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Reverse Zoonosis of Pandemic A(H1N1)pdm09 Influenza Viruses at the Swine/Human Interface

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Abstract

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Reverse Zoonosis of Pandemic A(H1N1)pdm09 Influenza Viruses at the Swine/Human Interface

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DEDICATION

This thesis is dedicated to my loving wife, Stephanie, who has unwaveringly loved and supported me through this long, long journey. Thank you for having such great patience with me. I am lucky to call you my wife!

I also want to dedicate this thesis to my parents. Thank you for always encouraging and motivating me to chase my dreams. I love you both!

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ABSTRACT

The 2009 pandemic influenza A(H1N1)pdm09 virus emerged from the swine population. Despite frequent zoonotic events, swine influenza viruses had not become established in humans previously and little is known about host-barriers which prevent swine influenza viruses from efficiently infecting humans. Thus, the emergence of the H1N1pdm09 viruses in humans and the subsequent reverse zoonoses back to swine offered an extremely valuable opportunity to expand current knowledge. We used our active swine farm surveillance platform in combination with viruses from the USDA surveillance program to look for evidence of interspecies transmission of H1N1pdm09 viruses in the US. We found phylogenetic evidence for multiple human to swine transmission events, all of which were transient suggesting that the human adapted viruses of swine origin had lost some fitness for swine. Based on our phylogenetic analysis we selected representative H1N1pdm09 viruses from the tips of swine and human sub-lineages for further study. Intriguingly, we found that after being re-introduced into the swine population, the human H1N1pdm09 viruses rapidly lost replicative fitness in human cells. Together these data provide support for a model where transmission of viruses from human to swine leads to rapid adaptation for the swine host which comes at the expense of optimal fitness for human.

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CHAPTER 1. INTRODUCTION

Influenza A

Influenza viruses are members of the *Orthomyxoviridae* family. They are currently classified into three distinct antigenic classes: A, B, and C.[1] Type A & B viruses have a negative-sense RNA (-ssRNA) genome comprised of eight gene segments which code for eleven major proteins; while type C viruses have seven gene segments.[1] Influenza A viruses are divided into subtypes based on the surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Currently 18 HA[2] (H1 – H18) and 11 NA (N1 – N11) subtypes have been identified. Influenza A viruses are subtyped based on which HA and NA are present on the surface of the virus (e.g. H1N1, H5N1).

Viral Structure and Protein Components

The RNA genome of Influenza viruses contains the following gene segments: 1-polymerase basic 2 (PB2), 2-polymerase basic 1 (PB1), 3-polymerase acidic (PA), 4-hemagglutinin (HA), 5-nucleoprotein (NP), 6-neruaminidase (NA), 7-matirx (M), and 8-nonstructural (NS). Some gene segments code for multiple proteins utilizing multiple open reading frames: for example, PB1 can code for both PB1 and PB1-F2, M can code for M1 and M2 proteins, NS can code for NS1 and NEP (**Figure 1-1**). The HA protein is responsible for binding the virus to the proper host target cell through specific sialic acid interactions.[3] Human influenza viruses preferentially bind to α2-6 linked sialic acid while avian influenza viruses preferentially bind to α2-3 sialic acid. Once the virus has infected a host cell through processes of endocytosis and pH-dependent release of nucleoprotein encapsulated viral RNA (RNPs) into the cytoplasm, the RNPs are transported to the nucleus where the polymerase complex, made up of PB2, PB1, and PA, begin replicating the viral genome with the aid of host cell machinery (see below for more detail). The M gene codes for M1 and M2 proteins.[4] M1 is a structural component of the virion and lines the inside of the virion. M2 is an ion channel that acts to acidify the virus during replication.[5] After replication, NA allows the new progeny viruses to bud off from the host cell by cleaving HA-sialic acid bonds through its sialidase activity.

Viral Replication

Influenza A virus replication starts by binding of HA to the correct sialic acid receptor on the host cell surface.[6] As detailed, and although a simplification, typically, human influenza viruses bind to sialic acids with α2-6 linkage while avian influenza viruses bind to sialic acid with α2-3 linkage.[1] The entire Influenza virus is then internalized via clathrin receptor mediated endocytosis into the host cell.[7] The increasingly acidic environment inside the endosome causes the HA protein to undergo a conformational change, releasing the fusion peptide which leads to fusion of virus and cell membranes.[8] The acidification of the virion interior releases RNPs from M1 binding

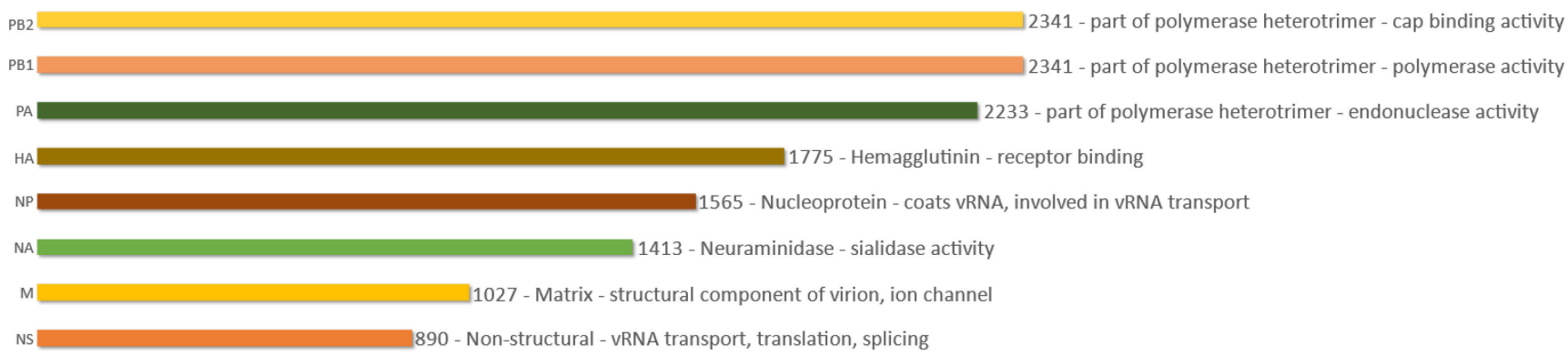

Figure 1-1. ssRNA Genome of Influenza A Viruses.

and subsequently delivers them into the cell cytoplasm.[7] RNPs are transported to the nucleus where transcription and replication of the vRNAs occurs in the host cell nucleus. vRNAs are the source for both species of positive sense RNAs; mRNA and complementary RNA (cRNA).[8] Capped mRNA, generated through a host cap-snatching mechanism utilizing PB2 cap binding and PA endonuclease activities, is exported to the cytoplasm for generation of additional viral proteins. cRNA acts as full-length template for progeny vRNA molecules. After replication and delivery of viral components to the host cell membrane, virions bud off in a M2-dependent manner.[9] NA cleaves the progeny virus from the host infected cell. Lastly, new viral HA proteins must undergo proteolytic cleavage for subsequent fusion to occur upon entry into a new cell. This process is achieved using host cell proteases which play a role in determining the anatomical location in which a virus can replicate with most viruses utilizing trypsin-like proteases confined to the respiratory tract.

Mechanisms of Antigenic/Genetic Diversity

Influenza viruses continue to pose threats to veterinary and human health despite the availability of vaccines.[10] A major reason for this is the ability of the virus to evade existing immunity through antigenic evolution.[11] Influenza viruses can change into distinct antigenic variants via two primary mechanisms: antigenic drift and antigenic shift. Antigenic drift is a slow, gradual accumulation of amino acid (AA) changes introduced by the infidelity of the viral polymerase during replication. By chance some of these mutations can occur in antigenically important sites on HA or NA (the two major antigenic proteins of the influenza virus).[12] In the face of existing immunity these variants are rapidly selected for, eventually leading to an antigenically distinct virus. Antigenic shift, however, is a rapid and more sudden process driven by the acquisition of completely new gene segments during replication. Antigenic shift can occur when two different Influenza viruses bind to and replicate in one single cell. During replication it is possible for gene segments from one virus to combine with gene segments from the other virus thereby producing a new virus called a reassortant[13] (**Figure 1-2**). This mechanism is what led to the creation of the 2009 A(H1N1)pdm09 pandemic virus. The 2009 virus was created through at least three independent and temporally distinct reassortant events involving swine and avian viruses.

Swine as Mixing Vessel

Unlike humans and most avian species, swine have both α2-6 and α2-3 sialic acid residues in approximately equal proportion along their respiratory tract[14] (**Figure 1-3**). Having both types of sialic acid theoretically allows a wider array of influenza viruses to infect the pig population than the human population. As mentioned in the section above, if an avian influenza virus and a human or swine virus were replicating in the same cell, there is the potential to create an array of reassortants, some of which that may have unique abilities to transmit and cause disease in humans. One of the worst influenza pandemics on record was the 1918 H1N1 pandemic. This pandemic originated from a

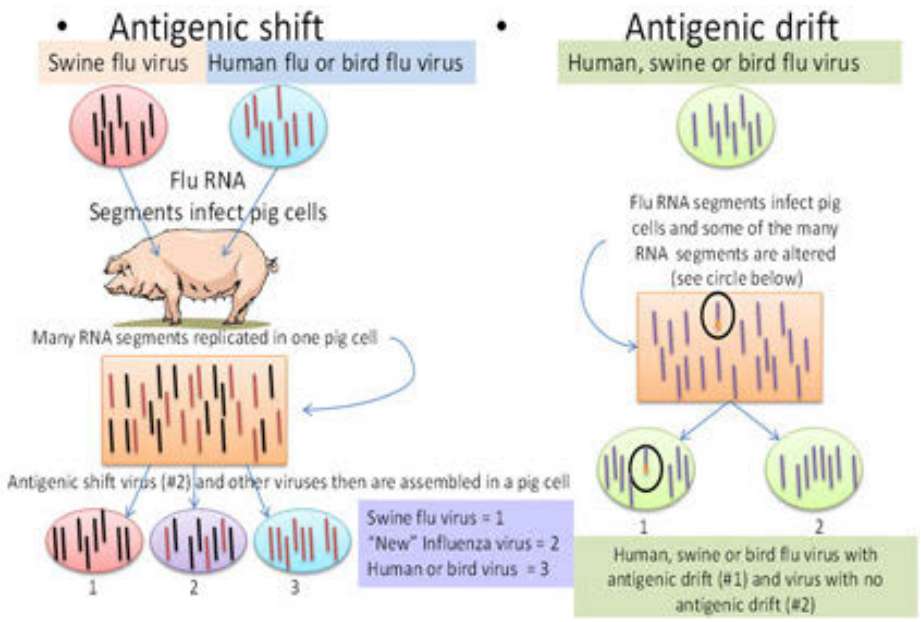

Figure 1-2. Mechanisms of Genetic Diversity in Influenza Viruses.

