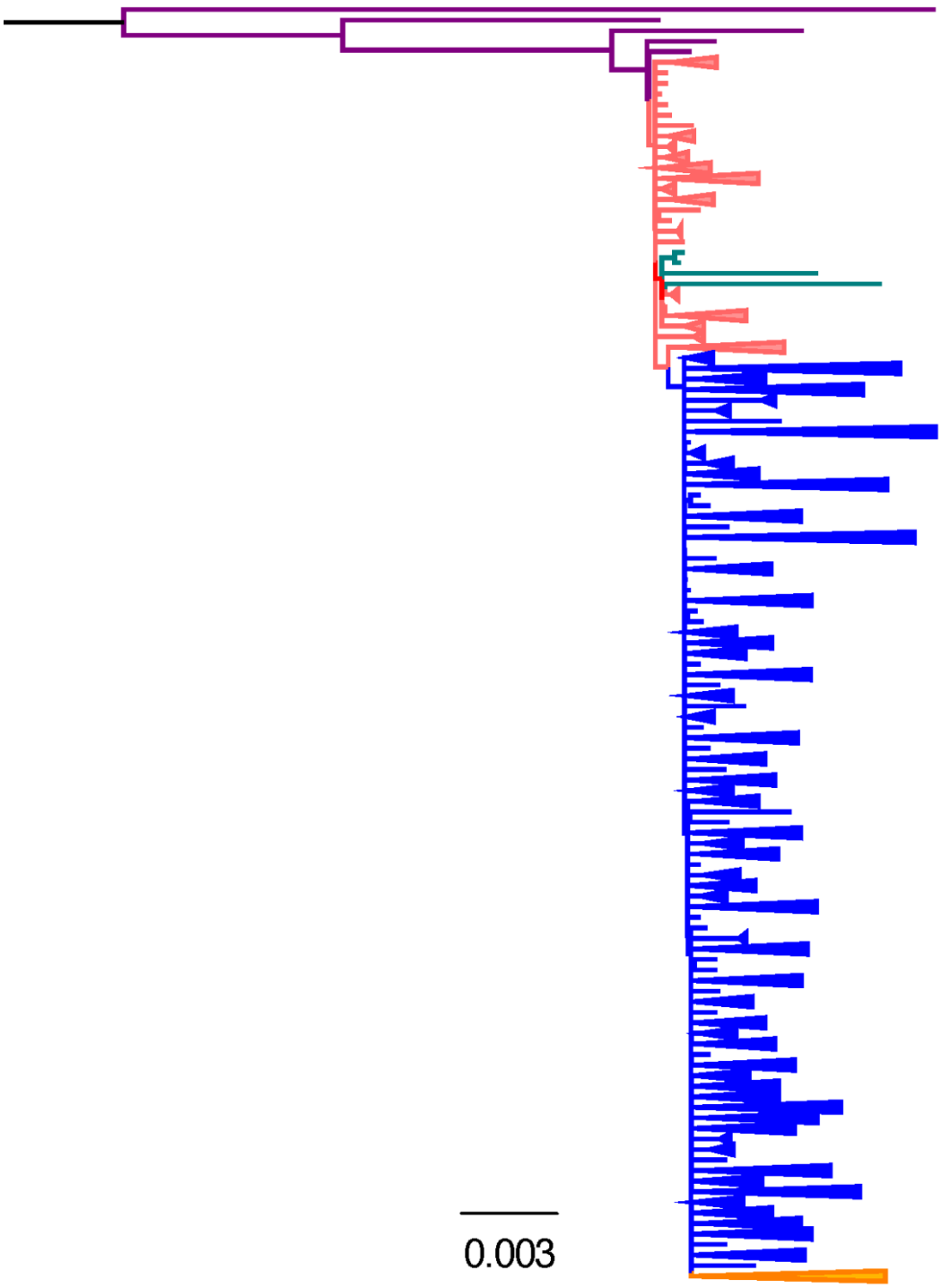
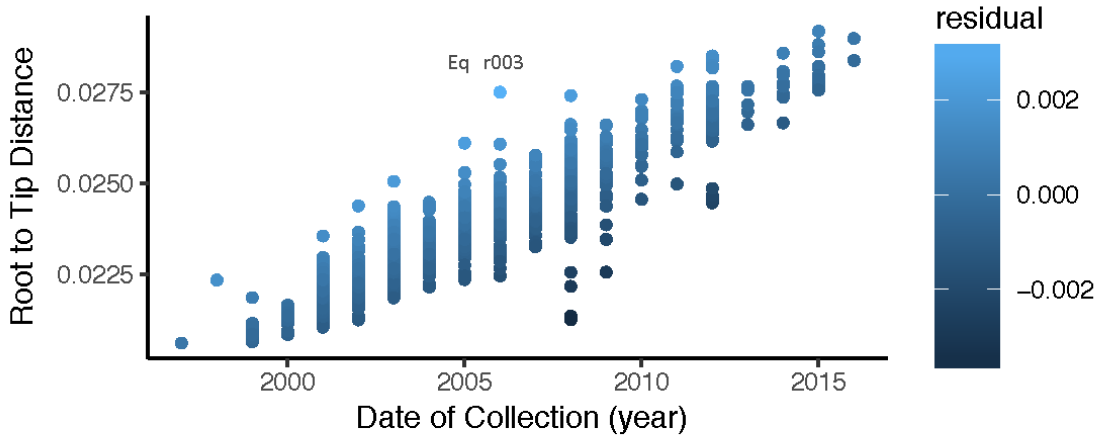


Figure 6.6 Analysis of Temporal Structure.
Root-to tip distance was determined for all Lineage 1 cluster 4 isolates from the maximum likelihood tree. Linear regression was performed to assess the correlation between Root-to-tip distance and date of isolation.



The North American isolates were independently down-sampled in triplicate and run with four well-characterized Old-World isolates (IS-98 STD, WNV_0043h_ISR00 [Israel 2000, Accession: HM152773], PaH001 [Tunisia 1997, Accession: AY268133], goose-Hungary/03 [Hungary 2003, Accession: DQ118127]) (total =209). To summarize the results of the Bayesian analysis, a maximum clade credibility (MCC) tree was generated from multiple independent runs of each dataset (Figure 6.7). The inferred mutation rates for each of the three phylogeny models was 4.72 $x10^{-4}$ (95% CI 4.29 $x10^{-4}$ - 5.16 $x10^{-4}$), 4.70 $x10^{-4}$ (95% CI 4.29 x 10^{-4} - 5.00 $x10^{-4}$) and 4.65 $x10^{-4}$ (95% CI 4.22 $x10^{-4}$ - 5.11 $x10^{-4}$), respectively. However, the mutation rate along the ArEq003 branch was significantly above the mutation rate of the tree (1.04 $x10^{-3}$, 95% CI 7.30 $x10^{-4}$ - 1.36 x 10^{-3} s/s/y).

The introduction of WNV into North America was estimated to occur between 1997 and 1999 and the South American introduction occurred soon after (between 1999 and 2001) (Table 6.5). In all trees, both Argentinian and Colombian isolates cluster together in a distinct clade within the NY99 genotype with isolates collected from Connecticut during 1999 (2471, Accession: AF206518) and 2001(BID-V4200, Accession: HM488136), and an isolate from Texas collected in 2002 (TX02A, Accession: AY289214) belonging to the extinct SECT genotype. This relationship was well supported in all trees (posterior > 0.80). The clustering patterns confirms that the Argentinian and Colombian sequences belong to the NY99 genotype.

Interestingly, South American isolates are basal to North American isolates BID-V4200, and TX02A in two of the three phylogenies, which would suggest two introductions from North America into South America or back migration from South America into North America. However, the branching pattern was weakly supported (posterior < 10) so no conclusion can be

Figure 6.7 Maximum clade credibility trees prepared using the Bayesian method. Sequences were down-sampled independently (n=209) in triplicate (A-C) and run separately for 100 million **Markov Chain Monte Carlo** steps. Multiple independent runs were combined to ensure topological convergence and adequate ESS values were achieved. Tree files were combined and annotated to generate three maximum clade credibility trees, each with a unique set of down-sampled sequences. Circles are present at each node to indicate posterior support. purple-red color indicates high support and green-brown indicated poor support. A red box indicated taxa from Colombian and Argentina.

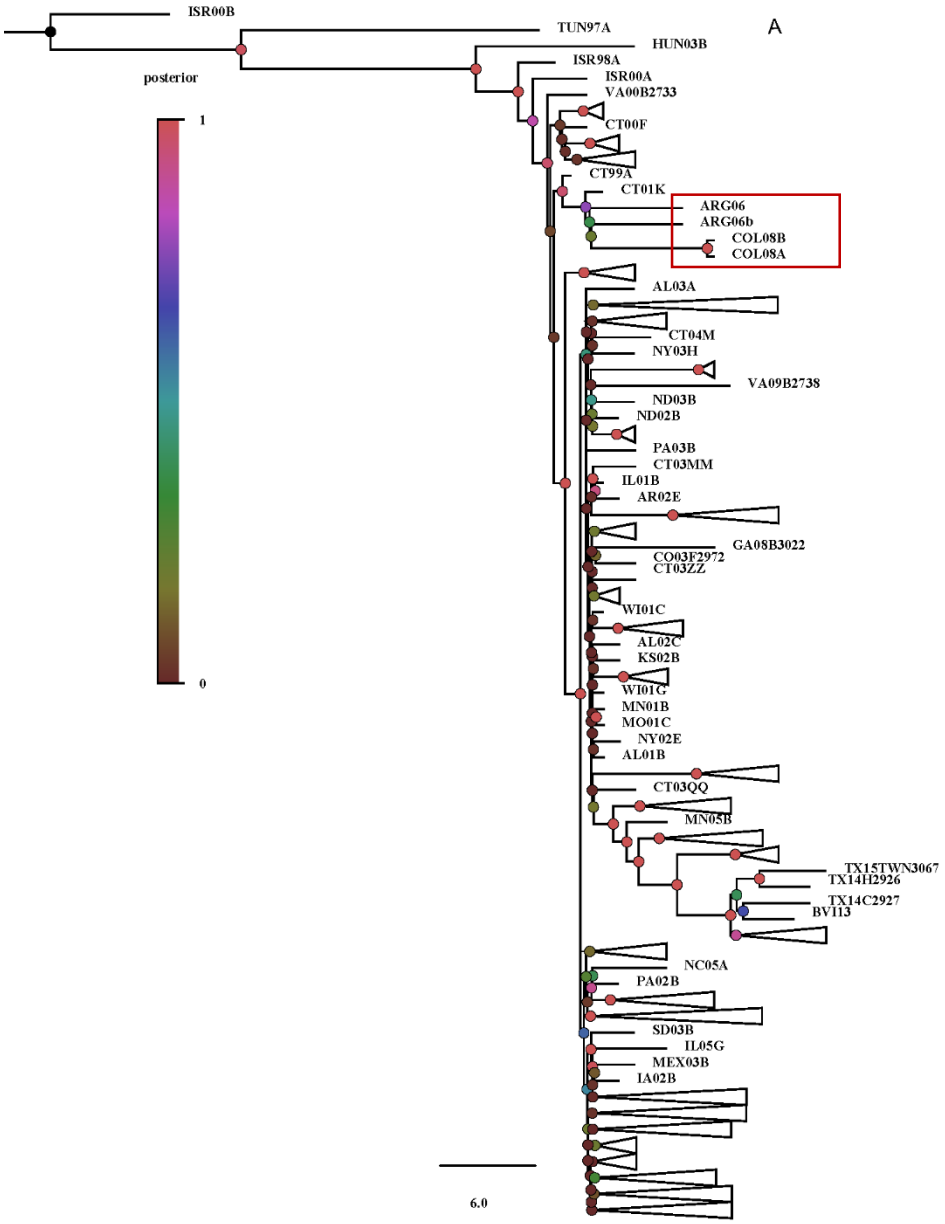


Figure 6.7B Maximum clade credibility trees prepared using the Bayesian method.

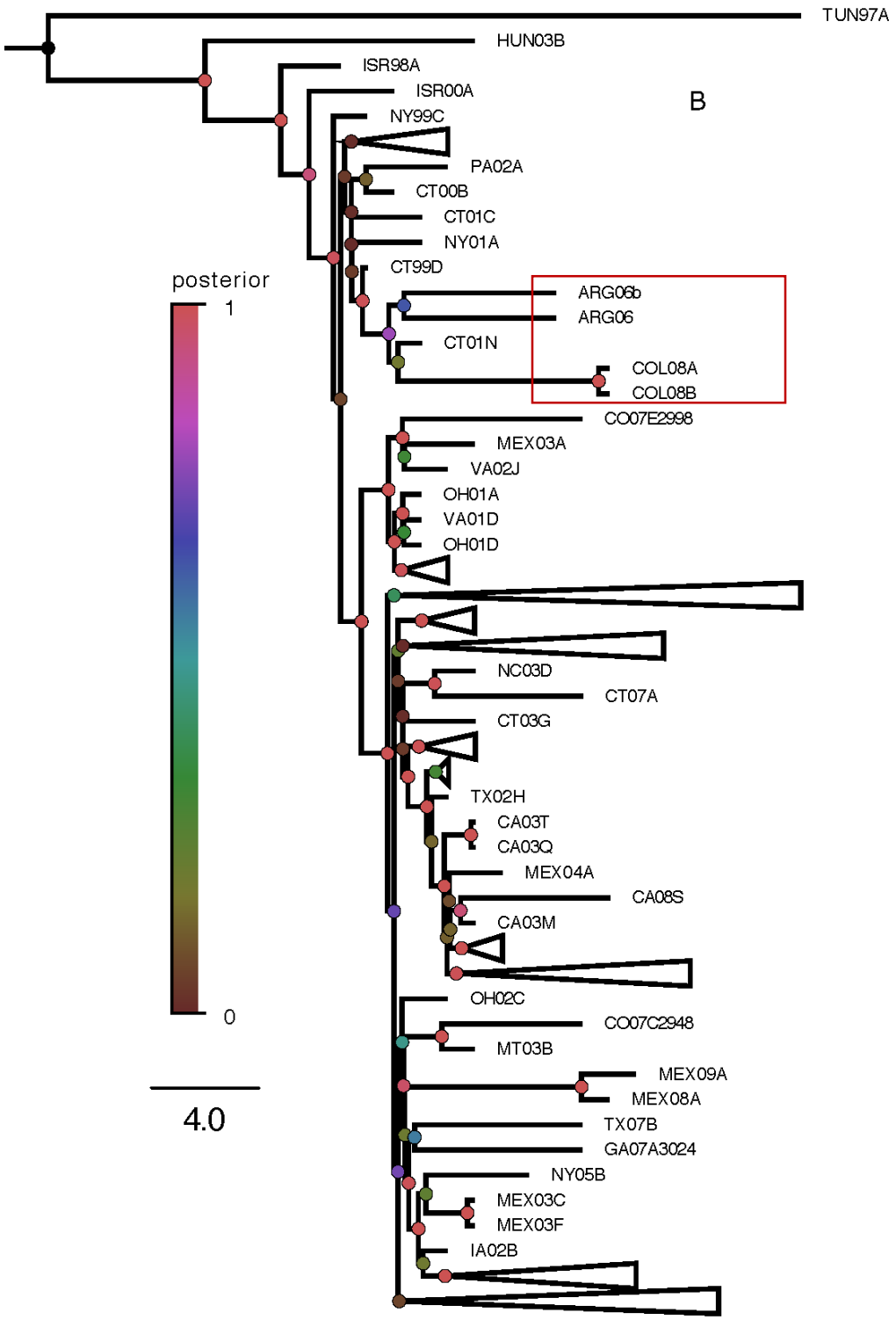


Figure 6.7C Maximum clade credibility trees prepared using the Bayesian method.

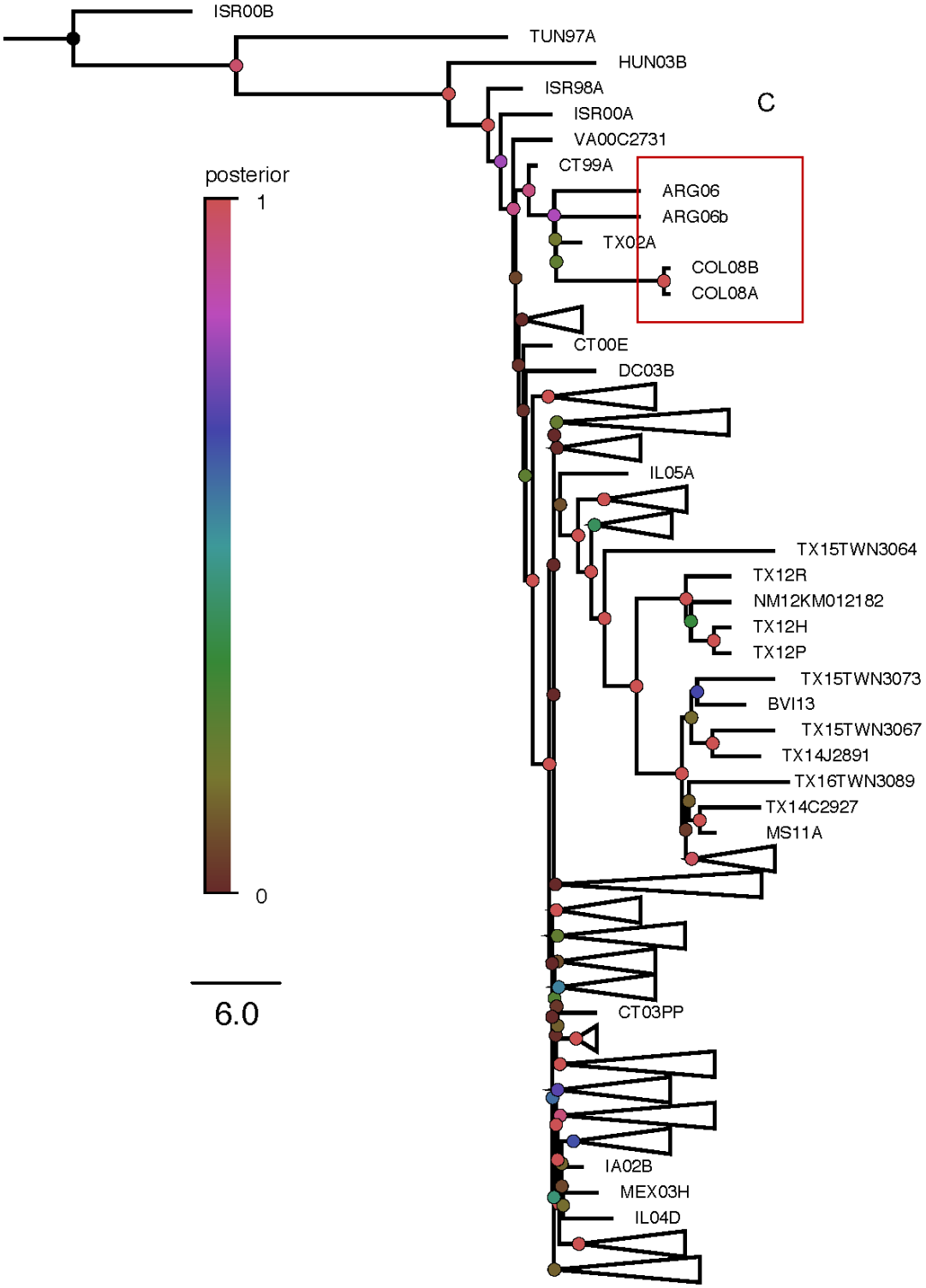


Table 6.5 The Most Recent Common Ancestors (MRCA).

The MRCA of the North American, South American isolates are summarized.

	MRCA (95% CI)			
Data set	North America		South America	
	mean	95% CI	mean	95% CI
1	1997.32	1996.49-1998.06	1999.90	1998.78-2000.52
2	1998.70	1997.91-1999.93	2000.68	1999.84-2001.31
3	1998.45	1997.59-1999.23	2000.97	1999.72-2001.33

drawn either way. Notably, isolates from the British Virgin Islands (2013) and Mexico (2003-2004 and 2008-2009) did not cluster with the South American isolates, indicating that WNV was introduced to each of these locations separately.

6.3 Discussion

Little is known about the evolution and epidemiology of WNV in South America. While serological evidence suggests the presence of WNV in South America, some investigators have suggested that positive serological results may have actually been due to cross reaction with other flaviviruses because virus isolation is rare and outbreaks of human or animal disease occur less frequently than in North America.[188] Thus far, previous studies have shown that WNV circulating in Colombia and Argentina as recently as 2008 clusters closely with sequences from the extinct SECT genotype [189] and belongs to the NY99 genotype,[86,87] which was displaced in North America in 2002. However, while the South American sequences cluster closely with the SECT genotype, they do not share any of the substitutions that define the SECT genotype. Previous studies also suggested that multiple introductions were responsible for the circulation of WNV in South America; one originating in North America, and another possibly from outside North America. To investigate the evolution of WNV in South America further, four WNV isolates collected from the sera of asymtomatic flamingos in the Medellin Zoo in Colombia were sequenced using NGS and compared to all previously reported WNV sequences (n=1705) using phylogenetic analyses.

The phylogeny estimates introduction of WNV in to North America to have occurred between 1997 and 1999, which is consistent with previous epidemiological and phylogenetic reports.[90,110] The introduction of WNV into South America occurred during the first few years following introduction in to North America (1999-2001). During this time, the dominant

genotype circulating North America was the NY99 genotype, which explains the presence of the NY99 genotype in South America.

Despite being members of the NY99 genotype, the Colombian isolates shared two amino acid substitutions, NS3-I188V and NS4A-A85T. Paradoxically, the NS4A-A85T substitution is associated with the SW/WN03 genotype, which was first identified in the US in 2003 and has been observed in previous isolates only with the accompanying E-V159A substitution of the NA/WN02 genotype. The NS4A-A85T substitution is known to have arisen multiple times independently among US isolates within the NA/WN02 genotype.[110] This example of convergent evolution strongly suggests that the NS4A-A85T substitution confers a fitness advantage for WNV and reinforces the potential importance of the NS4A-A85T substitution for the evolution of WNV in the Americas.

The maximum likelihood and Bayesian phylogenies presented here illustrate similar phylogenetic clustering patterns (Figure 6.4 and 6.5). All Colombian isolates clustered within the NY99 genotype suggesting an introduction event originating in North America. Two of the three phylogenies presented here demonstrate examples of North American sequences clustering within the South American clade, which could be explained by multiple introductions from North America into South America or back migration from South America into North America. However, these results should be interpreted with caution since the posterior support on these branches is very weak. Interestingly, isolates collected in Mexico[190–193] and the Caribbean (the British Virgin Islands) in 2013 [194] do not appear to be related to the South American isolates analyzed here from 2006 and 2008.

In addition, the clustering pattern of ArEq003 in the phylogenies presented here is not consistent with the previously reported phylogenetic studies in which that isolate clustered with

the Israeli sequences.[86,87] The previous study suggested that multiple introductions of WNV occurred into South America, including at least one introduction originating in the Old World that was not descended from North American WNV.[86] No evidence was found in this study to support this conclusion. Here, both Argentinian sequences (ArEq001and ArEq003) and the four Colombian isolates cluster together within the NY99 genotype. The results and analysis in this chapter support the hypothesis that WNV circulating in South America is descended from North American isolates, i.e. at least one introduction from North America into South America.

The topological discrepancies between phylogenies reported here and in previous studies may be explained by differences in the number of sequences included in the analysis (46-57 vs 209) or parameters included in the phylogenetic model (nucleotide substitution model, clock model, tree prior). Specifically, suboptimal models can result in a well-defined phylogenetic artifact, called long branch attraction (LBA), in which rapidly evolving sequences erroneously cluster at the root of the phylogeny.[195] In this study, Bayesian analyses showed the mutation rate along the ArEq003 branch was elevated compared to the overall tree, and the authors of previous studies noted that the Argentinian sequences (ArEq001 and ArEq003) differed much more than could be expected base on the similar time and location these isolates were collected.[86] Given the elevated mutation rate, it is likely that studies reporting ArEq003 clustering near the phylogenetic root with Old World isolates were influenced by LBA and do not reflect the true evolutionary history of WNV. The confounding effects of LBA were mitigated in the phylogenies reported here by extensive model selection, especially among models that allow estimation of invariable sites.

NGS analysis revealed prominent intra-host diversity in the prM, NS4B and NS5 genes. As nonstructural genes are associated with viral replication and modulation of the host innate

immune response, it is likely that diversity in NS4B and NS5 genes is the result of host adaptation. There was also evidence of reduced quasispecies diversity in the E and NS1 genes, as they had a smaller proportion of sites with SNVs than other genes. NS2A and NS2B genes had the lowest mean entropy of all regions across the genome. This suggests the presence of strong purifying selection pressure. Unfortunately, the passage histories of the Colombian virus isolates (3x C6/36 mosquito cells and 1x Vero cells) were very different from the North American isolates (1-2x Vero cells only) limiting NGS comparison between isolates collected on the two continents.

Overall, the genomic characterization of WNV isolates from Colombia has provided insight to the evolutionary history and ongoing evolution of the NY99 genotype in South America. The early introduction of WNV from North America into South America can explain the persistent circulation of the NY99 genotype in Colombia and Argentina. It has been suggested that the NY99 genotype was displaced in North America by the NA/WN02 genotype because it was less fit in mosquitoes. The presence of the less fit NY99 genotype in South America may account for the relatively infrequent outbreaks of WNV. However, the Colombian isolates did possess the NS4A-A85T substitution that is associated with the SW/WN03 genotype and found in multiple Lineages thorough the USA. While there is no phenotypic evidence to indicate a fitness benefit of the NS4A-A85T substitution, this example of convergent evolution suggests that it confers a selective advantage and is worthy of further study. These results highlight the impact of regional differences of WNV adaptation and reinforces the need to investigate WNV evolution within South America.

CHAPTER 7 DISCUSSION

7.1. Identification of Gaps

As recently illustrated by ZIKV and CHIKV, the introduction and subsequent spread of emerging arboviruses can have a dramatic impact on human health and the economy. The introduction of WNV into the USA is an important example of an emerging pathogen in the New World that may provide insight into the general patterns of viral evolution of RNA viruses within novel environments.

Following the emergence of WNV into New York in 1999, virologists and public health officials throughout North and South America sought to monitor WNV activity, including sequencing viral genomes. However, the dramatic variation in the location, timing, and variable efforts of WNV sequencing has left the field with a limited understanding of the broad patterns of WNV evolution, and no efficient way of monitoring the ongoing evolution. To address this gap, the studies presented here were undertaken to ascertain the evolutionary patterns and demographic history of WNV in the New World.

Prior to the work undertaken in this dissertation, several major gaps existed to the understanding of WNV in the New World, especially relating to the patterns of virus circulation and the extent in which selection pressure(s) continues to drive evolution. Previous studies have interpreted the "star-like" pattern of WNV phylogenies and the limited number of sites detected by standard dN/dS methods (FEL, IFEL, SLAC and MEME) as evidence that WNV evolution in the New World has reached homeostasis and recent mutations arise in response to stochastic variation alone and not positive selection pressure.[83,90,98,108,109] However, selection pressure is likely difficult to detect in WNV as arboviruses are known to the display reduced mutation rates compared to many RNA viruses due to the obligate host switching life cycle requiring insects

and vertebrate hosts.[10] Furthermore, standard dN/dS methods rely on naïve phylogenetic approaches, like neighbor-joining and maximum likelihood methods. As seen in Figures 3.1, 4.1, 5.3, 6.4 and 6.5 maximum likelihood phylogenies provide poor topological resolution and contain large polytomies, suggesting the relationships between WNV sequences cannot be resolved with this method, and the inferred ancestral sequences used to generate the dN/dS ratios are not likely to be accurate. Furthermore, these methods can be biased if particular subpopulations have been over or under sampled, as seen with WNV. In particular, a major limitation of WNV studies to date, including this study, is that interpretation is based on sampling very few areas for WNV isolates in the USA, which will potentially introduce sampling bias.

In addition, as little is known about the patterns of WNV circulation within the New World, there is no way to predict the flow of novel genotypes from one location to another. Several studies have used mathematical and phylogenetic methods to infer the rate at which the geographic range of WNV expanded.[135,139,196] Importantly, one study demonstrated that the dissemination of WNV through the USA followed a heterogeneous pattern, suggesting that both contagious diffusion and long-distance movement played a role.[139] While other studies have suggested that the heterogeneous pattern of diffusion can be explained by the differential diffusion rates of WNV belonging to different genotypes,[110] it does not explain the accelerated spread of WNV in the years immediately following the introduction in to the New World. In particular, WNV expanded throughout the entire east coast and west to the Mississippi river by 2001, prior to the selection of the NA/WN02 or SW/WN03 genotypes.[60]

Initial studies suggested that the movement of migratory birds was the most likely factor driving the accelerated pattern of WNV dissemination.[138] This hypothesis is supported by

evidence of phylogenetic structure based on avian flyways, i.e. WNV isolates collected in one flyway tended to cluster more closely with isolates collected within the same avian flyway.[108] Furthermore, serological evidence of WNV was reported in birds undertaking northward migration in the Fall,[137] but not during southward migration in the Spring.[134,137] This led some to suggest that birds were only important for the southward expansion of WNV.[197] However, these studies only considered birds flying on the east coast (east of the Mississippi River) and on the West Coast in California. Notably, birds flying through the central USA were not considered. Furthermore, all studies considering the relationship between migratory birds and the pattern of WNV circulation is the USA have focused on the flyways defined by waterfowl.[108,134,137] Studies based on waterfowl migration are less likely to be informative because passerines, and not waterfowl, are important hosts for WNV amplification or transmission.[123]

Finally, while there is ample serological evidence of WNV in South America, little is known about the genotype and phenotype of WNV isolates in South America.[198] This is in part because so few WNV isolates have been collected and even fewer have been fully sequenced. To date, only six full ORF sequences are available from South America: two from Argentina[69,86] and four from Colombia; three of the four Colombian isolates had identical consensus sequences.[87]

Interestingly, previous phylogenetic studies characterizing WNV in South America have suggested that WNV was introduced into North America and South America separately from related sources in the Old World.[86,87] However, it is not clear if these conclusions are reliable as these reports were vague regarding what phylogenetic models were used or how the models were selected.

The purpose of this dissertation was to address the gaps outlined above with the hypothesis that **evolution of WNV in the New World has been enhanced by long distance**

movement and concurrent genetic adaptation. In doing so, some general patterns of likely WNV circulation were identified in the USA and additional information was obtained regarding the introduction and subsequent maintenance of WNV in South America. The results of these studies are described below.

7.2. THE FINDINGS

After considering the variable geographic distribution of WNV sequences available in Genbank, 66 additional low-passage WNV isolates were identified in the WRCEVA from three U.S. locations (VA, GA, CO) between 2000-2010 and sequenced using NGS. The quasispecies profile of each of these isolates is described in chapter 3.

In chapter 4, the sequences obtained for 66 WNV isolates collected from CO, VA, and GA were combined with an additional 289 previously reported sequences in Genbank from NY, VA, GA, IL, ND, SD, TX and CO for phylogeographic analysis and evaluation of selection pressure. A Bayesian phylogenetic approach revealed that three geographic locations, NY, IL and TX, accounted for 88.5% of the total WNV movement events observed. Furthermore, it was observed that movement of WNV originating within the eastern USA (NY, VA, GA) traveled southward while WNV movement in the central USA (TX, ND, SD, CO) traveled northward. The exception to this observation was observed for WNV isolates originating in IL. Northward migration was observed from IL to NY and southward migration from IL to TX. Together, the results observed reveal an elliptical pattern of WNV circulation that correlated with the annual migration of terrestrial birds, which prefer to take easterly routes during fall migration and westerly routes when migrating in the spring.[151]

These findings are especially important because natural and man-made events, such as climate change, atmospheric fluctuations, habitat destruction, etc., can drastically alter host

migration which, in turn, affects the circulation patterns of infectious agents, such as WNV.[199,200] Correlating WNV movement with terrestrial bird migration may allow public health officials to anticipate changes in WNV circulation due to altered bird migration.

Furthermore, these results support the construction of efficient surveillance and vector mitigation strategies. Based on the information presented here, NY, IL and TX are the optimal sites to efficiently monitor ongoing WNV evolution. However, the limitation here is that given the variable number of sequences collected across the USA, only eight geographical locations could be included in this study. As additional sequences become available in the future, phylogenies with finer geographic resolution may reveal new locations that are important sources of WNV circulation. It may be especially interesting to analyze WNV isolates collected from locations where the Eastern and Central Flyways overlap. Like IL, these locations may support WNV circulation in all directions. If so, these locations could also be important contributors to WNV circulation in the US.

In chapter 5, one of the major sources of WNV movement, TX, was analyzed further. It was not surprising to observe that TX was an important source for WNV movement as it is known for long summers and mild winters, ensuring year-round mosquito activity and WNV transmission.[183] Texas is also an important location for migratory birds throughout the US. In fact, 98.5% of all migratory bird species in the USA have been identified in TX.[141] The studies in this dissertation were designed to look specifically at WNV in Harris County as it is the largest and most densely populated region in TX, and it is situated along the circa-Gulf route of birds, which is an important point of convergence for migratory birds traveling between the USA and Mexico. The large human population, annual influx of migratory birds, and year-round mosquito activity makes Harris County an important location for WNV surveillance.

Special attention was given to WNV isolates obtained during 2014 because it was largest outbreak of human WN disease reported to date in Harris County. Analysis of consensus sequences showed two amino acid substitutions, NS2A-R188K and NS4B-I240M, were identified among the 2014 Harris County isolates. A distinct genotype, NE/WN08, was identified containing WNV isolates with the NS2A-R188K substitution collected as early as 2008 in the northeastern region of the USA and subsequently throughout the continental USA and the British Virgin Islands (Figure 5.3). The NS2A-R188K substitution also appeared to arise independently in Colorado in 2006, Connecticut between 2006-2008, and New Mexico during 2010, as well as Africa, Europe and Russia as early as 1958 (Figure 5.4). Based on WNV sequences from Europe in Genbank, the substitution appears to be occurring with increased frequency among isolates collected after 2013, but as with the US only a limited number of geographic locations have been sampled. In the US, additional studies are needed to investigate the NS2A-R188K substitution further as TX is one of the very few areas where WNV isolates are still collected and sequenced each year; thus, additional areas need to be sampled to determine if this substitution is becoming a dominant genotype. While the phenotype of the NS2A-R188K substitution is not known, worldwide selection of the NS2A-R188K substitution suggests that the NS2A-R188K substitution confers a significant fitness advantage for WNV. As the importance of this substitution was identified while monitoring WNV evolution in Harris County, the study reinforces the importance of continued WNV surveillance in TX and supports the conclusion that WNV in TX can serve as a national model for WNV evolution.

Finally, in chapter 6, the evolution of WNV in South America was evaluated. As all South American isolates examined clustered within the NY99 genotype (Figure 6.5), it can be concluded that WNV from both North and South America are the result of a single introduction

from the Old World and that the NY99 genotype remained circulating in South America at least as recently as 2008. Although the clustering patterns in phylogenies presented here are not consistent with the previously reported topologies,[86,87] discrepancies may be attributed to previous studies utilizing poor model selection resulting in LBA. Unfortunately, the models used in previous studies were not clearly defined so they cannot be replicated. The phylogenetic studies presented here estimated the introduction of WNV into South America occurred prior to 2001, which was only a few years after WNV was first identified in NYC during 1999. Interestingly, despite being members of the NY99 genotype, the Colombian isolates shared the amino acid substitution NS4A-A85T, which is associated with the SW/WN03 genotype. The NS4A-A85T substitution is known to have arisen multiple times independently among US isolates within the NA/WN02 genotype.[110] Again, this example of convergent evolution suggests that the NS4A-A85T substitution confers a fitness advantage for WNV.

7.3 LIMITATIONS OF THESE STUDIES

The location, timing and intensity of WNV surveillance and sequencing efforts have varied dramatically throughout the USA. Although considerable effort was made to mitigate the effects of the variable sampling on the results presented in this dissertation, some unavoidable limitations remain. Namely, WNV sequences from only a handful of states could be included in this study. For instance, the movement of WNV in the western USA was not considered in this dissertation. This is because California was the only location in the western USA with a sufficient number of sequences for analysis. The Rocky Mountains provide a natural barrier between the western USA and the remainder of the country, and WNV movement in California has been described elsewhere.[110] Also, very few WNV isolates have been sequenced or were available from the USA after 2012, outside of TX. Therefore, recent virus circulation could not

be assessed. As new sequences become available in the future, it may be possible to evaluate the circulation of WNV in other geographic areas, including the western USA.

While chapter 5 of this dissertation proposes that TX could be a national model of WNV evolution, IL may be an important secondary location to consider in the future. Like TX, IL was also identified as a major source of WNV activity (Figure 4.6). Furthermore, as WNV movement in IL appears to be transported in all directions, it is possible that IL may even be more informative than TX, which only supports migration in the north and west directions. However, no sequences are available from IL after 2011 preventing any consideration of recent evolution. In addition, it may be worth considering locations south of IL that are located in the same overlapping region of terrestrial bird flyways.

Several additional locations may also be very interesting to considered as potential models of WNV evolution. For instance, other locations in the Gulf Coast region may be important because, like Harris County, TX, the Gulf Coast region is situated along the circa-Gulf route, which is an important point of convergence for migratory birds. In particular, WNV in southern Mississippi and Louisiana may be very important to evaluate as these locations are situated between the Eastern and Central Flyways as well as along the circa-Gulf route.

7.4 Patterns of WNV Evolution compared with other avian arboviruses

The role of birds in the spread of disease agents is well documented in the USA.[140] Birds are the primary hosts of WNV, and, as demonstrated here, they are likely the driving force facilitating WNV dissemination. In addition to WNV, birds have also been implicated in the spread of other arboviruses, including SLEV, USUV, and EEEV. Interestingly, many ecological and evolutionary patterns are shared between avian arboviruses circulating around the world.

Like WNV, SLEV is a flavivirus that is transmitted by *Culex* mosquitoes and predominately infects birds in the orders Passeriformes and Columbiformes.[123,201] SLEV appears to have arisen in South America and then spread to North America; however, SLEV outbreaks have resulted in more significant disease in North America than South America.[202] SLEV is similar to WNV in the following ways: (1) geographic isolation of SLEV in CA has been reported, which suggests that the general mechanisms for avian-arbovirus evolution in CA maybe different than in the remainder of the USA, (2) migratory birds were the most likely drivers of SLEV circulation between North and South America, and (3) migratory birds flying through the Gulf-of-Mexico along the circa-Gulf route were likely responsible for the distribution of SLEV throughout the USA.[202]

Notably, the role of migratory birds in dissemination of arboviruses is not restricted to the Americas. USUV is a flavivirus that is also transmitted by birds and Culex mosquitoes. USUV was originally identified in South Africa; however, in 1996, USUV emerged in Europe resulting in an outbreaks among Eurasian blackbirds, and some human infections.[203] Like WNV, recent studies have found that the geographic movement of USUV is consistent with avian flyways in Europe and Africa.[204]

Birds may also be responsible for the geographic movement of arboviruses in other families. EEEV is an avian-arbovirus belonging to the family *Togaviridae* that is transmitted by *Culiseta melanura* in freshwater swamps.[205] Outbreaks of EEEV also appear to be less frequent or less severe in South and Central America than in North America.[205] Similar to WNV, EEEV also involves passerine birds and is capable of causing neurological disease in humans.[205] This is likely due to the feeding preferences of the EEEV enzootic mosquito vector. Phylogenetic studies have also shown significant isolation of EEEV in North and South America and have

estimated that the two populations diverged between 1,600 to 2,300 years ago.[206] Additional studies have also shown significant antigenic differences among EEEV isolates collected in North and South America.[207] Interestingly, long distance movement appears to be less frequent in EEEV than WNV as studies of EEEV in the northeastern USA have demonstrated restricted movement between New Hampshire, Connecticut and New York.[208] This difference may be explained by WNV having a broader host range and is more promiscuously vectored than EEEV.

Taken together, migratory birds appear to be important drivers of the circulation of some arboviruses. In all cases, there appears to be limited movement between the continents in the northern and southern hemispheres. This could be due to differences in seasonal weather patterns or the behavior/abundance of host and vectors. As stated above, geographic isolation was observed in California among both WNV[110] and SLEV.[202] Several important differences exist between the eastern and western USA that may account for this observation. Notably, the seasonal shifts in atmospheric conditions that encourage looped bird migration are less pronounced in the west,[151] the climate is more arid,[150] and the topology is more varied, as the Rocky Mountains in the western USA have higher elevation than the Appalachian Mountains in the east. Also, *Cx. tarsalis* is the main WNV vector in CA; but *Cx. pipiens, Cx. restuans* and *Cx. quinquefasciatus* are the principle vectors in the eastern and central US. Studies have also shown that the Rocky Mountains provide a significant barrier for mosquito populations.[209]

Given these ecological differences, it is not surprising that evolution of both WNV and SLEV in California is distinct from WNV in other parts of the USA. Additional studies are needed to evaluate WNV among locations west of the Rocky Mountains. Furthermore, the avian arboviruses discussed here, WNV, SLEV and EEEV, appear to cause more significant outbreaks of human disease in North America than in South America. This observation is in stark contrast

with primate-associated arboviruses, including DENV, YFV, CHIKV and ZIKV, which cause significant human disease in South America, but not in the USA or Canada. It is possible that vertebrate host-to-human spillover events occur less frequently in South America due to an unidentified ecological factor(s), such as vector feeding preferences, host behavior, and preexisting immunity, avian host diversity, etc. However, as DENV, YFV and ZIKV are all transmitted *Aedes* spp. mosquitoes, the possibility cannot be excluded that *Aedes* mosquitoes have an advantage over *Culex* and *Culiseta* mosquitoes for transmission of viruses in South America. Finally, it is also possible that misdiagnosis and serological cross reactivity may create the perception that the viruses cause larger outbreaks in one location, while in fact true incidence may be similar.

7.5 Potential Implications of this Work

The results of these studies rely on several disciplines, including phylogenetic, virological and ecological sampling methods to enhance the theoretical field of viral evolution, and provide tangible recommendations for public health officials and policy makers. By defining the spatial and temporal patterns of WNV evolution, these studies support the identification of geographic locations for efficient WNV monitoring (NY, IL and TX). As WNV surveillance is actively continuing in Harris County, TX, this dissertation proposed and confirmed that TX can serve as a national model to study WNV evolution.

A potential limitation of this study is that WNV in California appears to be evolving independently from the remaining parts of the US. As WNV in CA appears to be unique, no location outside of CA can be used to model WNV in that state. Furthermore, WNV circulation in South America cannot be modeled by WNV in TX, as in seems that only limited viral movement has occurred between the two continents. This dissertation also revealed the probable

timeframe during which WNV was introduced into South America and demonstrated that all American WNV isolates are the result of a single introduction from the Old World. This is important as it corrects a previously reported error in the literature which relied on inappropriate phylogenetic parameters.[86]

Finally, during the course of these studies, two amino acid substitutions were identified that appear to be arising in response to convergent evolution, NS2A-R188K and NS4A-A85T. It is possible that one or both of these substitutions may confer a fitness advantage in either avian host or mosquito vectors. It is important that future studies evaluate the phenotypic advantage driving selection of these two substitutions in *in vitro* and *in vivo* mosquito and avian models so that the field may fully appreciate their relationship with WNV evolution.

APPENDICES

Appendix I

Summary of virus isolates sequenced for used in Chapter 3 and Chapter 4. The number of length polymorphisms (LPs) and nucleotide substitutions (Nsubs) identified by Vphaser2 are provided along with the isolate name, sequence identification number, virus isolate number (TWN) and all available source information (Host, Year, State, County).

TWN	Isolate Name	Sequence ID	Species	Year	State	County	Number of LPs	Number of NSubs
2672	VA AV 321-00	VA00A	Avian	2000	VA	Norfolk	6	64
2673	VA B 037-02	VA02A	Avian	2002	VA	Norfolk	6	18
2674	VA 1909-04	VA04A	Mosquito	2004	VA	Norfolk	6	19
2675	VA 3920	VA09A	Mosquito	2009	VA	Norfolk	2	4
2693	VA TC 2535-01	VA01A	Mosquito	2001	VA	Norfolk	3	26
2694	VA TC 1500	VA02C	Mosquito	2002	VA	Norfolk	3	6
2696	VA P 3321-05	VA05A	Mosquito	2005	VA	Norfolk	5	3
2697	VA P 4485-06	VA06A	Mosquito	2006	VA	Norfolk	5	8
2698	VA SP 5645-06	VA06C	Mosquito	2006	VA	Norfolk	5	13
2699	VA 1660	VA07A	Mosquito	2007	VA	Norfolk	5	6
2700	VA SP 1202-08	VA08B	Mosquito	2008	VA	Norfolk	4	6
2701	VA TC 2020-10	VA10C	Mosquito	2010	VA	Norfolk	5	9
2711	VA 2327	VA07B	Mosquito	2007	VA	Norfolk	4	9
2713	VA P 4209	VA05B	Mosquito	2005	VA	Norfolk	4	1
2714	VA SN 5859-09	VA09C	Mosquito	2009	VA	Norfolk	5	3
2715	VA TC 1117-10	VA10B	Mosquito	2010	VA	Norfolk	4	10
2716	VA TC 1368-08	VA08A	Mosquito	2008	VA	Norfolk	4	5
2717	VA TC 1500-02	VA02D	Mosquito	2002	VA	Norfolk	5	13
2718	VA TC 4043	VA03D	Mosquito	2003	VA	Norfolk	7	24
2719	VA AV 573-00	VA00D	Avian	2000	VA	Norfolk	5	53
2730	VA 2191	VA10A	Mosquito	2010	VA	Norfolk	6	7
2731	VA AV 380	VA00C	Avian	2000	VA	Norfolk	6	18
2733	VA AV 593	VA00B	Avian	2000	VA	Norfolk	9	46
2734	VA BD 37	VA02B	Avian	2002	VA	Norfolk	6	70
2735	VA TC 2790-03	VA03C	Mosquito	2003	VA	Norfolk	5	49
2736	VA P 4770-06	VA06B	Mosquito	2006	VA	Norfolk	3	9
2737	VA SN 3082-05	VA05C	Mosquito	2005	VA	Norfolk	5	3

2738	VA SN 3222-09	VA09B	Mosquito	2009	VA	Norfolk	5	17
2756	VA TC 4177	VA06D	Mosquito	2006	VA	Norfolk	4	10
2757	VA TC 2076	VA02E	Mosquito	2002	VA	Norfolk	4	3
2758	VA TC 3278	VA03E	Mosquito	2003	VA	Norfolk	5	9
2759	VA TC 1155	VA04C	Mosquito	2004	VA	Norfolk	4	14
2760	VA SN 4826-09	VA09E	Mosquito	2009	VA	Norfolk	4	15
2761	VA TC 2147	VA02F	Mosquito	2002	VA	Norfolk	4	15
2762	VA TC 1184-10	VA10D	Mosquito	2010	VA	Norfolk	6	14
2763	VA TC 1272	VA04D	Mosquito	2004	VA	Norfolk	4	8
2764	VA TC 1732-08	VA08D	Mosquito	2008	VA	Norfolk	5	8
2775	VA TC 1597	VA04B	Mosquito	2004	VA	Norfolk	2	2
2776	VA TC 1732-09	VA09D	Mosquito	2009	VA	Norfolk	5	9
2777	VA TC 2045-08	VA08C	Mosquito	2008	VA	Norfolk	4	4
2940	Laco 3008	CO03C	Avian	2003	CO	Fort Collins (80526)	1	13
2941	CO1862	CO04E	Mosquito	2004	CO	Larimer Co.	2	5
2942	GA lwn 50 4936	GA05B	Mosquito	2005	GA	Lawnder Co.	4	3
2945	AIDL-M-015	CO03D	Mosquito	2003	CO	Larimer Co.	6	5
2946	CO 06-7390	CO06A	Mosquito	2006	CO	Weld Co.	7	34
2947	M07-087	GA07B	Mosquito	2007	GA	Garden City, Chatham	4	19
2948	CO 07-8779	CO07C	Mosquito	2007	CO	Weld Co.	2	77
2949	FNT 09-199	GA09A	Mosquito	2009	GA	Atlanta, Fulton Co	4	15
2960	FNT 09-144	GA09C	Mosquito	2009	GA	Atlanta, Fulton Co	5	3
2961	DKB 08-0403	GA08A	Mosquito	2008	GA	Decatur, Dekalb	6	7
2962	Lwn 09-846	GA09B	Mosquito	2009	GA	Valdasta, Lowndes	3	3
2963	LACO-3041	CO03E	Avian	2003	CO	Fort Collins (80525)	5	53
2964	CO 06-608	CO06B	Mosquito	2006	CO	Weld Co.	5	21
2970	DB 4217	CO04H	Avian	2004	CO	Loveland, LarimerCo.	3	33
2971	DES 566-01	GA01C	Avian	2001	GA	Wane Co	5	10
2972	Laco 3038	CO03F	Avian	2003	CO	Fort Collins (80526)	6	10
2973	CO 06-10725	CO06C	Mosquito	2006	CO	Weld Co.	4	7
2974	CO 07-11032	CO06D	Mosquito	2006	CO	Weld Co.	6	10
2980	AIDL-M-012	CO03G	Mosquito	2003	CO	Larimer Co.	4	51
2981	Laco 3020	CO03H	Avian	2003	CO	Fort Collins	5	7
2982	CO 06-10723	CO06E	Mosquito	2006	CO	Weld Co.	6	62
2983	CO 08-13382	CO08A	Mosquito	2008	CO	Wellington, Larimer Co.	4	5

2984	DES 107-01	GA01D	Avian	2001	GA	Lowndes Co.	5	47
2997	CO 07-10970	CO07D	Mosquito	2007	CO	Weld Co.	2	2
2998	GT 02566	CO07E	Mosquito	2007	CO	colorado	6	10
2999	CO 06-584	CO06F	Mosquito	2006	CO	Weld Co.	7	31
3000	CO 07-8778	CO07F	Mosquito	2007	CO	Weld Co.	3	18
3001	CO 08-13386	CO08B	Mosquito	2008	CO	Wellington, Larimer Co.	3	6
3002	CO 2572	CO04F	Mosquito	2004	CO	Weld Co.	6	19
3003	CO-13363	CO08C	Mosquito	2008	CO	Wellington, Larimer Co.	8	8
3004	DB 4218	CO04G	Avian	2004	CO	Wellington, Larimer Co.	3	8
3005	CO 06-10716	CO06G	Mosquito	2006	CO	Weld Co.	6	13
3006	CO 08-13401	CO08D	Mosquito	2008	CO	Wellington, Larimer Co.	2	12
3007	CO 07-11027	CO07G	Mosquito	2007	CO	Weld Co.	3	1
3008	CO 07-9340	CO07H	Mosquito	2007	CO	Weld Co.	6	88
3009	CO 08-13787	CO08E	Mosquito	2008	CO	Wellington, Larimer Co.	3	9
3010	Laco 3022	CO03I	Avian	2003	CO	Ft. Collins (80526)	3	12
3011	CO 08-13410	CO08F	Mosquito	2008	CO	Scarborough Forth Collins	7	20
3012	DES 1191-02	GA02C	Avian	2002	GA	Fulton Co.	4	31
3013	DES 160-02	GA02D	Avian	2002	GA	Dekalb Co	3	7
3014	DES 1476-01	GA01E	Avian	2001	GA	Dekalb Co	2	20
3015	DES 1201-02	GA02E	Avian	2002	GA	Muscogee Co.	6	26
3016	GA Chc 04-1485	GA04B	Mosquito	2004	GA	Chattron Co.	5	15
3017	M07-086	GA07C	Mosquito	2007	GA	Atlanta, Fulton Co	4	4
3018	DES 07-53	GA07D	Avian	2007	GA	Norcross, Gwinnett	3	12
3019	DES 07-62	GA07E	Avian	2007	GA	Savannah, Chatham	3	12
3020	GA 05-179	GA05A	Avian	2005	GA	Dekalb Co	4	24
3021	GA 04-230	GA04A	Avian	2004	GA	Henry Co.	5	7
3022	DBK 08-0491	GA08B	Mosquito	2008	GA	Decatur, Dekalb	4	12
3023	laco 3030	CO03J	Avian	2003	CO	Fort Collins	2	83
3024	M07-069	GA07A	Mosquito	2007	GA	Garden City, Chatham	4	3

Appendix II

Summary of SNVs identified in Chapter 3. The position of each SNV was provided including the genome position, and gene position. Gene-AA indicated the amino acid position in each gene and Gene-N indicated the nucleotide position. SNV type was also provided (LP or NSUB) along with details about the consensus nucleotide (Cons) and the variant nucleotide (Var). Inserted nucleotides were indicated as I and deletions were indicated as d.

TWN	Genome Position	Gene-AA	Gene-N	Var	Cons	Strd bias pval	Type	Frequency (%)
2672	9006	NS5-442	NS5-1326	IC	d	1.00	LP	0.22
2672	8413	NS5-245	NS5-733	IA	d	0.40	LP	0.55
2672	7960	NS5-94	NS5-280	IA	d	0.40	LP	1.38
2672	4109	NS2A-195	NS2A-584	IA	d	0.16	LP	1.41
2672	6203	NS3-531	NS3-1592	IA	d	0.64	LP	6.22
2672	7267	NS4B-118	NS4B-352	IT	d	0.05	LP	8.94
2672	9002	NS5-441	NS5-1322	A	G	1.00	NSUB	0.21
2672	10048	NS5-790	NS5-2368	C	G	1.00	NSUB	0.27
2672	10047	NS5-789	NS5-2367	C	T	1.00	NSUB	0.27
2672	9623	NS5-648	NS5-1943	C	T	0.24	NSUB	0.47
2672	9628	NS5-650	NS5-1948	T	A	0.23	NSUB	0.49
2672	7773	NS5-31	NS5-93	T	C	1.00	NSUB	0.53
2672	10349	NS5-890	NS5-2669	T	C	0.67	NSUB	0.61
2672	6516	NS4A-16	NS4A-48	T	C	0.93	NSUB	0.65
2672	1494	E-176	E-528	T	C	1.00	NSUB	0.66
2672	6681	NS4A-71	NS4A-213	T	C	0.40	NSUB	0.68
2672	649	prM-62	prM-184	T	C	0.67	NSUB	0.79
2672	6385	NS3-592	NS3-1774	T	C	0.18	NSUB	0.83
2672	9573	NS5-631	NS5-1893	A	G	1.00	NSUB	0.84
2672	282	C-62	C-186	T	C	0.29	NSUB	0.86
2672	4129	NS2A-202	NS2A-604	T	C	1.00	NSUB	0.94
2672	3570	NS2A-15	NS2A-45	T	C	0.68	NSUB	0.96
2672	1200	E-78	E-234	G	A	0.95	NSUB	1.03
2672	3720	NS2A-65	NS2A-195	T	C	0.18	NSUB	1.03
2672	7519	NS4B-202	NS4B-604	G	A	0.83	NSUB	1.04
2672	4686	NS3-25	NS3-75	T	C	0.38	NSUB	1.17
2672	6204	NS3-531	NS3-1593	G	A	0.87	NSUB	1.23
2672	2165	E-400	E-1199	T	C	0.57	NSUB	1.37
2672	7627	NS4B-238	NS4B-712	T	C	0.69	NSUB	1.37
2672	1702	E-246	E-736	T	C	0.55	NSUB	1.38

2672	2881	NS1-138	NS1-412	T	C	0.07	NSUB	1.43
2672	4272	NS2B-18	NS2B-54	T	C	0.76	NSUB	1.45
2672	10558	3'UTR-163	3'UTR-163	T	G	1.00	NSUB	1.48
2672	5039	NS3-143	NS3-428	G	A	0.59	NSUB	1.53
2672	8472	NS5-264	NS5-792	T	C	1.00	NSUB	1.56
2672	3018	NS1-183	NS1-549	T	C	0.37	NSUB	1.60
2672	1062	E-32	E-96	C	T	0.63	NSUB	1.60
2672	6675	NS4A-69	NS4A-207	T	C	0.69	NSUB	1.61
2672	2359	E-465	E-1393	T	C	0.84	NSUB	1.66
2672	4815	NS3-68	NS3-204	T	C	0.31	NSUB	1.74
2672	395	C-100	C-299	T	C	0.39	NSUB	1.79
2672	666	prM-67	prM-201	C	T	0.81	NSUB	1.86
2672	2562	NS1-31	NS1-93	T	G	0.08	NSUB	1.86
2672	10536	3'UTR-141	3'UTR-141	C	T	1.00	NSUB	2.10
2672	4825	NS3-72	NS3-214	A	G	0.62	NSUB	2.11
2672	4230	NS2B-4	NS2B-12	G	A	0.14	NSUB	2.27
2672	6238	NS3-543	NS3-1627	T	C	0.33	NSUB	2.30
2672	6870	NS4A-134	NS4A-402	T	C	0.86	NSUB	2.32
2672	1599	E-211	E-633	T	C	0.42	NSUB	2.70
2672	10110	NS5-810	NS5-2430	G	A	0.81	NSUB	2.81
2672	9136	NS5-486	NS5-1456	T	C	0.88	NSUB	2.85
2672	6871	NS4A-135	NS4A-403	A	G	0.70	NSUB	2.92
2672	7155	NS4B-80	NS4B-240	T	C	0.74	NSUB	2.93
2672	736	prM-91	prM-271	A	C	0.64	NSUB	3.09
2672	1945	E-327	E-979	C	T	0.73	NSUB	3.78
2672	9996	NS5-772	NS5-2316	T	C	0.83	NSUB	3.98
2672	4735	NS3-42	NS3-124	A	G	0.63	NSUB	4.06
2672	9955	NS5-759	NS5-2275	T	C	0.37	NSUB	4.18
2672	7015	NS4B-34	NS4B-100	T	C	0.21	NSUB	4.28
2672	950	prM-162	prM-485	C	T	0.44	NSUB	4.58
2672	6798	NS4A-110	NS4A-330	T	C	0.32	NSUB	4.93
2672	7152	NS4B-79	NS4B-237	G	A	0.59	NSUB	5.23
2672	7233	NS4B-106	NS4B-318	T	C	0.50	NSUB	5.24
2672	10435	3'UTR-40	3'UTR-40	T	C	0.83	NSUB	5.52
2672	6138	NS3-509	NS3-1527	T	C	0.35	NSUB	5.81
2672	8670	NS5-330	NS5-990	T	C	0.34	NSUB	6.61
2672	5976	NS3-455	NS3-1365	T	C	0.59	NSUB	6.90
2672	10408	3'UTR-13	3'UTR-13	T	C	0.40	NSUB	8.00
2672	7183	NS4B-90	NS4B-268	A	G	0.67	NSUB	14.65

2672	8319	NS5-213	NS5-639	G	A	0.37	NSUB	24.95
2673	7319	NS4B-135	NS4B-404	D1	i	1.00	LP	0.19
2673	7311	NS4B-132	NS4B-396	IC	d	1.00	LP	0.19
2673	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.62
2673	4109	NS2A-195	NS2A-584	IA	d	0.95	LP	1.68
2673	9063	NS5-461	NS5-1383	IA	d	0.12	LP	3.93
2673	7267	NS4B-118	NS4B-352	IT	d	0.10	LP	11.93
2673	7315	NS4B-134	NS4B-400	T	A	1.00	NSUB	0.19
2673	9623	NS5-648	NS5-1943	C	T	0.38	NSUB	0.36
2673	7711	NS5-11	NS5-31	T	G	0.69	NSUB	0.51
2673	683	prM-73	prM-218	T	C	0.23	NSUB	0.56
2673	1599	E-211	E-633	T	C	0.71	NSUB	0.75
2673	977	E-4	E-11	C	T	0.31	NSUB	0.82
2673	10871	3'UTR-476	3'UTR-476	A	G	0.64	NSUB	0.94
2673	5166	NS3-185	NS3-555	A	G	0.36	NSUB	1.23
2673	8301	NS5-207	NS5-621	T	C	0.49	NSUB	1.36
2673	1356	E-130	E-390	T	C	0.26	NSUB	1.71
2673	10341	NS5-887	NS5-2661	T	C	0.93	NSUB	1.77
2673	3864	NS2A-113	NS2A-339	T	C	0.67	NSUB	2.00
2673	5076	NS3-155	NS3-465	G	A	0.18	NSUB	2.87
2673	4068	NS2A-181	NS2A-543	T	C	0.88	NSUB	4.32
2673	2037	E-357	E-1071	C	T	0.63	NSUB	6.48
2673	6888	NS4A-140	NS4A-420	T	C	0.25	NSUB	7.70
2673	3649	NS2A-42	NS2A-124	T	C	0.93	NSUB	8.49
2673	3290	NS1-274	NS1-821	G	A	0.67	NSUB	10.53
2674	7960	NS5-94	NS5-280	IA	d	0.41	LP	0.67
2674	5166	NS3-185	NS3-555	IA	d	0.29	LP	0.95
2674	4109	NS2A-195	NS2A-584	IA	d	0.07	LP	0.97
2674	9063	NS5-461	NS5-1383	IA	d	0.86	LP	1.57
2674	6203	NS3-531	NS3-1592	IA	d	0.45	LP	2.16
2674	7267	NS4B-118	NS4B-352	IT	d	0.30	LP	7.87
2674	5036	NS3-142	NS3-425	G	A	0.66	NSUB	0.51
2674	9019	NS5-447	NS5-1339	A	G	1.00	NSUB	0.56
2674	10198	NS5-840	NS5-2518	A	G	0.22	NSUB	0.56
2674	3977	NS2A-151	NS2A-452	T	C	1.00	NSUB	0.58
2674	1656	E-230	E-690	A	T	0.73	NSUB	0.63
2674	9917	NS5-746	NS5-2237	T	C	1.00	NSUB	0.66
2674	3825	NS2A-100	NS2A-300	T	C	1.00	NSUB	0.75
2674	1795	E-277	E-829	G	A	0.70	NSUB	0.79

2674	7012	NS4B-33	NS4B-97	T	C	0.70	NSUB	0.80
2674	6632	NS4A-55	NS4A-164	T	C	0.70	NSUB	0.81
2674	1580	E-205	E-614	T	C	0.70	NSUB	1.21
2674	1167	E-67	E-201	C	T	0.07	NSUB	2.05
2674	2317	E-451	E-1351	T	C	0.49	NSUB	2.09
2674	7155	NS4B-80	NS4B-240	T	C	0.86	NSUB	2.51
2674	1569	E-201	E-603	T	C	0.09	NSUB	3.82
2674	10486	3'UTR-91	3'UTR-91	G	A	0.48	NSUB	4.05
2674	2910	NS1-147	NS1-441	C	T	0.30	NSUB	5.70
2674	2101	E-379	E-1135	G	T	0.93	NSUB	6.30
2674	1500	E-178	E-534	G	A	0.24	NSUB	10.46
2675	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.84
2675	6203	NS3-531	NS3-1592	IA	d	0.55	LP	1.54
2675	4029	NS2A-168	NS2A-504	T	C	1.00	NSUB	0.44
2675	810	prM-115	prM-345	T	C	0.40	NSUB	0.50
2675	3078	NS1-203	NS1-609	G	A	0.69	NSUB	0.70
2675	5691	NS3-360	NS3-1080	C	T	0.32	NSUB	4.44
2693	4109	NS2A-195	NS2A-584	IA	d	0.69	LP	0.67
2693	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.94
2693	7267	NS4B-118	NS4B-352	IT	d	0.29	LP	6.43
2693	6516	NS4A-16	NS4A-48	T	C	1.00	NSUB	0.68
2693	3128	NS1-220	NS1-659	C	T	0.73	NSUB	0.70
2693	10314	NS5-878	NS5-2634	T	C	1.00	NSUB	0.76
2693	1083	E-39	E-117	C	T	1.00	NSUB	0.84
2693	8849	NS5-390	NS5-1169	G	A	0.19	NSUB	0.85
2693	8623	NS5-315	NS5-943	T	C	0.70	NSUB	0.89
2693	7452	NS4B-179	NS4B-537	T	C	0.50	NSUB	0.97
2693	10305	NS5-875	NS5-2625	T	C	0.73	NSUB	0.97
2693	5431	NS3-274	NS3-820	T	C	0.18	NSUB	0.99
2693	1569	E-201	E-603	T	C	0.49	NSUB	1.14
2693	1649	E-228	E-683	T	C	0.33	NSUB	1.29
2693	10341	NS5-887	NS5-2661	T	C	0.38	NSUB	1.30
2693	469	prM-2	prM-4	G	A	0.46	NSUB	1.38
2693	5391	NS3-260	NS3-780	T	C	0.63	NSUB	1.46
2693	10422	3'UTR-27	3'UTR-27	T	C	0.76	NSUB	1.68
2693	1428	E-154	E-462	T	C	0.16	NSUB	1.69
2693	2982	NS1-171	NS1-513	T	C	0.83	NSUB	2.21
2693	3018	NS1-183	NS1-549	T	C	0.27	NSUB	2.43
2693	5478	NS3-289	NS3-867	T	C	0.64	NSUB	2.93

2693	3570	NS2A-15	NS2A-45	T	C	0.21	NSUB	3.37
2693	6450	NS3-613	NS3-1839	T	C	0.09	NSUB	3.44
2693	10408	3'UTR-13	3'UTR-13	T	C	0.61	NSUB	4.35
2693	4255	NS2B-13	NS2B-37	T	C	0.35	NSUB	10.59
2693	6453	NS3-614	NS3-1842	T	C	0.05	NSUB	17.62
2693	5938	NS3-443	NS3-1327	G	A	0.20	NSUB	29.32
2693	729	prM-88	prM-264	G	A	0.90	NSUB	32.18
2694	4109	NS2A-195	NS2A-584	IA	d	0.70	LP	0.62
2694	7960	NS5-94	NS5-280	IA	d	0.60	LP	0.78
2694	9063	NS5-461	NS5-1383	IA	d	1.20	LP	0.91
2694	10157	NS5-826	NS5-2477	G	A	1.00	NSUB	0.20
2694	10160	NS5-827	NS5-2480	G	A	1.00	NSUB	0.22
2694	5932	NS3-441	NS3-1321	T	A	0.64	NSUB	0.37
2694	3436	NS1-323	NS1-967	C	T	1.00	NSUB	0.38
2694	5927	NS3-439	NS3-1316	C	A	0.22	NSUB	0.50
2694	4725	NS3-38	NS3-114	A	G	0.72	NSUB	4.88
2696	4109	NS2A-195	NS2A-584	IA	d	0.08	LP	0.69
2696	7960	NS5-94	NS5-280	IA	d	0.11	LP	0.86
2696	9063	NS5-461	NS5-1383	IA	d	0.54	LP	0.96
2696	5166	NS3-185	NS3-555	IA	d	0.36	LP	1.22
2696	6203	NS3-531	NS3-1592	IA	d	0.18	LP	2.08
2696	2624	NS1-52	NS1-155	G	A	0.13	NSUB	0.44
2696	2853	NS1-128	NS1-384	T	C	1.00	NSUB	0.56
2696	5166	NS3-185	NS3-555	A	G	0.45	NSUB	0.80
2697	4109	NS2A-195	NS2A-584	IA	d	0.06	LP	1.18
2697	7960	NS5-94	NS5-280	IA	d	0.38	LP	1.35
2697	5166	NS3-185	NS3-555	IA	d	0.21	LP	1.52
2697	9063	NS5-461	NS5-1383	IA	d	0.89	LP	1.56
2697	7267	NS4B-118	NS4B-352	IT	d	0.05	LP	11.20
2697	7711	NS5-11	NS5-31	T	G	0.69	NSUB	0.57
2697	7212	NS4B-99	NS4B-297	G	A	0.47	NSUB	0.73
2697	1821	E-285	E-855	C	T	0.74	NSUB	0.89
2697	9378	NS5-566	NS5-1698	C	T	1.00	NSUB	1.10
2697	10683	3'UTR-288	3'UTR-288	T	C	0.69	NSUB	1.43
2697	3042	NS1-191	NS1-573	T	C	0.25	NSUB	1.46
2697	4413	NS2B-65	NS2B-195	C	T	0.87	NSUB	1.63
2697	9909	NS5-743	NS5-2229	C	T	0.82	NSUB	2.34
2698	4650	NS3-13	NS3-39	IA	d	1.00	LP	0.83
2698	4109	NS2A-195	NS2A-584	IA	d	0.10	LP	1.23

2698	7960	NS5-94	NS5-280	IA	d	0.26	LP	1.57
2698	9063	NS5-461	NS5-1383	IA	d	0.38	LP	1.99
2698	7267	NS4B-118	NS4B-352	IT	d	0.13	LP	6.39
2698	2222	E-419	E-1256	T	C	1.00	NSUB	0.43
2698	7934	NS5-85	NS5-254	A	G	1.00	NSUB	0.52
2698	1260	E-98	E-294	T	C	1.00	NSUB	0.53
2698	3021	NS1-184	NS1-552	C	T	0.69	NSUB	0.56
2698	7711	NS5-11	NS5-31	T	G	0.71	NSUB	0.62
2698	10458	3'UTR-63	3'UTR-63	C	G	0.65	NSUB	0.62
2698	9125	NS5-482	NS5-1445	T	G	1.00	NSUB	0.72
2698	8838	NS5-386	NS5-1158	C	T	1.00	NSUB	0.77
2698	6393	NS3-594	NS3-1782	A	G	1.00	NSUB	0.79
2698	433	C-113	C-337	A	G	0.32	NSUB	0.82
2698	5166	NS3-185	NS3-555	A	G	0.23	NSUB	1.27
2698	7226	NS4B-104	NS4B-311	G	A	0.19	NSUB	1.77
2698	8972	NS5-431	NS5-1292	A	C	0.28	NSUB	4.22
2699	10489	3'UTR-94	3'UTR-94	D1	i	0.68	LP	0.77
2699	4650	NS3-13	NS3-39	IA	d	1.01	LP	0.87
2699	4109	NS2A-195	NS2A-584	IA	d	0.34	LP	1.07
2699	7960	NS5-94	NS5-280	IA	d	0.76	LP	1.27
2699	9063	NS5-461	NS5-1383	IA	d	0.74	LP	1.29
2699	7733	NS5-18	NS5-53	G	A	1.00	NSUB	0.48
2699	5166	NS3-185	NS3-555	A	G	0.43	NSUB	0.72
2699	1056	E-30	E-90	T	C	1.00	NSUB	0.80
2699	7711	NS5-11	NS5-31	T	G	0.50	NSUB	0.84
2699	9609	NS5-643	NS5-1929	A	G	0.95	NSUB	0.98
2699	1369	E-135	E-403	G	A	0.88	NSUB	3.88
2700	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.69
2700	4109	NS2A-195	NS2A-584	IA	d	0.81	LP	1.23
2700	5166	NS3-185	NS3-555	IA	d	0.13	LP	1.60
2700	7960	NS5-94	NS5-280	IA	d	0.73	LP	1.94
2700	8799	NS5-373	NS5-1119	T	G	0.41	NSUB	0.39
2700	7804	NS5-42	NS5-124	T	C	1.01	NSUB	0.47
2700	7587	NS4B-224	NS4B-672	T	C	1.00	NSUB	0.48
2700	1839	E-291	E-873	A	G	0.65	NSUB	0.51
2700	5166	NS3-185	NS3-555	A	G	0.72	NSUB	0.53
2700	2222	E-419	E-1256	T	C	0.74	NSUB	0.73
2701	5166	NS3-185	NS3-555	IA	d	0.49	LP	1.01
2701	5812	NS3-401	NS3-1201	D3	i	0.18	LP	1.16

2701	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	1.16
2701	7960	NS5-94	NS5-280	IA	d	0.40	LP	1.47
2701	9063	NS5-461	NS5-1383	IA	d	0.50	LP	1.61
2701	5419	NS3-270	NS3-808	T	C	0.67	NSUB	0.66
2701	3725	NS2A-67	NS2A-200	G	A	1.07	NSUB	0.70
2701	2913	NS1-148	NS1-444	T	C	0.46	NSUB	0.71
2701	3117	NS1-216	NS1-648	C	T	0.75	NSUB	0.79
2701	5874	NS3-421	NS3-1263	T	C	0.44	NSUB	1.11
2701	5166	NS3-185	NS3-555	A	G	1.00	NSUB	1.12
2701	3255	NS1-262	NS1-786	G	A	0.41	NSUB	1.48
2701	9781	NS5-701	NS5-2101	T	C	0.22	NSUB	2.30
2701	10850	3'UTR-455	3'UTR-455	T	G	0.36	NSUB	3.08
2711	4650	NS3-13	NS3-39	IA	d	0.38	LP	0.56
2711	7960	NS5-94	NS5-280	IA	d	0.71	LP	0.77
2711	4109	NS2A-195	NS2A-584	IA	d	0.11	LP	1.13
2711	9063	NS5-461	NS5-1383	IA	d	0.52	LP	1.66
2711	4401	NS2B-61	NS2B-183	T	C	1.00	NSUB	0.89
2711	5181	NS3-190	NS3-570	C	T	0.94	NSUB	1.06
2711	3318	NS1-283	NS1-849	C	T	1.00	NSUB	1.07
2711	306	C-70	C-210	G	A	0.81	NSUB	1.26
2711	9336	NS5-552	NS5-1656	G	A	0.85	NSUB	1.55
2711	5924	NS3-438	NS3-1313	A	G	0.75	NSUB	2.10
2711	7065	NS4B-50	NS4B-150	T	G	0.36	NSUB	2.12
2711	6996	NS4B-27	NS4B-81	T	G	0.84	NSUB	34.25
2711	4083	NS2A-186	NS2A-558	G	A	0.33	NSUB	36.06
2713	9063	NS5-461	NS5-1383	IA	d	0.69	LP	0.65
2713	4109	NS2A-195	NS2A-584	IA	d	0.69	LP	0.75
2713	7960	NS5-94	NS5-280	IA	d	0.71	LP	0.95
2713	6203	NS3-531	NS3-1592	IA	d	0.30	LP	1.78
2713	4758	NS3-49	NS3-147	C	T	1.00	NSUB	0.52
2714	7960	NS5-94	NS5-280	IA	d	0.48	LP	0.84
2714	5166	NS3-185	NS3-555	IA	d	0.30	LP	1.14
2714	4109	NS2A-195	NS2A-584	IA	d	0.10	LP	1.41
2714	6203	NS3-531	NS3-1592	IA	d	0.74	LP	2.27
2714	7267	NS4B-118	NS4B-352	IT	d	0.26	LP	8.38
2714	7604	NS4B-230	NS4B-689	C	T	0.76	NSUB	0.96
2714	10481	3'UTR-86	3'UTR-86	C	T	0.91	NSUB	3.60
2714	2318	E-451	E-1352	A	G	0.31	NSUB	5.75
2715	7960	NS5-94	NS5-280	IA	d	0.25	LP	0.77

2715	4109	NS2A-195	NS2A-584	IA	d	0.12	LP	1.04
2715	9063	NS5-461	NS5-1383	IA	d	0.79	LP	1.10
2715	6203	NS3-531	NS3-1592	IA	d	0.18	LP	4.21
2715	9066	NS5-462	NS5-1386	G	A	1.00	NSUB	0.19
2715	9072	NS5-464	NS5-1392	T	C	1.00	NSUB	0.28
2715	7267	NS4B-118	NS4B-352	T	C	1.00	NSUB	0.52
2715	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.62
2715	9997	NS5-773	NS5-2317	T	C	1.00	NSUB	0.72
2715	1700	E-245	E-734	T	C	0.69	NSUB	1.26
2715	1065	E-33	E-99	T	C	0.29	NSUB	1.48
2715	3808	NS2A-95	NS2A-283	C	T	0.68	NSUB	3.26
2715	6936	NS4B-7	NS4B-21	C	T	0.35	NSUB	3.99
2715	3817	NS2A-98	NS2A-292	G	A	0.42	NSUB	15.20
2716	7960	NS5-94	NS5-280	IA	d	0.48	LP	0.96
2716	4109	NS2A-195	NS2A-584	IA	d	0.41	LP	1.28
2716	9063	NS5-461	NS5-1383	IA	d	0.73	LP	1.35
2716	6203	NS3-531	NS3-1592	IA	d	0.13	LP	3.79
2716	10813	3'UTR-418	3'UTR-418	A	G	1.00	NSUB	0.41
2716	5166	NS3-185	NS3-555	A	G	0.68	NSUB	0.61
2716	1033	E-23	E-67	G	T	0.71	NSUB	0.62
2716	1143	E-59	E-177	C	T	0.85	NSUB	0.73
2716	865	prM-134	prM-400	C	T	0.75	NSUB	1.16
2717	4109	NS2A-195	NS2A-584	IA	d	0.71	LP	0.71
2717	5166	NS3-185	NS3-555	IA	d	0.73	LP	1.00
2717	9063	NS5-461	NS5-1383	IA	d	0.76	LP	1.13
2717	7960	NS5-94	NS5-280	IA	d	0.53	LP	1.34
2717	7267	NS4B-118	NS4B-352	IT	d	0.11	LP	8.62
2717	8496	NS5-272	NS5-816	C	T	1.00	NSUB	0.20
2717	8502	NS5-274	NS5-822	G	A	1.00	NSUB	0.22
2717	5935	NS3-442	NS3-1324	T	G	1.00	NSUB	0.25
2717	5932	NS3-441	NS3-1321	T	A	1.00	NSUB	0.26
2717	7332	NS4B-139	NS4B-417	A	G	0.43	NSUB	0.64
2717	3135	NS1-222	NS1-666	C	A	1.00	NSUB	0.77
2717	6204	NS3-531	NS3-1593	A	G	1.00	NSUB	0.80
2717	5709	NS3-366	NS3-1098	T	C	0.60	NSUB	0.88
2717	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.99
2717	2574	NS1-35	NS1-105	T	C	0.13	NSUB	1.02
2717	6936	NS4B-7	NS4B-21	C	T	0.23	NSUB	3.32
2717	2446	E-494	E-1480	C	T	0.34	NSUB	3.63

2717	4093	NS2A-190	NS2A-568	G	A	0.07	NSUB	12.57
2718	7960	NS5-94	NS5-280	IA	d	0.94	LP	0.77
2718	4109	NS2A-195	NS2A-584	IA	d	0.47	LP	0.83
2718	5166	NS3-185	NS3-555	IA	d	0.53	LP	1.27
2718	9063	NS5-461	NS5-1383	IA	d	0.64	LP	1.44
2718	7186	NS4B-91	NS4B-271	IG	d	0.57	LP	3.05
2718	7183	NS4B-90	NS4B-268	D1	i	0.42	LP	3.08
2718	6203	NS3-531	NS3-1592	IA	d	0.39	LP	3.19
2718	732	prM-89	prM-267	T	C	1.00	NSUB	0.38
2718	371	C-92	C-275	T	C	0.65	NSUB	0.43
2718	433	C-113	C-337	A	G	0.65	NSUB	0.46
2718	9480	NS5-600	NS5-1800	C	T	0.79	NSUB	0.63
2718	3159	NS1-230	NS1-690	T	C	1.00	NSUB	0.66
2718	3177	NS1-236	NS1-708	T	C	0.69	NSUB	0.74
2718	1595	E-210	E-629	T	C	1.00	NSUB	0.84
2718	8820	NS5-380	NS5-1140	T	C	1.00	NSUB	0.91
2718	3898	NS2A-125	NS2A-373	T	C	1.00	NSUB	1.03
2718	5166	NS3-185	NS3-555	A	G	0.47	NSUB	1.04
2718	8691	NS5-337	NS5-1011	T	C	1.02	NSUB	1.09
2718	8051	NS5-124	NS5-371	C	T	0.90	NSUB	1.59
2718	2930	NS1-154	NS1-461	G	A	0.09	NSUB	1.60
2718	8700	NS5-340	NS5-1020	C	T	0.46	NSUB	1.91
2718	2803	NS1-112	NS1-334	A	G	0.64	NSUB	2.05
2718	2348	E-461	E-1382	T	C	0.39	NSUB	3.06
2718	7226	NS4B-104	NS4B-311	A	G	0.22	NSUB	4.99
2718	7947	NS5-89	NS5-267	T	C	0.63	NSUB	6.49
2718	2213	E-416	E-1247	G	A	0.83	NSUB	9.22
2718	7419	NS4B-168	NS4B-504	T	C	0.23	NSUB	10.09
2718	3774	NS2A-83	NS2A-249	T	C	0.14	NSUB	11.65
2718	2572	NS1-35	NS1-103	C	T	0.62	NSUB	12.02
2718	8967	NS5-429	NS5-1287	G	A	0.42	NSUB	17.30
2718	1900	E-312	E-934	T	C	0.09	NSUB	19.08
2719	9063	NS5-461	NS5-1383	IA	d	0.45	LP	0.72
2719	7960	NS5-94	NS5-280	IA	d	0.36	LP	1.08
2719	4109	NS2A-195	NS2A-584	IA	d	0.39	LP	1.20
2719	5166	NS3-185	NS3-555	IA	d	0.07	LP	2.20
2719	6203	NS3-531	NS3-1592	IA	d	0.50	LP	5.07
2719	5943	NS3-444	NS3-1332	A	G	1.00	NSUB	0.39
2719	544	prM-27	prM-79	G	A	1.00	NSUB	0.48