Reprinted with permission from AAAS. Garten, R.J., et al., *Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans.* Science, 2009. **325**(5937): p. 197-201.[13]

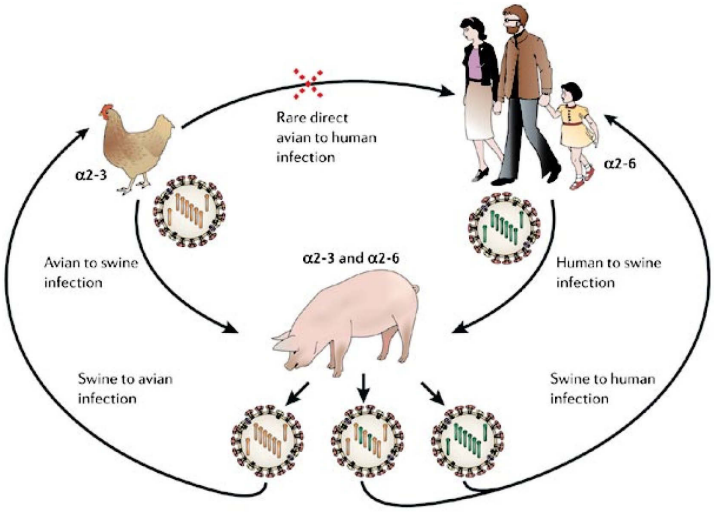

Figure 1-3. Sialic Acid Linkages in Swine, Human, and Avian Hosts.

Reprinted with permission from Springer Nature. Stevens, J., et al., *Glycan microarray technologies: tools to survey host specificity of influenza viruses.* Nature Reviews Microbiology, 2006. **4**(11): p. 857-864.[14]

combination of human, swine, and avian influenza viruses. It is thought that multiple reassortment events led to the creation of the 1918 pandemic virus. In the case of the 2009 H1N1pdm09 virus, the final reassortment event between swine viruses of the Eurasian and American lineages led to a virus that has transmission properties not inherent in either parental virus.[15,16]

Seasonal Influenza Outbreaks

Seasonal influenza outbreaks occur every year and vary in timing based on geographical location. For the United States, the typical flu season runs from November to April. The CDC estimates that in the US alone between 12,000 and 56,000 deaths occur annually from influenza infection.[17] The virulence, morbidity, and mortality depend partially on the virus itself but also the pre-existing immunity of the population. Influenza infections typically impact two age groups most severely: the young and the old. Immunocompromised patients also face more severe infections due to their weakened immune response. The strains that circulate seasonally are H1N1, H3N2, and B viruses, although it is typical for only one or two to be dominant in a given region in a given season.[18] For this reason and our inability to predict which strain might dominate, the seasonal vaccine typically includes one representative from each strain above. The WHO hosts an influenza vaccine composition meeting (VCM) biannually where data from influenza labs around the world is analyzed to determine the optimal combination of strains to include in the seasonal vaccine.

Pandemic Potential of Influenza Viruses

Pandemic influenza is one of the largest infectious disease threats to the human population.[19] Pandemics occur when a novel influenza A virus enters the human population and spreads; such events have the potential to cause catastrophic disease.[20] Two scenarios must be present for a pandemic to emerge. First, the human population must have low overall immunity to the virus to aid in its spread through communities. Antigenic shift is a major factor in the genesis of pandemic influenza viruses. If a reassortant virus emerges with a new gene combination than the population has previously been exposed to, there will be little population immunity to that virus.[21] Additionally, the virus must be able to transmit efficiently from human to human.[22] The catastrophic potential of influenza pandemics is highlighted by the 1918 Spanish influenza pandemic that swept the globe, infecting 25-40% of the world's population[23] and killing 20-100 million people.[24] Influenza pandemics again emerged in 1957 and in 1968, each killing an estimated 1 million people during their first waves.

Host Range Determinants

Interspecies transmission is a central component of influenza ecology. While there have been a number of documented interspecies transmission events, influenza

viruses typically have defined host ranges with transmission events the exception rather than the rule. It is also clear that the virologic markers that regulate zoonotic infection are different than those that regulate subsequent human-to-human spread.[25] Zoonosis occurs when an influenza virus transmits from animals to humans. Reverse zoonosis occurs when an influenza virus transmits from humans to animals.[26] Interspecies transmission, although rare, can also lead to pandemic viruses[27] with zoonotic events mostly linked to birds and pigs. Transmission from birds to humans has remained confined to isolated cases in situations where individuals came into close contact with the infected birds.[28] Swine to human transmission occurs slightly more often and was partially responsible for initiating the 2009 H1N1 pandemic.

Several molecular determinants of host range specificity have previously been identified.[28] One of the largest single amino acid residues that controls host range is the polymerase (PB2) 627K.[29] This PB2 position plays a key role in the overall host-associated genetic signature. A glutamic acid (E) is present in this position most avian isolates; alternatively, a lysine (K) at this position can facilitate a virus of avian origin to replicate in mammalian cells and increase pathogenicity in mice.[30] Another key determinant of host range is the HA protein. Specific amino acid substitutions within the receptor-binding site of HA can shift the receptor preference from α2-3 to α2-6 sialic acid (**Figure 1-4**). This receptor binding preference dictates where the virus will ultimately replicate in the host thereby also affecting transmission likelihood.[3] For instance, human influenza viruses typically bind to α2-6 linkages which are present in the upper respiratory tract of humans.[31] This makes transmission occur more readily than a virus that binds to α2-3 linkages which are more prevalent in the lower respiratory tract. The polymerase protein PA has also previously been shown to be a determinant of host range.[32] Residues T85I, G186S and L336M have all been identified as host-associated signatures.[32] One specific amino acid in the PB1 protein, AA 375, has previously been identified as a host-range signature.[33] Most avian viruses have an asparagine (N) at this position, whereas most human influenza viruses have a serine (S).[28] Although not an exhaustive list of all identified host-range signatures, these are some of the critically important host-range determinants. For an extensive overview of many previously identified host-range signatures, please refer to Cauldwell, Long [28].

2009 H1N1 Influenza Pandemic

The 2009 H1N1pdm09 pandemic was caused by a novel influenza virus which emerged from the swine population with the direct ability to infect and transmit in humans (reassortant and zoonosis event).[34] This pandemic virus emerged in Mexico in early 2009 (although it was first detected in Texas and California) but soon spread to the US and across the globe.[35] The genome contained a unique combination of gene segments from presently circulating swine viruses[13] (**Figure 1-5**). PB2, PB1, PA, HA, NP, and NS segments all originated from the triple reassortant/classical swine lineage of swine virus that had been widespread in the Americas and Asia.[36] NA and M segments, however, originated from Eurasian swine lineage viruses that had previously only been detected in Asia and Europe. This virus combined gene segments from both lineages

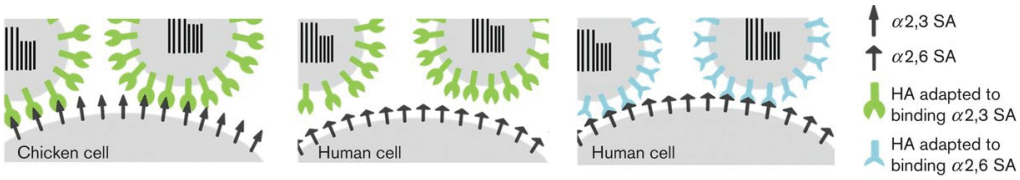

Figure 1-4. Host Range Determinants.

Reprinted with permission. Cauldwell, A.V., et al., *Viral determinants of influenza A virus host range.* Journal of General Virology, 2014. **95**(6): p. 1193-1210.[28]

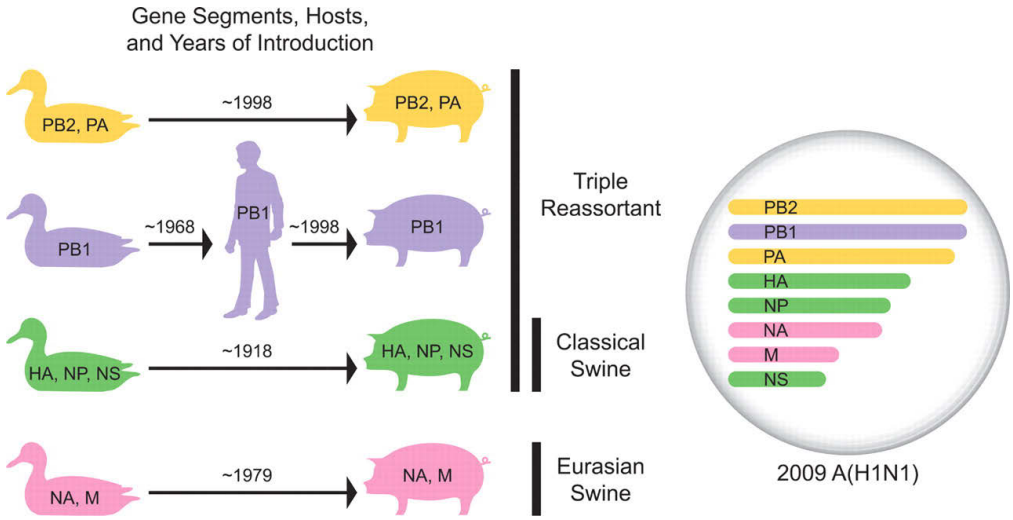

Figure 1-5. Origins of the 2009 H1N1 Pandemic Virus.