2719	6807	NS4A-113	NS4A-339	T	C	0.41	NSUB	0.48
2719	10317	NS5-879	NS5-2637	T	C	1.00	NSUB	0.51
2719	10349	NS5-890	NS5-2669	T	C	1.00	NSUB	0.52
2719	8529	NS5-283	NS5-849	C	T	1.00	NSUB	0.52
2719	759	prM-98	prM-294	G	A	1.00	NSUB	0.53
2719	4129	NS2A-202	NS2A-604	T	C	1.00	NSUB	0.54
2719	4054	NS2A-177	NS2A-529	T	C	1.00	NSUB	0.54
2719	4430	NS2B-71	NS2B-212	T	C	0.39	NSUB	0.66
2719	7419	NS4B-168	NS4B-504	T	C	0.71	NSUB	0.67
2719	1790	E-275	E-824	T	C	0.79	NSUB	0.73
2719	6901	NS4A-145	NS4A-433	G	A	1.00	NSUB	0.73
2719	10603	3'UTR-208	3'UTR-208	T	C	1.00	NSUB	0.74
2719	5148	NS3-179	NS3-537	T	C	0.73	NSUB	0.74
2719	1956	E-330	E-990	C	T	0.25	NSUB	0.79
2719	789	prM-108	prM-324	A	G	0.18	NSUB	0.83
2719	8661	NS5-327	NS5-981	T	C	1.00	NSUB	0.85
2719	9996	NS5-772	NS5-2316	T	C	1.00	NSUB	0.86
2719	6138	NS3-509	NS3-1527	T	C	0.52	NSUB	0.86
2719	6385	NS3-592	NS3-1774	T	C	1.00	NSUB	0.87
2719	2535	NS1-22	NS1-66	T	C	0.43	NSUB	0.89
2719	7382	NS4B-156	NS4B-467	T	C	0.42	NSUB	0.90
2719	1191	E-75	E-225	A	G	0.11	NSUB	0.93
2719	4536	NS2B-106	NS2B-318	T	G	1.00	NSUB	0.95
2719	8403	NS5-241	NS5-723	G	A	1.00	NSUB	0.95
2719	3254	NS1-262	NS1-785	T	C	0.12	NSUB	0.98
2719	679	prM-72	prM-214	A	T	0.71	NSUB	1.02
2719	3762	NS2A-79	NS2A-237	T	C	0.19	NSUB	1.11
2719	1515	E-183	E-549	C	T	0.72	NSUB	1.12
2719	4674	NS3-21	NS3-63	T	C	0.34	NSUB	1.14
2719	2758	NS1-97	NS1-289	C	T	0.33	NSUB	1.18
2719	3018	NS1-183	NS1-549	T	C	0.94	NSUB	1.19
2719	3864	NS2A-113	NS2A-339	T	C	0.22	NSUB	1.21
2719	5526	NS3-305	NS3-915	T	C	0.57	NSUB	1.29
2719	5191	NS3-194	NS3-580	T	C	0.68	NSUB	1.30
2719	10026	NS5-782	NS5-2346	T	C	1.00	NSUB	1.32
2719	8988	NS5-436	NS5-1308	T	G	1.00	NSUB	1.32
2719	10131	NS5-817	NS5-2451	T	C	0.82	NSUB	1.33
2719	5166	NS3-185	NS3-555	A	G	0.24	NSUB	1.35
2719	10871	3'UTR-476	3'UTR-476	A	G	0.69	NSUB	1.36

2719	638	prM-58	prM-173	T	C	0.49	NSUB	1.43
2719	1844	E-293	E-878	G	A	1.01	NSUB	1.44
2719	7392	NS4B-159	NS4B-477	T	C	0.34	NSUB	1.60
2719	3177	NS1-236	NS1-708	T	C	0.41	NSUB	1.60
2719	7068	NS4B-51	NS4B-153	T	C	0.97	NSUB	1.69
2719	6405	NS3-598	NS3-1794	T	C	0.67	NSUB	1.69
2719	10445	3'UTR-50	3'UTR-50	C	T	0.79	NSUB	1.77
2719	3984	NS2A-153	NS2A-459	T	C	0.90	NSUB	2.20
2719	4080	NS2A-185	NS2A-555	T	C	0.70	NSUB	2.25
2719	10305	NS5-875	NS5-2625	T	C	0.39	NSUB	2.27
2719	10023	NS5-781	NS5-2343	T	C	0.14	NSUB	2.55
2719	93	5'UTR-93	5'UTR-93	T	C	1.00	NSUB	4.09
2730	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.53
2730	4109	NS2A-195	NS2A-584	IA	d	0.71	LP	0.86
2730	9063	NS5-461	NS5-1383	IA	d	0.32	LP	1.20
2730	5166	NS3-185	NS3-555	IA	d	0.48	LP	1.33
2730	6203	NS3-531	NS3-1592	IA	d	0.33	LP	2.72
2730	7267	NS4B-118	NS4B-352	IT	d	0.69	LP	8.92
2730	4036	NS2A-171	NS2A-511	C	T	1.00	NSUB	0.18
2730	4038	NS2A-171	NS2A-513	G	A	1.00	NSUB	0.19
2730	5838	NS3-409	NS3-1227	A	G	0.63	NSUB	0.33
2730	916	prM-151	prM-451	T	C	1.00	NSUB	0.49
2730	5002	NS3-131	NS3-391	T	C	1.00	NSUB	0.51
2730	7848	NS5-56	NS5-168	C	T	1.00	NSUB	0.60
2730	7711	NS5-11	NS5-31	T	G	0.96	NSUB	0.91
2731	8413	NS5-245	NS5-733	IA	d	1.00	LP	0.53
2731	7960	NS5-94	NS5-280	IA	d	0.66	LP	0.60
2731	5166	NS3-185	NS3-555	IA	d	0.31	LP	1.21
2731	4109	NS2A-195	NS2A-584	IA	d	0.57	LP	1.44
2731	6203	NS3-531	NS3-1592	IA	d	0.71	LP	4.22
2731	7267	NS4B-118	NS4B-352	IT	d	0.24	LP	10.74
2731	7322	NS4B-136	NS4B-407	T	C	0.65	NSUB	0.47
2731	6938	NS4B-8	NS4B-23	G	A	0.38	NSUB	0.49
2731	7377	NS4B-154	NS4B-462	T	C	1.00	NSUB	0.53
2731	358	C-88	C-262	T	C	1.00	NSUB	0.63
2731	8640	NS5-320	NS5-960	C	T	0.26	NSUB	0.75
2731	8691	NS5-337	NS5-1011	T	C	1.16	NSUB	0.88
2731	2629	NS1-54	NS1-160	A	G	0.56	NSUB	1.04
2731	10386	NS5-902	NS5-2706	T	C	1.04	NSUB	1.22

2731	3388	NS1-307	NS1-919	C	T	0.32	NSUB	1.30
2731	5247	NS3-212	NS3-636	T	C	0.90	NSUB	1.98
2731	3864	NS2A-113	NS2A-339	T	C	0.18	NSUB	2.34
2731	10349	NS5-890	NS5-2669	T	C	0.89	NSUB	2.84
2731	1781	E-272	E-815	C	T	0.53	NSUB	2.89
2731	10437	3'UTR-42	3'UTR-42	C	T	0.68	NSUB	3.25
2731	1599	E-211	E-633	T	C	0.20	NSUB	3.61
2731	10044	NS5-788	NS5-2364	T	C	0.79	NSUB	4.39
2731	395	C-100	C-299	T	C	0.58	NSUB	5.00
2731	10408	3'UTR-13	3'UTR-13	T	C	0.74	NSUB	8.76
2733	7445	NS4B-177	NS4B-530	D1	i	1.00	LP	0.21
2733	7447	NS4B-178	NS4B-532	IA	d	1.00	LP	0.22
2733	8413	NS5-245	NS5-733	IA	d	0.38	LP	0.58
2733	4109	NS2A-195	NS2A-584	IA	d	0.58	LP	1.18
2733	7960	NS5-94	NS5-280	IA	d	0.57	LP	1.21
2733	5166	NS3-185	NS3-555	IA	d	0.45	LP	1.73
2733	9063	NS5-461	NS5-1383	IA	d	0.26	LP	2.20
2733	6203	NS3-531	NS3-1592	IA	d	0.45	LP	5.92
2733	7267	NS4B-118	NS4B-352	IT	d	0.10	LP	9.35
2733	5927	NS3-439	NS3-1316	C	A	1.00	NSUB	0.16
2733	5932	NS3-441	NS3-1321	T	A	1.00	NSUB	0.18
2733	775	prM-104	prM-310	T	C	1.00	NSUB	0.43
2733	9525	NS5-615	NS5-1845	T	C	0.62	NSUB	0.52
2733	1041	E-25	E-75	T	C	1.00	NSUB	0.52
2733	987	E-7	E-21	T	C	1.00	NSUB	0.55
2733	8849	NS5-390	NS5-1169	G	A	0.69	NSUB	0.55
2733	5224	NS3-205	NS3-613	T	C	0.48	NSUB	0.57
2733	4080	NS2A-185	NS2A-555	T	C	1.00	NSUB	0.57
2733	10017	NS5-779	NS5-2337	T	C	1.00	NSUB	0.61
2733	6203	NS3-531	NS3-1592	A	G	0.71	NSUB	0.69
2733	10373	NS5-898	NS5-2693	T	C	0.71	NSUB	0.71
2733	4036	NS2A-171	NS2A-511	T	C	1.00	NSUB	0.72
2733	1799	E-278	E-833	T	C	0.22	NSUB	0.73
2733	4290	NS2B-24	NS2B-72	C	T	0.70	NSUB	0.74
2733	628	prM-55	prM-163	A	G	0.65	NSUB	0.76
2733	4473	NS2B-85	NS2B-255	T	C	0.45	NSUB	0.81
2733	8253	NS5-191	NS5-573	T	C	0.80	NSUB	0.86
2733	3690	NS2A-55	NS2A-165	G	A	1.09	NSUB	0.91
2733	4518	NS2B-100	NS2B-300	T	C	0.45	NSUB	0.95

2733	1498	E-178	E-532	T	C	0.77	NSUB	0.96
2733	6214	NS3-535	NS3-1603	T	C	0.21	NSUB	0.96
2733	3699	NS2A-58	NS2A-174	T	C	0.75	NSUB	0.98
2733	5148	NS3-179	NS3-537	T	C	1.03	NSUB	1.06
2733	5166	NS3-185	NS3-555	A	G	1.01	NSUB	1.10
2733	4215	NS2A-230	NS2A-690	G	A	0.76	NSUB	1.12
2733	7341	NS4B-142	NS4B-426	A	G	0.24	NSUB	1.17
2733	10349	NS5-890	NS5-2669	T	C	0.37	NSUB	1.18
2733	5076	NS3-155	NS3-465	G	A	0.85	NSUB	1.25
2733	2712	NS1-81	NS1-243	A	G	0.88	NSUB	1.29
2733	10393	NS5-905	NS5-2713	T	C	0.73	NSUB	1.29
2733	9136	NS5-486	NS5-1456	T	C	1.00	NSUB	1.31
2733	5859	NS3-416	NS3-1248	C	T	0.70	NSUB	1.33
2733	2981	NS1-171	NS1-512	C	T	0.96	NSUB	1.40
2733	3339	NS1-290	NS1-870	T	C	0.74	NSUB	1.41
2733	7068	NS4B-51	NS4B-153	T	C	0.94	NSUB	1.48
2733	1790	E-275	E-824	T	C	0.12	NSUB	1.52
2733	1473	E-169	E-507	T	C	0.90	NSUB	1.59
2733	183	C-29	C-87	G	A	0.77	NSUB	1.73
2733	3011	NS1-181	NS1-542	T	C	0.61	NSUB	1.99
2733	3147	NS1-226	NS1-678	C	T	0.42	NSUB	2.05
2733	6138	NS3-509	NS3-1527	T	C	0.55	NSUB	2.17
2733	3018	NS1-183	NS1-549	T	C	0.58	NSUB	2.18
2733	6238	NS3-543	NS3-1627	T	C	0.57	NSUB	2.22
2733	93	5'UTR-93	5'UTR-93	T	C	0.19	NSUB	3.81
2733	10023	NS5-781	NS5-2343	T	C	0.17	NSUB	5.39
2734	8413	NS5-245	NS5-733	IA	d	1.00	LP	0.70
2734	4109	NS2A-195	NS2A-584	IA	d	0.11	LP	1.05
2734	7960	NS5-94	NS5-280	IA	d	0.54	LP	1.39
2734	9063	NS5-461	NS5-1383	IA	d	0.91	LP	1.45
2734	5166	NS3-185	NS3-555	IA	d	0.08	LP	1.49
2734	7267	NS4B-118	NS4B-352	IT	d	0.16	LP	8.81
2734	5935	NS3-442	NS3-1324	T	G	1.00	NSUB	0.34
2734	5932	NS3-441	NS3-1321	T	A	1.00	NSUB	0.34
2734	6573	NS4A-35	NS4A-105	G	A	0.68	NSUB	0.36
2734	6598	NS4A-44	NS4A-130	T	C	1.00	NSUB	0.40
2734	9600	NS5-640	NS5-1920	T	C	0.43	NSUB	0.40
2734	10211	NS5-844	NS5-2531	T	C	1.00	NSUB	0.41
2734	340	C-82	C-244	T	C	1.00	NSUB	0.45

2734	10349	NS5-890	NS5-2669	T	C	0.64	NSUB	0.47
2734	377	C-94	C-281	T	C	0.41	NSUB	0.48
2734	6780	NS4A-104	NS4A-312	T	C	0.24	NSUB	0.49
2734	266	C-57	C-170	T	C	1.00	NSUB	0.49
2734	1986	E-340	E-1020	T	C	0.65	NSUB	0.49
2734	3702	NS2A-59	NS2A-177	T	C	0.67	NSUB	0.51
2734	573	prM-36	prM-108	T	C	0.22	NSUB	0.53
2734	4288	NS2B-24	NS2B-70	T	C	0.66	NSUB	0.54
2734	1953	E-329	E-987	T	C	1.00	NSUB	0.57
2734	1356	E-130	E-390	T	C	1.00	NSUB	0.59
2734	7015	NS4B-34	NS4B-100	T	C	1.00	NSUB	0.60
2734	1227	E-87	E-261	T	C	1.00	NSUB	0.63
2734	7185	NS4B-90	NS4B-270	C	T	0.22	NSUB	0.64
2734	5517	NS3-302	NS3-906	T	C	0.71	NSUB	0.65
2734	5909	NS3-433	NS3-1298	T	C	1.00	NSUB	0.67
2734	4195	NS2A-224	NS2A-670	A	G	0.49	NSUB	0.70
2734	1883	E-306	E-917	T	C	1.06	NSUB	0.72
2734	1819	E-285	E-853	T	C	1.00	NSUB	0.73
2734	3018	NS1-183	NS1-549	T	C	1.02	NSUB	0.73
2734	842	prM-126	prM-377	T	C	0.72	NSUB	0.74
2734	2615	NS1-49	NS1-146	T	C	0.93	NSUB	0.74
2734	6648	NS4A-60	NS4A-180	A	G	0.29	NSUB	0.75
2734	7184	NS4B-90	NS4B-269	G	A	1.00	NSUB	0.75
2734	9114	NS5-478	NS5-1434	T	C	1.00	NSUB	0.77
2734	725	prM-87	prM-260	T	C	0.19	NSUB	0.79
2734	3033	NS1-188	NS1-564	T	C	0.75	NSUB	0.79
2734	8313	NS5-211	NS5-633	T	C	0.73	NSUB	0.81
2734	6204	NS3-531	NS3-1593	G	A	0.45	NSUB	0.82
2734	2493	NS1-8	NS1-24	T	C	0.77	NSUB	0.83
2734	3997	NS2A-158	NS2A-472	T	C	1.00	NSUB	0.84
2734	5391	NS3-260	NS3-780	T	C	0.96	NSUB	0.85
2734	687	prM-74	prM-222	T	C	1.00	NSUB	0.86
2734	6672	NS4A-68	NS4A-204	T	C	0.17	NSUB	0.88
2734	1844	E-293	E-878	G	A	0.78	NSUB	0.91
2734	4129	NS2A-202	NS2A-604	T	C	0.34	NSUB	1.07
2734	4548	NS2B-110	NS2B-330	T	C	0.42	NSUB	1.09
2734	8055	NS5-125	NS5-375	T	C	0.35	NSUB	1.11
2734	9028	NS5-450	NS5-1348	T	C	1.00	NSUB	1.11
2734	1287	E-107	E-321	G	A	0.75	NSUB	1.17

2734	3864	NS2A-113	NS2A-339	T	C	0.34	NSUB	1.22
2734	4746	NS3-45	NS3-135	C	T	1.00	NSUB	1.24
2734	6385	NS3-592	NS3-1774	T	C	1.01	NSUB	1.25
2734	10422	3'UTR-27	3'UTR-27	T	C	0.70	NSUB	1.26
2734	6888	NS4A-140	NS4A-420	T	C	0.80	NSUB	1.27
2734	7891	NS5-71	NS5-211	T	C	0.92	NSUB	1.28
2734	8649	NS5-323	NS5-969	T	C	0.46	NSUB	1.31
2734	6320	NS3-570	NS3-1709	T	C	0.59	NSUB	1.45
2734	1194	E-76	E-228	T	C	0.21	NSUB	1.45
2734	3010	NS1-181	NS1-541	C	T	0.47	NSUB	1.52
2734	7627	NS4B-238	NS4B-712	T	C	0.68	NSUB	1.56
2734	5145	NS3-178	NS3-534	C	A	0.52	NSUB	1.58
2734	10598	3'UTR-203	3'UTR-203	C	T	0.37	NSUB	1.62
2734	9204	NS5-508	NS5-1524	C	T	0.41	NSUB	1.64
2734	10009	NS5-777	NS5-2329	T	C	0.13	NSUB	1.74
2734	10065	NS5-795	NS5-2385	T	C	0.64	NSUB	1.82
2734	10305	NS5-875	NS5-2625	T	C	0.41	NSUB	1.85
2734	2523	NS1-18	NS1-54	G	A	0.94	NSUB	2.05
2734	8883	NS5-401	NS5-1203	T	C	0.93	NSUB	2.15
2734	2037	E-357	E-1071	C	T	0.07	NSUB	2.25
2734	7950	NS5-90	NS5-270	C	T	0.56	NSUB	2.36
2734	10408	3'UTR-13	3'UTR-13	T	C	0.97	NSUB	2.45
2734	1988	E-341	E-1022	T	C	0.59	NSUB	4.77
2734	10393	NS5-905	NS5-2713	T	C	0.96	NSUB	4.91
2735	8413	NS5-245	NS5-733	IA	d	0.50	LP	0.56
2735	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.93
2735	5166	NS3-185	NS3-555	IA	d	0.19	LP	1.00
2735	7960	NS5-94	NS5-280	IA	d	0.24	LP	1.38
2735	7267	NS4B-118	NS4B-352	IT	d	0.49	LP	9.28
2735	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.64
2735	8910	NS5-410	NS5-1230	C	T	0.78	NSUB	0.84
2735	5166	NS3-185	NS3-555	A	G	0.74	NSUB	0.99
2735	4341	NS2B-41	NS2B-123	A	T	0.12	NSUB	1.08
2735	5635	NS3-342	NS3-1024	A	G	0.56	NSUB	1.17
2735	3699	NS2A-58	NS2A-174	T	C	0.58	NSUB	1.19
2735	7113	NS4B-66	NS4B-198	T	C	0.87	NSUB	1.54
2735	6072	NS3-487	NS3-1461	T	C	0.64	NSUB	1.60
2735	1032	E-22	E-66	C	T	0.15	NSUB	1.73
2735	2904	NS1-145	NS1-435	C	T	0.76	NSUB	1.87

2735	6597	NS4A-43	NS4A-129	T	C	0.15	NSUB	1.92
2735	4362	NS2B-48	NS2B-144	G	A	0.22	NSUB	2.21
2735	4017	NS2A-164	NS2A-492	T	C	0.69	NSUB	2.37
2735	10029	NS5-783	NS5-2349	T	C	0.11	NSUB	2.80
2735	6008	NS3-466	NS3-1397	T	C	0.74	NSUB	2.85
2735	2826	NS1-119	NS1-357	G	A	0.11	NSUB	2.88
2735	4882	NS3-91	NS3-271	A	G	0.14	NSUB	3.20
2735	5231	NS3-207	NS3-620	T	A	0.85	NSUB	3.54
2735	5224	NS3-205	NS3-613	T	C	0.88	NSUB	3.62
2735	9408	NS5-576	NS5-1728	C	T	0.81	NSUB	3.91
2735	10550	3'UTR-155	3'UTR-155	T	C	0.67	NSUB	5.07
2735	3956	NS2A-144	NS2A-431	A	G	0.41	NSUB	6.22
2735	10203	NS5-841	NS5-2523	T	C	0.86	NSUB	6.58
2735	10047	NS5-789	NS5-2367	A	T	0.58	NSUB	7.26
2735	4644	NS3-11	NS3-33	A	G	0.15	NSUB	7.75
2735	3654	NS2A-43	NS2A-129	T	C	0.84	NSUB	7.79
2735	6771	NS4A-101	NS4A-303	G	A	0.68	NSUB	8.05
2735	9993	NS5-771	NS5-2313	T	C	0.56	NSUB	8.53
2735	660	prM-65	prM-195	T	C	0.10	NSUB	8.72
2735	4959	NS3-116	NS3-348	T	C	0.60	NSUB	9.76
2735	3942	NS2A-139	NS2A-417	C	T	0.61	NSUB	9.98
2735	4132	NS2A-203	NS2A-607	T	C	0.96	NSUB	10.14
2735	9325	NS5-549	NS5-1645	C	T	0.31	NSUB	10.20
2735	10408	3'UTR-13	3'UTR-13	C	T	0.37	NSUB	10.28
2735	1557	E-197	E-591	T	C	0.19	NSUB	10.31
2735	5832	NS3-407	NS3-1221	T	C	0.78	NSUB	10.76
2735	3300	NS1-277	NS1-831	C	T	0.99	NSUB	10.89
2735	6238	NS3-543	NS3-1627	T	C	0.20	NSUB	11.03
2735	2529	NS1-20	NS1-60	T	C	0.50	NSUB	11.22
2735	5976	NS3-455	NS3-1365	T	C	0.72	NSUB	11.60
2735	3138	NS1-223	NS1-669	T	C	0.77	NSUB	11.86
2735	10454	3'UTR-59	3'UTR-59	A	G	0.32	NSUB	12.42
2735	8568	NS5-296	NS5-888	C	T	0.33	NSUB	12.67
2735	438	C-114	C-342	G	A	0.45	NSUB	12.70
2735	10393	NS5-905	NS5-2713	C	T	0.20	NSUB	13.21
2735	4323	NS2B-35	NS2B-105	T	C	0.09	NSUB	14.10
2735	5253	NS3-214	NS3-642	C	T	0.38	NSUB	17.42
2735	4212	NS2A-229	NS2A-687	T	C	0.16	NSUB	22.12
2735	2844	NS1-125	NS1-375	A	T	0.77	NSUB	25.50

2736	9063	NS5-461	NS5-1383	IA	d	0.32	LP	0.90
2736	4109	NS2A-195	NS2A-584	IA	d	0.76	LP	1.79
2736	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.86
2736	10871	3'UTR-476	3'UTR-476	A	G	1.00	NSUB	0.66
2736	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.66
2736	5166	NS3-185	NS3-555	A	G	0.47	NSUB	1.09
2736	1629	E-221	E-663	T	C	0.52	NSUB	4.25
2736	1983	E-339	E-1017	C	T	0.71	NSUB	4.50
2736	6688	NS4A-74	NS4A-220	T	C	0.64	NSUB	10.93
2736	3903	NS2A-126	NS2A-378	T	C	0.73	NSUB	21.67
2736	865	prM-134	prM-400	C	T	0.29	NSUB	29.65
2736	6066	NS3-485	NS3-1455	C	T	0.52	NSUB	41.62
2737	4109	NS2A-195	NS2A-584	IA	d	0.25	LP	0.77
2737	7960	NS5-94	NS5-280	IA	d	0.14	LP	1.49
2737	5166	NS3-185	NS3-555	IA	d	0.07	LP	1.58
2737	9063	NS5-461	NS5-1383	IA	d	0.18	LP	1.87
2737	7267	NS4B-118	NS4B-352	IT	d	0.38	LP	10.92
2737	1368	E-134	E-402	T	C	1.00	NSUB	0.48
2737	10888	3'UTR-493	3'UTR-493	A	T	1.00	NSUB	3.03
2737	8778	NS5-366	NS5-1098	A	T	0.11	NSUB	22.64
2738	7960	NS5-94	NS5-280	IA	d	1.02	LP	1.19
2738	9063	NS5-461	NS5-1383	IA	d	0.18	LP	1.36
2738	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	1.52
2738	5166	NS3-185	NS3-555	IA	d	0.18	LP	2.27
2738	7267	NS4B-118	NS4B-352	IT	d	0.25	LP	9.59
2738	9620	NS5-647	NS5-1940	A	G	1.00	NSUB	0.25
2738	9624	NS5-648	NS5-1944	C	T	1.25	NSUB	0.34
2738	6726	NS4A-86	NS4A-258	C	T	1.00	NSUB	0.38
2738	1442	E-159	E-476	T	C	1.00	NSUB	0.40
2738	6204	NS3-531	NS3-1593	A	G	1.00	NSUB	0.43
2738	10415	3'UTR-20	3'UTR-20	G	A	1.00	NSUB	0.49
2738	1422	E-152	E-456	C	T	0.63	NSUB	0.59
2738	9744	NS5-688	NS5-2064	C	T	0.70	NSUB	0.60
2738	10408	3'UTR-13	3'UTR-13	C	T	1.00	NSUB	0.63
2738	6747	NS4A-93	NS4A-279	C	T	1.08	NSUB	0.65
2738	8827	NS5-383	NS5-1147	T	C	1.00	NSUB	0.77
2738	3703	NS2A-60	NS2A-178	T	C	0.13	NSUB	0.80
2738	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.84
2738	692	prM-76	prM-227	C	T	0.34	NSUB	9.98

2738	7516	NS4B-201	NS4B-601	C	T	0.91	NSUB	11.59
2738	10755	3'UTR-360	3'UTR-360	G	C	0.86	NSUB	14.52
2738	735	prM-90	prM-270	C	T	0.09	NSUB	20.63
2756	4650	NS3-13	NS3-39	IA	d	0.71	LP	0.84
2756	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	0.96
2756	9063	NS5-461	NS5-1383	IA	d	0.76	LP	1.05
2756	7960	NS5-94	NS5-280	IA	d	0.77	LP	1.66
2756	2046	E-360	E-1080	C	T	1.00	NSUB	0.54
2756	6891	NS4A-141	NS4A-423	C	G	1.22	NSUB	0.66
2756	7672	NS4B-253	NS4B-757	T	C	1.15	NSUB	0.80
2756	7078	NS4B-55	NS4B-163	T	C	0.94	NSUB	0.86
2756	803	prM-113	prM-338	G	A	0.73	NSUB	0.96
2756	2974	NS1-169	NS1-505	T	C	1.00	NSUB	1.00
2756	1155	E-63	E-189	C	T	0.61	NSUB	1.47
2756	961	prM-166	prM-496	C	T	0.93	NSUB	2.02
2756	9285	NS5-535	NS5-1605	C	T	0.06	NSUB	2.63
2756	10865	3'UTR-470	3'UTR-470	C	T	0.38	NSUB	36.63
2757	7960	NS5-94	NS5-280	IA	d	0.21	LP	1.21
2757	9063	NS5-461	NS5-1383	IA	d	0.87	LP	1.88
2757	6203	NS3-531	NS3-1592	IA	d	0.34	LP	2.42
2757	7267	NS4B-118	NS4B-352	IT	d	0.11	LP	9.85
2757	6108	NS3-499	NS3-1497	T	C	0.69	NSUB	0.49
2757	10888	3'UTR-493	3'UTR-493	A	T	0.50	NSUB	3.34
2757	3243	NS1-258	NS1-774	C	T	0.10	NSUB	37.74
2758	7960	NS5-94	NS5-280	IA	d	0.47	LP	0.89
2758	4109	NS2A-195	NS2A-584	IA	d	0.95	LP	1.65
2758	9063	NS5-461	NS5-1383	IA	d	0.10	LP	1.90
2758	6203	NS3-531	NS3-1592	IA	d	0.10	LP	3.63
2758	7267	NS4B-118	NS4B-352	IT	d	0.07	LP	9.85
2758	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.23
2758	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.44
2758	5927	NS3-439	NS3-1316	C	A	0.22	NSUB	0.44
2758	2619	NS1-50	NS1-150	C	T	0.48	NSUB	0.56
2758	5166	NS3-185	NS3-555	A	G	0.68	NSUB	0.67
2758	6204	NS3-531	NS3-1593	G	A	0.75	NSUB	1.04
2758	6675	NS4A-69	NS4A-207	T	C	0.56	NSUB	1.15
2758	10489	3'UTR-94	3'UTR-94	G	A	0.73	NSUB	1.81
2758	10830	3'UTR-435	3'UTR-435	C	T	0.59	NSUB	17.85
2759	7267	NS4B-118	NS4B-352	ITT	d	1.00	LP	0.71

2759	8972	NS5-431	NS5-1292	IA	d	1.00	LP	0.84
2759	8413	NS5-245	NS5-733	IA	d	0.76	LP	2.33
2759	7267	NS4B-118	NS4B-352	IT	d	0.07	LP	13.23
2759	755	prM-97	prM-290	C	A	1.00	NSUB	0.43
2759	10072	NS5-798	NS5-2392	G	A	1.00	NSUB	0.61
2759	422	C-109	C-326	T	C	0.48	NSUB	0.61
2759	10625	3'UTR-230	3'UTR-230	C	T	0.12	NSUB	0.64
2759	6204	NS3-531	NS3-1593	G	A	0.22	NSUB	0.66
2759	933	prM-156	prM-468	T	C	0.13	NSUB	0.66
2759	10558	3'UTR-163	3'UTR-163	A	G	1.00	NSUB	0.66
2759	8280	NS5-200	NS5-600	T	C	1.00	NSUB	0.91
2759	4109	NS2A-195	NS2A-584	A	C	0.12	NSUB	1.03
2759	9063	NS5-461	NS5-1383	A	G	0.51	NSUB	1.53
2759	10755	3'UTR-360	3'UTR-360	G	C	0.98	NSUB	2.04
2759	3963	NS2A-146	NS2A-438	G	A	0.44	NSUB	2.30
2759	10888	3'UTR-493	3'UTR-493	A	T	1.00	NSUB	2.68
2759	1814	E-283	E-848	T	C	0.13	NSUB	15.36
2760	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.07
2760	5166	NS3-185	NS3-555	IA	d	0.12	LP	1.59
2760	9063	NS5-461	NS5-1383	IA	d	0.52	LP	1.74
2760	6203	NS3-531	NS3-1592	IA	d	0.14	LP	4.18
2760	10248	NS5-856	NS5-2568	T	C	1.00	NSUB	0.31
2760	9352	NS5-558	NS5-1672	C	T	1.00	NSUB	0.60
2760	10035	NS5-785	NS5-2355	T	C	1.00	NSUB	1.01
2760	6669	NS4A-67	NS4A-201	G	A	0.39	NSUB	1.07
2760	7711	NS5-11	NS5-31	T	G	0.56	NSUB	1.07
2760	279	C-61	C-183	T	G	0.38	NSUB	1.20
2760	7179	NS4B-88	NS4B-264	T	C	0.72	NSUB	1.29
2760	9703	NS5-675	NS5-2023	A	C	0.21	NSUB	1.37
2760	3450	NS1-327	NS1-981	T	C	0.94	NSUB	1.41
2760	4431	NS2B-71	NS2B-213	A	G	0.24	NSUB	2.68
2760	682	prM-73	prM-217	T	G	0.31	NSUB	2.96
2760	3840	NS2A-105	NS2A-315	C	T	0.66	NSUB	7.49
2760	129	C-11	C-33	T	C	0.18	NSUB	10.73
2760	6755	NS4A-96	NS4A-287	C	T	0.75	NSUB	10.96
2760	4869	NS3-86	NS3-258	A	G	0.90	NSUB	16.41
2761	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.67
2761	5166	NS3-185	NS3-555	IA	d	0.29	LP	0.88

2761	4109	NS2A-195	NS2A-584	IA	d	0.34	LP	1.09
2761	7267	NS4B-118	NS4B-352	IT	d	0.36	LP	11.67
2761	4914	NS3-101	NS3-303	C	A	1.00	NSUB	0.21
2761	4910	NS3-100	NS3-299	A	T	1.00	NSUB	0.21
2761	504	prM-13	prM-39	A	G	1.00	NSUB	0.57
2761	5166	NS3-185	NS3-555	A	G	0.67	NSUB	0.57
2761	8157	NS5-159	NS5-477	C	T	1.00	NSUB	0.73
2761	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.87
2761	606	prM-47	prM-141	C	T	1.00	NSUB	1.01
2761	5340	NS3-243	NS3-729	T	C	0.65	NSUB	1.21
2761	4194	NS2A-223	NS2A-669	C	T	0.84	NSUB	1.30
2761	7637	NS4B-241	NS4B-722	T	C	0.19	NSUB	1.65
2761	10888	3'UTR-493	3'UTR-493	A	T	1.00	NSUB	2.95
2761	2109	E-381	E-1143	T	C	0.73	NSUB	13.08
2761	2838	NS1-123	NS1-369	G	A	0.49	NSUB	13.52
2761	4479	NS2B-87	NS2B-261	T	C	0.69	NSUB	13.95
2761	9907	NS5-743	NS5-2227	T	G	0.55	NSUB	14.53
2762	8413	NS5-245	NS5-733	IA	d	0.18	LP	0.90
2762	9063	NS5-461	NS5-1383	IA	d	0.10	LP	1.15
2762	4109	NS2A-195	NS2A-584	IA	d	0.94	LP	1.26
2762	7960	NS5-94	NS5-280	IA	d	0.86	LP	1.29
2762	5166	NS3-185	NS3-555	IA	d	0.17	LP	1.70
2762	7267	NS4B-118	NS4B-352	IT	d	0.41	LP	11.07
2762	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.16
2762	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.16
2762	433	C-113	C-337	A	G	0.41	NSUB	0.57
2762	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.63
2762	6018	NS3-469	NS3-1407	G	T	0.49	NSUB	0.73
2762	5958	NS3-449	NS3-1347	G	A	0.96	NSUB	0.79
2762	3102	NS1-211	NS1-633	A	G	0.94	NSUB	3.33
2762	10888	3'UTR-493	3'UTR-493	A	T	0.55	NSUB	3.58
2762	1251	E-95	E-285	G	A	0.88	NSUB	4.32
2762	9942	NS5-754	NS5-2262	T	C	0.87	NSUB	8.01
2762	1631	E-222	E-665	G	A	0.93	NSUB	8.22
2762	6214	NS3-535	NS3-1603	T	C	0.46	NSUB	8.42
2762	6069	NS3-486	NS3-1458	T	C	0.85	NSUB	8.89
2762	10393	NS5-905	NS5-2713	T	C	0.33	NSUB	19.15
2763	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	0.94
2763	7960	NS5-94	NS5-280	IA	d	0.57	LP	1.36

2763	5166	NS3-185	NS3-555	IA	d	0.24	LP	1.45
2763	6203	NS3-531	NS3-1592	IA	d	0.11	LP	4.29
2763	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.16
2763	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.16
2763	7711	NS5-11	NS5-31	T	G	0.71	NSUB	0.60
2763	10334	NS5-885	NS5-2654	C	T	0.71	NSUB	1.58
2763	3701	NS2A-59	NS2A-176	C	T	0.82	NSUB	1.90
2763	10888	3'UTR-493	3'UTR-493	A	T	0.23	NSUB	4.49
2763	1494	E-176	E-528	T	C	0.09	NSUB	10.99
2763	2576	NS1-36	NS1-107	T	C	0.75	NSUB	14.48
2764	4109	NS2A-195	NS2A-584	IA	d	0.39	LP	0.55
2764	9063	NS5-461	NS5-1383	IA	d	0.13	LP	0.76
2764	7960	NS5-94	NS5-280	IA	d	1.24	LP	0.83
2764	5166	NS3-185	NS3-555	IA	d	0.13	LP	1.46
2764	6203	NS3-531	NS3-1592	IA	d	0.06	LP	3.53
2764	5927	NS3-439	NS3-1316	C	A	1.00	NSUB	0.15
2764	397	C-101	C-301	T	A	1.00	NSUB	0.15
2764	5935	NS3-442	NS3-1324	T	G	1.00	NSUB	0.16
2764	394	C-100	C-298	C	T	1.00	NSUB	0.25
2764	10814	3'UTR-419	3'UTR-419	T	A	0.30	NSUB	1.25
2764	8627	NS5-316	NS5-947	T	C	1.00	NSUB	1.84
2764	6214	NS3-535	NS3-1603	T	C	0.62	NSUB	5.99
2764	8484	NS5-268	NS5-804	C	A	0.21	NSUB	42.20
2775	6203	NS3-531	NS3-1592	IA	d	1.00	LP	0.62
2775	7267	NS4B-118	NS4B-352	IT	d	0.70	LP	1.39
2775	7626	NS4B-237	NS4B-711	C	T	0.09	NSUB	0.87
2775	2928	NS1-153	NS1-459	G	A	0.73	NSUB	2.18
2776	4109	NS2A-195	NS2A-584	IA	d	0.73	LP	0.88
2776	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.97
2776	9063	NS5-461	NS5-1383	IA	d	0.70	LP	1.18
2776	5166	NS3-185	NS3-555	IA	d	0.31	LP	1.24
2776	7267	NS4B-118	NS4B-352	IT	d	0.16	LP	8.08
2776	5224	NS3-205	NS3-613	T	C	0.41	NSUB	0.38
2776	7754	NS5-25	NS5-74	T	C	1.00	NSUB	0.50
2776	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.67
2776	8544	NS5-288	NS5-864	C	T	1.00	NSUB	0.71
2776	8627	NS5-316	NS5-947	T	C	0.42	NSUB	1.10
2776	6214	NS3-535	NS3-1603	T	C	0.90	NSUB	6.22
2776	3411	NS1-314	NS1-942	A	G	0.90	NSUB	22.31