Reprinted with permission from AAAS. Garten, R.J., et al., *Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans.* Science, 2009. **325**(5937): p. 197-201.[13]

thereby producing an antigenically distinct virus via reassortment (antigenic shift). This reassortant event is thought to have occurred before the pandemic, possibly a number of years prior, but did not transmit to humans until April 2009 despite presumed circulation in the swine population.[37] Due to a lack of swine influenza surveillance and subsequent virus sequencing from this timeframe (and prior) in Mexico and countries in Central and South America, it is not known exactly how long this virus was circulating prior to the first zoonosis event. From December 2005 – January 2009 (pre-pandemic) there were only 12 reported cases of humans directly infected with swine influenza viruses. By mid-April of 2009 however, it was clear that a novel virus had emerged from the swine population with the ability for human transmission. On June 11, 2009 the WHO raised the threat level- signifying a global pandemic was occurring. This was the first influenza pandemic of the 21st century.

Luckily, these viruses had low pathogenesis and only lead to approximately 77,000 cases resulting in 332 deaths worldwide. Subsequent genetic analysis of these viruses determined very few of the molecular markers predicted to facilitate human transmission or increase virulence were present in the viral genomes of these viruses.

Influenza Phylogeny

Although several methods exist to study the phylogeny of influenza viruses, Bayesian Evolutionary Analysis Sampling Trees (BEAST)[38] is one of the most robust. Unlike traditional, nucleotide-only analyses, BEAST factors in a multitude of parameters to determine phylogenetic relationships. BEAST trees can be created based on time, geographical location, host, or any other parameter of interest. Bayesian analyses rely on Markov Chain Monte Carlo (MCMC) so that every tree generated is weighted based on posterior probability. BEAST is designed to create rooted, time-measured phylogenetic trees based on either strict or relaxed molecular clock models. It can be used to reconstruct prior phylogenies but is also a framework for testing evolutionary hypotheses.

Scope of Thesis and Research Aims

Host range specificity between human and swine influenza viruses is dictated via specific, identifiable nucleotide and amino acid residues that likely change rapidly during replication and transmission in each respective host. Despite the similarity of key host range markers between human and swine viruses as well as relatively frequent zoonotic events, it is clear that swine viruses require further changes to successfully establish in humans and that unidentified swine and human host range determinants exist. The 2009 pandemic has provided a setting with which to try and identify these markers. I propose the following Specific Aims to do so.

Specific Aim 1

To conduct a thorough phylogenetic analysis of human and swine influenza viruses.

Specific Aim 1 hypothesis: There are distinct phylogenetic differences between H1N1pdm09 viruses of human and swine origin, caused by genetic changes associated with their host range specificity.

Specific Aim 2

To determine the phenotypic differences between human and swine influenza viruses.

Specific Aim 2 hypothesis: There are identifiable phenotypic differences between human and swine influenza viruses that can be observed *in vitro* despite identical sialic acid linkage preference.

CHAPTER 2. MATERIALS AND METHODS

Computational Methodology

Data was obtained from the NIAID Influenza Research Database (IRD) [Zhang Y, et al. (2017)] through the web site at http://www.fludb.org. Complete influenza A genomes were restricted to only include 2009 pH1N1 sequences. The timeframe downloaded was from 2009-2018 (downloaded updated dataset on 10/11/2018). Refer to **Appendix B** for a representative dataset. Repetitive sequences were excluded so as to allow efficient data analysis without unnecessary redundancy. Genomes downloaded were both human and swine pH1N1 influenza viruses. Gene-by-gene a master datafile was generated in Microsoft Excel that contained all the influenza sequences for that gene. A random number generator in Excel was used to reduce the database size. 20% of each year's sequences were kept thus producing the final FASTA file necessary to align and build the preliminary phylogenetic tree.

Prior to generation of the preliminary tree, the sequences were aligned in MUltiple Sequence Comparison by Log- Expectation (or MUSCLE for short).[39] MUSCLE was called as follows:

```
1. /Users/daniel/MUSCLE/muscle3.8.31_i86darwin64 -in
   <input_filename>.fasta  -out <output_filename_Aligned>.fasta
```

The preliminary tree was produced in RAxML using the following call code:

```
1. /Applications/raxml/raxmlHPC-SSE3-Mac -s <input_filename>.fasta -
   n <output_filename>.tree -m GTRGAMMA -p 123 -# 3
```

Clockliness of the tree was assessed and verified using TempEst v.1.5.1. Precise date calculations were included in the input FASTA file to allow for TempEst and BEAST to use time data as well as sequence data in evaluating and building the tree. A best-fitting root was calculated in TempEst to help identify any outliers present in the dataset. If any clear outliers were found, they were excluded from the downstream analyses and a new preliminary tree was produced in RAxML. Again, the best fitting root was calculated and if the date now matched what was expected (a root date of ~2008-2009) then the dataset was deemed ready for BEAST.

The FASTA file used to generate the preliminary tree was imported into BEAUti v.1.10.1. Tip dates were parsed from the input FASTA file. Traits were added to identify the host of each sequence as either human or swine. A GTR substitution model was used and the site heterogeneity model was chosen as Gamma + Invariant Sites.[40] The best-fitting clock estimate was determined to be the uncorrelated relaxed clock model. The best tree model was determined to be Bayesian Skyride.[41,42] A random starting tree was calculated by BEAST rather than leading BEAST with a user-specified starting tree. The

chain length was specified as 150,000,000 with a screen echo of 15,000 and a write to log every 15,000. Finally, the BEAST XML file was generated.

BEAST was run from the command line as follows:

```
1. java -Xmx8g -jar beast.jar -beagle -beagle_SSE -beagle_instances
   2 -overwrite <input_BEAST_filename>.xml
```

The log was monitored over the course of several days to verify the run was proceeding as expected. Multiple runs of 150,000,000 chains were combined to generate the final maximum clade credibility (MCC) tree. In the case of both HA and NA the additional step of creating an empirical dataset and tree was performed to map the amino acid mutations identified in earlier work onto the tree. The specifics involved changing the XML code to prevent overall tree computation and focus solely on the discrete traits provided (i.e. which amino acid was present in the sequence). See **Appendix C** for relevant code snippets.

Biological Methodology

Surveillance Swab Collection

Our lab at St. Jude Children's Research Hospital (SJCRH) had established a collaboration with swine farmers in Georgia, Illinois, Oklahoma, and Nebraska. These farms collected nasal swabs in Phosphate Buffered Saline (PBS) and antibiotics from 30-60 pigs each month and sent them to SJCRH for further testing by real-time PCR (rtPCR).

Surveillance Sequencing

Next generation sequencing technologies (illumina and Roche) have made it possible to sequence large numbers of samples with a short and simple method. Briefly, RNA was extracted from the swab samples sent to SJCRH using the KingFisher (ThermoFisher Scientific Inc., Worcester, MA, USA). The extracted RNA was tested for the presence of Influenza M gene via rtPCR using CDC approved primers and probes. All positive samples were sequenced using illumina MiSeq technology. The RNA from positive swab samples was converted to DNA using the SuperScript III RT-PCR kit (LifeTechnologies, Grand Island, NY, USA). DNA was then enzymatically fragmented in a process called tagmentation. Transposases, which include adapter sequences, were added to the sample DNA. The transposases both fragmented the DNA as well as added adapter tags to each sample in preparation for the addition of unique barcodes (or indices). Next, a PCR reaction added sample specific barcodes (a 12-nucleotide sequence) to the adapter tag making it possible to identify each sample after sequencing. Finally, the DNA-Adapter-Barcode construct was PCR purified using a MinElute PCR

Purification Kit© (QIAGEN / Germantown, MD). Sequencing was conducted on the illumina MiSeq platform at the Hartwell Center for Bioinformatics and Biotechnology at SJCRH.