2776	10404	3'UTR-9	3'UTR-9	C	T	0.89	NSUB	23.61
2776	8484	NS5-268	NS5-804	C	A	0.51	NSUB	42.08
2777	4109	NS2A-195	NS2A-584	IA	d	0.18	LP	2.04
2777	7960	NS5-94	NS5-280	IA	d	0.34	LP	2.42
2777	9063	NS5-461	NS5-1383	IA	d	0.12	LP	4.25
2777	7267	NS4B-118	NS4B-352	IT	d	0.50	LP	8.59
2777	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.26
2777	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.36
2777	7267	NS4B-118	NS4B-352	T	C	1.00	NSUB	0.78
2777	5166	NS3-185	NS3-555	A	G	0.76	NSUB	1.14
2940	7960	NS5-94	NS5-280	IA	d	0.75	LP	1.01
2940	4109	NS2A-195	NS2A-584	IA	d	0.18	LP	1.21
2940	1755	E-263	E-789	C	T	1.00	NSUB	0.51
2940	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.73
2940	2652	NS1-61	NS1-183	T	C	0.76	NSUB	0.74
2940	2192	E-409	E-1226	T	C	1.00	NSUB	0.78
2940	6927	NS4B-4	NS4B-12	C	T	0.40	NSUB	0.87
2940	6807	NS4A-113	NS4A-339	T	C	1.20	NSUB	0.92
2940	5166	NS3-185	NS3-555	A	G	0.18	NSUB	1.05
2940	7524	NS4B-203	NS4B-609	A	G	0.89	NSUB	1.11
2940	10365	NS5-895	NS5-2685	G	A	0.40	NSUB	1.32
2940	435	C-113	C-339	T	C	0.97	NSUB	1.55
2940	1020	E-18	E-54	G	A	1.00	NSUB	1.98
2940	10401	3'UTR-6	3'UTR-6	G	A	0.85	NSUB	2.03
2940	10888	3'UTR-493	3'UTR-493	A	T	0.32	NSUB	2.62
2940	6982	NS4B-23	NS4B-67	A	G	0.80	NSUB	2.80
2940	1095	E-43	E-129	A	G	0.10	NSUB	3.10
2940	9045	NS5-455	NS5-1365	T	C	0.47	NSUB	3.85
2940	5526	NS3-305	NS3-915	T	C	0.22	NSUB	6.20
2940	7824	NS5-48	NS5-144	C	T	0.52	NSUB	8.33
2940	8340	NS5-220	NS5-660	C	T	0.21	NSUB	14.21
2940	1436	E-157	E-470	T	C	0.55	NSUB	15.82
2940	7296	NS4B-127	NS4B-381	T	C	0.75	NSUB	23.07
2940	2547	NS1-26	NS1-78	A	G	0.38	NSUB	44.95
2941	7960	NS5-94	NS5-280	IA	d	0.44	LP	0.76
2941	7267	NS4B-118	NS4B-352	IT	d	0.58	LP	2.92
2941	1756	E-264	E-790	T	C	1.00	NSUB	0.44
2941	3153	NS1-228	NS1-684	T	G	0.41	NSUB	0.58
2941	5310	NS3-233	NS3-699	C	T	1.00	NSUB	0.89

2941	10871	3'UTR-476	3'UTR-476	A	G	0.42	NSUB	1.06
2941	10695	3'UTR-300	3'UTR-300	T	C	0.91	NSUB	6.43
2942	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.02
2942	9063	NS5-461	NS5-1383	IA	d	0.30	LP	1.27
2942	6203	NS3-531	NS3-1592	IA	d	0.05	LP	2.16
2942	7267	NS4B-118	NS4B-352	IT	d	0.33	LP	3.59
2942	2347	E-461	E-1381	G	A	1.00	NSUB	0.46
2942	162	C-22	C-66	T	C	1.00	NSUB	0.64
2942	7188	NS4B-91	NS4B-273	A	T	0.26	NSUB	3.28
2945	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	0.43
2945	8413	NS5-245	NS5-733	IA	d	1.00	LP	0.53
2945	9063	NS5-461	NS5-1383	IA	d	0.48	LP	0.64
2945	7960	NS5-94	NS5-280	IA	d	0.39	LP	1.06
2945	6203	NS3-531	NS3-1592	IA	d	0.06	LP	2.12
2945	7267	NS4B-118	NS4B-352	IT	d	0.59	LP	4.01
2945	3185	NS1-239	NS1-716	A	G	1.00	NSUB	0.48
2945	7015	NS4B-34	NS4B-100	T	C	0.72	NSUB	1.14
2945	9125	NS5-482	NS5-1445	T	G	0.38	NSUB	1.41
2945	8861	NS5-394	NS5-1181	A	G	0.95	NSUB	1.54
2945	4446	NS2B-76	NS2B-228	C	T	0.27	NSUB	8.47
2946	9091	NS5-471	NS5-1411	D1	i	0.43	LP	0.24
2946	9063	NS5-461	NS5-1383	IA	d	0.14	LP	1.30
2946	4109	NS2A-195	NS2A-584	IA	d	0.47	LP	1.50
2946	7960	NS5-94	NS5-280	IA	d	0.48	LP	1.54
2946	5166	NS3-185	NS3-555	IA	d	0.07	LP	1.55
2946	6203	NS3-531	NS3-1592	IA	d	0.35	LP	3.15
2946	998	E-11	E-32	C	T	1.00	NSUB	0.17
2946	994	E-10	E-28	C	G	1.00	NSUB	0.18
2946	9094	NS5-472	NS5-1414	A	G	0.41	NSUB	0.24
2946	8769	NS5-363	NS5-1089	G	T	1.00	NSUB	0.29
2946	2355	E-463	E-1389	G	A	0.62	NSUB	0.33
2946	2356	E-464	E-1390	C	T	0.62	NSUB	0.33
2946	8784	NS5-368	NS5-1104	G	A	1.00	NSUB	0.42
2946	1116	E-50	E-150	G	A	1.00	NSUB	0.44
2946	6615	NS4A-49	NS4A-147	C	T	0.41	NSUB	0.44
2946	3018	NS1-183	NS1-549	T	C	1.00	NSUB	0.50
2946	7179	NS4B-88	NS4B-264	C	T	1.00	NSUB	0.53
2946	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.59
2946	3880	NS2A-119	NS2A-355	T	C	0.68	NSUB	0.59

2946	10458	3'UTR-63	3'UTR-63	C	G	1.08	NSUB	0.87
2946	3942	NS2A-139	NS2A-417	C	T	0.18	NSUB	0.89
2946	9125	NS5-482	NS5-1445	T	G	0.73	NSUB	0.93
2946	637	prM-58	prM-172	A	G	0.45	NSUB	0.95
2946	9279	NS5-533	NS5-1599	T	C	0.77	NSUB	1.03
2946	7636	NS4B-241	NS4B-721	G	A	0.12	NSUB	1.30
2946	3147	NS1-226	NS1-678	C	T	0.51	NSUB	1.31
2946	5166	NS3-185	NS3-555	A	G	0.78	NSUB	1.42
2946	4134	NS2A-203	NS2A-609	G	A	0.75	NSUB	1.80
2946	1579	E-205	E-613	G	A	0.15	NSUB	2.20
2946	7382	NS4B-156	NS4B-467	T	C	0.93	NSUB	2.24
2946	2834	NS1-122	NS1-365	C	T	0.76	NSUB	2.32
2946	2052	E-362	E-1086	C	T	0.86	NSUB	2.88
2946	7274	NS4B-120	NS4B-359	T	G	0.19	NSUB	4.08
2946	7962	NS5-94	NS5-282	G	A	0.64	NSUB	4.39
2946	2583	NS1-38	NS1-114	A	G	0.86	NSUB	4.60
2946	6296	NS3-562	NS3-1685	G	A	0.21	NSUB	8.46
2946	849	prM-128	prM-384	C	T	0.68	NSUB	12.78
2946	3193	NS1-242	NS1-724	G	A	0.64	NSUB	13.12
2946	8382	NS5-234	NS5-702	C	T	0.80	NSUB	15.68
2946	6493	NS4A-9	NS4A-25	C	T	0.78	NSUB	17.39
2947	2702	NS1-78	NS1-233	D1	i	1.00	LP	0.27
2947	7960	NS5-94	NS5-280	IA	d	0.72	LP	0.81
2947	5166	NS3-185	NS3-555	IA	d	0.53	LP	1.40
2947	9063	NS5-461	NS5-1383	IA	d	0.84	LP	1.51
2947	9258	NS5-526	NS5-1578	C	T	1.00	NSUB	0.21
2947	9264	NS5-528	NS5-1584	C	T	1.00	NSUB	0.24
2947	6721	NS4A-85	NS4A-253	A	G	0.67	NSUB	0.40
2947	10119	NS5-813	NS5-2439	T	C	0.23	NSUB	0.43
2947	9601	NS5-641	NS5-1921	A	G	0.67	NSUB	0.47
2947	6720	NS4A-84	NS4A-252	C	T	0.54	NSUB	0.50
2947	7674	NS4B-253	NS4B-759	A	G	0.69	NSUB	0.56
2947	7677	NS4B-254	NS4B-762	G	A	0.70	NSUB	0.58
2947	6765	NS4A-99	NS4A-297	C	T	1.00	NSUB	0.59
2947	6492	NS4A-8	NS4A-24	T	C	0.31	NSUB	0.59
2947	9639	NS5-653	NS5-1959	T	C	0.13	NSUB	0.60
2947	10065	NS5-795	NS5-2385	C	T	1.00	NSUB	0.61
2947	8550	NS5-290	NS5-870	T	C	0.69	NSUB	0.62
2947	3754	NS2A-77	NS2A-229	C	T	0.74	NSUB	0.64

2947	7711	NS5-11	NS5-31	T	G	0.46	NSUB	0.69
2947	8458	NS5-260	NS5-778	T	C	1.00	NSUB	0.69
2947	6750	NS4A-94	NS4A-282	T	C	0.95	NSUB	0.70
2947	3286	NS1-273	NS1-817	A	G	1.00	NSUB	0.71
2947	7806	NS5-42	NS5-126	C	T	0.33	NSUB	0.76
2948	7960	NS5-94	NS5-280	IA	d	0.36	LP	0.59
2948	9063	NS5-461	NS5-1383	IA	d	0.60	LP	1.53
2948	2226	E-420	E-1260	C	T	0.41	NSUB	0.50
2948	7979	NS5-100	NS5-299	C	T	0.49	NSUB	0.54
2948	9768	NS5-696	NS5-2088	T	C	1.00	NSUB	0.54
2948	5080	NS3-157	NS3-469	G	C	0.73	NSUB	0.58
2948	2616	NS1-49	NS1-147	C	T	0.30	NSUB	0.62
2948	4735	NS3-42	NS3-124	A	G	1.00	NSUB	0.67
2948	1844	E-293	E-878	G	A	1.00	NSUB	0.67
2948	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.72
2948	3132	NS1-221	NS1-663	G	A	0.35	NSUB	0.73
2948	716	prM-84	prM-251	T	C	0.62	NSUB	1.08
2948	2998	NS1-177	NS1-529	G	A	1.00	NSUB	1.09
2948	6237	NS3-542	NS3-1626	C	T	1.00	NSUB	1.19
2948	9834	NS5-718	NS5-2154	T	C	0.26	NSUB	1.45
2948	6088	NS3-493	NS3-1477	C	A	0.28	NSUB	1.53
2948	7125	NS4B-70	NS4B-210	T	C	0.46	NSUB	1.56
2948	9624	NS5-648	NS5-1944	C	T	0.37	NSUB	1.80
2948	1881	E-305	E-915	C	T	0.99	NSUB	1.83
2948	3399	NS1-310	NS1-930	C	T	0.98	NSUB	1.85
2948	4674	NS3-21	NS3-63	C	T	0.84	NSUB	1.90
2948	1329	E-121	E-363	C	T	0.68	NSUB	1.92
2948	5559	NS3-316	NS3-948	T	A	0.81	NSUB	1.94
2948	348	C-84	C-252	C	T	0.52	NSUB	2.01
2948	1320	E-118	E-354	G	A	0.71	NSUB	2.03
2948	9264	NS5-528	NS5-1584	C	A	0.08	NSUB	2.08
2948	9579	NS5-633	NS5-1899	T	C	0.18	NSUB	2.14
2948	4590	NS2B-124	NS2B-372	G	A	0.93	NSUB	2.19
2948	4036	NS2A-171	NS2A-511	C	T	0.79	NSUB	2.20
2948	1410	E-148	E-444	T	C	0.70	NSUB	2.23
2948	6642	NS4A-58	NS4A-174	T	C	0.16	NSUB	2.26
2948	1947	E-327	E-981	G	A	0.97	NSUB	2.30
2948	9510	NS5-610	NS5-1830	T	C	0.45	NSUB	2.32
2948	1285	E-107	E-319	C	T	0.55	NSUB	2.32

2948	8550	NS5-290	NS5-870	T	C	0.26	NSUB	2.38
2948	1065	E-33	E-99	C	T	0.20	NSUB	2.41
2948	2880	NS1-137	NS1-411	C	T	0.55	NSUB	2.44
2948	1974	E-336	E-1008	T	C	0.36	NSUB	2.46
2948	4929	NS3-106	NS3-318	T	A	0.64	NSUB	2.47
2948	9660	NS5-660	NS5-1980	T	C	1.00	NSUB	2.47
2948	10747	3'UTR-352	3'UTR-352	T	A	0.82	NSUB	2.49
2948	5995	NS3-462	NS3-1384	G	A	0.95	NSUB	2.53
2948	6720	NS4A-84	NS4A-252	C	T	0.43	NSUB	2.63
2948	4272	NS2B-18	NS2B-54	C	T	0.07	NSUB	2.65
2948	8514	NS5-278	NS5-834	G	A	0.34	NSUB	2.68
2948	9471	NS5-597	NS5-1791	T	C	0.14	NSUB	2.68
2948	9690	NS5-670	NS5-2010	C	T	0.29	NSUB	2.77
2948	5991	NS3-460	NS3-1380	A	G	0.66	NSUB	2.78
2948	7029	NS4B-38	NS4B-114	A	G	0.78	NSUB	2.80
2948	7021	NS4B-36	NS4B-106	C	T	0.84	NSUB	2.82
2948	8292	NS5-204	NS5-612	A	G	0.40	NSUB	2.82
2948	6598	NS4A-44	NS4A-130	T	C	0.09	NSUB	2.85
2948	10296	NS5-872	NS5-2616	T	C	0.46	NSUB	2.87
2948	10281	NS5-867	NS5-2601	T	C	0.56	NSUB	2.92
2948	1425	E-153	E-459	A	G	0.64	NSUB	2.98
2948	10471	3'UTR-76	3'UTR-76	C	T	0.71	NSUB	3.01
2948	1878	E-304	E-912	C	T	0.17	NSUB	3.02
2948	9729	NS5-683	NS5-2049	T	C	0.55	NSUB	3.03
2948	2913	NS1-148	NS1-444	T	C	0.96	NSUB	3.05
2948	1887	E-307	E-921	A	G	0.36	NSUB	3.06
2948	4347	NS2B-43	NS2B-129	T	C	0.88	NSUB	3.07
2948	6983	NS4B-23	NS4B-68	C	T	0.40	NSUB	3.16
2948	3850	NS2A-109	NS2A-325	T	C	0.51	NSUB	3.16
2948	7269	NS4B-118	NS4B-354	T	C	0.34	NSUB	3.17
2948	4230	NS2B-4	NS2B-12	A	G	0.22	NSUB	3.21
2948	3816	NS2A-97	NS2A-291	G	A	0.72	NSUB	3.22
2948	9180	NS5-500	NS5-1500	T	C	0.84	NSUB	3.27
2948	9801	NS5-707	NS5-2121	T	C	0.08	NSUB	3.32
2948	2697	NS1-76	NS1-228	C	T	0.94	NSUB	3.41
2948	10829	3'UTR-434	3'UTR-434	T	C	0.74	NSUB	3.52
2948	3138	NS1-223	NS1-669	C	T	0.24	NSUB	3.53
2948	3900	NS2A-125	NS2A-375	A	G	0.70	NSUB	3.65
2948	8368	NS5-230	NS5-688	A	G	0.94	NSUB	3.69

2948	10408	3'UTR-13	3'UTR-13	C	T	1.00	NSUB	4.04
2948	10407	3'UTR-12	3'UTR-12	C	T	1.00	NSUB	4.09
2948	10435	3'UTR-40	3'UTR-40	C	T	0.52	NSUB	4.76
2948	3363	NS1-298	NS1-894	A	T	0.13	NSUB	5.80
2949	7960	NS5-94	NS5-280	IA	d	0.75	LP	1.69
2949	4109	NS2A-195	NS2A-584	IA	d	0.13	LP	1.85
2949	5166	NS3-185	NS3-555	IA	d	0.15	LP	2.40
2949	6203	NS3-531	NS3-1592	IA	d	0.42	LP	5.73
2949	10157	NS5-826	NS5-2477	G	A	1.00	NSUB	0.19
2949	10160	NS5-827	NS5-2480	G	A	1.00	NSUB	0.32
2949	4935	NS3-108	NS3-324	T	C	0.65	NSUB	0.45
2949	10119	NS5-813	NS5-2439	T	C	0.23	NSUB	0.46
2949	4782	NS3-57	NS3-171	C	T	1.00	NSUB	0.48
2949	9799	NS5-707	NS5-2119	A	G	0.38	NSUB	0.53
2949	4779	NS3-56	NS3-168	T	C	1.00	NSUB	0.54
2949	8199	NS5-173	NS5-519	T	C	0.69	NSUB	0.54
2949	6203	NS3-531	NS3-1592	A	G	1.00	NSUB	0.54
2949	8814	NS5-378	NS5-1134	A	G	1.00	NSUB	0.56
2949	4776	NS3-55	NS3-165	A	T	0.23	NSUB	0.63
2949	5166	NS3-185	NS3-555	A	G	0.36	NSUB	1.08
2949	9125	NS5-482	NS5-1445	T	G	0.51	NSUB	1.12
2949	4946	NS3-112	NS3-335	G	A	0.77	NSUB	1.19
2949	10888	3'UTR-493	3'UTR-493	A	T	0.38	NSUB	2.99
2960	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.65
2960	7960	NS5-94	NS5-280	IA	d	0.71	LP	0.78
2960	5166	NS3-185	NS3-555	IA	d	0.29	LP	0.90
2960	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	1.19
2960	6203	NS3-531	NS3-1592	IA	d	0.80	LP	2.40
2960	8849	NS5-390	NS5-1169	G	A	1.00	NSUB	0.52
2960	5187	NS3-192	NS3-576	A	G	0.22	NSUB	0.52
2960	2217	E-417	E-1251	G	A	1.00	NSUB	0.56
2961	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.66
2961	6740	NS4A-91	NS4A-272	IT	d	0.25	LP	0.68
2961	9063	NS5-461	NS5-1383	IA	d	0.12	LP	1.15
2961	4109	NS2A-195	NS2A-584	IA	d	0.94	LP	1.43
2961	5166	NS3-185	NS3-555	IA	d	0.12	LP	1.55
2961	6203	NS3-531	NS3-1592	IA	d	0.27	LP	2.82
2961	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.47
2961	2149	E-395	E-1183	T	C	1.00	NSUB	0.51

2961	9364	NS5-562	NS5-1684	A	G	1.00	NSUB	0.63
2961	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.71
2961	9125	NS5-482	NS5-1445	T	G	1.00	NSUB	0.83
2961	10888	3'UTR-493	3'UTR-493	A	T	0.49	NSUB	4.06
2961	10437	3'UTR-42	3'UTR-42	T	C	0.35	NSUB	32.78
2962	4109	NS2A-195	NS2A-584	IA	d	0.69	LP	0.71
2962	6203	NS3-531	NS3-1592	IA	d	0.10	LP	2.91
2962	7267	NS4B-118	NS4B-352	IT	d	0.42	LP	6.36
2962	7728	NS5-16	NS5-48	T	C	0.40	NSUB	0.37
2962	6204	NS3-531	NS3-1593	G	A	1.00	NSUB	0.60
2962	2662	NS1-65	NS1-193	A	C	0.57	NSUB	4.32
2963	5367	NS3-252	NS3-756	D1	i	1.00	LP	0.14
2963	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.77
2963	4109	NS2A-195	NS2A-584	IA	d	0.07	LP	1.24
2963	7960	NS5-94	NS5-280	IA	d	0.54	LP	1.90
2963	6203	NS3-531	NS3-1592	IA	d	0.77	LP	3.50
2963	5368	NS3-253	NS3-757	C	A	1.00	NSUB	0.14
2963	7125	NS4B-70	NS4B-210	G	C	1.00	NSUB	0.18
2963	7124	NS4B-70	NS4B-209	G	C	1.00	NSUB	0.18
2963	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.21
2963	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.21
2963	10179	NS5-833	NS5-2499	T	C	0.69	NSUB	0.47
2963	790	prM-109	prM-325	A	G	0.22	NSUB	0.53
2963	6876	NS4A-136	NS4A-408	T	C	0.29	NSUB	0.56
2963	7637	NS4B-241	NS4B-722	T	C	0.47	NSUB	0.58
2963	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.59
2963	3178	NS1-237	NS1-709	T	C	1.00	NSUB	0.71
2963	10435	3'UTR-40	3'UTR-40	T	C	0.73	NSUB	0.98
2963	3714	NS2A-63	NS2A-189	G	A	0.09	NSUB	1.13
2963	4033	NS2A-170	NS2A-508	G	A	0.87	NSUB	1.44
2963	7509	NS4B-198	NS4B-594	T	C	0.87	NSUB	2.54
2963	10422	3'UTR-27	3'UTR-27	T	C	0.81	NSUB	2.72
2963	1443	E-159	E-477	C	T	0.98	NSUB	5.24
2963	10688	3'UTR-293	3'UTR-293	C	T	0.07	NSUB	5.43
2963	5784	NS3-391	NS3-1173	C	T	0.07	NSUB	6.02
2963	2182	E-406	E-1216	G	A	0.50	NSUB	6.20
2963	5868	NS3-419	NS3-1257	G	A	0.19	NSUB	6.30
2963	7752	NS5-24	NS5-72	T	C	0.46	NSUB	6.85
2963	10338	NS5-886	NS5-2658	T	C	0.17	NSUB	6.97

2963	2040	E-358	E-1074	C	T	0.60	NSUB	7.01
2963	8535	NS5-285	NS5-855	G	A	0.35	NSUB	7.14
2963	101	C-2	C-5	T	C	1.00	NSUB	7.33
2963	1410	E-148	E-444	T	A	0.39	NSUB	7.42
2963	5224	NS3-205	NS3-613	C	T	0.86	NSUB	7.61
2963	7635	NS4B-240	NS4B-720	A	G	0.51	NSUB	7.74
2963	7299	NS4B-128	NS4B-384	C	T	0.37	NSUB	7.77
2963	10373	NS5-898	NS5-2693	C	T	0.21	NSUB	7.78
2963	7392	NS4B-159	NS4B-477	C	T	0.19	NSUB	8.00
2963	1698	E-244	E-732	A	G	0.36	NSUB	8.12
2963	2619	NS1-50	NS1-150	T	C	0.48	NSUB	8.17
2963	10651	3'UTR-256	3'UTR-256	G	A	0.14	NSUB	8.17
2963	2371	E-469	E-1405	T	C	0.87	NSUB	8.94
2963	2994	NS1-175	NS1-525	T	C	0.06	NSUB	8.96
2963	10393	NS5-905	NS5-2713	T	C	0.85	NSUB	9.67
2963	933	prM-156	prM-468	C	T	0.80	NSUB	9.72
2963	3702	NS2A-59	NS2A-177	T	C	0.23	NSUB	9.74
2963	8301	NS5-207	NS5-621	T	C	0.51	NSUB	10.09
2963	10484	3'UTR-89	3'UTR-89	T	G	0.94	NSUB	10.13
2963	10607	3'UTR-212	3'UTR-212	T	C	0.62	NSUB	10.16
2963	9510	NS5-610	NS5-1830	T	C	0.99	NSUB	10.54
2963	52	5'UTR-52	5'UTR-52	T	C	0.06	NSUB	10.71
2963	7539	NS4B-208	NS4B-624	A	G	0.13	NSUB	10.89
2963	7494	NS4B-193	NS4B-579	A	G	0.28	NSUB	12.54
2963	3963	NS2A-146	NS2A-438	A	G	0.49	NSUB	17.02
2963	6063	NS3-484	NS3-1452	G	A	0.46	NSUB	18.61
2963	5737	NS3-376	NS3-1126	C	T	0.32	NSUB	20.07
2963	10461	3'UTR-66	3'UTR-66	T	C	0.48	NSUB	21.54
2964	10492	3'UTR-97	3'UTR-97	IT	d	0.38	LP	0.56
2964	4109	NS2A-195	NS2A-584	IA	d	0.45	LP	1.54
2964	7960	NS5-94	NS5-280	IA	d	0.23	LP	1.68
2964	6982	NS4B-23	NS4B-67	C	G	0.65	NSUB	0.44
2964	7239	NS4B-108	NS4B-324	T	C	0.64	NSUB	0.46
2964	5871	NS3-420	NS3-1260	A	G	0.25	NSUB	0.46
2964	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.50
2964	7284	NS4B-123	NS4B-369	A	C	0.69	NSUB	0.60
2964	5612	NS3-334	NS3-1001	T	C	1.00	NSUB	0.62
2964	8640	NS5-320	NS5-960	C	T	1.00	NSUB	0.70
2964	7014	NS4B-33	NS4B-99	C	T	0.70	NSUB	0.73

2964	8787	NS5-369	NS5-1107	C	A	0.76	NSUB	0.77
2964	10612	3'UTR-217	3'UTR-217	T	C	0.24	NSUB	0.83
2964	7711	NS5-11	NS5-31	T	G	0.76	NSUB	0.90
2964	10545	3'UTR-150	3'UTR-150	T	C	1.00	NSUB	0.97
2964	7255	NS4B-114	NS4B-340	A	G	0.33	NSUB	1.00
2964	1080	E-38	E-114	A	G	0.20	NSUB	1.01
2964	402	C-102	C-306	G	A	0.39	NSUB	1.01
2964	3465	NS1-332	NS1-996	C	T	1.00	NSUB	1.08
2964	9797	NS5-706	NS5-2117	G	A	0.75	NSUB	1.15
2964	7662	NS4B-249	NS4B-747	G	A	0.38	NSUB	1.22
2964	2572	NS1-35	NS1-103	C	T	0.71	NSUB	2.73
2970	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.38
2970	6203	NS3-531	NS3-1592	IA	d	0.44	LP	1.72
2970	7267	NS4B-118	NS4B-352	IT	d	0.80	LP	4.21
2970	7161	NS4B-82	NS4B-246	C	T	1.00	NSUB	0.25
2970	7158	NS4B-81	NS4B-243	A	G	1.00	NSUB	0.38
2970	5741	NS3-377	NS3-1130	G	A	1.00	NSUB	0.48
2970	8823	NS5-381	NS5-1143	T	C	0.67	NSUB	0.56
2970	9898	NS5-740	NS5-2218	A	G	0.70	NSUB	0.60
2970	6408	NS3-599	NS3-1797	T	C	0.61	NSUB	0.71
2970	8538	NS5-286	NS5-858	T	C	1.00	NSUB	0.77
2970	4167	NS2A-214	NS2A-642	T	C	1.00	NSUB	0.78
2970	2496	NS1-9	NS1-27	T	C	0.75	NSUB	0.81
2970	7203	NS4B-96	NS4B-288	T	C	0.45	NSUB	0.82
2970	4691	NS3-27	NS3-80	T	C	1.00	NSUB	0.83
2970	7115	NS4B-67	NS4B-200	T	C	0.27	NSUB	0.86
2970	2724	NS1-85	NS1-255	T	C	0.39	NSUB	0.98
2970	2186	E-407	E-1220	T	C	0.41	NSUB	1.01
2970	10317	NS5-879	NS5-2637	T	C	1.00	NSUB	1.06
2970	4887	NS3-92	NS3-276	A	G	0.11	NSUB	1.18
2970	7449	NS4B-178	NS4B-534	A	G	1.00	NSUB	1.51
2970	4533	NS2B-105	NS2B-315	T	C	0.18	NSUB	1.69
2970	1155	E-63	E-189	C	T	0.22	NSUB	2.20
2970	2991	NS1-174	NS1-522	T	C	0.23	NSUB	2.71
2970	2481	NS1-4	NS1-12	C	T	0.05	NSUB	2.85
2970	9501	NS5-607	NS5-1821	C	T	0.48	NSUB	3.11
2970	3687	NS2A-54	NS2A-162	A	G	0.65	NSUB	3.20
2970	4308	NS2B-30	NS2B-90	T	C	0.34	NSUB	3.40
2970	5370	NS3-253	NS3-759	C	T	0.75	NSUB	3.70

2970	124	C-10	C-28	G	A	0.71	NSUB	4.08
2970	2784	NS1-105	NS1-315	T	C	0.29	NSUB	5.32
2970	2871	NS1-134	NS1-402	A	G	0.89	NSUB	7.10
2970	3246	NS1-259	NS1-777	A	G	0.09	NSUB	10.22
2970	2851	NS1-128	NS1-382	T	C	0.94	NSUB	11.06
2971	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.51
2971	9063	NS5-461	NS5-1383	IA	d	0.69	LP	0.62
2971	4109	NS2A-195	NS2A-584	IA	d	0.08	LP	1.80
2971	6203	NS3-531	NS3-1592	IA	d	0.60	LP	2.47
2971	7267	NS4B-118	NS4B-352	IT	d	0.52	LP	9.01
2971	10458	3'UTR-63	3'UTR-63	C	G	0.49	NSUB	0.90
2971	804	prM-113	prM-339	T	C	0.99	NSUB	2.56
2971	3850	NS2A-109	NS2A-325	C	T	0.81	NSUB	3.93
2971	381	C-95	C-285	T	C	0.83	NSUB	4.01
2971	4194	NS2A-223	NS2A-669	C	T	0.80	NSUB	4.51
2971	2204	E-413	E-1238	G	A	0.07	NSUB	6.74
2971	6165	NS3-518	NS3-1554	C	T	0.20	NSUB	7.12
2971	9255	NS5-525	NS5-1575	T	C	0.29	NSUB	14.39
2971	9687	NS5-669	NS5-2007	T	C	0.93	NSUB	28.57
2971	93	5'UTR-93	5'UTR-93	T	C	0.33	NSUB	40.84
2972	4354	NS2B-46	NS2B-136	IC	d	0.65	LP	0.42
2972	9063	NS5-461	NS5-1383	IA	d	0.29	LP	1.10
2972	7960	NS5-94	NS5-280	IA	d	0.23	LP	1.20
2972	4109	NS2A-195	NS2A-584	IA	d	0.49	LP	1.50
2972	783	prM-106	prM-318	IA	d	0.16	LP	1.62
2972	6203	NS3-531	NS3-1592	IA	d	0.74	LP	1.75
2972	2152	E-396	E-1186	T	C	0.25	NSUB	0.42
2972	10620	3'UTR-225	3'UTR-225	T	C	0.64	NSUB	0.44
2972	2337	E-457	E-1371	A	G	0.40	NSUB	0.49
2972	1420	E-152	E-454	T	C	1.00	NSUB	0.58
2972	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.63
2972	6039	NS3-476	NS3-1428	A	G	0.22	NSUB	0.63
2972	10871	3'UTR-476	3'UTR-476	A	G	0.44	NSUB	1.32
2972	7637	NS4B-241	NS4B-722	T	C	0.85	NSUB	1.76
2972	786	prM-107	prM-321	G	A	0.74	NSUB	2.87
2973	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.73
2973	5166	NS3-185	NS3-555	IA	d	0.74	LP	1.19
2973	9063	NS5-461	NS5-1383	IA	d	0.81	LP	1.70
2973	6203	NS3-531	NS3-1592	IA	d	0.97	LP	2.63

2973	3850	NS2A-109	NS2A-325	C	T	1.00	NSUB	0.52
2973	3179	NS1-237	NS1-710	C	T	0.22	NSUB	0.83
2973	4108	NS2A-195	NS2A-583	T	G	0.23	NSUB	2.37
2973	4968	NS3-119	NS3-357	C	T	0.30	NSUB	3.70
2973	3187	NS1-240	NS1-718	A	G	0.35	NSUB	4.74
2973	10050	NS5-790	NS5-2370	A	G	0.10	NSUB	8.25
2973	7449	NS4B-178	NS4B-534	A	G	0.96	NSUB	10.80
2974	8413	NS5-245	NS5-733	IA	d	0.23	LP	0.57
2974	5166	NS3-185	NS3-555	IA	d	0.23	LP	1.03
2974	9063	NS5-461	NS5-1383	IA	d	0.44	LP	1.75
2974	4109	NS2A-195	NS2A-584	IA	d	0.98	LP	1.92
2974	6203	NS3-531	NS3-1592	IA	d	0.49	LP	3.87
2974	6970	NS4B-19	NS4B-55	IA	d	0.20	LP	5.89
2974	3774	NS2A-83	NS2A-249	T	C	0.90	NSUB	1.08
2974	8094	NS5-138	NS5-414	A	G	0.73	NSUB	2.12
2974	2472	NS1-1	NS1-3	T	C	0.95	NSUB	2.64
2974	9984	NS5-768	NS5-2304	A	G	0.32	NSUB	2.79
2974	9873	NS5-731	NS5-2193	G	T	0.98	NSUB	2.95
2974	9969	NS5-763	NS5-2289	C	T	0.09	NSUB	3.13
2974	2832	NS1-121	NS1-363	C	T	0.28	NSUB	3.21
2974	3242	NS1-258	NS1-773	T	C	0.87	NSUB	7.15
2974	3353	NS1-295	NS1-884	C	G	0.38	NSUB	7.25
2974	7341	NS4B-142	NS4B-426	A	G	0.99	NSUB	9.60
2980	9063	NS5-461	NS5-1383	IA	d	1.17	LP	0.92
2980	7960	NS5-94	NS5-280	IA	d	0.35	LP	1.04
2980	4109	NS2A-195	NS2A-584	IA	d	0.35	LP	1.74
2980	6203	NS3-531	NS3-1592	IA	d	0.69	LP	4.20
2980	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.16
2980	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.24
2980	1157	E-64	E-191	T	C	1.00	NSUB	0.61
2980	5002	NS3-131	NS3-391	T	C	1.00	NSUB	0.70
2980	2899	NS1-144	NS1-430	T	C	1.00	NSUB	0.89
2980	6203	NS3-531	NS3-1592	A	G	0.52	NSUB	0.95
2980	7761	NS5-27	NS5-81	T	C	0.42	NSUB	1.02
2980	7806	NS5-42	NS5-126	T	C	0.83	NSUB	1.07
2980	5166	NS3-185	NS3-555	A	G	0.51	NSUB	1.09
2980	10404	3'UTR-9	3'UTR-9	C	T	1.00	NSUB	1.14
2980	7627	NS4B-238	NS4B-712	T	C	0.66	NSUB	1.44
2980	7365	NS4B-150	NS4B-450	G	A	0.95	NSUB	1.45

2980	912	prM-149	prM-447	T	C	0.79	NSUB	1.65
2980	8895	NS5-405	NS5-1215	T	C	0.44	NSUB	1.65
2980	4026	NS2A-167	NS2A-501	G	A	0.70	NSUB	1.69
2980	6238	NS3-543	NS3-1627	C	T	0.75	NSUB	1.76
2980	4088	NS2A-188	NS2A-563	A	G	0.91	NSUB	1.77
2980	660	prM-65	prM-195	C	T	0.70	NSUB	1.77
2980	1506	E-180	E-540	C	T	0.60	NSUB	1.82
2980	4584	NS2B-122	NS2B-366	C	T	0.79	NSUB	1.85
2980	789	prM-108	prM-324	A	G	0.55	NSUB	2.11
2980	946	prM-161	prM-481	C	T	0.77	NSUB	2.41
2980	6721	NS4A-85	NS4A-253	G	A	0.25	NSUB	12.78
2980	1698	E-244	E-732	G	A	0.06	NSUB	13.01
2980	10408	3'UTR-13	3'UTR-13	T	C	0.05	NSUB	14.21
2980	9207	NS5-509	NS5-1527	T	C	0.12	NSUB	14.41
2980	8621	NS5-314	NS5-941	A	G	0.08	NSUB	14.66
2980	2040	E-358	E-1074	T	C	0.43	NSUB	15.14
2980	5784	NS3-391	NS3-1173	T	C	0.18	NSUB	15.25
2980	5224	NS3-205	NS3-613	T	C	0.96	NSUB	15.76
2980	10607	3'UTR-212	3'UTR-212	C	T	0.84	NSUB	15.86
2980	6192	NS3-527	NS3-1581	G	A	0.35	NSUB	15.94
2980	6820	NS4A-118	NS4A-352	C	T	0.91	NSUB	16.48
2980	10373	NS5-898	NS5-2693	T	C	0.17	NSUB	17.33
2980	2880	NS1-137	NS1-411	T	C	0.80	NSUB	17.34
2980	5868	NS3-419	NS3-1257	A	G	0.37	NSUB	17.35
2980	9072	NS5-464	NS5-1392	C	T	0.11	NSUB	17.38
2980	7392	NS4B-159	NS4B-477	T	C	0.30	NSUB	17.49
2980	1320	E-118	E-354	A	G	0.94	NSUB	17.83
2980	3876	NS2A-117	NS2A-351	T	C	0.32	NSUB	18.30
2980	7635	NS4B-240	NS4B-720	G	A	0.14	NSUB	19.57
2980	7410	NS4B-165	NS4B-495	C	T	0.26	NSUB	20.78
2980	8550	NS5-290	NS5-870	C	T	0.67	NSUB	21.04
2980	9264	NS5-528	NS5-1584	T	C	0.62	NSUB	26.71
2980	804	prM-113	prM-339	T	C	0.76	NSUB	29.82
2981	9063	NS5-461	NS5-1383	IA	d	0.30	LP	0.94
2981	7960	NS5-94	NS5-280	IA	d	0.75	LP	1.10
2981	5166	NS3-185	NS3-555	IA	d	0.12	LP	1.28
2981	4109	NS2A-195	NS2A-584	IA	d	0.06	LP	1.29
2981	6203	NS3-531	NS3-1592	IA	d	0.71	LP	3.10
2981	6871	NS4A-135	NS4A-403	A	G	1.00	NSUB	0.40

2981	5166	NS3-185	NS3-555	A	G	0.66	NSUB	0.57
2981	3672	NS2A-49	NS2A-147	C	T	0.41	NSUB	0.76
2981	6741	NS4A-91	NS4A-273	T	C	0.22	NSUB	0.93
2981	6433	NS3-608	NS3-1822	T	C	0.61	NSUB	1.74
2981	4621	NS3-4	NS3-10	C	T	0.09	NSUB	1.96
2981	340	C-82	C-244	T	C	0.44	NSUB	2.55
2982	7267	NS4B-118	NS4B-352	IT	d	0.67	LP	0.76
2982	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.05
2982	9063	NS5-461	NS5-1383	IA	d	0.80	LP	1.45
2982	6203	NS3-531	NS3-1592	IA	d	0.62	LP	3.35
2982	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.16
2982	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.24
2982	3898	NS2A-125	NS2A-373	T	C	1.00	NSUB	0.79
2982	4393	NS2B-59	NS2B-175	A	G	0.19	NSUB	0.90
2982	9013	NS5-445	NS5-1333	T	C	0.51	NSUB	1.15
2982	4255	NS2B-13	NS2B-37	T	C	0.44	NSUB	2.83
2982	1443	E-159	E-477	C	T	0.83	NSUB	2.88
2982	9796	NS5-706	NS5-2116	C	T	0.76	NSUB	3.05
2982	10443	3'UTR-48	3'UTR-48	A	T	0.25	NSUB	3.81
2982	6132	NS3-507	NS3-1521	C	T	0.34	NSUB	4.46
2982	2638	NS1-57	NS1-169	T	C	0.99	NSUB	4.50
2982	6993	NS4B-26	NS4B-78	C	T	0.08	NSUB	5.15
2982	5088	NS3-159	NS3-477	T	C	0.88	NSUB	5.19
2982	2833	NS1-122	NS1-364	G	A	0.58	NSUB	5.81
2982	10203	NS5-841	NS5-2523	T	C	0.83	NSUB	5.91
2982	10771	3'UTR-376	3'UTR-376	T	C	0.61	NSUB	6.34
2982	7548	NS4B-211	NS4B-633	G	A	0.80	NSUB	6.42
2982	6780	NS4A-104	NS4A-312	C	A	0.14	NSUB	6.55
2982	5530	NS3-307	NS3-919	T	C	0.19	NSUB	6.64
2982	6129	NS3-506	NS3-1518	C	A	0.67	NSUB	7.00
2982	5135	NS3-175	NS3-524	C	T	0.16	NSUB	7.36
2982	5995	NS3-462	NS3-1384	A	G	0.39	NSUB	7.52
2982	6238	NS3-543	NS3-1627	C	T	0.09	NSUB	7.81
2982	1320	E-118	E-354	A	G	0.64	NSUB	8.03
2982	8621	NS5-314	NS5-941	A	G	0.92	NSUB	8.13
2982	3399	NS1-310	NS1-930	T	C	0.85	NSUB	8.24
2982	6790	NS4A-108	NS4A-322	C	T	0.80	NSUB	8.32
2982	10131	NS5-817	NS5-2451	C	T	0.12	NSUB	8.43
2982	5631	NS3-340	NS3-1020	C	A	0.15	NSUB	8.65