Stock Virus Growth

All stock viruses (**Table 2-1**) used in this study were grown in Madin Darby Canine Kidney (MDCK) cells. Each virus was diluted to 1:100 in infection media (Gibco Minimum Essential Media + 1% vitamins + 1% antibiotics + 1% glutamine + 5% BSA). The infection media was supplemented with TPCK trypsin (ThermoFisher Scientific catalog 20233) at a concentration of 1:2000 as MDCK cells do not produce an endogenous protease. Viruses were incubated in a flask containing MDCK cells for 1 hour at 37ºC / 5% CO_2. After 1 hour, the virus dilution inoculum was removed, and the cells were washed twice with PBS. New infection media containing TPCK trypsin was added to the flask. Cells were incubated at 37ºC / 5% CO_2 for two days and virus was harvested on day 2. Titers were determined as $\log_{10}$ $TCID_{50}$/mL using the Reed and Muench method.[43]

Growth Kinetics

Normal human bronchial epithelial (NHBE) cells (EpiAirway kit (AIR-100) MatTek Corp) were grown on 6.5-mm-diameter inserts and placed above 1 mL of growth medium (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with epidermal growth factors, gentamicin 5 µg/ml, Amphotericin B 0.25 µg/ml, phenol red) in a 6-well tissue culture plate. The cells are grown such that the apical surfaces are exposed to air while the basal surfaces are exposed to the growth medium. NHBE cells were washed with sterile PBS to remove mucus secretions from the apical surface prior to infection. MOI was calculated by counting trypsinized cells using the Countess cell counter (Invitrogen catalog number C10227). Viruses were diluted accordingly to reach an MOI of 0.01. Cells were then inoculated on the apical side with each virus dilution at 37ºC. After a 1-hour incubation, the inoculum was removed. Exogenous trypsin addition was unnecessary as NHBE cells secrete a protease similar to trypsin that allows for hemagglutinin cleavage. Progeny viruses released into the apical compartment of NHBE cells were harvested at 24, 48, and 72 hours post-infection by the addition and collection of 150 µl of medium to the apical surface. The media was allowed to equilibrate for 30 min at 37°C before it was collected and stored at -80ºC for titration via $TCID_{50}$ in MDCK cells. Titers were determined as $\log_{10}$ $TCID_{50}$/mL using the Reed and Muench method.[43]

Swine trachea explants were derived using previously described methods.[44] Tracheal explants produced by punch biopsies were cultured in bronchial epithelial cell basal medium (BEBM) on transwell inserts (Corning, Tewksbury, MA, USA). Prior to infection, explants were washed four times with sterile PBS. Three explants were randomly selected for cell counting using the Countess cell counter (Invitrogen catalog number C10227) and averaged to calculate the MOI for each virus. Explants were then

Table 2-1. Swine Viruses Used in Growth Kinetics Experiments.

Virus	Accession Number
A/swine/Illinois/A01047715/2010	CY114668
A/swine/Illinois/10-001551-2/2009	GU984402
A/swine/Illinois/21IL1207/2009	SJCRH sequence
A/swine/Illinois/35572/2009	GU984390
A/swine/Illinois/A01049981/2011	JX045997
A/swine/Indiana/30IN0428/2010	SJCRH sequence
A/swine/Iowa/21IA1207/2010	SJCRH sequence
A/swine/Iowa/44837-1/2009	HQ424885
A/swine/Iowa/A01049128/2010	JF833337
A/swine/Iowa/A01049980/2011	JN863540
A/swine/Iowa/A01202854/2011	JX092451
A/swine/Minnesota/130A/2009	HQ840306
A/swine/Minnesota/25618/2011	JN193422
A/swine/Minnesota/36MN1026/2011	SJCRH sequence
A/swine/Minnesota/36MN2142/2012	SJCRH sequence
A/swine/Minnesota/54354/2010	HQ622586
A/swine/Minnesota/8762-2/2010	GU984417
A/swine/Missouri/15534/2010	HM219624
A/swine/North Carolina/38/2009	JQ638657
A/swine/North Carolina/A01049174/2010	JF833344
A/swine/Oregon/A00700068/2011	JN193425
A/swine/Texas/A01202511/2011	JX092296
A/Tennessee/F2090/2011	SJCRH sequence
A/Tennessee/F3004/2010	SJCRH sequence
A/Tennessee/F3013/2012	SJCRH sequence

incubated for 1 hour, in triplicate per virus, with an MOI of 0.01. Following the incubation period, explants were washed in triplicate with sterile PBS. At 24, 48, 72 hours post-infection (hpi), 300 µL of infection media was added to the apical chamber of all trachea explants. The media was allowed to equilibrate for 30 min at 37°C before it was collected and stored at -80ºC for titration via $TCID_{50}$ in MDCK cells. Titers were determined as $\log_{10}$ $TCID_{50}$/mL using the Reed and Muench method.[43]

Virus Titration ($TCID_{50}$)

All of the growth kinetics time points collected previously were stored at -80°C until viral $TCID_{50}$ titers could be determined. All $TCID_{50}$ titers were determined using MDCK cells by making 10-fold dilutions of each time point and infecting a single well of a 96 well plate with one dilution. Each time point was measured in quadruplicate using 0.5% (v/v) turkey red blood cells in PBS.

CHAPTER 3. RESULTS

Swine Surveillance Overview

In an effort to enhance our computational power, we utilized an existing swine surveillance program to increase the number of sequences from H1N1pdm09 viruses in swine. The swab study conducted in collaboration with Lowe Consulting Ltd. sent 14,954 swabs to SJCRH for study over a one-year period. The epidemiologic aspects of this program have been previously published.[45] From these nearly 15,000 swabs, 741 (~5.0%) tested positive for influenza M gene via rtPCR. Approximately 230 were sequenced and deposited into IRD and those that were H1N1pdm09 viruses were added to the dataset for this thesis.[45]

Bayesian Phylogeny

The primary purpose of our phylogenetic analysis was to identify regions of a combined tree that suggested interspecies transmission events where swine H1N1pdm09 viruses transmitted to humans and vice-versa. This was achieved by producing Bayesian phylogenies based on available human and swine sequences in public databases supplemented with additional swine virus sequences from our own surveillance. The phylogenies provided by BEAST gave us a unique perspective on the genetic diversity present in these influenza viruses. We were able to note several reverse zoonotic transmission events where the phylogenies strongly supported the likelihood that a human virus was re-introduced back into the swine population (**Figures 3-1** through **3-4)**. Transmission events like these are a key component of producing potentially pandemic influenza viruses. Within the scope of this thesis, it appears that most reverse zoonotic events are transient, and the viruses do not become enzootic within swine. These data suggest that the human-adapted viruses have a reduced fitness for swine, consistent with our hypotheses. Similar findings have also previously been described.[46] These transient events can be observed on the phylogenetic trees where a swine virus (green) appears with a red root node, signifying the original (ancestor) virus was from a human host. We identified approximately twenty reverse zoonotic events during our analyses of the HA gene. The majority of these events seem to be single human-to-swine transmission events that did not transmit to other swine. Of course, there is a chance that more transmission occurred than we are able to detect due to a lack of sensitivity of swine surveillance. Each reverse zoonotic event that we identified lasted only for one season/year and did not establish well enough to infect or transmit for longer periods. We were unable to find any phylogenetic evidence for swine-to-human (zoonotic) transmission.

Over time there was genetic drift in the 2009 A(H1N1)pdm09 pandemic virus genes as is to be expected for influenza viruses. Interestingly, this drift was limited in the first couple of years of the circulation of the virus with a marked increase from 2011

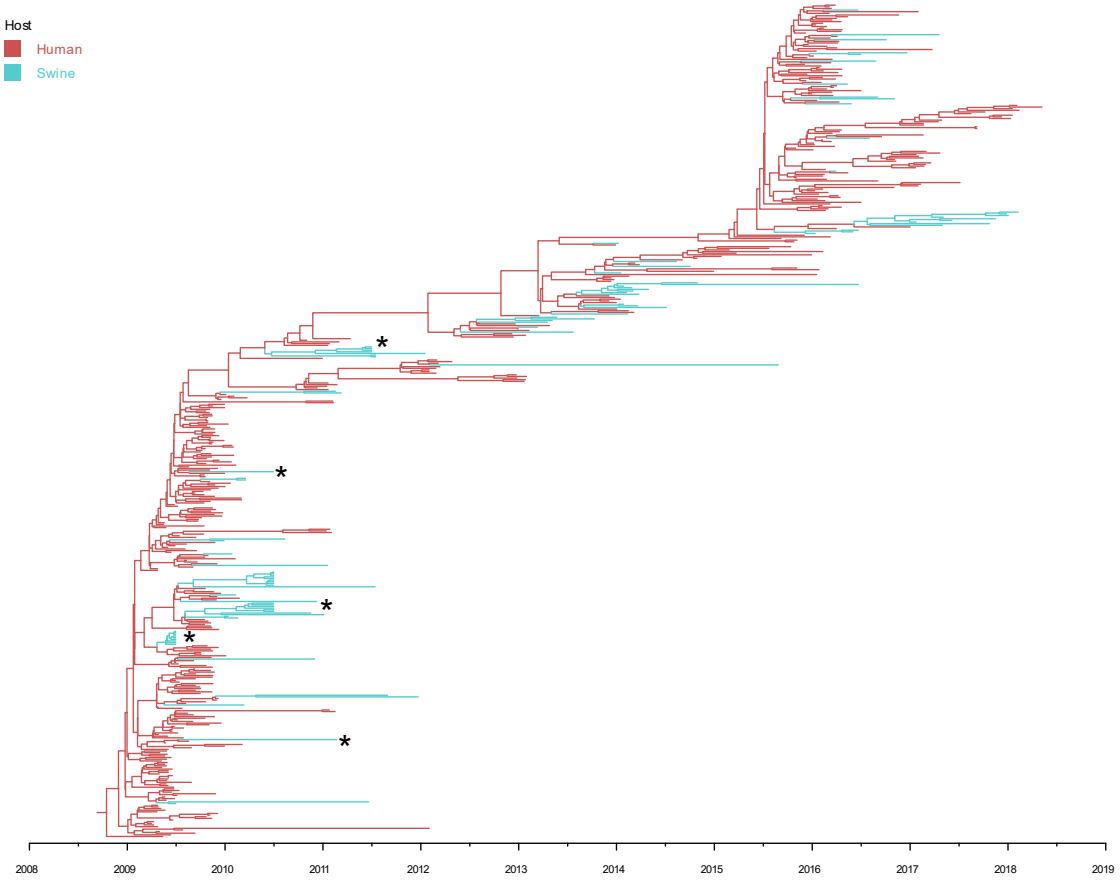

Figure 3-1. Phylogenetic BEAST Tree for HA.

Human sequences are colored in red and swine sequences are colored in green. Viruses used in growth characterization experiments are denoted with an asterisk (*). Diversity is present amongst both the swine and human sequences. There are several introduction events where humans introduce an influenza virus back into swine (reverse zoonosis).