2982	10062	NS5-794	NS5-2382	T	C	0.30	NSUB	8.67
2982	3414	NS1-315	NS1-945	T	C	0.82	NSUB	8.74
2982	9210	NS5-510	NS5-1530	A	G	0.52	NSUB	8.77
2982	6936	NS4B-7	NS4B-21	T	C	0.82	NSUB	8.80
2982	10496	3'UTR-101	3'UTR-101	T	C	0.77	NSUB	8.92
2982	660	prM-65	prM-195	C	T	0.22	NSUB	8.92
2982	9453	NS5-591	NS5-1773	T	C	0.09	NSUB	9.11
2982	8550	NS5-290	NS5-870	C	T	0.22	NSUB	9.23
2982	6721	NS4A-85	NS4A-253	G	A	0.13	NSUB	9.39
2982	1293	E-109	E-327	T	C	0.78	NSUB	9.51
2982	6105	NS3-498	NS3-1494	C	T	0.22	NSUB	9.58
2982	7179	NS4B-88	NS4B-264	T	C	0.70	NSUB	9.58
2982	3474	NS1-335	NS1-1005	T	C	0.62	NSUB	9.62
2982	1380	E-138	E-414	A	T	0.63	NSUB	9.75
2982	8261	NS5-194	NS5-581	G	A	0.15	NSUB	10.02
2982	7830	NS5-50	NS5-150	T	C	0.47	NSUB	10.33
2982	8883	NS5-401	NS5-1203	C	T	0.88	NSUB	10.47
2982	6072	NS3-487	NS3-1461	T	C	0.48	NSUB	10.51
2982	4536	NS2B-106	NS2B-318	G	A	0.06	NSUB	10.71
2982	5391	NS3-260	NS3-780	C	A	0.18	NSUB	10.76
2982	7587	NS4B-224	NS4B-672	T	C	0.44	NSUB	10.79
2982	4899	NS3-96	NS3-288	G	A	0.17	NSUB	11.15
2982	9264	NS5-528	NS5-1584	T	C	0.15	NSUB	11.23
2982	1116	E-50	E-150	A	G	0.18	NSUB	12.00
2983	4109	NS2A-195	NS2A-584	IA	d	0.11	LP	0.78
2983	9063	NS5-461	NS5-1383	IA	d	0.77	LP	0.80
2983	5166	NS3-185	NS3-555	IA	d	0.14	LP	0.88
2983	7960	NS5-94	NS5-280	IA	d	0.71	LP	0.89
2983	6998	NS4B-28	NS4B-83	C	G	0.38	NSUB	0.36
2983	7326	NS4B-137	NS4B-411	T	C	0.78	NSUB	0.57
2983	7711	NS5-11	NS5-31	T	G	0.38	NSUB	0.64
2983	5166	NS3-185	NS3-555	A	G	0.25	NSUB	0.93
2983	5943	NS3-444	NS3-1332	T	G	0.69	NSUB	0.94
2984	8413	NS5-245	NS5-733	IA	d	0.22	LP	0.67
2984	7960	NS5-94	NS5-280	IA	d	0.50	LP	0.85
2984	9063	NS5-461	NS5-1383	IA	d	0.83	LP	1.14
2984	4109	NS2A-195	NS2A-584	IA	d	0.16	LP	1.89
2984	6203	NS3-531	NS3-1592	IA	d	0.42	LP	4.26
2984	4914	NS3-101	NS3-303	C	A	0.54	NSUB	0.25

2984	4910	NS3-100	NS3-299	A	T	1.00	NSUB	0.25
2984	10113	NS5-811	NS5-2433	G	A	0.39	NSUB	0.41
2984	4424	NS2B-69	NS2B-206	G	C	0.40	NSUB	0.44
2984	2925	NS1-152	NS1-456	T	C	1.00	NSUB	0.44
2984	524	prM-20	prM-59	T	C	0.37	NSUB	0.47
2984	5224	NS3-205	NS3-613	T	C	0.25	NSUB	0.51
2984	3774	NS2A-83	NS2A-249	T	C	1.00	NSUB	0.52
2984	276	C-60	C-180	C	T	0.70	NSUB	0.56
2984	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.57
2984	7443	NS4B-176	NS4B-528	T	C	1.00	NSUB	0.58
2984	5166	NS3-185	NS3-555	A	G	1.00	NSUB	0.58
2984	3049	NS1-194	NS1-580	G	A	0.69	NSUB	0.59
2984	3409	NS1-314	NS1-940	G	A	1.00	NSUB	0.62
2984	10478	3'UTR-83	3'UTR-83	G	A	0.68	NSUB	0.63
2984	1881	E-305	E-915	C	T	0.70	NSUB	0.63
2984	9753	NS5-691	NS5-2073	A	G	0.26	NSUB	0.63
2984	595	prM-44	prM-130	G	A	0.71	NSUB	0.67
2984	4530	NS2B-104	NS2B-312	C	T	1.00	NSUB	0.68
2984	2018	E-351	E-1052	T	C	1.00	NSUB	0.69
2984	4533	NS2B-105	NS2B-315	T	C	0.69	NSUB	0.70
2984	8317	NS5-213	NS5-637	A	C	0.72	NSUB	0.71
2984	10481	3'UTR-86	3'UTR-86	C	T	0.69	NSUB	0.72
2984	7389	NS4B-158	NS4B-474	T	C	0.35	NSUB	0.74
2984	274	C-60	C-178	A	G	1.00	NSUB	0.74
2984	1188	E-74	E-222	T	C	0.74	NSUB	0.76
2984	72	5'UTR-72	5'UTR-72	A	G	0.13	NSUB	0.78
2984	7410	NS4B-165	NS4B-495	T	C	0.15	NSUB	0.79
2984	5613	NS3-334	NS3-1002	T	C	0.96	NSUB	0.80
2984	5325	NS3-238	NS3-714	G	A	1.00	NSUB	0.85
2984	1223	E-86	E-257	T	C	0.54	NSUB	0.88
2984	4060	NS2A-179	NS2A-535	C	T	0.75	NSUB	0.89
2984	9723	NS5-681	NS5-2043	T	C	1.00	NSUB	1.00
2984	4017	NS2A-164	NS2A-492	T	C	1.00	NSUB	1.07
2984	8388	NS5-236	NS5-708	T	C	1.00	NSUB	1.12
2984	10393	NS5-905	NS5-2713	G	T	0.73	NSUB	1.24
2984	3015	NS1-182	NS1-546	A	G	0.49	NSUB	1.26
2984	408	C-104	C-312	G	A	0.11	NSUB	1.73
2984	9630	NS5-650	NS5-1950	T	C	0.37	NSUB	2.16
2984	371	C-92	C-275	T	C	0.71	NSUB	2.49

2984	4272	NS2B-18	NS2B-54	T	C	0.10	NSUB	2.75
2984	8616	NS5-312	NS5-936	C	T	0.18	NSUB	2.87
2984	4340	NS2B-41	NS2B-122	T	C	0.81	NSUB	3.55
2984	1270	E-102	E-304	A	G	0.76	NSUB	3.85
2984	5040	NS3-143	NS3-429	T	C	0.10	NSUB	4.84
2984	7021	NS4B-36	NS4B-106	G	T	0.99	NSUB	4.97
2984	7821	NS5-47	NS5-141	T	C	0.73	NSUB	12.41
2997	7960	NS5-94	NS5-280	IA	d	0.28	LP	0.75
2997	9063	NS5-461	NS5-1383	IA	d	0.53	LP	0.98
2997	4699	NS3-30	NS3-88	T	C	0.76	NSUB	0.86
2997	8517	NS5-279	NS5-837	T	C	0.39	NSUB	2.05
2998	555	prM-30	prM-90	D1	i	1.00	LP	0.16
2998	917	prM-151	prM-452	D2	i	0.40	LP	0.40
2998	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.50
2998	7960	NS5-94	NS5-280	IA	d	0.06	LP	0.80
2998	7267	NS4B-118	NS4B-352	IT	d	0.67	LP	4.65
2998	558	prM-31	prM-93	T	G	1.00	NSUB	0.16
2998	6183	NS3-524	NS3-1572	T	C	0.41	NSUB	0.38
2998	10269	NS5-863	NS5-2589	C	G	1.00	NSUB	0.53
2998	1227	E-87	E-261	T	C	1.00	NSUB	0.53
2998	10458	3'UTR-63	3'UTR-63	C	G	1.00	NSUB	0.54
2998	4208	NS2A-228	NS2A-683	T	A	0.22	NSUB	0.62
2998	1468	E-168	E-502	G	A	0.88	NSUB	1.76
2998	4308	NS2B-30	NS2B-90	T	C	0.47	NSUB	2.87
2998	3320	NS1-284	NS1-851	T	C	0.89	NSUB	3.13
2998	120	C-8	C-24	T	C	0.26	NSUB	3.20
2999	8413	NS5-245	NS5-733	IA	d	0.38	LP	0.53
2999	9063	NS5-461	NS5-1383	IA	d	0.38	LP	0.58
2999	7960	NS5-94	NS5-280	IA	d	0.96	LP	1.31
2999	4109	NS2A-195	NS2A-584	IA	d	0.49	LP	1.45
2999	5166	NS3-185	NS3-555	IA	d	0.40	LP	1.62
2999	6203	NS3-531	NS3-1592	IA	d	0.77	LP	3.23
2999	7267	NS4B-118	NS4B-352	IT	d	0.05	LP	5.59
2999	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.16
2999	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.24
2999	6656	NS4A-63	NS4A-188	A	T	1.00	NSUB	0.27
2999	2787	NS1-106	NS1-318	T	C	1.00	NSUB	0.44
2999	10157	NS5-826	NS5-2477	G	A	0.38	NSUB	0.44
2999	2501	NS1-11	NS1-32	G	A	1.00	NSUB	0.47

2999	8413	NS5-245	NS5-733	A	G	1.00	NSUB	0.52
2999	3600	NS2A-25	NS2A-75	T	C	0.60	NSUB	0.53
2999	777	prM-104	prM-312	G	A	1.00	NSUB	0.54
2999	10364	NS5-895	NS5-2684	G	A	0.71	NSUB	0.60
2999	2733	NS1-88	NS1-264	T	C	0.73	NSUB	0.68
2999	2066	E-367	E-1100	T	C	0.73	NSUB	0.72
2999	5166	NS3-185	NS3-555	A	G	0.43	NSUB	0.74
2999	10123	NS5-815	NS5-2443	C	T	1.00	NSUB	0.75
2999	10592	3'UTR-197	3'UTR-197	T	G	0.26	NSUB	0.79
2999	5568	NS3-319	NS3-957	T	A	1.01	NSUB	0.81
2999	7711	NS5-11	NS5-31	T	G	0.75	NSUB	0.87
2999	4962	NS3-117	NS3-351	G	A	0.75	NSUB	0.87
2999	399	C-101	C-303	G	A	0.77	NSUB	1.00
2999	7950	NS5-90	NS5-270	C	T	0.39	NSUB	1.08
2999	10173	NS5-831	NS5-2493	T	C	0.78	NSUB	1.13
2999	932	prM-156	prM-467	C	T	0.63	NSUB	1.13
2999	10627	3'UTR-232	3'UTR-232	A	G	0.84	NSUB	1.66
2999	2795	NS1-109	NS1-326	G	A	0.33	NSUB	1.66
2999	9525	NS5-615	NS5-1845	T	C	0.12	NSUB	1.95
2999	8886	NS5-402	NS5-1206	G	A	0.73	NSUB	3.47
2999	9465	NS5-595	NS5-1785	C	T	0.72	NSUB	4.20
2999	7399	NS4B-162	NS4B-484	C	T	0.68	NSUB	5.10
2999	10641	3'UTR-246	3'UTR-246	G	A	0.81	NSUB	5.48
2999	1715	E-250	E-749	G	A	0.56	NSUB	7.01
3000	7960	NS5-94	NS5-280	IA	d	0.69	LP	0.64
3000	9063	NS5-461	NS5-1383	IA	d	0.53	LP	0.93
3000	5166	NS3-185	NS3-555	IA	d	0.18	LP	1.04
3000	10157	NS5-826	NS5-2477	G	A	1.00	NSUB	0.17
3000	10160	NS5-827	NS5-2480	G	A	1.00	NSUB	0.19
3000	544	prM-27	prM-79	G	A	1.00	NSUB	0.22
3000	551	prM-29	prM-86	T	C	0.64	NSUB	0.29
3000	7648	NS4B-245	NS4B-733	C	A	1.00	NSUB	0.47
3000	3374	NS1-302	NS1-905	T	C	0.37	NSUB	0.56
3000	5529	NS3-306	NS3-918	T	G	0.75	NSUB	0.63
3000	9375	NS5-565	NS5-1695	C	T	0.37	NSUB	0.69
3000	387	C-97	C-291	C	G	0.51	NSUB	0.98
3000	10427	3'UTR-32	3'UTR-32	C	T	0.75	NSUB	1.52
3000	5117	NS3-169	NS3-506	C	A	0.14	NSUB	2.10
3000	751	prM-96	prM-286	A	G	0.73	NSUB	2.46

3000	1570	E-202	E-604	C	T	0.22	NSUB	2.84
3000	4419	NS2B-67	NS2B-201	G	A	0.39	NSUB	5.40
3000	4736	NS3-42	NS3-125	C	T	0.10	NSUB	5.45
3000	10287	NS5-869	NS5-2607	A	G	0.12	NSUB	7.18
3000	9255	NS5-525	NS5-1575	T	C	0.19	NSUB	9.85
3000	3084	NS1-205	NS1-615	A	G	0.37	NSUB	15.99
3001	7960	NS5-94	NS5-280	IA	d	0.42	LP	0.68
3001	6203	NS3-531	NS3-1592	IA	d	0.31	LP	1.99
3001	7267	NS4B-118	NS4B-352	IT	d	0.87	LP	3.35
3001	4702	NS3-31	NS3-91	T	C	0.18	NSUB	0.44
3001	4698	NS3-29	NS3-87	G	A	0.14	NSUB	0.45
3001	8493	NS5-271	NS5-813	A	G	0.45	NSUB	0.66
3001	6015	NS3-468	NS3-1404	G	A	0.21	NSUB	0.90
3001	7593	NS4B-226	NS4B-678	T	C	0.77	NSUB	3.89
3001	348	C-84	C-252	C	T	0.46	NSUB	6.07
3002	5166	NS3-185	NS3-555	IA	d	0.46	LP	0.92
3002	4109	NS2A-195	NS2A-584	IA	d	0.54	LP	1.22
3002	9063	NS5-461	NS5-1383	IA	d	0.34	LP	1.39
3002	7960	NS5-94	NS5-280	IA	d	0.08	LP	1.39
3002	6203	NS3-531	NS3-1592	IA	d	0.82	LP	3.32
3002	7267	NS4B-118	NS4B-352	IT	d	0.91	LP	11.03
3002	7158	NS4B-81	NS4B-243	A	G	1.00	NSUB	0.36
3002	7161	NS4B-82	NS4B-246	C	T	1.00	NSUB	0.36
3002	7389	NS4B-158	NS4B-474	C	T	1.00	NSUB	0.47
3002	365	C-90	C-269	T	C	1.00	NSUB	0.47
3002	357	C-87	C-261	G	A	0.38	NSUB	0.49
3002	4875	NS3-88	NS3-264	G	A	1.01	NSUB	0.60
3002	6105	NS3-498	NS3-1494	C	T	1.01	NSUB	0.68
3002	10733	3'UTR-338	3'UTR-338	A	G	1.00	NSUB	1.24
3002	2598	NS1-43	NS1-129	T	C	0.73	NSUB	1.69
3002	1170	E-68	E-204	T	C	0.89	NSUB	1.76
3002	2020	E-352	E-1054	A	G	0.78	NSUB	3.39
3002	5184	NS3-191	NS3-573	G	A	0.41	NSUB	7.39
3002	897	prM-144	prM-432	A	G	0.43	NSUB	7.62
3002	1275	E-103	E-309	T	C	0.33	NSUB	10.47
3002	8670	NS5-330	NS5-990	T	C	0.15	NSUB	16.88
3002	4500	NS2B-94	NS2B-282	C	T	0.07	NSUB	17.08
3002	10339	NS5-887	NS5-2659	C	T	0.25	NSUB	20.36
3002	267	C-57	C-171	G	A	0.93	NSUB	26.89

3002	1452	E-162	E-486	T	C	0.15	NSUB	29.69
3003	7319	NS4B-135	NS4B-404	D1	i	1.00	LP	0.13
3003	7311	NS4B-132	NS4B-396	IC	d	1.00	LP	0.14
3003	7960	NS5-94	NS5-280	IA	d	0.73	LP	0.67
3003	5166	NS3-185	NS3-555	IA	d	0.45	LP	0.88
3003	9063	NS5-461	NS5-1383	IA	d	0.76	LP	0.90
3003	4109	NS2A-195	NS2A-584	IA	d	0.29	LP	1.17
3003	7267	NS4B-118	NS4B-352	IT	d	0.28	LP	7.95
3003	7315	NS4B-134	NS4B-400	T	A	1.00	NSUB	0.13
3003	7047	NS4B-44	NS4B-132	C	G	0.55	NSUB	0.21
3003	7046	NS4B-44	NS4B-131	A	T	0.54	NSUB	0.21
3003	5247	NS3-212	NS3-636	C	T	0.69	NSUB	0.39
3003	2795	NS1-109	NS1-326	G	A	0.46	NSUB	0.52
3003	10458	3'UTR-63	3'UTR-63	C	G	0.29	NSUB	0.80
3003	5329	NS3-240	NS3-718	G	C	0.14	NSUB	1.30
3003	10888	3'UTR-493	3'UTR-493	A	T	1.00	NSUB	2.35
3004	9063	NS5-461	NS5-1383	IA	d	0.18	LP	1.16
3004	7960	NS5-94	NS5-280	IA	d	0.84	LP	1.61
3004	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.41
3004	7648	NS4B-245	NS4B-733	G	A	1.00	NSUB	0.45
3004	5205	NS3-198	NS3-594	T	C	0.53	NSUB	0.63
3004	5166	NS3-185	NS3-555	A	G	0.18	NSUB	0.99
3004	10582	3'UTR-187	3'UTR-187	T	G	0.10	NSUB	1.00
3004	2195	E-410	E-1229	T	C	0.54	NSUB	1.91
3004	1371	E-135	E-405	T	C	0.85	NSUB	4.48
3004	8424	NS5-248	NS5-744	T	C	0.18	NSUB	19.65
3005	9063	NS5-461	NS5-1383	IA	d	0.18	LP	1.24
3005	4109	NS2A-195	NS2A-584	IA	d	0.12	LP	1.30
3005	7960	NS5-94	NS5-280	IA	d	0.82	LP	1.34
3005	7267	NS4B-118	NS4B-352	IT	d	0.53	LP	1.79
3005	5166	NS3-185	NS3-555	IA	d	0.17	LP	2.02
3005	3445	NS1-326	NS1-976	A	G	1.00	NSUB	0.27
3005	5927	NS3-439	NS3-1316	C	A	0.38	NSUB	0.36
3005	7711	NS5-11	NS5-31	T	G	0.22	NSUB	0.54
3005	10582	3'UTR-187	3'UTR-187	T	G	0.57	NSUB	1.04
3005	5166	NS3-185	NS3-555	A	G	0.57	NSUB	1.44
3005	3399	NS1-310	NS1-930	C	T	0.16	NSUB	1.50
3005	7855	NS5-59	NS5-175	G	A	0.99	NSUB	3.01
3005	9492	NS5-604	NS5-1812	C	T	0.87	NSUB	3.27

3005	5367	NS3-252	NS3-756	C	T	0.97	NSUB	4.53
3005	147	C-17	C-51	G	A	0.14	NSUB	4.74
3005	10439	3'UTR-44	3'UTR-44	G	A	0.88	NSUB	6.42
3006	7960	NS5-94	NS5-280	IA	d	0.19	LP	1.04
3006	6203	NS3-531	NS3-1592	IA	d	0.14	LP	1.23
3006	4802	NS3-64	NS3-191	A	G	0.66	NSUB	0.14
3006	4086	NS2A-187	NS2A-561	T	C	1.00	NSUB	0.21
3006	9532	NS5-618	NS5-1852	T	C	1.00	NSUB	0.23
3006	10458	3'UTR-63	3'UTR-63	C	G	0.68	NSUB	0.23
3006	994	E-10	E-28	C	G	0.25	NSUB	0.30
3006	5903	NS3-431	NS3-1292	G	A	0.55	NSUB	0.33
3006	2286	E-440	E-1320	T	G	0.13	NSUB	0.34
3006	2779	NS1-104	NS1-310	T	C	0.22	NSUB	0.35
3006	1386	E-140	E-420	T	C	0.52	NSUB	0.41
3006	8181	NS5-167	NS5-501	A	G	0.66	NSUB	0.42
3006	7884	NS5-68	NS5-204	A	G	0.41	NSUB	1.37
3006	10888	3'UTR-493	3'UTR-493	A	T	1.00	NSUB	1.70
3007	4109	NS2A-195	NS2A-584	IA	d	0.24	LP	0.64
3007	7960	NS5-94	NS5-280	IA	d	0.16	LP	0.79
3007	6203	NS3-531	NS3-1592	IA	d	0.14	LP	1.29
3007	1844	E-293	E-878	G	A	0.90	NSUB	5.88
3008	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.82
3008	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.09
3008	4109	NS2A-195	NS2A-584	IA	d	0.74	LP	1.12
3008	5166	NS3-185	NS3-555	IA	d	0.12	LP	1.16
3008	6203	NS3-531	NS3-1592	IA	d	0.90	LP	2.11
3008	1232	E-89	E-266	T	C	0.71	NSUB	0.56
3008	5369	NS3-253	NS3-758	C	A	1.07	NSUB	0.67
3008	6747	NS4A-93	NS4A-279	T	C	0.72	NSUB	0.68
3008	7742	NS5-21	NS5-62	G	A	1.00	NSUB	0.71
3008	8499	NS5-273	NS5-819	T	C	1.00	NSUB	0.72
3008	6068	NS3-486	NS3-1457	C	T	1.00	NSUB	0.89
3008	5094	NS3-161	NS3-483	T	C	0.49	NSUB	1.06
3008	8721	NS5-347	NS5-1041	T	C	1.00	NSUB	1.08
3008	3717	NS2A-64	NS2A-192	C	T	0.72	NSUB	1.08
3008	8601	NS5-307	NS5-921	C	T	1.00	NSUB	1.16
3008	9843	NS5-721	NS5-2163	A	G	1.00	NSUB	1.21
3008	7711	NS5-11	NS5-31	T	G	0.82	NSUB	1.41
3008	2262	E-432	E-1296	G	A	0.42	NSUB	1.56

3008	6735	NS4A-89	NS4A-267	C	T	0.32	NSUB	2.90
3008	4272	NS2B-18	NS2B-54	C	T	0.15	NSUB	3.92
3008	933	prM-156	prM-468	T	C	0.10	NSUB	3.95
3008	7419	NS4B-168	NS4B-504	T	C	0.23	NSUB	4.13
3008	1479	E-171	E-513	T	A	0.64	NSUB	4.13
3008	7173	NS4B-86	NS4B-258	C	T	0.48	NSUB	4.37
3008	6693	NS4A-75	NS4A-225	A	G	0.83	NSUB	4.47
3008	1750	E-262	E-784	T	C	0.43	NSUB	4.55
3008	2304	E-446	E-1338	T	C	0.73	NSUB	4.60
3008	7602	NS4B-229	NS4B-687	T	C	0.11	NSUB	4.61
3008	7395	NS4B-160	NS4B-480	C	A	0.78	NSUB	4.64
3008	8436	NS5-252	NS5-756	T	C	0.74	NSUB	4.76
3008	9711	NS5-677	NS5-2031	T	C	0.06	NSUB	4.80
3008	435	C-113	C-339	T	C	0.63	NSUB	4.84
3008	2853	NS1-128	NS1-384	T	C	0.54	NSUB	4.85
3008	5376	NS3-255	NS3-765	T	C	0.43	NSUB	4.87
3008	3498	NS1-343	NS1-1029	G	A	0.82	NSUB	4.92
3008	4569	NS2B-117	NS2B-351	C	T	0.97	NSUB	4.97
3008	6063	NS3-484	NS3-1452	T	G	0.51	NSUB	5.08
3008	6540	NS4A-24	NS4A-72	C	T	0.20	NSUB	5.13
3008	6069	NS3-486	NS3-1458	C	T	0.74	NSUB	5.22
3008	2559	NS1-30	NS1-90	T	C	0.26	NSUB	5.38
3008	7155	NS4B-80	NS4B-240	T	C	0.11	NSUB	5.43
3008	8487	NS5-269	NS5-807	A	G	0.29	NSUB	5.46
3008	963	prM-166	prM-498	C	T	0.39	NSUB	5.54
3008	5280	NS3-223	NS3-669	A	G	0.13	NSUB	5.55
3008	4140	NS2A-205	NS2A-615	G	A	0.51	NSUB	5.63
3008	5889	NS3-426	NS3-1278	T	C	0.90	NSUB	5.65
3008	6238	NS3-543	NS3-1627	T	C	0.56	NSUB	5.74
3008	9732	NS5-684	NS5-2052	C	T	0.32	NSUB	5.80
3008	7793	NS5-38	NS5-113	C	T	0.42	NSUB	5.83
3008	4699	NS3-30	NS3-88	C	T	0.11	NSUB	5.84
3008	9759	NS5-693	NS5-2079	C	T	0.61	NSUB	5.93
3008	1662	E-232	E-696	A	G	0.87	NSUB	5.93
3008	4344	NS2B-42	NS2B-126	T	C	0.41	NSUB	5.98
3008	7938	NS5-86	NS5-258	C	T	0.06	NSUB	6.04
3008	3969	NS2A-148	NS2A-444	T	C	0.19	NSUB	6.11
3008	3515	NS1-349	NS1-1046	G	A	0.35	NSUB	6.38
3008	732	prM-89	prM-267	C	T	0.41	NSUB	6.44

3008	3528	NS2A-1	NS2A-3	C	T	0.84	NSUB	6.45
3008	5703	NS3-364	NS3-1092	T	C	0.78	NSUB	6.49
3008	4824	NS3-71	NS3-213	C	T	0.55	NSUB	6.50
3008	6992	NS4B-26	NS4B-77	G	A	0.16	NSUB	6.57
3008	7059	NS4B-48	NS4B-144	A	G	0.10	NSUB	6.58
3008	6165	NS3-518	NS3-1554	C	T	0.95	NSUB	6.59
3008	7812	NS5-44	NS5-132	G	A	0.88	NSUB	6.59
3008	9381	NS5-567	NS5-1701	T	C	0.73	NSUB	6.61
3008	6786	NS4A-106	NS4A-318	C	A	0.19	NSUB	6.66
3008	10200	NS5-840	NS5-2520	C	T	0.92	NSUB	6.70
3008	660	prM-65	prM-195	C	T	0.27	NSUB	6.71
3008	4398	NS2B-60	NS2B-180	C	T	0.90	NSUB	6.83
3008	4212	NS2A-229	NS2A-687	T	C	0.90	NSUB	6.93
3008	10471	3'UTR-76	3'UTR-76	G	T	0.23	NSUB	6.97
3008	5331	NS3-240	NS3-720	C	T	0.06	NSUB	6.99
3008	5595	NS3-328	NS3-984	A	G	0.13	NSUB	7.02
3008	8505	NS5-275	NS5-825	C	T	0.81	NSUB	7.27
3008	8511	NS5-277	NS5-831	C	T	0.83	NSUB	7.35
3008	8778	NS5-366	NS5-1098	A	T	0.86	NSUB	7.49
3008	10248	NS5-856	NS5-2568	C	T	0.93	NSUB	7.53
3008	4479	NS2B-87	NS2B-261	C	T	0.84	NSUB	7.76
3008	381	C-95	C-285	T	C	0.08	NSUB	7.89
3008	10347	NS5-889	NS5-2667	T	C	0.25	NSUB	7.98
3008	394	C-100	C-298	T	G	0.61	NSUB	8.01
3008	6256	NS3-549	NS3-1645	C	T	0.52	NSUB	8.07
3008	10317	NS5-879	NS5-2637	C	T	0.42	NSUB	8.82
3008	5199	NS3-196	NS3-588	T	C	0.36	NSUB	11.90
3008	10427	3'UTR-32	3'UTR-32	C	T	0.08	NSUB	11.99
3008	2811	NS1-114	NS1-342	T	C	0.91	NSUB	20.72
3009	9063	NS5-461	NS5-1383	IA	d	0.29	LP	0.72
3009	6203	NS3-531	NS3-1592	IA	d	0.73	LP	1.14
3009	4263	NS2B-15	NS2B-45	C	T	0.13	NSUB	6.00
3009	6825	NS4A-119	NS4A-357	C	T	0.64	NSUB	6.40
3009	4129	NS2A-202	NS2A-604	C	T	0.85	NSUB	6.76
3009	9381	NS5-567	NS5-1701	T	C	0.11	NSUB	7.07
3009	3518	NS1-350	NS1-1049	C	T	0.19	NSUB	7.48
3009	2973	NS1-168	NS1-504	C	T	0.22	NSUB	8.19
3009	427	C-111	C-331	G	A	0.44	NSUB	8.71
3009	9462	NS5-594	NS5-1782	C	T	0.30	NSUB	9.42

3010	4109	NS2A-195	NS2A-584	IA	d	1.00	LP	1.18
3010	7960	NS5-94	NS5-280	IA	d	1.00	LP	1.75
3010	3969	NS2A-148	NS2A-444	T	C	1.00	NSUB	0.95
3010	9241	NS5-521	NS5-1561	T	C	1.00	NSUB	1.13
3010	10436	3'UTR-41	3'UTR-41	G	A	1.00	NSUB	1.21
3010	4668	NS3-19	NS3-57	T	C	0.74	NSUB	2.00
3010	4089	NS2A-188	NS2A-564	A	G	0.30	NSUB	3.21
3010	10408	3'UTR-13	3'UTR-13	T	C	0.35	NSUB	7.99
3010	3792	NS2A-89	NS2A-267	A	T	0.91	NSUB	8.67
3010	9616	NS5-646	NS5-1936	A	C	0.12	NSUB	8.68
3010	4677	NS3-22	NS3-66	T	C	0.32	NSUB	9.29
3010	7308	NS4B-131	NS4B-393	C	T	0.05	NSUB	14.46
3010	3861	NS2A-112	NS2A-336	C	T	0.94	NSUB	15.17
3010	951	prM-162	prM-486	T	G	0.70	NSUB	32.89
3011	5236	NS3-209	NS3-625	D1	i	1.00	LP	0.18
3011	3836	NS2A-104	NS2A-311	D1	i	1.00	LP	0.48
3011	4106	NS2A-194	NS2A-581	IA	d	1.00	LP	1.50
3011	9060	NS5-460	NS5-1380	IA	d	0.96	LP	1.67
3011	7957	NS5-93	NS5-277	IA	d	0.43	LP	1.72
3011	6200	NS3-530	NS3-1589	IA	d	0.50	LP	2.77
3011	5243	NS3-211	NS3-632	T	C	1.00	NSUB	0.19
3011	10464	3'UTR-69	3'UTR-69	T	G	1.00	NSUB	0.62
3011	5163	NS3-184	NS3-552	A	G	0.66	NSUB	0.69
3011	7264	NS4B-117	NS4B-349	T	C	0.41	NSUB	0.79
3011	2223	E-419	E-1257	C	T	0.73	NSUB	0.84
3011	8721	NS5-347	NS5-1041	C	T	0.42	NSUB	1.22
3011	6855	NS4A-129	NS4A-387	T	C	0.42	NSUB	1.44
3011	8805	NS5-375	NS5-1125	T	C	0.71	NSUB	1.73
3011	10411	3'UTR-16	3'UTR-16	C	T	0.41	NSUB	1.91
3011	10422	3'UTR-27	3'UTR-27	C	T	0.34	NSUB	1.95
3011	5967	NS3-452	NS3-1356	C	T	0.62	NSUB	2.18
3011	9522	NS5-614	NS5-1842	T	C	0.43	NSUB	2.22
3011	5296	NS3-229	NS3-685	A	G	0.81	NSUB	3.02
3011	2193	E-409	E-1227	T	C	0.82	NSUB	3.19
3011	1089	E-41	E-123	C	T	0.48	NSUB	3.28
3011	628	prM-55	prM-163	T	C	0.21	NSUB	3.56
3011	8187	NS5-169	NS5-507	T	C	0.95	NSUB	3.74
3011	5353	NS3-248	NS3-742	T	C	0.67	NSUB	4.22
3011	3897	NS2A-124	NS2A-372	A	G	0.07	NSUB	11.20

3011	593	prM-43	prM-128	C	T	0.38	NSUB	14.97
3012	9063	NS5-461	NS5-1383	IA	d	1.00	LP	0.75
3012	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.99
3012	4109	NS2A-195	NS2A-584	IA	d	0.74	LP	1.60
3012	6203	NS3-531	NS3-1592	IA	d	0.33	LP	3.05
3012	749	prM-95	prM-284	T	C	0.59	NSUB	0.37
3012	747	prM-94	prM-282	A	G	0.64	NSUB	0.46
3012	7462	NS4B-183	NS4B-547	T	C	0.63	NSUB	0.60
3012	6443	NS3-611	NS3-1832	A	T	1.00	NSUB	0.73
3012	9649	NS5-657	NS5-1969	A	G	0.64	NSUB	0.79
3012	2881	NS1-138	NS1-412	T	C	0.23	NSUB	0.87
3012	5373	NS3-254	NS3-762	G	A	0.69	NSUB	0.89
3012	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.89
3012	3547	NS2A-8	NS2A-22	T	C	1.00	NSUB	0.89
3012	953	prM-163	prM-488	T	C	1.00	NSUB	0.97
3012	3726	NS2A-67	NS2A-201	G	A	0.44	NSUB	1.02
3012	10317	NS5-879	NS5-2637	T	C	0.46	NSUB	1.08
3012	415	C-107	C-319	A	G	0.08	NSUB	1.15
3012	4517	NS2B-100	NS2B-299	C	T	0.69	NSUB	1.17
3012	4146	NS2A-207	NS2A-621	A	G	1.00	NSUB	1.24
3012	3598	NS2A-25	NS2A-73	T	C	0.68	NSUB	1.45
3012	4464	NS2B-82	NS2B-246	C	T	0.08	NSUB	1.47
3012	7891	NS5-71	NS5-211	T	C	1.00	NSUB	1.50
3012	1983	E-339	E-1017	C	T	1.00	NSUB	1.50
3012	3768	NS2A-81	NS2A-243	T	G	1.06	NSUB	1.62
3012	7173	NS4B-86	NS4B-258	T	C	0.55	NSUB	1.66
3012	6385	NS3-592	NS3-1774	T	C	1.02	NSUB	1.76
3012	7785	NS5-35	NS5-105	T	C	0.81	NSUB	1.98
3012	3441	NS1-324	NS1-972	G	A	1.00	NSUB	2.07
3012	1368	E-134	E-402	C	T	0.87	NSUB	2.14
3012	10338	NS5-886	NS5-2658	C	T	0.34	NSUB	2.17
3012	7389	NS4B-158	NS4B-474	T	C	0.75	NSUB	2.47
3012	6871	NS4A-135	NS4A-403	A	G	0.22	NSUB	2.67
3012	5812	NS3-401	NS3-1201	A	G	0.80	NSUB	2.86
3012	10626	3'UTR-231	3'UTR-231	T	C	0.39	NSUB	2.90
3012	489	prM-8	prM-24	A	G	0.07	NSUB	2.97
3013	7960	NS5-94	NS5-280	IA	d	0.52	LP	1.21
3013	5166	NS3-185	NS3-555	IA	d	0.76	LP	1.44
3013	6203	NS3-531	NS3-1592	IA	d	0.31	LP	3.15

3013	5199	NS3-196	NS3-588	T	C	0.59	NSUB	0.48
3013	4109	NS2A-195	NS2A-584	A	C	1.02	NSUB	0.59
3013	2913	NS1-148	NS1-444	T	C	1.01	NSUB	0.61
3013	2223	E-419	E-1257	T	C	0.24	NSUB	0.63
3013	5166	NS3-185	NS3-555	A	G	1.01	NSUB	0.64
3013	10349	NS5-890	NS5-2669	T	C	1.00	NSUB	0.65
3013	6238	NS3-543	NS3-1627	T	C	0.83	NSUB	0.76
3014	4109	NS2A-195	NS2A-584	IA	d	0.93	LP	1.62
3014	6203	NS3-531	NS3-1592	IA	d	1.12	LP	2.19
3014	5331	NS3-240	NS3-720	T	C	0.64	NSUB	0.65
3014	7711	NS5-11	NS5-31	T	G	1.00	NSUB	0.80
3014	10408	3'UTR-13	3'UTR-13	T	C	0.63	NSUB	0.93
3014	1496	E-177	E-530	T	C	1.00	NSUB	0.98
3014	2929	NS1-154	NS1-460	A	G	1.00	NSUB	0.98
3014	2574	NS1-35	NS1-105	T	C	1.00	NSUB	1.07
3014	5583	NS3-324	NS3-972	C	T	0.46	NSUB	1.08
3014	785	prM-107	prM-320	G	A	1.00	NSUB	1.17
3014	5166	NS3-185	NS3-555	A	G	0.43	NSUB	1.39
3014	6528	NS4A-20	NS4A-60	G	A	1.00	NSUB	1.43
3014	1467	E-167	E-501	T	C	0.11	NSUB	1.43
3014	3409	NS1-314	NS1-940	G	A	0.70	NSUB	1.61
3014	972	E-2	E-6	T	C	0.05	NSUB	1.78
3014	8322	NS5-214	NS5-642	C	T	0.87	NSUB	1.80
3014	3060	NS1-197	NS1-591	T	C	0.12	NSUB	1.97
3014	3880	NS2A-119	NS2A-355	T	C	0.57	NSUB	2.54
3014	6072	NS3-487	NS3-1461	T	C	0.79	NSUB	2.62
3014	6201	NS3-530	NS3-1590	A	G	0.74	NSUB	3.05
3014	8004	NS5-108	NS5-324	T	C	0.84	NSUB	3.08
3014	7891	NS5-71	NS5-211	T	C	0.62	NSUB	3.26
3015	9063	NS5-461	NS5-1383	IA	d	0.69	LP	0.70
3015	6740	NS4A-91	NS4A-272	IT	d	1.00	LP	0.75
3015	7960	NS5-94	NS5-280	IA	d	1.00	LP	0.78
3015	6741	NS4A-91	NS4A-273	D1	i	0.79	LP	1.36
3015	6203	NS3-531	NS3-1592	IA	d	0.71	LP	2.31
3015	7267	NS4B-118	NS4B-352	IT	d	0.09	LP	4.77
3015	1122	E-52	E-156	T	C	1.00	NSUB	0.44
3015	4792	NS3-61	NS3-181	A	G	0.70	NSUB	0.49
3015	2770	NS1-101	NS1-301	T	C	0.70	NSUB	0.55
3015	8838	NS5-386	NS5-1158	C	T	1.00	NSUB	0.55