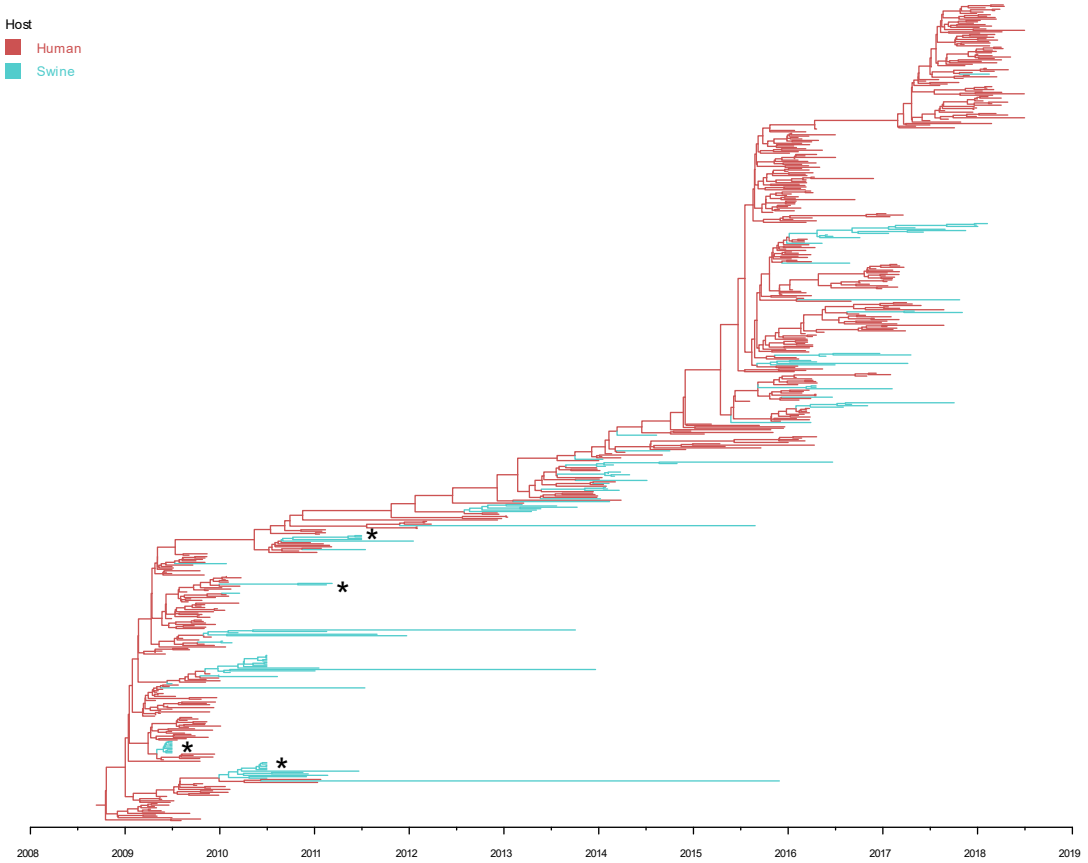

Figure 3-2. Phylogenetic BEAST Tree for NA.

Human sequences are colored in red and swine sequences are colored in green. Viruses used in growth characterization experiments are denoted with an asterisk (*). Diversity is present amongst both the swine and human sequences. There are several introduction events where humans introduce an influenza virus back into swine (reverse zoonosis).

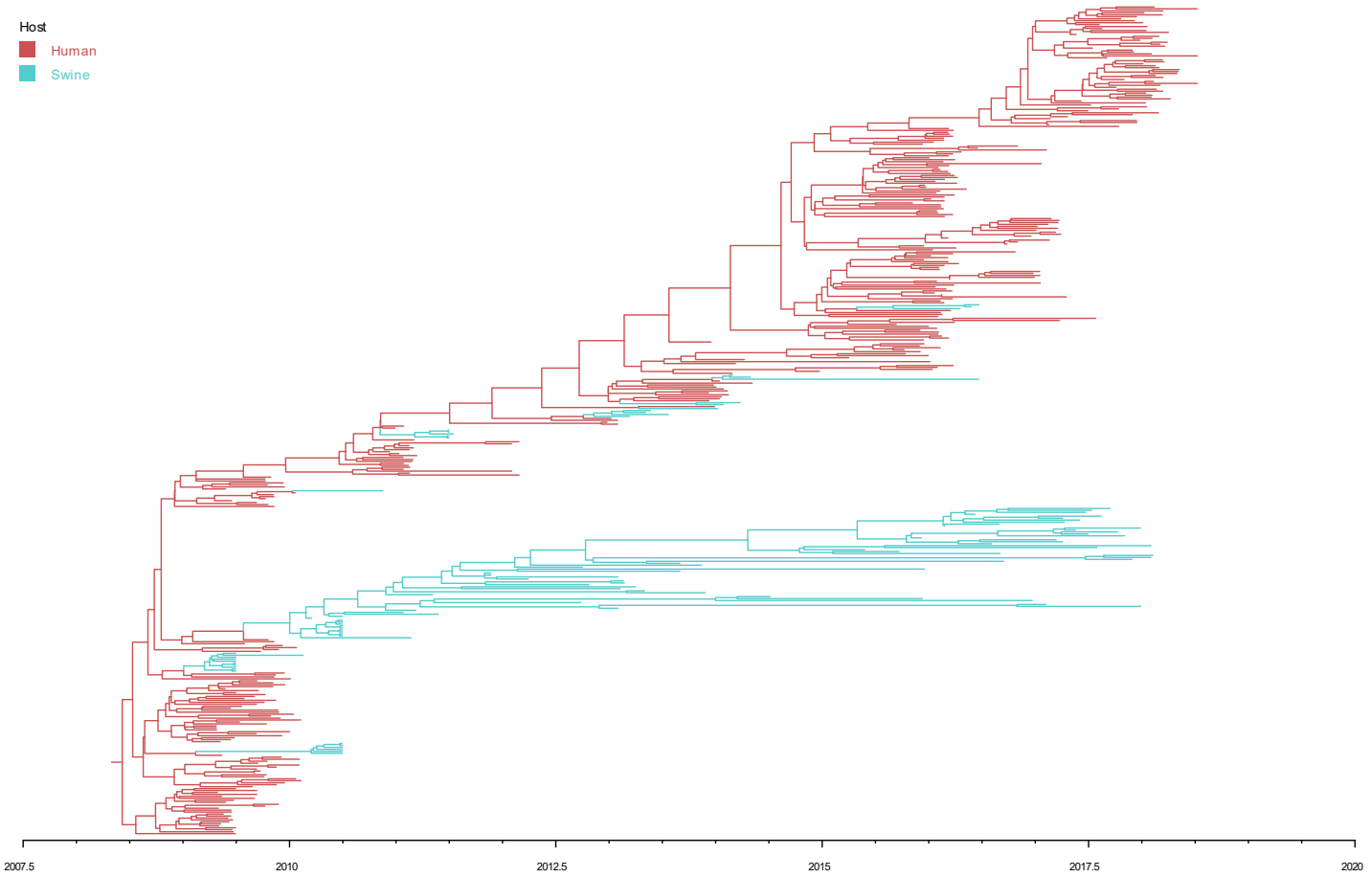

Figure 3-3. Phylogenetic BEAST Tree for M.

Human sequences are colored in red and swine sequences are colored in green. Diversity is present amongst both the swine and human sequences. There are several introduction events where humans introduce an influenza virus back into swine (reverse zoonosis).

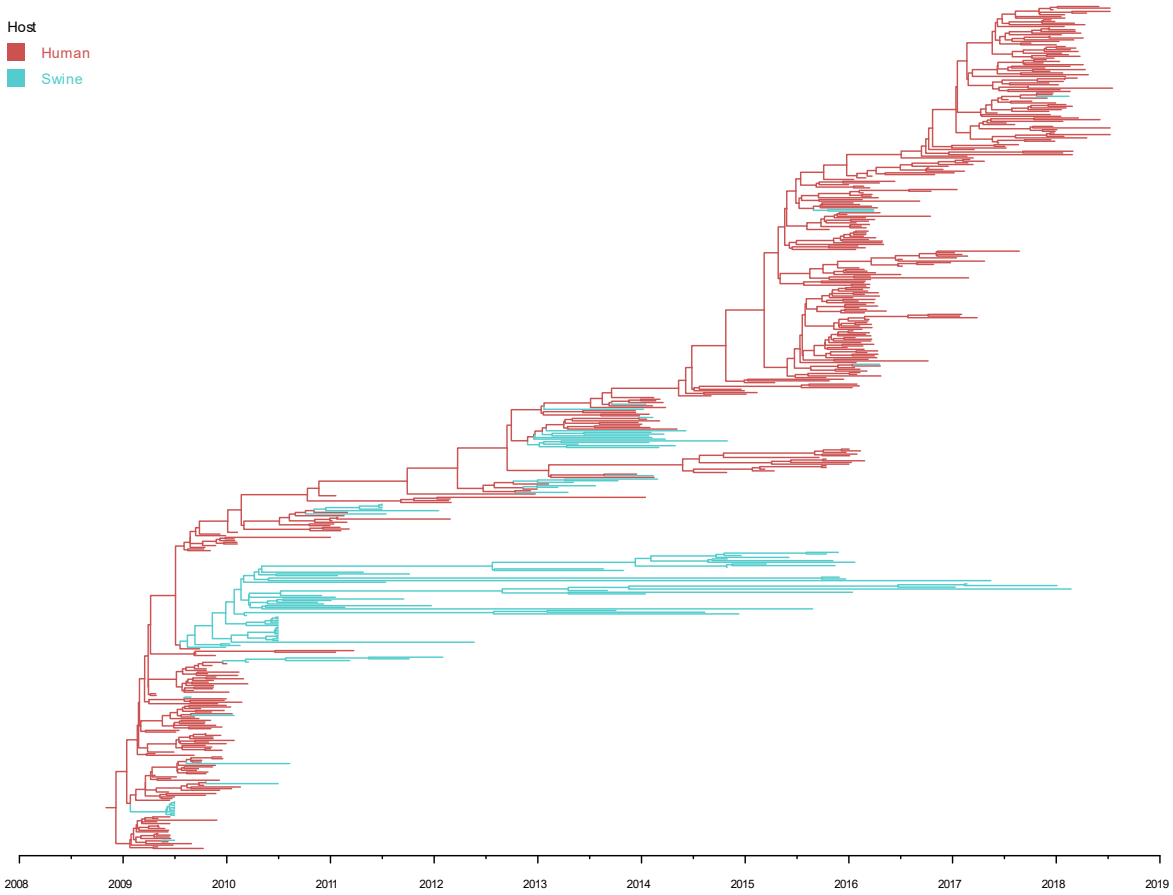

Figure 3-4. Phylogenetic BEAST Tree for NP.

Human sequences are colored in red and swine sequences are colored in green. Diversity is present amongst both the swine and human sequences. There are several introduction events where humans introduce an influenza virus back into swine (reverse zoonosis).

onwards. Although speculation, it is probably that this was due to an accumulation of human population immunity to the virus with many naïve hosts available in the first two years (2009-2011). There were no obvious temporal differences in the number of reverse zoonoses events with human-to-swine transmission events apparent throughout the HA and NA trees.

Growth Kinetics

To test our hypothesis that there are identifiable phenotypic differences between human and swine H1N1pdm09 viruses, we initially selected three human and three swine pandemic viruses to test in cells of swine and human origin. The purpose of this was to determine if growth and transmission (albeit limited for swine) in swine or human populations influenced viral replication in cells from these respective hosts. The initial viruses chosen for growth kinetic experiments were essentially random based on easily obtainable viruses at SJCRH. The data from this first experiment is summarized in (**Figure 3-5**). Each virus was inoculated at a low MOI (0.01) onto NHBE cells present on Transwell inserts. While all six viruses were able to grow in the NHBE cells, we detected two different phenotypes. The human viruses grew to higher titers than the swine viruses (average human titer 7.39, average swine titer 5.37). Statistical analysis using the Holm-Sidak method t test[47], with alpha = 0.05 revealed statistically significant changes in $TCID_{50}$ titer between the human and swine viruses (p values listed in brackets below) [24hpi $p < 0.001$, 48 and 72hpi $p < 0.01$]. The one exception was swine virus A/swine/IL/21IL1207/2009 which also grew an average titer of 6.7 (similar to the human viruses tested) [24hpi $p = 0.053535$, 48hpi $p = 0.176644$, 72hpi $p = 0.259422$]. This initial experiment was the first indication that two distinct growth phenotypes might exist in the 2009 A(H1N1)pdm09 viruses with swine-origin viruses replicating, in general, less well in human systems.

Based on the initial results in human cells, we next wanted to test the same six viruses in a swine cell model. A pig tracheal explant system was used. Each virus was again diluted to achieve a low MOI (0.01) and each explant was inoculated with one virus. The growth kinetics observed in the pig explants differed from the NHBE cells (**Figure 3-6**). All viruses (human and swine) grew to approximately equal titers (average human titer 5.96, average swine titer 6.26). Statistical analysis using the Holm-Sidak method t test[47], with alpha = 0.05 revealed no statistically significant difference between any swine virus and A/TN/F2090/2011.

After identifying the high and low growth phenotype in human cells we wanted to use our phylogenetic analyses to select an additional set of swine viruses to extend these observations. Going back to our BEAST trees (**Figures 3-1** and **3-2**) we selected another twenty swine viruses from throughout the HA tree to grow in NHBE cells. The selection of these viruses was chosen based on sequence similarity or dissimilarity to one of the initial three swine viruses (A/swine/IL/21IL1207/2009, A/swine/IN/30IN0428/2010, or A/swine/MN/36MN1026/2011). We were able to identify three more swine viruses with

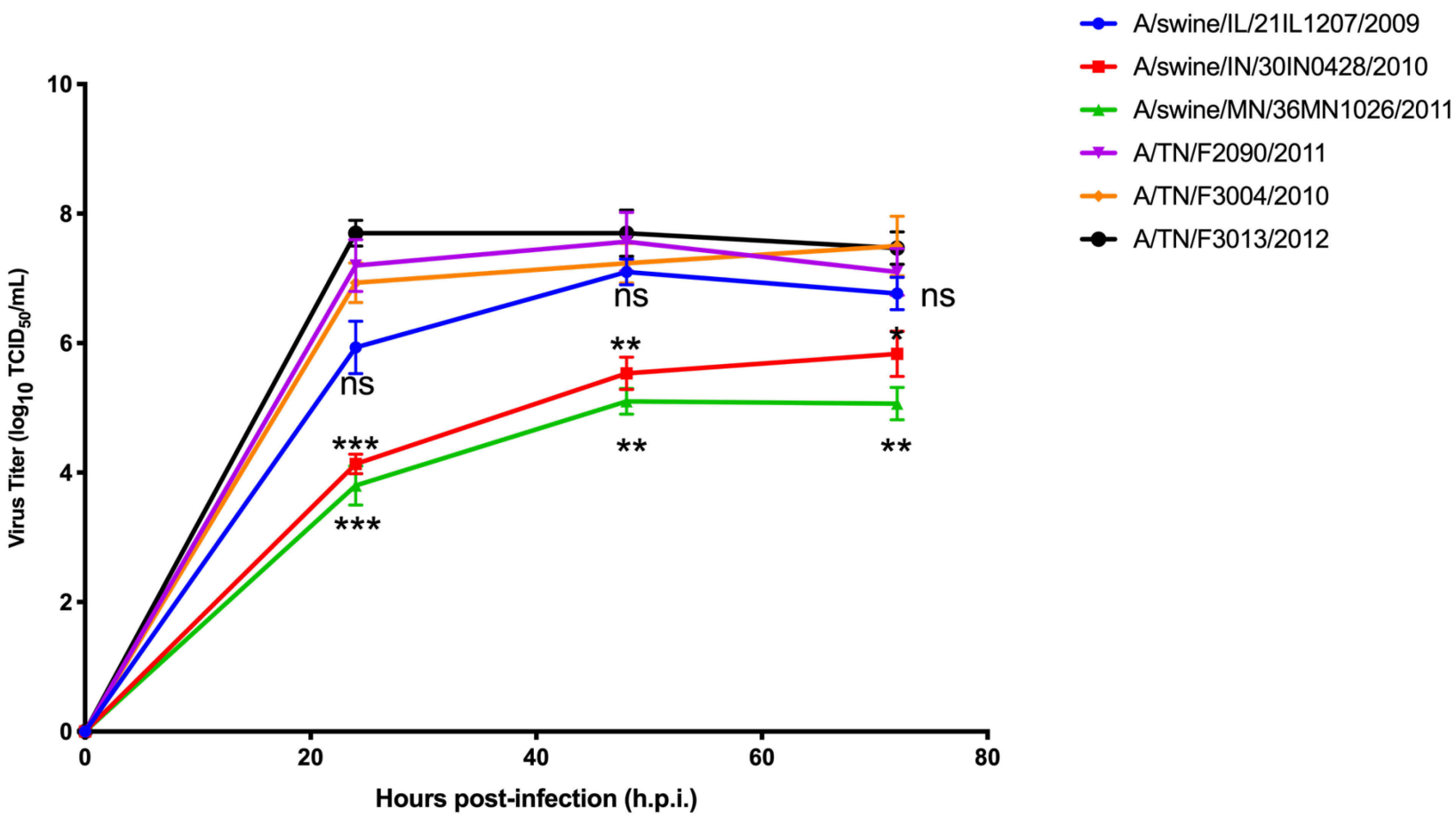

Figure 3-5. Initial NHBE Experiment.

MOI 0.01 at 37ºC (* indicate significance as compared to A/TN/F2090/2011. Statistical significance determined using the Holm-Sidak method t test[47], with alpha = 0.05.)