3015	2526	NS1-19	NS1-57	A	G	0.23	NSUB	0.55
3015	1213	E-83	E-247	A	G	1.00	NSUB	0.59
3015	8976	NS5-432	NS5-1296	G	A	0.45	NSUB	0.64
3015	7203	NS4B-96	NS4B-288	T	C	0.68	NSUB	0.64
3015	6530	NS4A-21	NS4A-62	T	G	0.74	NSUB	0.65
3015	7476	NS4B-187	NS4B-561	G	A	0.73	NSUB	0.70
3015	9513	NS5-611	NS5-1833	T	C	0.42	NSUB	0.70
3015	789	prM-108	prM-324	A	G	1.10	NSUB	0.76
3015	6901	NS4A-145	NS4A-433	G	A	1.00	NSUB	0.79
3015	8952	NS5-424	NS5-1272	T	C	1.00	NSUB	0.79
3015	4938	NS3-109	NS3-327	T	C	1.00	NSUB	0.99
3015	569	prM-35	prM-104	C	T	0.44	NSUB	1.08
3015	264	C-56	C-168	T	C	0.54	NSUB	1.09
3015	8075	NS5-132	NS5-395	C	T	0.72	NSUB	1.19
3015	8949	NS5-423	NS5-1269	T	C	0.18	NSUB	1.29
3015	5085	NS3-158	NS3-474	T	C	0.73	NSUB	1.40
3015	9960	NS5-760	NS5-2280	C	T	0.70	NSUB	1.47
3015	9297	NS5-539	NS5-1617	T	C	0.49	NSUB	1.79
3015	6735	NS4A-89	NS4A-267	T	C	0.67	NSUB	1.89
3015	3187	NS1-240	NS1-718	A	G	0.69	NSUB	2.27
3015	5088	NS3-159	NS3-477	T	C	0.22	NSUB	2.31
3015	9465	NS5-595	NS5-1785	C	T	0.67	NSUB	5.18
3016	9063	NS5-461	NS5-1383	IA	d	0.21	LP	1.00
3016	5166	NS3-185	NS3-555	IA	d	0.18	LP	1.13
3016	4109	NS2A-195	NS2A-584	IA	d	0.43	LP	1.28
3016	6203	NS3-531	NS3-1592	IA	d	0.61	LP	2.11
3016	7267	NS4B-118	NS4B-352	IT	d	0.69	LP	6.44
3016	566	prM-34	prM-101	C	G	0.65	NSUB	0.43
3016	10437	3'UTR-42	3'UTR-42	C	T	0.51	NSUB	0.44
3016	3120	NS1-217	NS1-651	T	G	0.77	NSUB	0.98
3016	6204	NS3-531	NS3-1593	G	A	0.76	NSUB	1.10
3016	539	prM-25	prM-74	C	T	0.88	NSUB	1.17
3016	3735	NS2A-70	NS2A-210	T	G	0.55	NSUB	2.35
3016	7661	NS4B-249	NS4B-746	G	A	0.12	NSUB	2.48
3016	6871	NS4A-135	NS4A-403	A	G	0.90	NSUB	3.40
3016	2799	NS1-110	NS1-330	G	A	0.87	NSUB	4.42
3016	5297	NS3-229	NS3-686	T	C	0.94	NSUB	5.07
3016	207	C-37	C-111	T	G	0.05	NSUB	5.97
3016	880	prM-139	prM-415	T	G	0.92	NSUB	7.07

3016	3631	NS2A-36	NS2A-106	A	G	0.98	NSUB	12.36
3016	3327	NS1-286	NS1-858	T	C	0.78	NSUB	20.94
3016	8115	NS5-145	NS5-435	C	T	0.33	NSUB	23.54
3017	7960	NS5-94	NS5-280	IA	d	0.08	LP	0.64
3017	4109	NS2A-195	NS2A-584	IA	d	0.12	LP	1.24
3017	6203	NS3-531	NS3-1592	IA	d	0.31	LP	2.24
3017	7267	NS4B-118	NS4B-352	IT	d	0.76	LP	3.78
3017	1166	E-67	E-200	C	A	1.00	NSUB	0.46
3017	8121	NS5-147	NS5-441	T	C	0.65	NSUB	0.60
3017	10888	3'UTR-493	3'UTR-493	A	T	0.31	NSUB	1.17
3017	4296	NS2B-26	NS2B-78	G	T	0.05	NSUB	11.82
3018	4109	NS2A-195	NS2A-584	IA	d	0.62	LP	0.53
3018	7960	NS5-94	NS5-280	IA	d	0.70	LP	0.80
3018	9063	NS5-461	NS5-1383	IA	d	0.48	LP	0.94
3018	378	C-94	C-282	C	T	0.24	NSUB	0.51
3018	2158	E-398	E-1192	T	C	0.67	NSUB	0.63
3018	1266	E-100	E-300	T	C	0.46	NSUB	0.71
3018	10408	3'UTR-13	3'UTR-13	T	C	0.19	NSUB	0.82
3018	6792	NS4A-108	NS4A-324	A	G	1.00	NSUB	1.00
3018	10688	3'UTR-293	3'UTR-293	C	T	1.00	NSUB	1.55
3018	3093	NS1-208	NS1-624	C	T	0.95	NSUB	1.65
3018	10092	NS5-804	NS5-2412	C	A	0.20	NSUB	2.17
3018	4230	NS2B-4	NS2B-12	G	A	0.11	NSUB	5.17
3018	1485	E-173	E-519	T	G	0.55	NSUB	7.22
3018	1860	E-298	E-894	G	A	0.95	NSUB	8.37
3018	93	5'UTR-93	5'UTR-93	T	C	1.00	NSUB	16.18
3019	7960	NS5-94	NS5-280	IA	d	0.61	LP	0.78
3019	9063	NS5-461	NS5-1383	IA	d	1.11	LP	1.03
3019	5166	NS3-185	NS3-555	IA	d	0.07	LP	1.46
3019	7893	NS5-71	NS5-213	A	C	1.00	NSUB	0.26
3019	7908	NS5-76	NS5-228	A	G	1.00	NSUB	0.44
3019	320	C-75	C-224	T	A	1.00	NSUB	0.56
3019	8529	NS5-283	NS5-849	C	T	1.00	NSUB	0.56
3019	3649	NS2A-42	NS2A-124	T	C	1.00	NSUB	0.58
3019	5166	NS3-185	NS3-555	A	G	0.49	NSUB	0.88
3019	5058	NS3-149	NS3-447	C	T	1.00	NSUB	0.96
3019	1728	E-254	E-762	G	A	0.76	NSUB	1.02
3019	7149	NS4B-78	NS4B-234	G	A	0.77	NSUB	1.31
3019	6687	NS4A-73	NS4A-219	A	G	0.14	NSUB	1.46

3019	1123	E-53	E-157	T	C	0.57	NSUB	6.32
3019	3969	NS2A-148	NS2A-444	T	C	0.73	NSUB	12.64
3020	9063	NS5-461	NS5-1383	IA	d	0.12	LP	0.81
3020	4109	NS2A-195	NS2A-584	IA	d	0.32	LP	1.30
3020	7960	NS5-94	NS5-280	IA	d	0.22	LP	1.90
3020	6203	NS3-531	NS3-1592	IA	d	0.31	LP	4.04
3020	9607	NS5-643	NS5-1927	A	G	1.00	NSUB	0.15
3020	9611	NS5-644	NS5-1931	G	A	1.00	NSUB	0.23
3020	10157	NS5-826	NS5-2477	G	A	1.00	NSUB	0.26
3020	4914	NS3-101	NS3-303	C	A	1.00	NSUB	0.34
3020	4910	NS3-100	NS3-299	A	T	1.00	NSUB	0.46
3020	7711	NS5-11	NS5-31	T	G	0.43	NSUB	1.02
3020	10495	3'UTR-100	3'UTR-100	C	T	1.07	NSUB	1.10
3020	10017	NS5-779	NS5-2337	T	C	0.52	NSUB	1.26
3020	2619	NS1-50	NS1-150	C	T	0.69	NSUB	1.42
3020	522	prM-19	prM-57	T	C	0.76	NSUB	1.61
3020	7392	NS4B-159	NS4B-477	T	C	0.42	NSUB	2.35
3020	3409	NS1-314	NS1-940	G	A	0.76	NSUB	2.79
3020	4227	NS2B-3	NS2B-9	A	C	0.80	NSUB	3.15
3020	3556	NS2A-11	NS2A-31	C	T	0.43	NSUB	4.28
3020	1481	E-172	E-515	T	C	0.31	NSUB	4.40
3020	6424	NS3-605	NS3-1813	T	C	0.45	NSUB	5.25
3020	1467	E-167	E-501	T	C	0.31	NSUB	6.68
3020	6331	NS3-574	NS3-1720	C	T	0.36	NSUB	8.00
3020	8687	NS5-336	NS5-1007	T	C	0.23	NSUB	8.70
3020	1776	E-270	E-810	C	T	0.72	NSUB	15.01
3020	5358	NS3-249	NS3-747	T	C	0.80	NSUB	17.16
3020	3579	NS2A-18	NS2A-54	A	G	0.90	NSUB	20.03
3020	5623	NS3-338	NS3-1012	G	A	0.05	NSUB	24.28
3020	93	5'UTR-93	5'UTR-93	T	C	0.08	NSUB	28.70
3021	4109	NS2A-195	NS2A-584	IA	d	0.39	LP	0.58
3021	7960	NS5-94	NS5-280	IA	d	0.48	LP	0.73
3021	9063	NS5-461	NS5-1383	IA	d	0.79	LP	1.26
3021	6203	NS3-531	NS3-1592	IA	d	0.26	LP	2.29
3021	7267	NS4B-118	NS4B-352	IT	d	0.70	LP	5.46
3021	10084	NS5-802	NS5-2404	T	A	1.00	NSUB	0.13
3021	10082	NS5-801	NS5-2402	T	C	1.00	NSUB	0.14
3021	2795	NS1-109	NS1-326	G	A	1.00	NSUB	0.36
3021	861	prM-132	prM-396	A	T	1.00	NSUB	0.73

3021	2737	NS1-90	NS1-268	A	G	0.83	NSUB	1.39
3021	3972	NS2A-149	NS2A-447	G	A	0.90	NSUB	2.64
3021	7326	NS4B-137	NS4B-411	T	C	0.17	NSUB	2.70
3022	7960	NS5-94	NS5-280	IA	d	0.22	LP	1.05
3022	5166	NS3-185	NS3-555	IA	d	0.31	LP	1.42
3022	9063	NS5-461	NS5-1383	IA	d	0.10	LP	1.73
3022	7267	NS4B-118	NS4B-352	IT	d	0.55	LP	6.65
3022	4149	NS2A-208	NS2A-624	T	C	0.50	NSUB	0.47
3022	4294	NS2B-26	NS2B-76	G	A	1.12	NSUB	0.56
3022	292	C-66	C-196	T	C	1.00	NSUB	0.57
3022	6348	NS3-579	NS3-1737	G	A	1.00	NSUB	0.61
3022	7866	NS5-62	NS5-186	T	G	0.75	NSUB	0.63
3022	7443	NS4B-176	NS4B-528	T	C	0.67	NSUB	0.71
3022	307	C-71	C-211	A	G	1.00	NSUB	0.96
3022	5166	NS3-185	NS3-555	A	G	0.69	NSUB	1.02
3022	1843	E-293	E-877	A	G	0.52	NSUB	1.06
3022	7164	NS4B-83	NS4B-249	A	G	0.54	NSUB	1.83
3022	5625	NS3-338	NS3-1014	C	T	0.06	NSUB	12.50
3022	5322	NS3-237	NS3-711	G	A	0.98	NSUB	12.54
3023	7960	NS5-94	NS5-280	IA	d	0.12	LP	0.75
3023	9063	NS5-461	NS5-1383	IA	d	0.42	LP	0.98
3023	381	C-95	C-285	T	C	0.70	NSUB	0.37
3023	5910	NS3-433	NS3-1299	T	C	0.71	NSUB	0.38
3023	1331	E-122	E-365	T	C	1.00	NSUB	0.40
3023	8667	NS5-329	NS5-987	T	C	0.69	NSUB	0.40
3023	6888	NS4A-140	NS4A-420	T	C	0.69	NSUB	0.41
3023	7419	NS4B-168	NS4B-504	T	C	0.41	NSUB	0.42
3023	10349	NS5-890	NS5-2669	T	C	1.00	NSUB	0.43
3023	4196	NS2A-224	NS2A-671	T	C	1.00	NSUB	0.44
3023	4749	NS3-46	NS3-138	T	C	0.22	NSUB	0.44
3023	2223	E-419	E-1257	T	C	0.71	NSUB	0.44
3023	6011	NS3-467	NS3-1400	T	C	0.45	NSUB	0.46
3023	7636	NS4B-241	NS4B-721	G	A	1.00	NSUB	0.48
3023	7203	NS4B-96	NS4B-288	T	C	0.69	NSUB	0.48
3023	9013	NS5-445	NS5-1333	T	C	0.70	NSUB	0.48
3023	3720	NS2A-65	NS2A-195	T	C	0.73	NSUB	0.49
3023	6493	NS4A-9	NS4A-25	T	C	1.09	NSUB	0.49
3023	6780	NS4A-104	NS4A-312	T	C	1.00	NSUB	0.50
3023	3808	NS2A-95	NS2A-283	T	C	0.25	NSUB	0.52

3023	6672	NS4A-68	NS4A-204	T	C	0.77	NSUB	0.52
3023	5709	NS3-366	NS3-1098	T	C	1.00	NSUB	0.52
3023	7452	NS4B-179	NS4B-537	T	C	1.00	NSUB	0.53
3023	9624	NS5-648	NS5-1944	T	C	1.01	NSUB	0.53
3023	7179	NS4B-88	NS4B-264	T	C	1.00	NSUB	0.54
3023	9468	NS5-596	NS5-1788	T	C	1.01	NSUB	0.55
3023	2973	NS1-168	NS1-504	T	C	0.40	NSUB	0.56
3023	3646	NS2A-41	NS2A-121	T	C	0.47	NSUB	0.56
3023	3969	NS2A-148	NS2A-444	T	C	0.45	NSUB	0.57
3023	5898	NS3-429	NS3-1287	C	T	0.76	NSUB	0.57
3023	5487	NS3-292	NS3-876	G	A	0.30	NSUB	0.58
3023	2388	E-474	E-1422	T	C	0.44	NSUB	0.58
3023	4086	NS2A-187	NS2A-561	T	C	0.72	NSUB	0.58
3023	9276	NS5-532	NS5-1596	T	C	1.01	NSUB	0.58
3023	8751	NS5-357	NS5-1071	T	C	0.75	NSUB	0.61
3023	6807	NS4A-113	NS4A-339	T	C	0.47	NSUB	0.62
3023	3654	NS2A-43	NS2A-129	T	C	0.75	NSUB	0.63
3023	2638	NS1-57	NS1-169	T	C	0.76	NSUB	0.63
3023	1790	E-275	E-824	T	C	1.00	NSUB	0.64
3023	3576	NS2A-17	NS2A-51	T	C	1.00	NSUB	0.65
3023	8670	NS5-330	NS5-990	T	C	0.36	NSUB	0.65
3023	775	prM-104	prM-310	T	C	1.00	NSUB	0.65
3023	10351	NS5-891	NS5-2671	T	C	0.73	NSUB	0.66
3023	6871	NS4A-135	NS4A-403	A	G	0.12	NSUB	0.66
3023	7593	NS4B-226	NS4B-678	T	C	0.37	NSUB	0.67
3023	4059	NS2A-178	NS2A-534	A	G	1.00	NSUB	0.68
3023	4473	NS2B-85	NS2B-255	T	C	0.53	NSUB	0.69
3023	594	prM-43	prM-129	T	C	1.15	NSUB	0.69
3023	4036	NS2A-171	NS2A-511	T	C	0.75	NSUB	0.70
3023	3774	NS2A-83	NS2A-249	T	C	0.32	NSUB	0.71
3023	435	C-113	C-339	T	C	1.00	NSUB	0.71
3023	849	prM-128	prM-384	T	C	0.41	NSUB	0.75
3023	1207	E-81	E-241	T	C	0.78	NSUB	0.76
3023	10624	3'UTR-229	3'UTR-229	T	C	1.00	NSUB	0.76
3023	1473	E-169	E-507	T	C	0.78	NSUB	0.79
3023	10305	NS5-875	NS5-2625	T	C	0.36	NSUB	0.81
3023	7627	NS4B-238	NS4B-712	T	C	0.37	NSUB	0.81
3023	10296	NS5-872	NS5-2616	T	C	1.00	NSUB	0.82
3023	450	C-118	C-354	T	C	0.52	NSUB	0.83

3023	5076	NS3-155	NS3-465	G	A	0.44	NSUB	0.83
3023	3864	NS2A-113	NS2A-339	T	C	0.99	NSUB	0.89
3023	5817	NS3-402	NS3-1206	C	T	1.00	NSUB	0.90
3023	8979	NS5-433	NS5-1299	C	T	0.34	NSUB	0.95
3023	1620	E-218	E-654	T	C	0.76	NSUB	0.96
3023	4527	NS2B-103	NS2B-309	T	C	0.64	NSUB	1.03
3023	8079	NS5-133	NS5-399	T	C	0.76	NSUB	1.07
3023	4347	NS2B-43	NS2B-129	T	C	0.91	NSUB	1.07
3023	5224	NS3-205	NS3-613	T	C	0.52	NSUB	1.10
3023	3702	NS2A-59	NS2A-177	T	C	0.90	NSUB	1.12
3023	452	C-119	C-356	T	C	0.39	NSUB	1.15
3023	6238	NS3-543	NS3-1627	T	C	0.56	NSUB	1.17
3023	7015	NS4B-34	NS4B-100	T	C	0.77	NSUB	1.24
3023	1900	E-312	E-934	T	C	0.84	NSUB	1.24
3023	5865	NS3-418	NS3-1254	T	C	0.75	NSUB	1.29
3023	7183	NS4B-90	NS4B-268	A	G	0.39	NSUB	1.34
3023	10435	3'UTR-40	3'UTR-40	T	C	0.75	NSUB	1.36
3023	10314	NS5-878	NS5-2634	T	C	0.69	NSUB	1.67
3023	3898	NS2A-125	NS2A-373	T	C	0.80	NSUB	2.08
3023	7637	NS4B-241	NS4B-722	T	C	0.49	NSUB	2.20
3023	816	prM-117	prM-351	T	C	0.73	NSUB	2.81
3023	2899	NS1-144	NS1-430	T	C	0.17	NSUB	3.72
3023	6270	NS3-553	NS3-1659	A	G	0.25	NSUB	3.91
3023	3844	NS2A-107	NS2A-319	C	T	0.41	NSUB	4.35
3023	10408	3'UTR-13	3'UTR-13	T	C	0.64	NSUB	11.80
3023	7950	NS5-90	NS5-270	C	T	0.06	NSUB	16.99
3024	7960	NS5-94	NS5-280	IA	d	0.16	LP	0.49
3024	4109	NS2A-195	NS2A-584	IA	d	0.46	LP	0.87
3024	6203	NS3-531	NS3-1592	IA	d	0.19	LP	3.12
3024	7267	NS4B-118	NS4B-352	IT	d	0.84	LP	4.26
3024	7779	NS5-33	NS5-99	T	C	0.46	NSUB	0.58
3024	10774	3'UTR-379	3'UTR-379	T	C	1.00	NSUB	1.20
3024	5096	NS3-162	NS3-485	C	T	0.84	NSUB	1.39

Appendix III

Summary of WNV sequences used in Chapter 4. Sequences were collected from Genbank and provided by the WRCEVA. Accession number was provided when available.

Strain	State	Host	Year	Accession
NY99-crow-V76/1	NY	Avian	1999	FJ151394
BSL106-06	ND	Human	2006	JF957167
BSL22-09	SD	Human	2009	JF957181
BSL24-09	TX	Human	2009	JF957182
BSL26-11	NY	Human	2011	JQ700442
BSL27-09	TX	Human	2009	JF957183
CO4-07	CO	Human	2007	JF957169
CO5-07	CO	Human	2007	JF957170
CO7-09	CO	Human	2009	JF957184
NY10-03	NY	Mosquito	2003	JQ700437
New York 99 isolate 4132	NY	Avian	1999	HQ596519
NY99-eqhs	NY	Equine	1999	AF260967
Bird 1153	TX	Avian	2003	AY712945
Bird 1171	TX	Avian	2003	AY712946
Bird 1461	TX	Avian	2003	AY712947
Mosquito v4369	TX	Mosquito	2003	AY712948
CO 2003 1	CO	Avian	2003	DQ164204
CO 2003 2	CO	Avian	2003	DQ164203
GA 2002 1	GA	Human	2002	DQ164196
GA 2002 2	GA	Human	2002	DQ164197
NY 2001 Suffolk	NY	Avian	2001	DQ164194
NY 2002 Broome	NY	Avian	2002	DQ164187
NY 2002 Clinton	NY	Avian	2002	DQ164193
NY 2002 Nassau	NY	Mosquito	2002	DQ164195
NY 2002 Queens	NY	Avian	2002	DQ164186
NY 2003 Albany	NY	Avian	2003	DQ164189
NY 2003 Chautauqua	NY	Avian	2003	DQ164191
NY 2003 Rockland	NY	Avian	2003	DQ164192
NY 2003 Suffolk	NY	Avian	2003	DQ164190
NY 2003 Westchester	NY	Avian	2003	DQ164188
TX 2002 1	TX	Human	2002	DQ164198
TX 2002 2	TX	Human	2002	DQ164205
TX 2003	TX	Human	2003	DQ164199

TX 2004 Harris 4/ Bird 3588	TX	Avian	2004	DQ164206
V6200_20470003	ND	Bird	2008	KJ501253
V6203_22212002	ND	Bird	2008	KJ501254
V6204_22225001	ND	Bird	2008	KJ501255
V6207_22728001	ND	Bird	2009	KJ501256
V6208_4820096	ND	Bird	2002	KJ501257
V6209_20501001	SD	Bird	2007	KJ501258
V6210_20500002	SD	Bird	2008	KJ501259
V6211_22171002	SD	Bird	2008	KJ501260
V6212_22171003	SD	Bird	2008	KJ501440
V6213_22284001	SD	Bird	2008	KJ501441
V6214_4788479	TX	Bird	2002	KJ501261
V6373_4737463	IL	Bird	2001	KJ501443
V6374_4745119	GA	Bird	2001	KJ501264
V6375_4813219	GA	Bird	2002	KJ501265
V6376_4813245	GA	Bird	2002	KJ501266
V6387_4855214	NY	Bird	2003	KJ501272
V6425_4737462	IL	Bird	2001	KJ501292
V6436_4820101	ND	Bird	2002	KJ501298
V6463_18739002	SD	Bird	2003	KJ501470
V6464_4855172	VA	Bird	2003	KJ501312
V6468_4855132	GA	Bird	2003	KJ501472
V6469_4737065	IL	Bird	2001	KJ501473
V6498_4820094	ND	Bird	2002	KJ501328
V6499_18721002	ND	Bird	2003	KJ501488
V6500_18738002	ND	Bird	2003	KJ501329
V6501_19504002	ND	Bird	2005	KJ501330
V6505_4743001	VA	Bird	2001	KJ501332
V6506_4813099	VA	Bird	2002	KJ501489
V6507_4813107	VA	Bird	2002	KJ501490
V6508_4813133	VA	Bird	2002	KJ501491
V6510_4813147	VA	Bird	2002	KJ501333
V6531_4737114	IL	Bird	2001	KJ501496
V6532_4737113	IL	Bird	2001	KJ501497
V6545_4820065	ND	Bird	2002	KJ501503
V6546_18735004	ND	Bird	2003	KJ501349
V6547_18742002	ND	Bird	2003	KJ501350
V6548_18755001	ND	Bird	2003	KJ501504

V6556_18320002	SD	Bird	2001	KJ501507
V6558_4788033	TX	Bird	2002	KJ501355
V6559_4855184	TX	Bird	2003	KJ501356
V6560_4867361	TX	Bird	2003	KJ501508
V6561_4867363	TX	Bird	2003	KJ501357
V6562_4867452	TX	Bird	2003	KJ501358
V6616_4745142	GA	Bird	2001	KJ501512
V6617_4813185	GA	Bird	2002	KJ501373
V6619_4813357	GA	Bird	2002	KJ501514
V6645_4927032	SD	Bird	2003	KJ501389
V6646_4927033	SD	Bird	2003	KJ501390
V6647_4927034	SD	Bird	2003	KJ501391
V6648_4927009	SD	Bird	2003	KJ501392
V6649_4745115	VA	Bird	2001	KJ501393
V6650_4743002	VA	Bird	2001	KJ501394
V6651_4745144	VA	Bird	2001	KJ501395
V6652_4813135	VA	Bird	2002	KJ501519
V6653_4855154	VA	Bird	2003	KJ501396
V6659_4737091	IL	Bird	2001	KJ501401
V6676_19168001	ND	Bird	2004	KJ501522
V6678_19504001	ND	Bird	2005	KJ501414
V6680_19847001	ND	Bird	2006	KJ501523
V6682_19935001	ND	Bird	2006	KJ501524
V6683	ND	Bird	2006	KJ501416
V6684	ND	Bird	2006	KJ501417
V6685_22304006	ND	Bird	2008	KJ501418
V6686_4927035	SD	Bird	2004	KJ501420
V6686_4927035	SD	Bird	2004	KJ501419
V6688_4927037	SD	Bird	2004	KJ501421
V6689_4927038	SD	Bird	2004	KJ501525
V6690_4927039	SD	Bird	2004	KJ501422
V6691_4927040	SD	Bird	2004	KJ501423
V6692_19815001	SD	Bird	2006	KJ501526
V6694_20373001	SD	Bird	2007	KJ501424
V6695_20373002	SD	Bird	2007	KJ501425
V6696_4867383	TX	Bird	2003	KJ501527
V6698_4788044	TX	Bird	2002	KJ501426
V6699_4788591	TX	Bird	2002	KJ501427

385-99	NY	Avian	1999	AY842931
AVA1202598	TX	Mosquito	2012	kc736486
AVA1202600	TX	Mosquito	2012	kc736487
AVA1202621	TX	Mosquito	2012	kc736490
AVA1202624	TX	Mosquito	2012	kc736491
AVA1202689	TX	Mosquito	2012	kc736492
AVA1202969	TX	Mosquito	2012	kc736493
AVA1204250	TX	Mosquito	2012	kc736494
AVA1204260	TX	Mosquito	2012	kc736502
AVA1204331	TX	Mosquito	2012	kc736495
AVA1204356	TX	Mosquito	2012	kc736496
AVA1204485	TX	Mosquito	2012	kc736497
AVA1204579	TX	Mosquito	2012	kc736498
AVA1204580	TX	Mosquito	2012	kc736499
AVA1204753	TX	Mosquito	2012	kc736500
AVA1204895	TX	Mosquito	2012	kc736501
Kuritz [Beaumont TVP8533]	TX	Human	2002	AY289214
BSL2-05	SD	Human	2005	DQ666452
FDA-Hu2002	NY	Human	2002	AY646354
GCTX1-2005	TX	Human	2005	DQ666449
GCTX2-2005	TX	Human	2005	DQ666450
WNV-1/US/BID-V4090/2007	NY	Avian	2007	HM488199
WNV-1/US/BID-V4092/2007	NY	Avian	2007	HM488200
WNV-1/US/BID-V4093/2007	NY	Avian	2007	HM488201
WNV-1/US/BID-V4094/2007	NY	Avian	2007	HM488202
WNV-1/US/BID-V4095/2007	NY	Avian	2007	HM756678
WNV-1/US/BID-V4096/2008	NY	Avian	2008	HM488203
WNV-1/US/BID-V4097/2008	NY	Avian	2008	HM756660
WNV-1/US/BID-V4098/2008	NY	Avian	2008	HM488204
WNV-1/US/BID-V4099/2008	NY	Avian	2008	HM488205
WNV-1/US/BID-V4100/2008	NY	Avian	2008	HM488206
WNV-1/US/BID-V4101/2008	NY	Avian	2008	HM488207
WNV-1/US/BID-V4336/2002	IL	Avian	2002	HM488177
WNV-1/US/BID-V4337/2002	IL	Avian	2002	HM488178
WNV-1/US/BID-V4338/2002	IL	Avian	2002	HM488179
WNV-1/US/BID-V4339/2002	IL	Avian	2002	HM488180
WNV-1/US/BID-V4340/2002	IL	Avian	2002	HM488181
WNV-1/US/BID-V4341/2002	IL	Avian	2002	HM488182

WNV-1/US/BID-V4342/2002	IL	Avian	2002	HQ705669
WNV-1/US/BID-V4343/2002	IL	Avian	2002	HQ671742
WNV-1/US/BID-V4344/2002	IL	Avian	2002	JN183891
WNV-1/US/BID-V4345/2002	IL	Avian	2002	HM488183
WNV-1/US/BID-V4346/2002	IL	Avian	2002	HM488184
WNV-1/US/BID-V4347/2003	IL	Avian	2003	HM488185
WNV-1/US/BID-V4349/2003	IL	Avian	2003	HM756676
WNV-1/US/BID-V4350/2003	IL	Avian	2003	HM488186
WNV-1/US/BID-V4351/2003	IL	Avian	2003	HM488187
WNV-1/US/BID-V4353/2003	IL	Avian	2004	HM488188
WNV-1/US/BID-V4368/2004	IL	Avian	2004	HM488190
WNV-1/US/BID-V4369/2004	IL	Avian	2004	HM488191
WNV-1/US/BID-V4371/2005	IL	Avian	2005	HM488192
WNV-1/US/BID-V4373/2005	IL	Avian	2005	HM488193
WNV-1/US/BID-V4374/2005	IL	Avian	2005	HM488194
WNV-1/US/BID-V4375/2005	IL	Avian	2005	HM488195
WNV-1/US/BID-V4376/2004	IL	Avian	2004	HM488189
WNV-1/US/BID-V4376/2005	IL	Avian	2005	HM488196
WNV-1/US/BID-V4377/2005	IL	Avian	2005	HM488197
WNV-1/US/BID-V4378/2005	IL	Mosquito	2005	HM488198
WNV-1/US/BID-V4379/2005	IL	Mosquito	2005	JN183892
WNV-1/US/BID-V4553/2006	IL	Mosquito	2006	HM488253
WNV-1/US/BID-V4559/2007	IL	Mosquito	2007	HM488254
WNV-1/US/BID-V4622/2008	NY	Avian	2008	HM488237
WNV-1/US/BID-V4623/2008	NY	Avian	2008	HM488238
WNV-1/US/BID-V4624/2008	NY	Avian	2008	HM488239
WNV-1/US/BID-V4625/2008	NY	Avian	2008	HQ671721
WNV-1/US/BID-V4626/2008	NY	Avian	2008	JN183885
WNV-1/US/BID-V4627/2008	NY	Avian	2008	HM488240
WNV-1/US/BID-V4628/2008	NY	Avian	2008	HM488241
WNV-1/US/BID-V4629/2008	NY	Avian	2008	JN183886
WNV-1/US/BID-V4631/2008	NY	Avian	2008	HM488242
WNV-1/US/BID-V4632/2008	NY	Avian	2008	HM488243
WNV-1/US/BID-V4634/2008	NY	Avian	2008	HM488244
WNV-1/US/BID-V4635/2008	NY	Avian	2008	HM488245
WNV-1/US/BID-V4689/2001	NY	Avian	2001	HM488246
WNV-1/US/BID-V4691/2001	NY	Avian	2001	HM488247
WNV-1/US/BID-V4692/2001	NY	Avian	2001	HM756661

WNV-1/US/BID-V4693/2001	NY	Avian	2001	HM756662
WNV-1/US/BID-V4694/2001	NY	Avian	2001	HM488248
WNV-1/US/BID-V4696/2001	NY	Avian	2001	HM488249
WNV-1/US/BID-V4697/2001	NY	Avian	2001	HM756663
WNV-1/US/BID-V4701/2002	NY	Avian	2002	HM756664
WNV-1/US/BID-V4704/2002	NY	Avian	2002	HQ671722
WNV-1/US/BID-V4706/2002	NY	Avian	2002	JN183887
WNV-1/US/BID-V4709/2002	NY	Avian	2002	HM756665
WNV-1/US/BID-V4711/2003	NY	Avian	2003	HM756666
WNV-1/US/BID-V4712/2003	NY	Avian	2003	HM756667
WNV-1/US/BID-V4715/2003	NY	Avian	2003	HQ671723
WNV-1/US/BID-V4716/2003	NY	Avian	2003	HM756668
WNV-1/US/BID-V4717/2003	NY	Avian	2003	HM488250
WNV-1/US/BID-V4718/2003	NY	Avian	2003	HM756669
WNV-1/US/BID-V4719/2003	NY	Avian	2003	HM488251
WNV-1/US/BID-V4720/2003	NY	Avian	2003	HM756670
WNV-1/US/BID-V4798/2004	NY	Avian	2004	HM756671
WNV-1/US/BID-V4799/2004	NY	Avian	2004	HM756672
WNV-1/US/BID-V4800/2004	NY	Avian	2004	JF899528
WNV-1/US/BID-V4801/2004	NY	Avian	2004	HM756673
WNV-1/US/BID-V4803/2004	NY	Avian	2004	JN367277
WNV-1/US/BID-V4805/2005	NY	Avian	2005	HM488252
WNV-1/US/BID-V4806/2005	NY	Avian	2005	HM756675
WNV-1/US/BID-V4808/2005	NY	Avian	2005	JF899529
WNV-1/US/BID-V4883/2005	NY	Avian	2005	HQ671724
WNV-1/US/BID-V4885/2005	NY	Avian	2005	HQ671725
WNV-1/US/BID-V4887/2005	NY	Avian	2005	HQ671726
WNV-1/US/BID-V4891/2006	NY	Avian	2006	HQ671728
WNV-1/US/BID-V4892/2006	NY	Avian	2006	HQ671729
WNV-1/US/BID-V4896/2006	NY	Avian	2006	JN183888
WNV-1/US/BID-V4897/2007	NY	Avian	2007	HQ671730
WNV-1/US/BID-V5147/2007	NY	Avian	2007	JF730042
WNV-1/US/BID-V5148/2007	NY	Avian	2007	JF488097
WNV-1/US/BID-V5150/2004	NY	Avian	2004	JF488094
WNV-1/US/BID-V5157/2009	NY	Avian	2009	JF488095
WNV-1/US/BID-V5159/2009	NY	Avian	2009	JF488096
WNV-1/US/BID-V4797/2004	NY	Avian	2004	HQ671738
03-20TX	TX	Human	2003	DQ431693

03-22TX	TX	Human	2003	DQ431694
03-82IL	IL	Human	2003	DQ431695
04-214CO	CO	Human	2004	DQ431701
04-216CO	CO	Human	2004	DQ431702
04-218CO	CO	Human	2004	DQ431703
04-219CO	CO	Human	2004	DQ431704
04-233ND	ND	Human	2004	DQ431705
HNY2001	NY	Human	2001	AF533540
HNY1999	NY	Human	1999	AF202541
TX 2002-HC	TX	Avian	2002	DQ176637
NY99-flamingo382-99	NY	Avian	1999	AF196835
WN NY 2000-crow3356	NY	Avian	2000	AF404756
WN NY 2000-grouse3282	NY	Avian	2000	AF404755
NY99iso-1	NY	-	1999	FJ411043
TX AR12-10674	TX	Mosquito	2012	KC711059
TX AR12-1648	TX	Mosquito	2012	KC711058
TX AR10-5718	TX	Mosquito	2010	JX015522
TX AR10-6572	TX	Mosquito	2010	JX015523
TX AR5-2686	TX	Mosquito	2005	JX015515
TX AR7-6745	TX	Mosquito	2007	JX015516
TX AR8-5947	TX	Mosquito	2008	JX015517
TX AR9-5282	TX	Mosquito	2009	JX015519
TX AR9-7465	TX	Mosquito	2009	JX015521
TX AR12-1486	TX	Mosquito	2012	KC711057
TX8546	TX	Avian	2012	KC333376
TX8551	TX	Avian	2012	KC333377
TX8559	TX	Avian	2012	KC333378
TX8560	TX	Avian	2012	KC333379
TX8562	TX	Avian	2012	KC333380
TX8567	TX	Avian	2012	KC333381
TX8571	TX	Avian	2012	KC333382
TX8572	TX	Avian	2012	KC333383
TX8589	TX	Avian	2012	KC333384
TX8590	TX	Avian	2012	KC333385
TX8599	TX	Avian	2012	KC333386
TX8604	TX	Avian	2012	KC333387
Bird114	TX	Avian	2002	GU827998
Bird1175	TX	Avian	2003	GU828000

Bird1519	TX	Avian	2003	GU828004
Bird1576	TX	Avian	2003	GU827999
Bird1881	TX	Avian	2003	GU828003
v4095	TX	Mosquito	2003	GU828002
v4380	TX	Mosquito	2003	GU828001
M12214	TX	Mosquito	2005	JF415914
M19433	TX	Mosquito	2007	JF415919
M20122	TX	Mosquito	2009	JF415928
M20140	TX	Mosquito	2009	JF415926
M20141	TX	Mosquito	2009	JF415927
M37012	TX	Mosquito	2009	JF415922
M37906	TX	Mosquito	2009	JF415923
M39488	TX	Mosquito	2009	JF415925
M6019	TX	Mosquito	2006	JF415930
TX 7558	TX	Avian	2008	JF415921
TX5058	TX	Avian	2005	JF415929
TX5810	TX	Avian	2006	JF415915
TX6276	TX	Avian	2006	JF415916
TX6647	TX	Avian	2007	JF415917
TX6747	TX	Avian	2007	JF415918
TX7191	TX	Avian	2007	JF415920
TX7827	TX	Avian	2009	JF415924
TX8092	TX	Avian	2010	KC333374
TX8349	TX	Avian	2011	KC333375
007WG-TX05EP	TX	Human	2005	GQ507468
011WG-TX06EP	TX	Human	2006	GQ507470
013WG-TX07EP	TX	Human	2007	GQ507471
AVA1202606	TX	Mosquito	2012	kc736488
AVA1202615	TX	Mosquito	2012	kc736489
AIDL-M-012	CO	mosquito	2003	
AIDL-M-015	CO	Mosquito	2003	
CO 06-10716	CO	mosquito pool(50)	2006	
CO 06-10723	CO	mosquito pool(50)	2006	
CO 06-10725	CO	mosquito	2006	
CO 06-584	CO	mosquito pool(50)	2006	
CO 06-608	CO	mosquito	2006	
CO 06-7390	CO	mosquito	2006	
CO 07-10970	CO	mosquito pool (33)	2007	

CO 07-11027	CO	mosquito pool 40	2007	
CO 07-11032	CO	mosquito	2006	
CO 07-8778	CO	mosquito pool (29)	2007	
CO 07-8779	CO	mosquito	2007	
CO 07-9340	CO	pool 24	2007	
CO 08-13382	CO	mosquito	2008	
CO 08-13386	CO	mosquito	2008	
CO 08-13401	CO	mosquito	2008	
CO 08-13410	CO	Moquito	2008	
CO 08-13787	CO	mosquito	2008	
CO 2572	CO	mosquito pool 5	2004	
CO-13363	CO	mosquito	2008	
CO1862	CO	Avian	2004	
DB 4217	CO	Avian	2004	
DB 4218	CO	bird	2004	
DBK 08-0491	GA	Mosquito	2008	
DES 07-53	GA	Avian	2007	
DES 07-62	GA	Avian	2007	
DES 107-01	GA	Avian	2001	
DES 1191-02	GA	Avian	2002	
DES 1201-02	GA	Avian	2002	
DES 1476-01	GA	Avian	2001	
DES 160-02	GA	Avian	2002	
DES 566-01	GA	Avian	2001	
DKB 08-0403	GA	Mosquito	2008	
DO0352	TX	Avian	2013	
DO329 TX9780	TX	Avian	2014	
FNT 09-144	GA	Mosquito	2009	
FNT 09-199	GA	Mosquito	2009	
GA 04-230	GA	Avian	2004	
GA 05-179	GA	Avian	2005	
GA Chc 04-1485	GA	Mosquito	2004	
GA lwn 50 4936	GA	Mosquito	2005	
GT 02566	CO	mosquito	2007	
Laco 3008	CO	Avian	2003	
Laco 3020	CO	Avian	2003	
Laco 3022	CO	Avian	2003	
laco 3030	CO	Avian	2003	

Laco 3038	CO	Avian	2003	
LACO-3041	CO	Avian	2003	
Lwn 09-846	GA	Mosquito	2009	
M07-069	GA	Mosquito	2007	
M07-086	GA	Mosquito	2007	
M07-087	GA	Mosquito	2007	
TVP 21075 TX9582 DO130	TX	Avian	2014	
TVP21082 TX9587	TX	Avian	2014	
TVP21083 TX 9589	TX	Avian	2014	
TVP21092 TX9601	TX	Avian	2014	
TVP21096 TX 9597	TX	Avian	2014	
TX AR12-3169	TX	Mosquito	2012	
TX AR12-7025	TX	Mosquito	2012	
TX AR12-7607	TX	Mosquito	2012	
TX AR12-8920	TX	Mosquito	2012	
TX AR12-9793	TX	Mosquito	2012	
TX8759	TX	Avian	2012	
TX8779	TX	Avian	2012	
TX8820	TX	Avian	2012	
TX9364 DO279	TX	Avian	2013	
TX9388 DO303	TX	Avian	2013	
TX9410 TVP20206 DO325	TX	Avian	2013	
TX9604 TVP21093	TX	Avian	2014	
TX9611 TVP21097	TX	Avian	2014	
TX9614 TVP 21100	TX	Avian	2014	
TX9631	TX	Avian	2014	
VA 1660	VA	Mosquito	2007	
VA 1909-04	VA	Mosquito	2004	
VA 2191	VA	Mosquito	2010	
VA 2327	VA	Mosquito	2007	
VA 3920	VA	Mosquito	2009	
VA AV 321-00	VA	Avian	2000	
VA AV 380	VA	Avian	2000	
VA AV 573-00	VA	Avian	2000	
VA AV 593	VA	Avian	2000	
VA B 037-02	VA	Avian	2002	
VA BD 37	VA	Avian	2002	
VA P 3321-05	VA	Mosquito	2005	

VA P 4209	VA	Mosquito	2005	
VA P 4485-06	VA	Mosquito	2006	
VA P 4770-06	VA	Mosquito	2006	
VA SN 3082-05	VA	Mosquito	2005	
VA SN 3222-09	VA	Mosquito	2009	
VA SN 4826-09	VA	Mosquito	2009	
VA SN 5859-09	VA	Mosquito	2009	
VA SP 1202-08	VA	Mosquito	2008	
VA SP 5645-06	VA	Mosquito	2006	
VA TC 1117-10	VA	Mosquito	2010	
VA TC 1155	VA	Mosquito	2004	
VA TC 1184-10	VA	Mosquito	2010	
VA TC 1272	VA	Mosquito	2004	
VA TC 1368-08	VA	Mosquito	2008	
VA TC 1500	VA	Mosquito	2002	
VA TC 1500-02	VA	Mosquito	2002	
VA TC 1597	VA	Mosquito	2004	
VA TC 1732-08	VA	Mosquito	2008	
VA TC 1732-09	VA	Mosquito	2009	
VA TC 2020-10	VA	Mosquito	2010	
VA TC 2045-08	VA	Mosquito	2008	
VA TC 2076	VA	Mosquito	2002	
VA TC 2147	VA	Mosquito	2002	
VA TC 2535-01	VA	Mosquito	2001	
VA TC 2790-03	VA	Mosquito	2003	
VA TC 3278	VA	Mosquito	2003	
VA TC 4043	VA	Mosquito	2003	
VA TC 4177	VA	Mosquito	2006	

Appendix IV

Summary of all SNVs identified among the WNV isolates collected in Harris County during 2014. The position of each SNV was provided including the genome position, and gene position. SNV type was also provided (LP or NSUB) along with details about the consensus nucleotide (Cons) and the variant nucleotide (Var). Inserted nucleotides were indicated as I and deletions were indicated as d. Variants with strand bias below 0.05 were excluded.