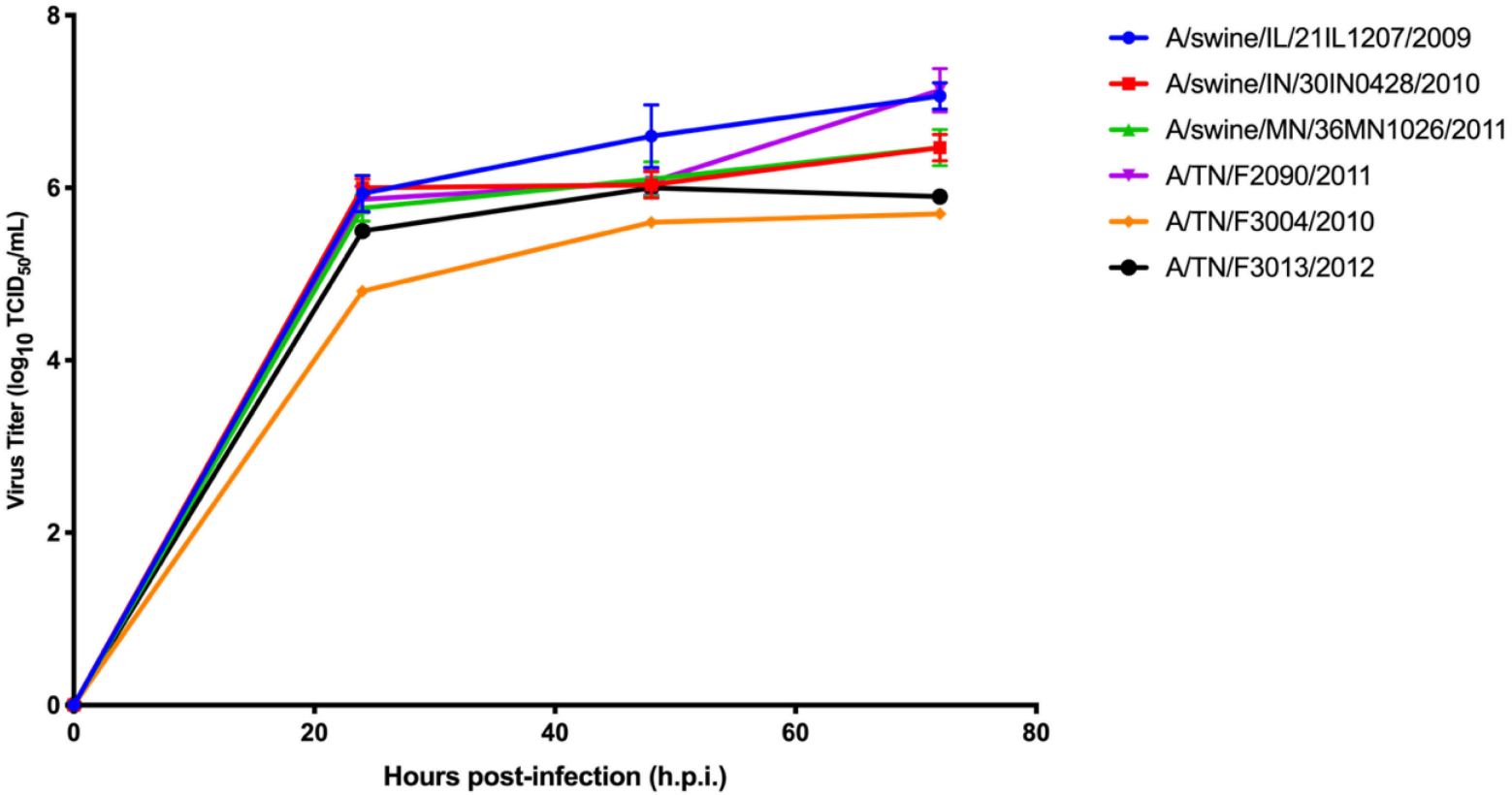

Figure 3-6. Pig Explant Growth Experiment.

MOI 0.01 at 37ºC (no significant differences detected as compared to A/TN/F2090/2011. Statistical significance determined using the Holm-Sidak method t test[47], with alpha = 0.05.)

the high-growth phenotype and seventeen with the low-growth phenotype (**Figure 3-7**). Together these data provide support for a model where replication of a human-origin virus in swine leads to rapid adaptation for swine which comes at the expense of optimal fitness for the previous human host.

To further test our hypothesis of host adaptation leading to a lack of fitness for the previous host, we conducted an amino acid alignment of both the HA and NA proteins from a human pandemic virus (A/Tennessee/F2090/2011) and a swine pandemic virus (A/swine/Minnesota/36MN1026/2011) (selected based on growth phenotype in human cells). We were able to identify four changes in the HA protein and three changes in the NA protein that might be linked with host range and replicative fitness (**Figures 3-8** and **3-9**). The amino acid changes observed in HA were: N38D, N125S, H138Y, and R259K (**Figure 3-8**). The amino acid changes observed in NA were: S14N, S258T, and N368T (**Figure 3-9**). One of the lowest growing swine viruses that we tested was A/swine/Minnesota/36MN1026/2011. This particular virus possessed all seven of the changes listed above. A comparison of all studied viruses and their associated changes is included in **Table 3-1**. The presence and effect of these changes were explored further using a modified BEAST XML file.

Empirical trees can be calculated in BEAST by modifying the tree calculation parameters. Refer to **Appendix C** for relevant code modifications. Briefly, the code is altered to prevent overall tree calculation (as the tree has already been calculated previously). Instead code is added to allow BEAST to assign nodes based on presence or absence of certain characteristics, in our case, which amino acid was present at a specified location. We programmed BEAST with the amino acid residues and positions identified above. The empirical trees produced via this method gave us insight into how prevalent these changes are in human and swine. While each changed AA was noted a few times throughout the HA and NA trees, none established themselves in the population. It is important to note that all predicted "low growth" changes were only found in swine-origin viruses. It is thought this is due to the lack of fitness conferred by the presence of these mutations. Further, the only virus with all seven mutations is the A/swine/Minnesota/36MN1026/2011 virus. Figures included are as follows: HA N38D (**Figure 3-10**), N125S (**Figure 3-11**), H138Y (**Figure 3-12**), R259K (**Figure 3-13**), and all HA changes (**Figure 3-14**). NA S14N (**Figure 3-15**), S258T (**Figure 3-16**), N368T (**Figure 3-17**), and all NA changes (**Figure 3-18**).

The tanglegram (**Figure 3-19**) provides a linked view of all matching swine viruses from tree to tree. The green connecting lines indicate swine viruses present in all of the trees indicating where clusters of sequences group across multiple trees. This allows us to compare relative position and temporal placement of multiple sequences in one figure.

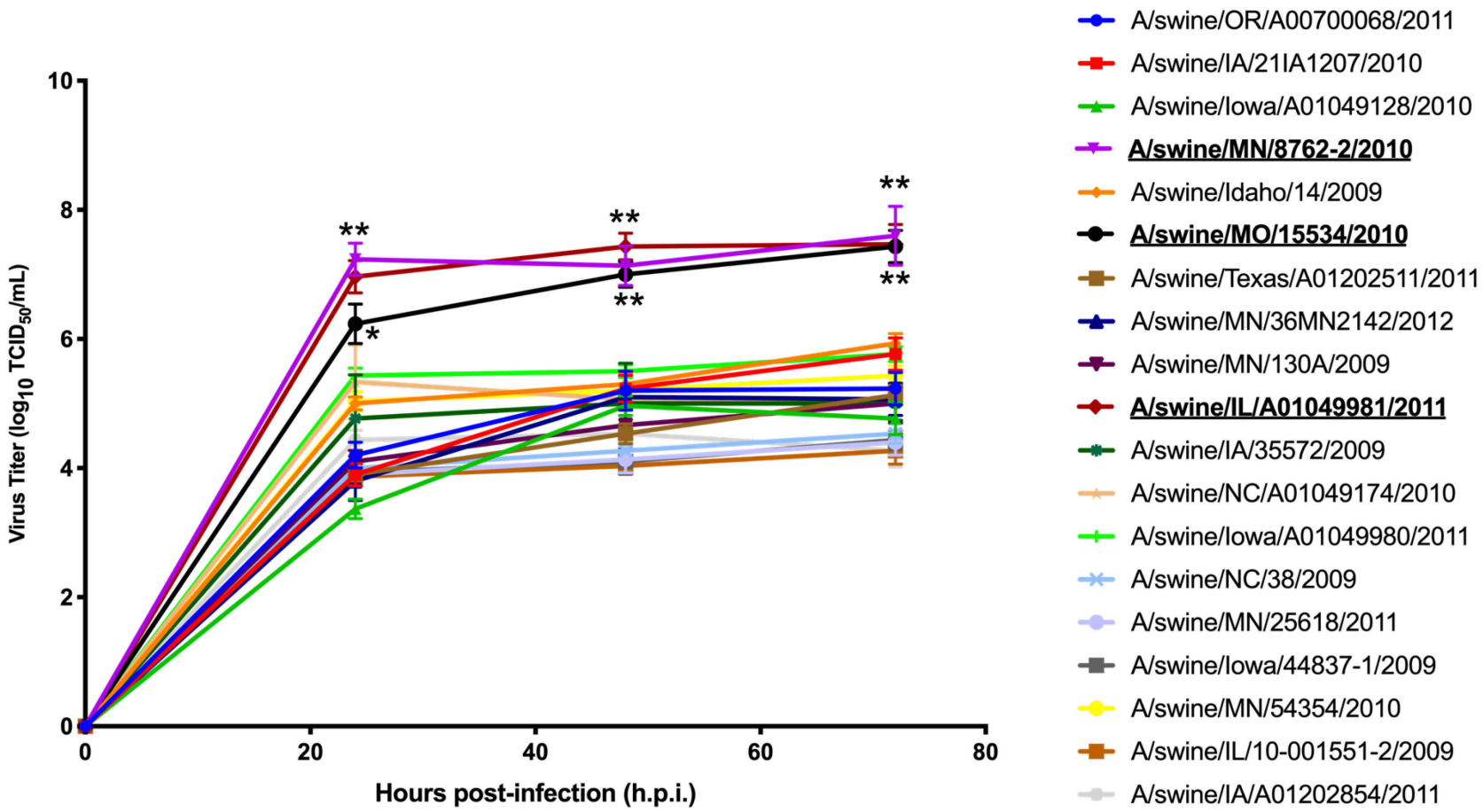

Figure 3-7. Follow-up NHBE Growth Experiment with Phylogenetically Chosen Viruses.

MOI 0.01 at 37ºC (* indicate significance as compared to A/TN/F2090/2011. Statistical significance determined using the Holm-Sidak method t test[47], with alpha = 0.05.) Statistically significant swine viruses are shown in bold font and underlined.

Figure 3-8. HA Amino Acid Alignment.

Hemagglutinin amino acid comparison between representative human and swine viruses.

Figure 3-9. NA Amino Acid Alignment.

Neuraminidase amino acid comparison between representative human and swine viruses.

Table 3-1. Amino Acid Residues Present in All Swine Viruses Tested.