Isolate	Genome Position	Gene	Nucleotide Position	Var	Cons	Type	Frequency (%)
TX 9582 (D0130)	93	5'utr	93	T	C	NSUB	33.19
TX 9604 (D0152)	93	5'utr	93	T	C	NSUB	6.292
TX 9780 (D0329)	93	5'utr	93	T	C	NSUB	10.05
TX 9614 (D0162)	161	C	65	T	C	NSUB	0.1755
TX 9780 (D0329)	161	C	65	T	C	NSUB	0.2472
TX 9631 (D0179)	161	C	65	T	C	NSUB	0.2445
TX 9589 (D0137)	450	C	354	T	C	NSUB	1.154
TX 9604 (D0152)	450	C	354	T	C	NSUB	0.258
TX 9780 (D0329)	450	C	354	T	C	NSUB	0.3365
TX 9597 (D0145)	450	C	354	T	C	NSUB	7.677
TX 9597 (D0145)	608	prM	143	T	C	NSUB	0.2442
TX 9631 (D0179)	608	prM	143	T	C	NSUB	0.1346
TX 9597 (D0145)	723	prM	258	T	C	NSUB	0.3124
TX 9597 (D0145)	723	prM	258	A	C	NSUB	0.04166
TX 9587 (D0135)	864	prM	399	D2	i	LP	0.1386
TX 9631 (D0179)	864	prM	399	D2	i	LP	0.1021
TX 9582 (D0130)	1307	E	341	D2	i	LP	0.1009
TX 9589 (D0137)	1307	E	341	D2	i	LP	0.09577
TX 9587 (D0135)	1307	E	341	D2	i	LP	0.1113
TX 9631 (D0179)	1307	E	341	D2	i	LP	0.08275
TX 9582 (D0130)	1844	E	878	D1	i	LP	0.132
TX 9582 (D0130)	1844	E	878	D2	i	LP	0.132
TX 9589 (D0137)	1844	E	878	D1	i	LP	0.1355
TX 9587 (D0135)	1844	E	878	D1	i	LP	0.147
TX 9614 (D0162)	1844	E	878	D1	i	LP	0.1338
TX 9582 (D0130)	1975	E	1009	D1	i	LP	0.1096
TX 9587 (D0135)	1975	E	1009	D1	i	LP	0.1668
TX 9601 (D0149)	1975	E	1009	D1	i	LP	0.1591
TX 9631 (D0179)	1975	E	1009	D1	i	LP	0.1808

TX 9589 (D0137)	2366	E	1400	T	C	NSUB	0.8529
TX 9631 (D0179)	2366	E	1400	T	C	NSUB	0.138
TX 9589 (D0137)	2717	NS1	248	D2	i	LP	0.09444
TX 9601 (D0149)	2717	NS1	248	A	G	NSUB	0.2208
TX 9614 (D0162)	2795	NS1	326	D1	i	LP	0.07884
TX 9614 (D0162)	2795	NS1	326	G	A	NSUB	0.1381
TX 9601 (D0149)	2795	NS1	326	D2	i	LP	0.08962
TX 9631 (D0179)	2795	NS1	326	D2	i	LP	0.08101
TX 9631 (D0179)	2795	NS1	326	D1	i	LP	0.09258
TX 9582 (D0130)	3355	NS1	886	T	C	NSUB	0.1884
TX 9604 (D0152)	3355	NS1	886	T	C	NSUB	0.2252
TX 9587 (D0135)	3504	NS1	1035	T	C	NSUB	0.2962
TX 9597 (D0145)	3504	NS1	1035	T	C	NSUB	2.375
TX 9631 (D0179)	3504	NS1	1035	T	C	NSUB	0.1698
TX 9780 (D0329)	3702	NS2A	177	T	C	NSUB	0.1203
TX 9631 (D0179)	3702	NS2A	177	T	C	NSUB	0.09153
TX 9614 (D0162)	3805	NS2A	280	D1	i	LP	0.09651
TX 9631 (D0179)	3805	NS2A	280	D1	i	LP	0.09159
TX 9589 (D0137)	3808	NS2A	283	T	C	NSUB	0.1502
TX 9587 (D0135)	3808	NS2A	283	T	C	NSUB	0.1112
TX 9597 (D0145)	3808	NS2A	283	T	C	NSUB	0.4033
TX 9582 (D0130)	3839	NS2A	314	D1	i	LP	0.08886
TX 9614 (D0162)	3839	NS2A	314	D1	i	LP	0.1198
TX 9589 (D0137)	3898	NS2A	373	T	C	NSUB	0.5018
TX 9587 (D0135)	3898	NS2A	373	T	C	NSUB	0.2325
TX 9604 (D0152)	3898	NS2A	373	T	C	NSUB	0.304
TX 9589 (D0137)	3966	NS2A	441	G	A	NSUB	0.4367
TX 9587 (D0135)	3966	NS2A	441	T	A	NSUB	0.1944
TX 9601 (D0149)	4422	NS2B	204	C	T	NSUB	0.5261
TX 9604 (D0152)	4422	NS2B	204	C	T	NSUB	0.3552
TX 9604 (D0152)	4563	NS2B	345	T	C	NSUB	0.4217
TX 9597 (D0145)	4563	NS2B	345	T	C	NSUB	0.8564
TX 9582 (D0130)	4602	NS2B	384	T	C	NSUB	0.1231
TX 9601 (D0149)	4602	NS2B	384	T	C	NSUB	12.97
TX 9587 (D0135)	4804	NS3	193	T	C	NSUB	1.087
TX 9631 (D0179)	4804	NS3	193	T	C	NSUB	0.17
TX 9587 (D0135)	4944	NS3	333	A	G	NSUB	0.1963
TX 9614 (D0162)	4944	NS3	333	G	A	NSUB	0.0232
TX 9587 (D0135)	4971	NS3	360	G	A	NSUB	0.144

TX 9614 (D0162)	4971	NS3	360	A	G	NSUB	0.03501
TX 9587 (D0135)	5166	NS3	555	A	G	NSUB	0.3625
TX 9614 (D0162)	5166	NS3	555	A	G	NSUB	0.3596
TX 9601 (D0149)	5166	NS3	555	A	G	NSUB	0.1919
TX 9604 (D0152)	5166	NS3	555	A	G	NSUB	0.4237
TX 9780 (D0329)	5166	NS3	555	A	G	NSUB	0.2417
TX 9780 (D0329)	5166	NS3	555	IA	d	LP	0.3566
TX 9597 (D0145)	5166	NS3	555	IA	d	LP	0.3489
TX 9611 (D0159)	5166	NS3	555	A	G	NSUB	0.6069
TX 9631 (D0179)	5166	NS3	555	A	G	NSUB	0.3587
TX 9589 (D0137)	5391	NS3	780	T	C	NSUB	0.1492
TX 9597 (D0145)	5391	NS3	780	T	C	NSUB	0.6504
TX 9587 (D0135)	5400	NS3	789	T	C	NSUB	0.254
TX 9597 (D0145)	5400	NS3	789	T	C	NSUB	7.52
TX 9589 (D0137)	5526	NS3	915	T	C	NSUB	0.2306
TX 9597 (D0145)	5526	NS3	915	T	C	NSUB	2.949
TX 9597 (D0145)	5553	NS3	942	C	T	NSUB	8.43
TX 9631 (D0179)	5553	NS3	942	T	C	NSUB	0.1981
TX 9597 (D0145)	5865	NS3	1254	T	C	NSUB	0.8041
TX 9631 (D0179)	5865	NS3	1254	T	C	NSUB	0.1365
TX 9587 (D0135)	5889	NS3	1278	C	T	NSUB	0.1535
TX 9597 (D0145)	5889	NS3	1278	C	T	NSUB	1.481
TX 9589 (D0137)	6203	NS3	1592	A	G	NSUB	0.2098
TX 9597 (D0145)	6203	NS3	1592	IA	d	LP	1.113
TX 9611 (D0159)	6203	NS3	1592	A	G	NSUB	0.3488
TX 9614 (D0162)	6310	NS3	1699	T	G	NSUB	0.03835
TX 9780 (D0329)	6310	NS3	1699	T	G	NSUB	0.07456
TX 9587 (D0135)	6540	NS4A	72	T	C	NSUB	0.1909
TX 9604 (D0152)	6540	NS4A	72	T	C	NSUB	0.5152
TX 9601 (D0149)	6871	NS4A	403	A	G	NSUB	0.1935
TX 9780 (D0329)	6871	NS4A	403	A	G	NSUB	0.1804
TX 9589 (D0137)	7267	NS4B	352	IT	d	LP	4.776
TX 9587 (D0135)	7267	NS4B	352	T	C	NSUB	0.1518
TX 9587 (D0135)	7267	NS4B	352	ITT	d	LP	0.1691
TX 9614 (D0162)	7267	NS4B	352	T	C	NSUB	0.2046
TX 9601 (D0149)	7267	NS4B	352	IT	d	LP	5.256
TX 9604 (D0152)	7267	NS4B	352	T	C	NSUB	0.2767
TX 9780 (D0329)	7267	NS4B	352	IT	d	LP	2.069
TX 9587 (D0135)	7419	NS4B	504	C	T	NSUB	0.1366

TX 9601 (D0149)	7419	NS4B	504	C	T	NSUB	1.569
TX 9587 (D0135)	7515	NS4B	600	C	T	NSUB	0.121
TX 9604 (D0152)	7515	NS4B	600	A	T	NSUB	0.2563
TX 9582 (D0130)	7711	NS5	31	T	G	NSUB	0.1497
TX 9589 (D0137)	7711	NS5	31	T	G	NSUB	0.1947
TX 9587 (D0135)	7711	NS5	31	T	G	NSUB	0.2501
TX 9614 (D0162)	7711	NS5	31	T	G	NSUB	0.312
TX 9601 (D0149)	7711	NS5	31	T	G	NSUB	0.1892
TX 9604 (D0152)	7711	NS5	31	T	G	NSUB	0.2488
TX 9582 (D0130)	7960	NS5	280	IA	d	LP	0.5095
TX 9589 (D0137)	7960	NS5	280	IA	d	LP	0.8537
TX 9587 (D0135)	7960	NS5	280	IA	d	LP	0.7665
TX 9614 (D0162)	7960	NS5	280	IA	d	LP	0.8243
TX 9601 (D0149)	7960	NS5	280	IA	d	LP	0.343
TX 9780 (D0329)	7960	NS5	280	IA	d	LP	0.4469
TX 9582 (D0130)	7961	NS5	281	D1	i	LP	0.1517
TX 9601 (D0149)	7961	NS5	281	D1	i	LP	0.2148
TX 9589 (D0137)	7992	NS5	312	D1	i	LP	0.09106
TX 9587 (D0135)	7992	NS5	312	D1	i	LP	0.1109
TX 9582 (D0130)	8413	NS5	733	IA	d	LP	0.2369
TX 9589 (D0137)	8413	NS5	733	IA	d	LP	0.231
TX 9587 (D0135)	8413	NS5	733	IA	d	LP	0.1393
TX 9614 (D0162)	8413	NS5	733	IA	d	LP	0.2799
TX 9604 (D0152)	8413	NS5	733	IA	d	LP	0.3424
TX 9587 (D0135)	8414	NS5	734	D2	i	LP	0.2136
TX 9601 (D0149)	8414	NS5	734	D2	i	LP	0.1259
TX 9614 (D0162)	8973	NS5	1293	D2	i	LP	0.104
TX 9601 (D0149)	8973	NS5	1293	D2	i	LP	0.1308
TX 9587 (D0135)	9016	NS5	1336	IG	d	LP	0.1718
TX 9614 (D0162)	9016	NS5	1336	IG	d	LP	0.1361
TX 9589 (D0137)	9063	NS5	1383	IA	d	LP	0.7806
TX 9587 (D0135)	9063	NS5	1383	IA	d	LP	0.5272
TX 9601 (D0149)	9063	NS5	1383	IA	d	LP	0.7069
TX 9604 (D0152)	9063	NS5	1383	IA	d	LP	0.8253
TX 9780 (D0329)	9063	NS5	1383	IA	d	LP	0.4125
TX 9601 (D0149)	9125	NS5	1445	T	G	NSUB	0.496
TX 9604 (D0152)	9125	NS5	1445	T	G	NSUB	0.2717
TX 9587 (D0135)	9760	NS5	2080	D1	i	LP	0.09056
TX 9614 (D0162)	9760	NS5	2080	D1	i	LP	0.07446

TX 9589 (D0137)	10160	NS5	2480	G	A	NSUB	0.0969
TX 9604 (D0152)	10160	NS5	2480	G	A	NSUB	0.161
TX 9589 (D0137)	10287	NS5	2607	A	G	NSUB	0.2771
TX 9587 (D0135)	10287	NS5	2607	A	G	NSUB	0.2359
TX 9582 (D0130)	10370	NS5	2690	T	C	NSUB	0.1222
TX 9589 (D0137)	10370	NS5	2690	T	C	NSUB	0.2212
TX 9587 (D0135)	10408	3'UTR	10408	T	C	NSUB	0.2369
TX 9604 (D0152)	10408	3'UTR	10408	T	C	NSUB	0.5089
TX 9604 (D0152)	10487	3'UTR	10487	G	A	NSUB	0.4628
TX 9604 (D0152)	10487	3'UTR	10487	D1	i	LP	0.2308
TX 9611 (D0159)	79	5'UTR	79	T	C	NSUB	17.9
TX 9780 (D0329)	160	C	64	T	C	NSUB	0.4354
TX 9631 (D0179)	246	C	150	T	C	NSUB	1.743
TX 9614 (D0162)	324	C	228	G	A	NSUB	0.3204
TX 9582 (D0130)	345	C	249	C	T	NSUB	0.1739
TX 9589 (D0137)	358	C	262	A	C	NSUB	9.607
TX 9587 (D0135)	363	C	267	A	G	NSUB	0.1372
TX 9601 (D0149)	378	C	282	C	T	NSUB	0.8592
TX 9614 (D0162)	399	C	303	G	A	NSUB	0.1173
TX 9587 (D0135)	401	C	305	D1	i	LP	0.1782
TX 9589 (D0137)	431	C	335	T	C	NSUB	0.7471
TX 9587 (D0135)	435	C	339	C	T	NSUB	0.2008
TX 9589 (D0137)	468	prM	3	C	T	NSUB	0.4286
TX 9631 (D0179)	476	prM	11	T	C	NSUB	0.4618
TX 9589 (D0137)	495	prM	30	A	G	NSUB	0.1774
TX 9589 (D0137)	583	prM	118	A	G	NSUB	1.16
TX 9631 (D0179)	611	prM	146	C	T	NSUB	1.717
TX 9589 (D0137)	725	prM	260	T	C	NSUB	0.1202
TX 9597 (D0145)	727	prM	262	T	A	NSUB	0.06333
TX 9587 (D0135)	757	prM	292	D2	i	LP	0.07557
TX 9631 (D0179)	801	prM	336	T	G	NSUB	0.2272
TX 9604 (D0152)	805	prM	340	G	A	NSUB	0.2936
TX 9597 (D0145)	823	prM	358	C	T	NSUB	0.6851
TX 9587 (D0135)	831	prM	366	D1	i	LP	0.09104
TX 9589 (D0137)	869	prM	404	T	C	NSUB	0.4243
TX 9597 (D0145)	895	prM	430	G	A	NSUB	0.4283
TX 9587 (D0135)	935	prM	470	C	T	NSUB	0.1756
TX 9589 (D0137)	967	E	1	C	T	NSUB	0.4092
TX 9597 (D0145)	987	E	21	C	T	NSUB	6.58

TX 9597 (D0145)	999	E	33	T	C	NSUB	1.915
TX 9597 (D0145)	1000	E	34	G	T	NSUB	0.8582
TX 9611 (D0159)	1002	E	36	A	G	NSUB	7.418
TX 9604 (D0152)	1005	E	39	G	A	NSUB	0.5335
TX 9631 (D0179)	1086	E	120	T	C	NSUB	0.1199
TX 9597 (D0145)	1151	E	185	C	T	NSUB	6.034
TX 9631 (D0179)	1164	E	198	T	C	NSUB	0.1507
TX 9604 (D0152)	1170	E	204	T	C	NSUB	0.2146
TX 9631 (D0179)	1173	E	207	T	C	NSUB	0.2428
TX 9597 (D0145)	1185	E	219	A	G	NSUB	5.872
TX 9587 (D0135)	1188	E	222	T	C	NSUB	0.2257
TX 9582 (D0130)	1200	E	234	G	A	NSUB	0.1872
TX 9597 (D0145)	1213	E	247	C	G	NSUB	1.495
TX 9587 (D0135)	1216	E	250	D1	i	LP	0.08421
TX 9614 (D0162)	1231	E	265	A	G	NSUB	0.3753
TX 9601 (D0149)	1233	E	267	D1	i	LP	0.09407
TX 9589 (D0137)	1248	E	282	G	A	NSUB	1.551
TX 9597 (D0145)	1263	E	297	G	A	NSUB	4.669
TX 9589 (D0137)	1343	E	377	C	T	NSUB	0.6051
TX 9631 (D0179)	1359	E	393	A	G	NSUB	1.933
TX 9614 (D0162)	1360	E	394	D1	i	LP	0.07579
TX 9597 (D0145)	1373	E	407	G	A	NSUB	0.7673
TX 9587 (D0135)	1401	E	435	G	A	NSUB	0.2116
TX 9589 (D0137)	1431	E	465	T	C	NSUB	0.1845
TX 9597 (D0145)	1454	E	488	G	A	NSUB	0.4253
TX 9597 (D0145)	1467	E	501	T	C	NSUB	0.4333
TX 9597 (D0145)	1470	E	504	T	C	NSUB	0.6543
TX 9631 (D0179)	1533	E	567	T	C	NSUB	0.1741
TX 9601 (D0149)	1562	E	596	G	A	NSUB	0.632
TX 9611 (D0159)	1572	E	606	T	C	NSUB	10.24
TX 9589 (D0137)	1618	E	652	C	T	NSUB	0.3112
TX 9597 (D0145)	1692	E	726	C	T	NSUB	1.24
TX 9589 (D0137)	1771	E	805	A	G	NSUB	0.1545
TX 9589 (D0137)	1814	E	848	T	C	NSUB	0.4761
TX 9631 (D0179)	1842	E	876	A	G	NSUB	0.3477
TX 9601 (D0149)	1886	E	920	G	A	NSUB	0.1489
TX 9582 (D0130)	1893	E	927	T	C	NSUB	0.2619
TX 9601 (D0149)	1895	E	929	G	A	NSUB	0.3153
TX 9631 (D0179)	1911	E	945	T	C	NSUB	0.1856

TX 9587 (D0135)	1999	E	1033	G	T	NSUB	1.705
TX 9589 (D0137)	2022	E	1056	A	G	NSUB	0.3629
TX 9597 (D0145)	2040	E	1074	C	T	NSUB	7.347
TX 9587 (D0135)	2109	E	1143	C	T	NSUB	0.1403
TX 9597 (D0145)	2172	E	1206	C	T	NSUB	7.618
TX 9597 (D0145)	2191	E	1225	G	A	NSUB	0.6926
TX 9631 (D0179)	2199	E	1233	T	C	NSUB	0.1113
TX 9601 (D0149)	2247	E	1281	T	C	NSUB	0.2929
TX 9589 (D0137)	2271	E	1305	T	C	NSUB	0.219
TX 9631 (D0179)	2293	E	1327	T	C	NSUB	0.1338
TX 9587 (D0135)	2304	E	1338	C	T	NSUB	0.138
TX 9587 (D0135)	2371	E	1405	T	C	NSUB	0.2182
TX 9601 (D0149)	2375	E	1409	C	T	NSUB	0.482
TX 9604 (D0152)	2390	E	1424	G	A	NSUB	0.2264
TX 9601 (D0149)	2419	E	1453	D1	i	LP	0.1301
TX 9597 (D0145)	2474	NS1	5	T	C	NSUB	0.4073
TX 9587 (D0135)	2550	NS1	81	T	C	NSUB	0.1565
TX 9597 (D0145)	2565	NS1	96	T	C	NSUB	0.7353
TX 9631 (D0179)	2573	NS1	104	G	A	NSUB	0.2739
TX 9589 (D0137)	2579	NS1	110	G	A	NSUB	0.3758
TX 9631 (D0179)	2583	NS1	114	T	G	NSUB	0.1732
TX 9587 (D0135)	2593	NS1	124	T	C	NSUB	0.1987
TX 9631 (D0179)	2595	NS1	126	G	A	NSUB	0.3866
TX 9631 (D0179)	2603	NS1	134	C	T	NSUB	0.2382
TX 9604 (D0152)	2620	NS1	151	G	A	NSUB	0.293
TX 9582 (D0130)	2648	NS1	179	D1	i	LP	0.07144
TX 9597 (D0145)	2656	NS1	187	T	C	NSUB	0.5135
TX 9597 (D0145)	2658	NS1	189	T	G	NSUB	0.3812
TX 9631 (D0179)	2666	NS1	197	D1	i	LP	0.09641
TX 9597 (D0145)	2711	NS1	242	G	A	NSUB	6.58
TX 9631 (D0179)	2712	NS1	243	A	G	NSUB	0.1837
TX 9589 (D0137)	2836	NS1	367	C	T	NSUB	0.3574
TX 9589 (D0137)	2843	NS1	374	T	C	NSUB	0.1813
TX 9631 (D0179)	2859	NS1	390	T	C	NSUB	0.1744
TX 9611 (D0159)	2899	NS1	430	T	C	NSUB	8.841
TX 9604 (D0152)	2928	NS1	459	G	A	NSUB	1.136
TX 9582 (D0130)	2947	NS1	478	D1	i	LP	0.07508
TX 9611 (D0159)	2974	NS1	505	A	C	NSUB	0.2022
TX 9604 (D0152)	2982	NS1	513	T	C	NSUB	0.3019

TX 9589 (D0137)	2993	NS1	524	D2	i	LP	0.1288
TX 9597 (D0145)	3029	NS1	560	T	C	NSUB	0.9771
TX 9631 (D0179)	3043	NS1	574	C	T	NSUB	0.3106
TX 9631 (D0179)	3047	NS1	578	T	C	NSUB	0.675
TX 9631 (D0179)	3061	NS1	592	T	C	NSUB	0.306
TX 9587 (D0135)	3093	NS1	624	C	T	NSUB	0.2482
TX 9614 (D0162)	3129	NS1	660	T	C	NSUB	0.3137
TX 9597 (D0145)	3138	NS1	669	C	T	NSUB	1.838
TX 9597 (D0145)	3168	NS1	699	C	T	NSUB	5.91
TX 9780 (D0329)	3177	NS1	708	T	C	NSUB	0.2029
TX 9589 (D0137)	3183	NS1	714	A	G	NSUB	1.068
TX 9597 (D0145)	3225	NS1	756	T	C	NSUB	0.628
TX 9589 (D0137)	3282	NS1	813	T	C	NSUB	0.329
TX 9604 (D0152)	3285	NS1	816	A	G	NSUB	0.3876
TX 9614 (D0162)	3356	NS1	887	T	C	NSUB	0.1638
TX 9589 (D0137)	3362	NS1	893	T	C	NSUB	0.1965
TX 9631 (D0179)	3485	NS1	1016	A	G	NSUB	0.1375
TX 9587 (D0135)	3501	NS1	1032	C	T	NSUB	0.2251
TX 9587 (D0135)	3528	NS2A	3	T	C	NSUB	0.227
TX 9589 (D0137)	3550	NS2A	25	C	T	NSUB	0.2505
TX 9631 (D0179)	3573	NS2A	48	A	G	NSUB	0.1443
TX 9597 (D0145)	3576	NS2A	51	T	C	NSUB	0.7397
TX 9582 (D0130)	3598	NS2A	73	T	C	NSUB	0.2004
TX 9589 (D0137)	3625	NS2A	100	G	A	NSUB	0.2476
TX 9601 (D0149)	3666	NS2A	141	A	G	NSUB	0.1592
TX 9589 (D0137)	3694	NS2A	169	D2	i	LP	0.1371
TX 9587 (D0135)	3699	NS2A	174	T	C	NSUB	0.1259
TX 9587 (D0135)	3720	NS2A	195	T	C	NSUB	0.2305
TX 9597 (D0145)	3754	NS2A	229	C	T	NSUB	0.493
TX 9780 (D0329)	3774	NS2A	249	T	C	NSUB	0.1781
TX 9631 (D0179)	3790	NS2A	265	D1	i	LP	0.1078
TX 9587 (D0135)	3792	NS2A	267	T	C	NSUB	0.1274
TX 9631 (D0179)	3810	NS2A	285	T	C	NSUB	0.2663
TX 9597 (D0145)	3867	NS2A	342	C	T	NSUB	0.4643
TX 9597 (D0145)	3888	NS2A	363	T	C	NSUB	3.402
TX 9587 (D0135)	3912	NS2A	387	C	T	NSUB	0.1695
TX 9589 (D0137)	3927	NS2A	402	C	T	NSUB	0.8941
TX 9587 (D0135)	3933	NS2A	408	A	G	NSUB	0.1968
TX 9582 (D0130)	3963	NS2A	438	G	A	NSUB	0.5103

TX 9589 (D0137)	3984	NS2A	459	T	C	NSUB	11.14
TX 9631 (D0179)	4005	NS2A	480	T	C	NSUB	0.1559
TX 9631 (D0179)	4009	NS2A	484	T	C	NSUB	0.1169
TX 9587 (D0135)	4088	NS2A	563	G	A	NSUB	0.14
TX 9589 (D0137)	4095	NS2A	570	A	G	NSUB	0.18
TX 9582 (D0130)	4109	NS2A	584	IAA	d	LP	0.1068
TX 9780 (D0329)	4129	NS2A	604	T	C	NSUB	0.2023
TX 9597 (D0145)	4137	NS2A	612	T	C	NSUB	5.557
TX 9631 (D0179)	4170	NS2A	645	T	C	NSUB	0.1118
TX 9589 (D0137)	4176	NS2A	651	T	C	NSUB	0.4603
TX 9587 (D0135)	4194	NS2A	669	C	T	NSUB	0.1923
TX 9597 (D0145)	4196	NS2A	671	T	C	NSUB	0.5237
TX 9597 (D0145)	4200	NS2A	675	C	T	NSUB	0.4009
TX 9587 (D0135)	4261	NS2B	43	D1	i	LP	0.09027
TX 9631 (D0179)	4266	NS2B	48	T	C	NSUB	0.3962
TX 9601 (D0149)	4296	NS2B	78	C	T	NSUB	6.304
TX 9631 (D0179)	4323	NS2B	105	T	C	NSUB	0.1551
TX 9597 (D0145)	4347	NS2B	129	T	C	NSUB	0.331
TX 9601 (D0149)	4398	NS2B	180	C	T	NSUB	0.1401
TX 9631 (D0179)	4526	NS2B	308	D1	i	LP	0.09527
TX 9587 (D0135)	4530	NS2B	312	T	C	NSUB	0.1574
TX 9597 (D0145)	4543	NS2B	325	T	G	NSUB	1.14
TX 9780 (D0329)	4564	NS2B	346	T	C	NSUB	0.2339
TX 9582 (D0130)	4582	NS2B	364	D1	i	LP	0.07676
TX 9631 (D0179)	4596	NS2B	378	T	C	NSUB	0.1284
TX 9587 (D0135)	4611	NS2B	393	G	A	NSUB	0.1785
TX 9589 (D0137)	4617	NS3	6	T	C	NSUB	0.2932
TX 9611 (D0159)	4643	NS3	32	G	A	NSUB	0.4364
TX 9597 (D0145)	4662	NS3	51	T	C	NSUB	0.4389
TX 9631 (D0179)	4686	NS3	75	T	C	NSUB	0.1158
TX 9631 (D0179)	4691	NS3	80	T	C	NSUB	0.1986
TX 9631 (D0179)	4731	NS3	120	T	G	NSUB	0.6093
TX 9631 (D0179)	4770	NS3	159	D1	i	LP	0.08547
TX 9631 (D0179)	4779	NS3	168	T	C	NSUB	0.3783
TX 9597 (D0145)	4791	NS3	180	T	C	NSUB	7.873
TX 9589 (D0137)	4795	NS3	184	A	G	NSUB	0.5257
TX 9589 (D0137)	4848	NS3	237	T	C	NSUB	0.4379
TX 9587 (D0135)	4909	NS3	298	A	G	NSUB	0.1555
TX 9589 (D0137)	4918	NS3	307	A	G	NSUB	1.591

TX 9597 (D0145)	4936	NS3	325	A	G	NSUB	0.3569
TX 9587 (D0135)	4986	NS3	375	T	C	NSUB	0.07094
TX 9631 (D0179)	5034	NS3	423	T	C	NSUB	0.183
TX 9587 (D0135)	5097	NS3	486	A	G	NSUB	0.4433
TX 9587 (D0135)	5132	NS3	521	T	C	NSUB	0.1964
TX 9780 (D0329)	5136	NS3	525	T	C	NSUB	0.1588
TX 9587 (D0135)	5148	NS3	537	T	C	NSUB	0.2668
TX 9631 (D0179)	5185	NS3	574	T	C	NSUB	0.3409
TX 9597 (D0145)	5199	NS3	588	T	C	NSUB	0.2728
TX 9780 (D0329)	5205	NS3	594	T	C	NSUB	0.1846
TX 9601 (D0149)	5228	NS3	617	D2	i	LP	0.08783
TX 9604 (D0152)	5239	NS3	628	D1	i	LP	0.09778
TX 9631 (D0179)	5271	NS3	660	T	C	NSUB	0.1391
TX 9631 (D0179)	5340	NS3	729	T	C	NSUB	0.1627
TX 9587 (D0135)	5367	NS3	756	D1	i	LP	0.0364
TX 9587 (D0135)	5368	NS3	757	C	A	NSUB	0.03608
TX 9601 (D0149)	5445	NS3	834	T	C	NSUB	0.4429
TX 9582 (D0130)	5455	NS3	844	D1	i	LP	0.07546
TX 9587 (D0135)	5457	NS3	846	C	T	NSUB	0.0819
TX 9587 (D0135)	5472	NS3	861	T	A	NSUB	0.1065
TX 9631 (D0179)	5522	NS3	911	G	A	NSUB	0.1671
TX 9597 (D0145)	5576	NS3	965	T	C	NSUB	0.4548
TX 9587 (D0135)	5611	NS3	1000	A	T	NSUB	0.1162
TX 9589 (D0137)	5617	NS3	1006	C	T	NSUB	1.722
TX 9589 (D0137)	5631	NS3	1020	T	C	NSUB	0.1601
TX 9587 (D0135)	5658	NS3	1047	T	C	NSUB	0.1371
TX 9587 (D0135)	5667	NS3	1056	T	C	NSUB	0.05657
TX 9587 (D0135)	5678	NS3	1067	C	T	NSUB	0.06967
TX 9589 (D0137)	5709	NS3	1098	T	C	NSUB	0.2876
TX 9589 (D0137)	5721	NS3	1110	A	T	NSUB	0.1878
TX 9589 (D0137)	5736	NS3	1125	T	C	NSUB	10.07
TX 9614 (D0162)	5755	NS3	1144	D1	i	LP	0.07567
TX 9589 (D0137)	5763	NS3	1152	T	C	NSUB	0.2082
TX 9601 (D0149)	5775	NS3	1164	G	A	NSUB	0.3263
TX 9587 (D0135)	5847	NS3	1236	T	C	NSUB	0.1317
TX 9597 (D0145)	5859	NS3	1248	C	T	NSUB	6.219
TX 9597 (D0145)	5868	NS3	1257	A	G	NSUB	6.793
TX 9780 (D0329)	5919	NS3	1308	G	A	NSUB	0.1869
TX 9589 (D0137)	5995	NS3	1384	G	A	NSUB	0.3212

TX 9582 (D0130)	6069	NS3	1458	T	C	NSUB	31.77
TX 9582 (D0130)	6085	NS3	1474	ICA	d	LP	0.07337
TX 9601 (D0149)	6089	NS3	1478	IA	d	LP	0.09339
TX 9587 (D0135)	6102	NS3	1491	T	C	NSUB	0.1465
TX 9614 (D0162)	6117	NS3	1506	D1	i	LP	0.0963
TX 9597 (D0145)	6161	NS3	1550	C	T	NSUB	0.8516
TX 9587 (D0135)	6165	NS3	1554	T	C	NSUB	0.163
TX 9597 (D0145)	6189	NS3	1578	T	C	NSUB	8.294
TX 9587 (D0135)	6204	NS3	1593	G	A	NSUB	0.3157
TX 9631 (D0179)	6209	NS3	1598	C	A	NSUB	0.1963
TX 9614 (D0162)	6223	NS3	1612	A	T	NSUB	0.06103
TX 9589 (D0137)	6281	NS3	1670	T	C	NSUB	0.1404
TX 9587 (D0135)	6312	NS3	1701	T	C	NSUB	0.1932
TX 9601 (D0149)	6319	NS3	1708	G	A	NSUB	0.1648
TX 9597 (D0145)	6363	NS3	1752	A	G	NSUB	0.5293
TX 9589 (D0137)	6396	NS3	1785	T	C	NSUB	0.7387
TX 9631 (D0179)	6401	NS3	1790	C	T	NSUB	0.2435
TX 9587 (D0135)	6468	NS3	1857	C	T	NSUB	0.2052
TX 9587 (D0135)	6471	NS4A	3	T	C	NSUB	0.2045
TX 9587 (D0135)	6493	NS4A	25	T	C	NSUB	0.1715
TX 9631 (D0179)	6498	NS4A	30	D1	i	LP	0.0839
TX 9587 (D0135)	6522	NS4A	54	A	G	NSUB	0.1099
TX 9597 (D0145)	6526	NS4A	58	G	A	NSUB	1.085
TX 9601 (D0149)	6534	NS4A	66	G	A	NSUB	0.2332
TX 9587 (D0135)	6574	NS4A	106	D1	i	LP	0.03418
TX 9589 (D0137)	6594	NS4A	126	A	G	NSUB	0.2837
TX 9587 (D0135)	6598	NS4A	130	T	C	NSUB	0.2351
TX 9631 (D0179)	6598	NS4A	130	T	C	NSUB	0.1473
TX 9587 (D0135)	6615	NS4A	147	T	C	NSUB	0.1184
TX 9587 (D0135)	6618	NS4A	150	T	C	NSUB	0.129
TX 9587 (D0135)	6639	NS4A	171	T	C	NSUB	0.235
TX 9631 (D0179)	6639	NS4A	171	T	C	NSUB	0.2496
TX 9597 (D0145)	6672	NS4A	204	T	C	NSUB	0.7773
TX 9587 (D0135)	6705	NS4A	237	A	G	NSUB	0.1819
TX 9587 (D0135)	6712	NS4A	244	C	T	NSUB	0.1508
TX 9780 (D0329)	6722	NS4A	254	T	C	NSUB	0.4527
TX 9597 (D0145)	6722	NS4A	254	T	C	NSUB	0.6148
TX 9587 (D0135)	6750	NS4A	282	C	T	NSUB	0.2977
TX 9604 (D0152)	6807	NS4A	339	T	C	NSUB	0.2285

TX 9589 (D0137)	6816	NS4A	348	C	T	NSUB	0.1571
TX 9597 (D0145)	6871	NS4A	403	A	G	NSUB	0.8909
TX 9587 (D0135)	6881	NS4A	413	D1	i	LP	0.07234
TX 9601 (D0149)	6901	NS4A	433	G	A	NSUB	0.27
TX 9587 (D0135)	6921	NS4B	6	A	G	NSUB	0.1517
TX 9587 (D0135)	6956	NS4B	41	T	G	NSUB	0.1612
TX 9587 (D0135)	6959	NS4B	44	G	A	NSUB	0.1574
TX 9601 (D0149)	6964	NS4B	49	D1	i	LP	0.0781
TX 9601 (D0149)	6968	NS4B	53	G	A	NSUB	0.2465
TX 9601 (D0149)	7006	NS4B	91	A	G	NSUB	0.2475
TX 9597 (D0145)	7038	NS4B	123	T	C	NSUB	2.634
TX 9597 (D0145)	7077	NS4B	162	A	G	NSUB	6.873
TX 9614 (D0162)	7113	NS4B	198	T	C	NSUB	0.143
TX 9780 (D0329)	7143	NS4B	228	T	A	NSUB	0.2466
TX 9631 (D0179)	7155	NS4B	240	T	C	NSUB	0.2562
TX 9589 (D0137)	7170	NS4B	255	T	C	NSUB	0.2891
TX 9604 (D0152)	7173	NS4B	258	T	C	NSUB	0.5173
TX 9597 (D0145)	7182	NS4B	267	T	C	NSUB	0.8853
TX 9604 (D0152)	7185	NS4B	270	C	T	NSUB	0.4595
TX 9631 (D0179)	7201	NS4B	286	T	C	NSUB	0.1832
TX 9589 (D0137)	7204	NS4B	289	T	C	NSUB	0.5119
TX 9780 (D0329)	7223	NS4B	308	A	G	NSUB	0.04421
TX 9631 (D0179)	7223	NS4B	308	D1	i	LP	0.05112
TX 9631 (D0179)	7225	NS4B	310	T	G	NSUB	0.06789
TX 9780 (D0329)	7231	NS4B	316	IC	d	LP	0.04709
TX 9589 (D0137)	7233	NS4B	318	T	C	NSUB	0.3515
TX 9582 (D0130)	7239	NS4B	324	T	C	NSUB	0.08351
TX 9582 (D0130)	7245	NS4B	330	A	T	NSUB	0.0658
TX 9597 (D0145)	7245	NS4B	330	T	C	NSUB	8.3
TX 9582 (D0130)	7249	NS4B	334	A	G	NSUB	0.1598
TX 9597 (D0145)	7267	NS4B	352	IT	d	LP	2.575
TX 9604 (D0152)	7274	NS4B	359	T	G	NSUB	0.9419
TX 9587 (D0135)	7284	NS4B	369	T	C	NSUB	0.188
TX 9589 (D0137)	7311	NS4B	396	A	G	NSUB	0.4574
TX 9631 (D0179)	7311	NS4B	396	IC	d	LP	0.0359
TX 9631 (D0179)	7315	NS4B	400	T	A	NSUB	0.04623
TX 9631 (D0179)	7319	NS4B	404	D1	i	LP	0.03546
TX 9582 (D0130)	7391	NS4B	476	IC	d	LP	0.08861
TX 9631 (D0179)	7392	NS4B	477	T	C	NSUB	0.273

TX 9587 (D0135)	7395	NS4B	480	A	C	NSUB	0.3789
TX 9587 (D0135)	7404	NS4B	489	T	G	NSUB	0.1785
TX 9587 (D0135)	7431	NS4B	516	A	G	NSUB	0.1547
TX 9597 (D0145)	7447	NS4B	532	T	C	NSUB	0.4109
TX 9631 (D0179)	7447	NS4B	532	T	C	NSUB	0.6821
TX 9597 (D0145)	7452	NS4B	537	T	C	NSUB	1.029
TX 9597 (D0145)	7509	NS4B	594	T	C	NSUB	0.2566
TX 9604 (D0152)	7539	NS4B	624	T	G	NSUB	0.368
TX 9587 (D0135)	7602	NS4B	687	C	T	NSUB	0.1616
TX 9582 (D0130)	7605	NS4B	690	A	G	NSUB	0.2625
TX 9631 (D0179)	7632	NS4B	717	T	C	NSUB	0.1858
TX 9587 (D0135)	7635	NS4B	720	A	G	NSUB	0.08669
TX 9601 (D0149)	7642	NS4B	727	D2	i	LP	0.1046
TX 9631 (D0179)	7647	NS4B	732	T	C	NSUB	0.1147
TX 9589 (D0137)	7656	NS4B	741	T	C	NSUB	0.2109
TX 9614 (D0162)	7661	NS4B	746	G	A	NSUB	0.116
TX 9601 (D0149)	7674	NS4B	759	G	A	NSUB	1.269
TX 9611 (D0159)	7711	NS5	31	T	G	NSUB	0.5695
TX 9631 (D0179)	7711	NS5	31	T	G	NSUB	0.2076
TX 9582 (D0130)	7721	NS5	41	D1	i	LP	0.07731
TX 9587 (D0135)	7731	NS5	51	C	T	NSUB	0.1608
TX 9631 (D0179)	7751	NS5	71	A	T	NSUB	0.1943
TX 9589 (D0137)	7752	NS5	72	T	C	NSUB	0.1558
TX 9587 (D0135)	7758	NS5	78	A	G	NSUB	0.198
TX 9589 (D0137)	7779	NS5	99	T	C	NSUB	0.4996
TX 9780 (D0329)	7809	NS5	129	T	C	NSUB	0.1473
TX 9587 (D0135)	7812	NS5	132	G	A	NSUB	0.1532
TX 9597 (D0145)	7824	NS5	144	C	T	NSUB	0.5449
TX 9582 (D0130)	7860	NS5	180	D1	i	LP	0.09232
TX 9780 (D0329)	7891	NS5	211	T	C	NSUB	0.2114
TX 9597 (D0145)	7902	NS5	222	T	C	NSUB	0.6185
TX 9597 (D0145)	7947	NS5	267	T	C	NSUB	0.4652
TX 9631 (D0179)	7947	NS5	267	T	C	NSUB	0.1274
TX 9611 (D0159)	7960	NS5	280	IA	d	LP	1.272
TX 9631 (D0179)	7960	NS5	280	IA	d	LP	0.4845
TX 9611 (D0159)	7961	NS5	281	D1	i	LP	0.4036
TX 9631 (D0179)	7961	NS5	281	D1	i	LP	0.09791
TX 9589 (D0137)	7971	NS5	291	T	C	NSUB	0.1968
TX 9589 (D0137)	7980	NS5	300	T	C	NSUB	0.2491

TX 9601 (D0149)	8070	NS5	390	A	G	NSUB	0.2934
TX 9597 (D0145)	8082	NS5	402	T	C	NSUB	1.579
TX 9631 (D0179)	8155	NS5	475	T	C	NSUB	0.2538
TX 9604 (D0152)	8166	NS5	486	T	C	NSUB	0.1886
TX 9614 (D0162)	8215	NS5	535	D1	i	LP	0.08785
TX 9589 (D0137)	8232	NS5	552	T	C	NSUB	0.233
TX 9604 (D0152)	8238	NS5	558	T	C	NSUB	0.742
TX 9601 (D0149)	8241	NS5	561	T	C	NSUB	0.7958
TX 9587 (D0135)	8248	NS5	568	D1	i	LP	0.092
TX 9631 (D0179)	8248	NS5	568	D1	i	LP	0.1129
TX 9614 (D0162)	8265	NS5	585	T	G	NSUB	0.2108
TX 9614 (D0162)	8287	NS5	607	D1	i	LP	0.1074
TX 9631 (D0179)	8413	NS5	733	IA	d	LP	0.2037
TX 9597 (D0145)	8453	NS5	773	C	T	NSUB	8.754
TX 9597 (D0145)	8478	NS5	798	A	G	NSUB	0.46
TX 9631 (D0179)	8511	NS5	831	C	T	NSUB	0.283
TX 9597 (D0145)	8514	NS5	834	G	A	NSUB	3.837
TX 9589 (D0137)	8540	NS5	860	A	G	NSUB	0.1744
TX 9597 (D0145)	8553	NS5	873	C	T	NSUB	1.113
TX 9614 (D0162)	8564	NS5	884	IC	d	LP	0.1138
TX 9587 (D0135)	8565	NS5	885	C	T	NSUB	0.1514
TX 9589 (D0137)	8566	NS5	886	T	C	NSUB	1.045
TX 9597 (D0145)	8566	NS5	886	T	C	NSUB	0.354
TX 9587 (D0135)	8631	NS5	951	T	C	NSUB	0.1697
TX 9587 (D0135)	8644	NS5	964	T	C	NSUB	0.2213
TX 9587 (D0135)	8661	NS5	981	T	C	NSUB	0.1778
TX 9587 (D0135)	8673	NS5	993	D1	i	LP	0.07342
TX 9631 (D0179)	8751	NS5	1071	T	C	NSUB	0.1824
TX 9631 (D0179)	8760	NS5	1080	A	G	NSUB	0.1394
TX 9631 (D0179)	8849	NS5	1169	G	A	NSUB	1.993
TX 9597 (D0145)	8861	NS5	1181	A	G	NSUB	1.09
TX 9597 (D0145)	8883	NS5	1203	T	C	NSUB	0.4799
TX 9582 (D0130)	8889	NS5	1209	D1	i	LP	0.06925
TX 9780 (D0329)	8898	NS5	1218	T	C	NSUB	0.1477
TX 9631 (D0179)	8967	NS5	1287	G	A	NSUB	0.2281
TX 9597 (D0145)	8973	NS5	1293	D2	i	LP	0.1667
TX 9631 (D0179)	8973	NS5	1293	D2	i	LP	0.0798
TX 9589 (D0137)	8977	NS5	1297	D1	i	LP	0.1144
TX 9597 (D0145)	8991	NS5	1311	A	G	NSUB	9.664

TX 9597 (D0145)	9063	NS5	1383	IA	d	LP	0.513
TX 9631 (D0179)	9063	NS5	1383	IA	d	LP	0.7547
TX 9582 (D0130)	9064	NS5	1384	D1	i	LP	0.1064
TX 9631 (D0179)	9064	NS5	1384	D1	i	LP	0.129
TX 9614 (D0162)	9073	NS5	1393	IGA	d	LP	0.0749
TX 9614 (D0162)	9094	NS5	1414	A	G	NSUB	0.08839
TX 9587 (D0135)	9147	NS5	1467	G	A	NSUB	0.1563
TX 9604 (D0152)	9151	NS5	1471	T	C	NSUB	0.3423
TX 9597 (D0145)	9171	NS5	1491	T	C	NSUB	0.7736
TX 9587 (D0135)	9224	NS5	1544	D1	i	LP	0.09777
TX 9597 (D0145)	9237	NS5	1557	T	C	NSUB	5.063
TX 9631 (D0179)	9246	NS5	1566	C	T	NSUB	0.2135
TX 9631 (D0179)	9276	NS5	1596	T	C	NSUB	0.1114
TX 9587 (D0135)	9279	NS5	1599	C	T	NSUB	0.1481
TX 9587 (D0135)	9381	NS5	1701	C	T	NSUB	0.137
TX 9582 (D0130)	9399	NS5	1719	C	T	NSUB	44.97
TX 9587 (D0135)	9468	NS5	1788	T	C	NSUB	0.163
TX 9631 (D0179)	9522	NS5	1842	C	T	NSUB	0.2571
TX 9587 (D0135)	9591	NS5	1911	A	G	NSUB	0.08377
TX 9587 (D0135)	9594	NS5	1914	A	G	NSUB	0.09393
TX 9631 (D0179)	9596	NS5	1916	G	A	NSUB	0.2618
TX 9587 (D0135)	9603	NS5	1923	D1	i	LP	0.1101
TX 9614 (D0162)	9671	NS5	1991	T	C	NSUB	0.1632
TX 9587 (D0135)	9696	NS5	2016	A	G	NSUB	0.1514
TX 9597 (D0145)	9744	NS5	2064	C	T	NSUB	8.473
TX 9587 (D0135)	9750	NS5	2070	D1	i	LP	0.1137
TX 9631 (D0179)	9768	NS5	2088	T	C	NSUB	0.1557
TX 9597 (D0145)	9788	NS5	2108	T	C	NSUB	0.5291
TX 9631 (D0179)	9788	NS5	2108	T	C	NSUB	0.161
TX 9597 (D0145)	9846	NS5	2166	T	C	NSUB	6.726
TX 9582 (D0130)	9912	NS5	2232	G	C	NSUB	0.6128
TX 9780 (D0329)	9917	NS5	2237	T	C	NSUB	0.1977
TX 9631 (D0179)	9936	NS5	2256	T	C	NSUB	0.2364
TX 9587 (D0135)	9955	NS5	2275	C	T	NSUB	0.3935
TX 9780 (D0329)	9996	NS5	2316	T	C	NSUB	0.2618
TX 9597 (D0145)	10035	NS5	2355	C	T	NSUB	8.606
TX 9587 (D0135)	10059	NS5	2379	A	C	NSUB	0.1474
TX 9614 (D0162)	10071	NS5	2391	G	A	NSUB	0.066
TX 9582 (D0130)	10082	NS5	2402	T	C	NSUB	0.04897

TX 9582 (D0130)	10084	NS5	2404	T	A	NSUB	0.02866
TX 9587 (D0135)	10120	NS5	2440	A	C	NSUB	0.2391
TX 9614 (D0162)	10136	NS5	2456	IC	d	LP	0.07479
TX 9631 (D0179)	10160	NS5	2480	G	A	NSUB	0.1385
TX 9597 (D0145)	10211	NS5	2531	T	C	NSUB	0.4588
TX 9614 (D0162)	10233	NS5	2553	D2	i	LP	0.1328
TX 9604 (D0152)	10248	NS5	2568	T	C	NSUB	0.3036
TX 9597 (D0145)	10248	NS5	2568	T	C	NSUB	0.6434
TX 9582 (D0130)	10264	NS5	2584	A	G	NSUB	0.186
TX 9582 (D0130)	10277	NS5	2597	D1	i	LP	0.066
TX 9589 (D0137)	10305	NS5	2625	T	C	NSUB	0.8668
TX 9589 (D0137)	10314	NS5	2634	T	C	NSUB	0.1777
TX 9587 (D0135)	10317	NS5	2637	C	T	NSUB	0.1662
TX 9587 (D0135)	10351	NS5	2671	C	T	NSUB	0.2898
TX 9631 (D0179)	10353	NS5	2673	D1	i	LP	0.08812
TX 9631 (D0179)	10359	NS5	2679	D2	i	LP	0.1191
TX 9631 (D0179)	10364	NS5	2684	G	A	NSUB	0.2612
TX 9614 (D0162)	10367	NS5	2687	D2	i	LP	0.1523
TX 9631 (D0179)	10367	NS5	2687	D2	i	LP	0.12
TX 9604 (D0152)	10368	NS5	2688	T	C	NSUB	0.3389
TX 9589 (D0137)	10390	NS5	2710	A	G	NSUB	0.2959
TX 9587 (D0135)	10393	NS5	2713	T	C	NSUB	0.2828
TX 9601 (D0149)	10402	3'UTR	10402	D1	i	LP	0.109
TX 9589 (D0137)	10405	3'UTR	10405	G	A	NSUB	0.5393
TX 9597 (D0145)	10408	3'UTR	10408	C	T	NSUB	8.406
TX 9631 (D0179)	10408	3'UTR	10408	T	C	NSUB	0.4442
TX 9601 (D0149)	10422	3'UTR	10422	T	C	NSUB	0.1987
TX 9587 (D0135)	10435	3'UTR	10435	T	C	NSUB	0.2816
TX 9631 (D0179)	10435	3'UTR	10435	T	C	NSUB	0.2773
TX 9631 (D0179)	10458	3'UTR	10458	C	G	NSUB	0.1541
TX 9587 (D0135)	10459	3'UTR	10459	T	C	NSUB	0.1807
TX 9597 (D0145)	10485	3'UTR	10485	C	T	NSUB	7.13
TX 9597 (D0145)	10489	3'UTR	10489	G	A	NSUB	2.158
TX 9597 (D0145)	10520	3'UTR	10520	T	C	NSUB	8.232
TX 9780 (D0329)	10521	3'UTR	10521	T	C	NSUB	0.4815
TX 9589 (D0137)	10607	3'UTR	10607	C	T	NSUB	0.8897
TX 9780 (D0329)	10623	3'UTR	10623	T	C	NSUB	0.1534
TX 9597 (D0145)	10726	3'UTR	10726	G	A	NSUB	1.012
TX 9582 (D0130)	10761	3'UTR	10761	A	G	NSUB	0.2309

TX 9631 (D0179)	10863	3'UTR	10863	A	G	NSUB	0.3295
TX 9604 (D0152)	10869	3'UTR	10869	A	G	NSUB	0.6465
TX 9631 (D0179)	10871	3'UTR	10871	A	G	NSUB	0.6193
TX 9780 (D0329)	10887	3'UTR	10887	A	T	NSUB	1.58

Appendix V

Summary of all SNVs identified in the Colombian isolates collected during 2008.

Isolate	Position	SNV Percent	Gene	Gene Position
COL524/08	864	0.1824	prM	prM-133
COL524/08	1844	0.3033	E	E-293
COL524/08	1950	6.032	E	E-328
COL524/08	2727	0.8776	NS1	NS1-86
COL524/08	3310	4.981	NS1	NS1-281
COL524/08	4109	1.054	NS2A	NS2A-195
COL524/08	4110	0.1614	NS2A	NS2A-195
COL524/08	5166	0.6883	NS3	NS3-185
COL524/08	6203	0.3545	NS3	NS3-531
COL524/08	6203	2.993	NS3	NS3-531
COL524/08	6204	0.2912	NS3	NS3-531
COL524/08	6340	0.6757	NS3	NS3-577
COL524/08	6966	0.04969	NS4B	NS4B-17
COL524/08	6970	0.05493	NS4B	NS4B-19
COL524/08	7636	3.646	NS4B	NS4B-241
COL524/08	7643	4.178	NS4B	NS4B-243
COL524/08	7661	4.686	NS4B	NS4B-249
COL524/08	7711	0.6231	NS5	NS5-11
COL524/08	7960	1.015	NS5	NS5-94
COL524/08	8289	1.038	NS5	NS5-203
COL524/08	8413	0.2705	NS5	NS5-245
COL524/08	8414	0.1982	NS5	NS5-245
COL524/08	9063	0.9721	NS5	NS5-461
COL524/08	10123	5.296	NS5	NS5-815
COL524/08	10554	0.3711	3'UTR	3'UTR-159
COL739/08	190	0.1511	C	C-32
COL739/08	202	0.09955	C	C-36
COL739/08	219	0.7963	C	C-41
COL739/08	545	0.2754	prM	prM-27
COL739/08	759	0.5955	prM	prM-98
COL739/08	777	48.55	prM	prM-104
COL739/08	1320	1.2	E	E-118
COL739/08	1451	0.2872	E	E-162

COL739/08	2380	0.3481	E	E-472
COL739/08	2390	0.1322	E	E-475
COL739/08	2620	0.5651	NS1	NS1-51
COL739/08	2882	1.699	NS1	NS1-138
COL739/08	2915	0.3838	NS1	NS1-149
COL739/08	3128	0.3706	NS1	NS1-220
COL739/08	3526	1.565	NS2A	NS2A-1
COL739/08	3624	0.5564	NS2A	NS2A-33
COL739/08	3644	1.126	NS2A	NS2A-40
COL739/08	4038	0.3954	NS2A	NS2A-171
COL739/08	4038	0.3345	NS2A	NS2A-171
COL739/08	4040	0.1191	NS2A	NS2A-172
COL739/08	4048	0.1485	NS2A	NS2A-175
COL739/08	4109	1.127	NS2A	NS2A-195
COL739/08	4428	0.3967	NS2B	NS2B-70
COL739/08	4671	0.4796	NS3	NS3-20
COL739/08	4910	0.1604	NS3	NS3-100
COL739/08	4914	0.1584	NS3	NS3-101
COL739/08	5166	0.7145	NS3	NS3-185
COL739/08	5199	0.6133	NS3	NS3-196
COL739/08	5355	0.4072	NS3	NS3-248
COL739/08	5551	0.6436	NS3	NS3-314
COL739/08	5576	0.3427	NS3	NS3-322
COL739/08	5724	0.3461	NS3	NS3-371
COL739/08	5932	0.0908	NS3	NS3-441
COL739/08	5935	0.08803	NS3	NS3-442
COL739/08	5944	0.6368	NS3	NS3-445
COL739/08	6138	0.2779	NS3	NS3-509
COL739/08	6203	0.3693	NS3	NS3-531
COL739/08	6203	3.559	NS3	NS3-531
COL739/08	6236	0.08292	NS3	NS3-542
COL739/08	6239	0.0814	NS3	NS3-543
COL739/08	6240	0.08065	NS3	NS3-543
COL739/08	6241	0.07984	NS3	NS3-544
COL739/08	6256	0.4594	NS3	NS3-549
COL739/08	6263	0.6297	NS3	NS3-551
COL739/08	6465	0.4483	NS3	NS3-618
COL739/08	6628	0.8246	NS4A	NS4A-54
COL739/08	6648	0.2435	NS4A	NS4A-60

COL739/08	6839	0.46	NS4A	NS4A-124
COL739/08	6880	0.1009	NS4A	NS4A-138
COL739/08	6892	0.08274	NS4A	NS4A-142
COL739/08	6901	0.1659	NS4A	NS4A-145
COL739/08	7161	0.4035	NS4B	NS4B-82
COL739/08	7529	0.9798	NS4B	NS4B-205
COL739/08	7547	1.501	NS4B	NS4B-211
COL739/08	7635	0.5422	NS4B	NS4B-240
COL739/08	7636	2.791	NS4B	NS4B-241
COL739/08	7648	0.5289	NS4B	NS4B-245
COL739/08	7651	1.681	NS4B	NS4B-246
COL739/08	7661	14.73	NS4B	NS4B-249
COL739/08	7711	0.636	NS5	NS5-11
COL739/08	8085	0.4678	NS5	NS5-135
COL739/08	8086	24.82	NS5	NS5-136
COL739/08	8117	0.1539	NS5	NS5-146
COL739/08	8183	0.7306	NS5	NS5-168
COL739/08	8197	0.4217	NS5	NS5-173
COL739/08	8198	0.3618	NS5	NS5-173
COL739/08	8666	0.153	NS5	NS5-329
COL739/08	8804	0.3331	NS5	NS5-375
COL739/08	9571	0.5424	NS5	NS5-631
COL739/08	9603	0.1381	NS5	NS5-641
COL739/08	9684	0.6095	NS5	NS5-668
COL739/08	10052	0.1794	NS5	NS5-791
COL739/08	10063	0.2031	NS5	NS5-795
COL739/08	10110	3.926	NS5	NS5-810
COL739/08	10115	0.6105	NS5	NS5-812
COL739/08	10117	1.33	NS5	NS5-813
COL739/08	10120	1.297	NS5	NS5-814
COL739/08	10123	3.434	NS5	NS5-815
COL739/08	10227	0.4167	NS5	NS5-849
COL739/08	10462	0.547	3'UTR	3'UTR-67
COL739/08	10477	1.228	3'UTR	3'UTR-82
COL739/08	10478	1.254	3'UTR	3'UTR-83
COL739/08	10489	0.3116	3'UTR	3'UTR-94
COL928/08	397	0.2724	C	C-101
COL928/08	606	1.065	prM	prM-47
COL928/08	777	41.9	prM	prM-104

COL928/08	928	3.646	prM	prM-155
COL928/08	1320	0.6978	E	E-118
COL928/08	1377	0.3393	E	E-137
COL928/08	1897	0.2934	E	E-311
COL928/08	2025	0.4805	E	E-353
COL928/08	2506	0.2914	NS1	NS1-13
COL928/08	2797	0.3505	NS1	NS1-110
COL928/08	3032	0.3887	NS1	NS1-188
COL928/08	3128	0.522	NS1	NS1-220
COL928/08	3363	1.28	NS1	NS1-298
COL928/08	3494	0.5168	NS1	NS1-342
COL928/08	3526	1.487	NS2A	NS2A-1
COL928/08	3667	0.4603	NS2A	NS2A-48
COL928/08	4109	1.315	NS2A	NS2A-195
COL928/08	4212	0.5446	NS2A	NS2A-229
COL928/08	4408	0.5945	NS2B	NS2B-64
COL928/08	4910	0.1311	NS3	NS3-100
COL928/08	4914	0.1258	NS3	NS3-101
COL928/08	5094	0.2868	NS3	NS3-161
COL928/08	5166	0.7192	NS3	NS3-185
COL928/08	5496	0.4797	NS3	NS3-295
COL928/08	5724	0.3691	NS3	NS3-371
COL928/08	5745	1.081	NS3	NS3-378
COL928/08	5944	2.47	NS3	NS3-445
COL928/08	6182	1.501	NS3	NS3-524
COL928/08	6203	2.957	NS3	NS3-531
COL928/08	6204	0.2798	NS3	NS3-531
COL928/08	6493	0.4168	NS4A	NS4A-9
COL928/08	6615	0.09039	NS4A	NS4A-49
COL928/08	6936	0.5573	NS4B	NS4B-7
COL928/08	6982	0.2768	NS4B	NS4B-23
COL928/08	7547	0.9652	NS4B	NS4B-211
COL928/08	7627	0.4448	NS4B	NS4B-238
COL928/08	7754	0.5193	NS5	NS5-25
COL928/08	7873	1.027	NS5	NS5-65
COL928/08	7960	0.9604	NS5	NS5-94
COL928/08	8088	0.2968	NS5	NS5-136
COL928/08	8189	0.9189	NS5	NS5-170
COL928/08	8325	0.4952	NS5	NS5-215

COL928/08	8413	0.2699	NS5	NS5-245
COL928/08	8508	2.717	NS5	NS5-276
COL928/08	8529	0.7721	NS5	NS5-283
COL928/08	8690	1.678	NS5	NS5-337
COL928/08	8697	0.5351	NS5	NS5-339
COL928/08	8911	0.7364	NS5	NS5-411
COL928/08	9063	0.8554	NS5	NS5-461
COL928/08	9438	0.9236	NS5	NS5-586
COL928/08	9885	0.8919	NS5	NS5-735
COL928/08	10110	2.244	NS5	NS5-810
COL928/08	10117	0.5696	NS5	NS5-813
COL928/08	10117	0.8408	NS5	NS5-813
COL928/08	10120	0.6366	NS5	NS5-814
COL928/08	10123	3.476	NS5	NS5-815
COL928/08	10347	0.3218	NS5	NS5-889
COL928/08	10477	0.6874	3'UTR	3'UTR-82
COL928/08	10852	0.1355	3'UTR	3'UTR-457
COL928/08	10853	0.1378	3'UTR	3'UTR-458
COL9835/08	69	1.315	5'UTR	5'UTR-69
COL9835/08	699	0.5055	prM	prM-78
COL9835/08	732	0.2608	prM	prM-89
COL9835/08	975	0.4074	E	E-3
COL9835/08	1238	0.2749	E	E-91
COL9835/08	1320	1.057	E	E-118
COL9835/08	2075	0.4486	E	E-370
COL9835/08	2202	0.5972	E	E-412
COL9835/08	2295	0.6236	E	E-443
COL9835/08	2352	0.6082	E	E-462
COL9835/08	2795	0.191	NS1	NS1-109
COL9835/08	2797	0.5922	NS1	NS1-110
COL9835/08	2847	0.308	NS1	NS1-126
COL9835/08	3010	0.8003	NS1	NS1-181
COL9835/08	3128	0.4736	NS1	NS1-220
COL9835/08	3526	1.674	NS2A	NS2A-1
COL9835/08	4109	0.952	NS2A	NS2A-195
COL9835/08	4573	0.3859	NS2B	NS2B-119
COL9835/08	5496	0.3089	NS3	NS3-295
COL9835/08	5551	0.7103	NS3	NS3-314
COL9835/08	5617	0.2907	NS3	NS3-336

COL9835/08	5724	0.4362	NS3	NS3-371
COL9835/08	5733	0.4261	NS3	NS3-374
COL9835/08	5927	0.2703	NS3	NS3-439
COL9835/08	6203	0.3458	NS3	NS3-531
COL9835/08	6203	3.038	NS3	NS3-531
COL9835/08	6204	0.5536	NS3	NS3-531
COL9835/08	6579	0.4169	NS4A	NS4A-37
COL9835/08	6628	0.5202	NS4A	NS4A-54
COL9835/08	6839	0.3183	NS4A	NS4A-124
COL9835/08	6983	0.3305	NS4B	NS4B-23
COL9835/08	7267	0.5952	NS4B	NS4B-118
COL9835/08	7527	7.109	NS4B	NS4B-204
COL9835/08	7547	1.047	NS4B	NS4B-211
COL9835/08	7635	0.5245	NS4B	NS4B-240
COL9835/08	7636	1.257	NS4B	NS4B-241
COL9835/08	7643	15.07	NS4B	NS4B-243
COL9835/08	7651	1.42	NS4B	NS4B-246
COL9835/08	7661	15.63	NS4B	NS4B-249
COL9835/08	7960	1.154	NS5	NS5-94
COL9835/08	8690	0.6447	NS5	NS5-337
COL9835/08	9063	0.5354	NS5	NS5-461
COL9835/08	9393	0.3109	NS5	NS5-571
COL9835/08	9465	0.4652	NS5	NS5-595
COL9835/08	10110	2.958	NS5	NS5-810
COL9835/08	10117	1.495	NS5	NS5-813
COL9835/08	10119	29.24	NS5	NS5-813
COL9835/08	10120	1.121	NS5	NS5-814
COL9835/08	10123	3.961	NS5	NS5-815
COL9835/08	10260	1.037	NS5	NS5-860
COL9835/08	10477	1.87	3'UTR	3'UTR-82
COL9835/08	10478	0.5091	3'UTR	3'UTR-83
COL9835/08	10855	0.5443	3'UTR	3'UTR-460

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Yoshimura, T., Helm, B., Briceño, V. F., Harris-Pascal, D., Nicotra, A. B., Williams, E., Ball, M. C., Cooper, E. J., Ernakovich, J. G., Hopping, K. A., Berdanier, A. B., Simpson, R. T., Kachergis, E. J., Steltzer, H., Wallenstein, M. D., LEINGÄRTNER, A., KRAUSS, J., Steffan-Dewenter, I., Lecomte, N., Gauthier, G., Giroux, J.-F., Lessard-Therrien, M., Bolmgren, K., Davies, T. J., Liebezeit, J. R., Gurney, K. E. B., Budde, M., Zack, S., Ward, D., Pickering, C., Green, K., Barros, A. A., Venn, S., Sweet, S. K., Gough, L., Griffin, K. L., Boelman, N. T., Sweet, S. K., Gough, L., Griffin, K. L., Boelman, N. T., Tennant, C. J., Crosby, B. T., Godsey, S. E., Williams, C. M., Henry, H. A. L., Sinclair, B. J., Callaghan, T. V, Jonasson, C., Thierfelder, T., Yang, Z., Hedenås, H., Johansson, M., Molau, U., Van Bogaert, R., Michelsen, A., Olofsson, J., Gwynn-Jones, D., Bokhorst, S., Phoenix, G., Bjerke, J. W., Tømmervik, H., Christensen, T. R., Hanna, E., Koller, E. K., Sloan, V. L., Clark, G. F., Stark, J. S., Johnston, E. B. E. L., Runcie, J. W., Goldsworthy, P. M., Raymond, B., Riddle, M. J., Gauthier, G., Bêty, J., Cadieux, M.-C. M., Legagneux, P., Doiron, M., Chevallier, C., Lai, S., Tarroux, A., Berteaux, D., Høye, T. T., Post, E., Schmidt, N. M., Trøjelsgaard, K., Forchhammer, M. C., Ide, R., Oguma, H., Ornes, S., Varidaki, A., Mitsi, V., Ghose, S., Magida, J., Dias, C., Russo, S. J., Vialou, V., Caldarone, B. J., Carol, A., Nestler, E. J., Zachariou, V., Zimova, M., Oyler, J., Running, S., Abatzoglou, J. T., Paul, M., Robroek, B. J. M., Heijboer, A., Jassey, V. E. J., Hefting, M. M., Rouwenhorst, T. G., Buttler, A., Bragazza, L., Assini, J., Young, K. L., Jamieson, M. A., Trowbridge, A. M., Raffa, K. F., Lindroth, R. L., Kivinen, S., Kaarlejärvi, E., Jylhä, K., Räisänen, J., Marshall, K. E., Sinclair, B. J., Sheriff, M. J., Kenagy, G. J., Richter, M., Lee, T., Toien, O., Kohl, F., Buck, C. L., Barnes, B. M., Iler, A. M., Inouye, D. W., Høye, T. T., Miller-Rushing, A. J., Burkle, L. A., Johnston, E. B. E. L., Bale, J. S., Hayward, S. A. L., Torp, M., Olofsson, J., Witzell, J., Baxter, R., Valéry, L., Cadieux, M.-C. M., Gauthier, G., Walther, G. R., Wipf, S., Rixen, C., Post, E., Forchhammer, M. C., Bret-Harte, M. S., Callaghan, T. V, Christensen, T. R., Elberling, B., Fox, A. D., Gilg, O., Hik, D. S., Høye, T. T., Ims, R. A., Jeppesen, E., Klein, D. R., Madsen, J., McGuire, A. D., Rysgaard, S., Schindler, D. E., Stirling, I., Tamstorf, M. P., Tyler, N. J. C., van der Wal, R., Welker, J., Wookey, P. A., Schmidt, N. M., Aastrup, P., Petrucco-Toffolo, E., Battisti, B., Brown, R., Derksen, C., Wang, L., Mernild, S. H., Liston, G. E., Hasholt, B., Reale, D., McAdam, A. G., Boutin, S., Berteaux, D., Hinkler, J., Pedersen, S. B., Rasch, M., Hansen, B. U., Liston, G. E., Sturm, M., König, M., Sturm, M., Liston, G. E., Ovaskainen, O., Skorokhodova, S., Yakovleva, M., Sukhov, A., Kutenkov, A., Julitta, T., Cremonese, E., Migliavacca, M., Colombo, R., Galvagno, M., Siniscalco, C., Rossini, M., Fava, F., Cogliati, S., Morra, U., Menzel, A., Rautio, M., Dufresne, F., Laurion, I., Bonilla, S., Vincent, W. F., Christoffersen, K. S., Rautio, M., Bolduc, E., Casajus, N., Legagneux, P., Mckinnon, L., Gilchrist, H. G., Leung, M., Morrison, R. I. G., Reid, D., Smith, P. A., Buddle, C. M., Parker, S. M., Huryn, A. D., Museum, H., Lafferty, P. A. K. D., Baiser, B., Buckley, H. L., Gotelli, N. J., Ellison, A. M., Williams, C. M., Henry, H. A. L., Sinclair, B. J., Lbert, K. A. A., Jolla, L., Sciences, P., Fields, D., Road, L., Legagneux, P., Gauthier, G., Lecomte, N., Schmidt, N. M., Reid, D., Cadieux, M.-C. M., Berteaux, D., Bêty, J., Krebs, C. J., Ims, R. A., Yoccoz, N. G., Morrison, R. I. G. & Leroux, S. J. Community-level phenological response to climate change. *Glob. Chang. Biol.* **3,** 33–41 (2013).

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VITA

Daniele Swetnam was born on April 19, 1986 in Ft. Lauderdale. FL. She graduated from Northeast High School in 2004, and she received a Bachelor's degree in biological sciences with minor in chemistry from Florida State university in 2008. While in college, she worked as a laboratory assistant with Frederick Ronquist studying the evolution of Hymenoptera (bees, wasps and ants), and later she also completed an Honor's Thesis Project with David Balkwill for the purpose of identifying novel mutations associated with ciproflaxicin resistance in deep subsurface bacteria. After graduation, she accepted a position at the University of Florida supporting the study of poxvirus pathogenesis with Dick Moyer. Then, in 2010, she joined the threat characterization team at the Nation Biodefense Analysis and Countermeasure Center in Frederick, MD. In 2012, she enrolled in the Graduate School of Biomedical Sciences at the University of Texas Medical Branch and joined the lab of Alan Barrett. Her primary interests are molecular epidemiology and the evolution of viruses, especially in the context of novel environments or hosts. The focus of her dissertation was to characterize the evolution of West Nile Virus in the New World. During graduate school, she also completed two internships with the World Health Organization. Her long-terms goals are to understand the evolutionary mechanisms that facilitate pathogen emergence to improve public health responses.

EDUCATION

B.S., May 2008, Florida State University, Tallahassee, FL

PUBLICATIONS

IN PREPARATION

1. Daniele Swetnam, Debra A. Simmons, Hilda Guzman, Robert B. Tesh, Alan Barrett. Migration of Terrestrial Birds Drives West Nile Virus Circulation in the USA. (In Submission)

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IN PRESS

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Virus, a Model for Orthopoxvirus Infections of Humans. Viruses. 2011 January; 1;3(1):47-62. PMID: 21373379

7. Zupanska AK, Drummond PB, Swetnam DM, Al-Khedery B, Allred DR. Universal primers suitable to assess population dynamics reveal apparent mutually exclusive transcription of the Babesia bovis ves1α gene. Molecular and Biochemical Parasitology. 2009 July; 166 (1), pp. 47-53. PMID: 19428672

REVIEWS

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ABSTRACTS

1. Daniele M. Swetnam, Brian R. Mann, Robert B Tesh, Alan D.T. Barrett. Geospatial Evolution of West Nile Virus Beneath the Consensus Level. 12 July 2015 [Oral Presentation] American Society for Virology Conference, Western University, London, Ontario, Canada

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5. Daniele M. Swetnam, Brian R. Mann, Dreda A. Symonds, Robert B. Tesh and Alan D.T. Barrett. Patterns of West Nile Virus Emergence in the United States. 21 June 2014 [Oral Presentation] American Society for Virology Conference, Colorado State University, Fort Collins, CO.

6. Daniele M. Swetnam, Brian R. Mann, Dreda A. Symonds, Robert B. Tesh and Alan D.T. Barrett. Patterns of West Nile Virus Emergence in the United States. 21 June 2014 [Poster Presentation] IHII/McLaughlin Colloquium, The University of Texas Medical Branch, Galveston, TX

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2016	**Risk Assessment and Surveillance Intern** Mentor: Johannes Schnitzler World Health Organization (WHO), Geneva, Switzerland
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2013	**Student** Quantitative Systems Immunology Summer School, Boston University
2010-2012	**Biological Threat Characterization Research Assistant** National Biodefense Analysis and Countermeasure Center (NBACC)
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2006-2008	**Honors Research Student** Mentor: David Balkwill Florida State University, College of Medicine
2005-2007	**Laboratory Technician** Mentor: Frederick Ronquist Florida State University, School of Computational Science
2003-2004	**Student Volunteer** Florida Atlantic University, College of Science

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2015-2016: **Senate Body Chair and Executive Committee Member**

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- Cultivating Learning and Safe Environments (CLASE) Working Group

2014-2015: **Chair**, Academic Resources and Facilities Committee (SGA)

- Student Health Governance Committee
- Academic Resources Advisory Board

2013-2015: **Senator**, Student Government Association

2013-2014: **Member**, Students Today Alumni Tomorrow Advisory Committee

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2004-2008 **Chair**, Alternative Break Corps, Center for Civic Education

2006- 2007 **Board of Directors**, International Medical Outreach, Center for Civic Education,

SCIENTIFIC SESSIONS ORGANIZED

2013 **Abstract Chai**r, Conference Committee, Houston Global Health Collaborative

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Graduate School (GSBS): 2014 Grant Writing Mini Lecture

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MEMBERSHIP IN SCIENTIFIC SOCIETIES/PROFESSIONAL ORGANIZATIONS:

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HONORS:

2016	Zhou-Geng Endowment Scholarship
2015	Arthur and Dorothy Barrett Scholarship
2015	American Society of Virology Student Travel Grant
2015	Sigma Xi Award for Best Overall Poster
2015	McLaughlin Colloquium Travel Award
2014	Zho Sisters Great Expectations Scholarship
2014	American Society of Virology Student Travel Grant
2013	Quantitative Systems Immunology Summer School Travel Scholarship
2007	Bess Ward Honors Research Grant
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