Virus	Growth Phenotype	HA Pos. 38	HA Pos. 125	HA Pos. 138	HA Pos. 259	NA Pos. 14	NA Pos. 258	NA Pos. 368
A/swine/Illinois/A01047715/2010	Low	N	N	R	R	N	S	N
A/swine/Illinois/10-001551-2/2009	Low	N	S	H	R	S	T	N
A/swine/Illinois/21IL1207/2009	High	N	N	H	R	S	S	N
A/swine/Illinois/35572/2009	Low	D	N	H	R	N	S	N
A/swine/Illinois/A01049981/2011	High	N	N	H	R	S	S	N
A/swine/Indiana/30IN0428/2010	Low	N	N	Y	R	S	S	T
A/swine/Iowa/21IA1207/2010	Low	N	S	H	K	S	T	N
A/swine/Iowa/44837-1/2009	Low	N	N	H	R	S	S	N
A/swine/Iowa/A01049128/2010	Low	N	S	H	K	N	S	N
A/swine/Iowa/A01049980/2011	Low	D	N	H	R	S	T	N
A/swine/Iowa/A01202854/2011	Low	N	N	Y	K	S	S	T
A/swine/Minnesota/130A/2009	Low	N	S	H	R	N	S	T
A/swine/Minnesota/25618/2011	Low	N	S	Y	R	N	S	N
A/swine/Minnesota/36MN1026/2011	Low	D	S	Y	K	N	T	T
A/swine/Minnesota/36MN2142/2012	Low	D	S	H	K	S	T	N
A/swine/Minnesota/54354/2010	Low	N	S	H	K	N	S	N
A/swine/Minnesota/8762-2/2010	High	N	N	H	R	S	S	N
A/swine/Missouri/15534/2010	High	N	N	H	R	S	S	N
A/swine/North Carolina/38/2009	Low	D	N	H	R	S	S	N
A/swine/North Carolina/A01049174/2010	Low	N	N	Y	R	S	T	T
A/swine/Oregon/A00700068/2011	Low	N	S	H	K	N	S	N
A/swine/Texas/A01202511/2011	Low	D	N	H	K	S	T	N

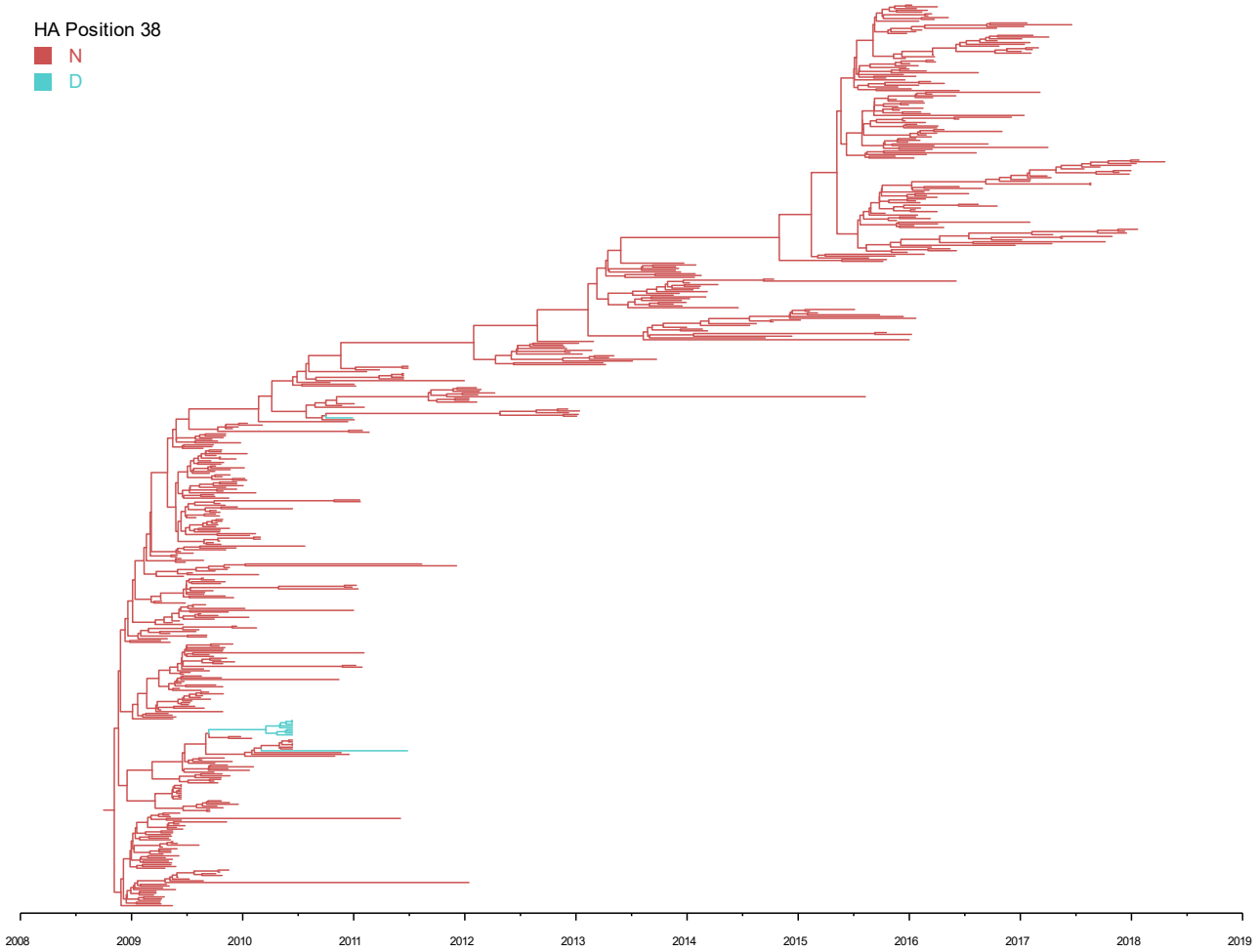

Figure 3-10. Empirical BEAST Tree for HA Position 38.

Annotated based on presence of amino acid at position 38. 38N is colored in red and 38D is colored in green.

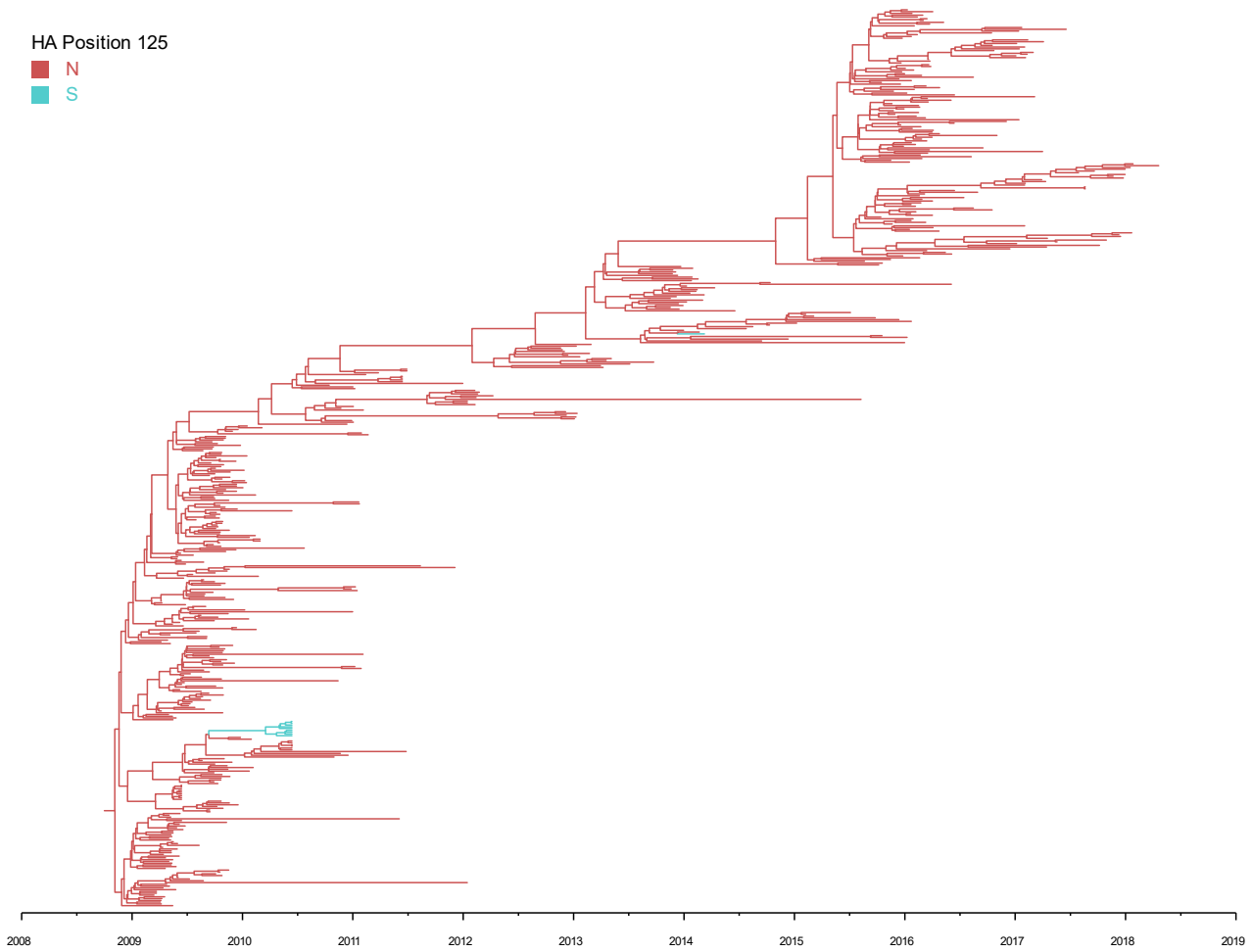

Figure 3-11. Empirical BEAST Tree for HA Position 125.

Annotated based on presence of amino acid at position 125. 125N is colored in red and 125S is colored in green.

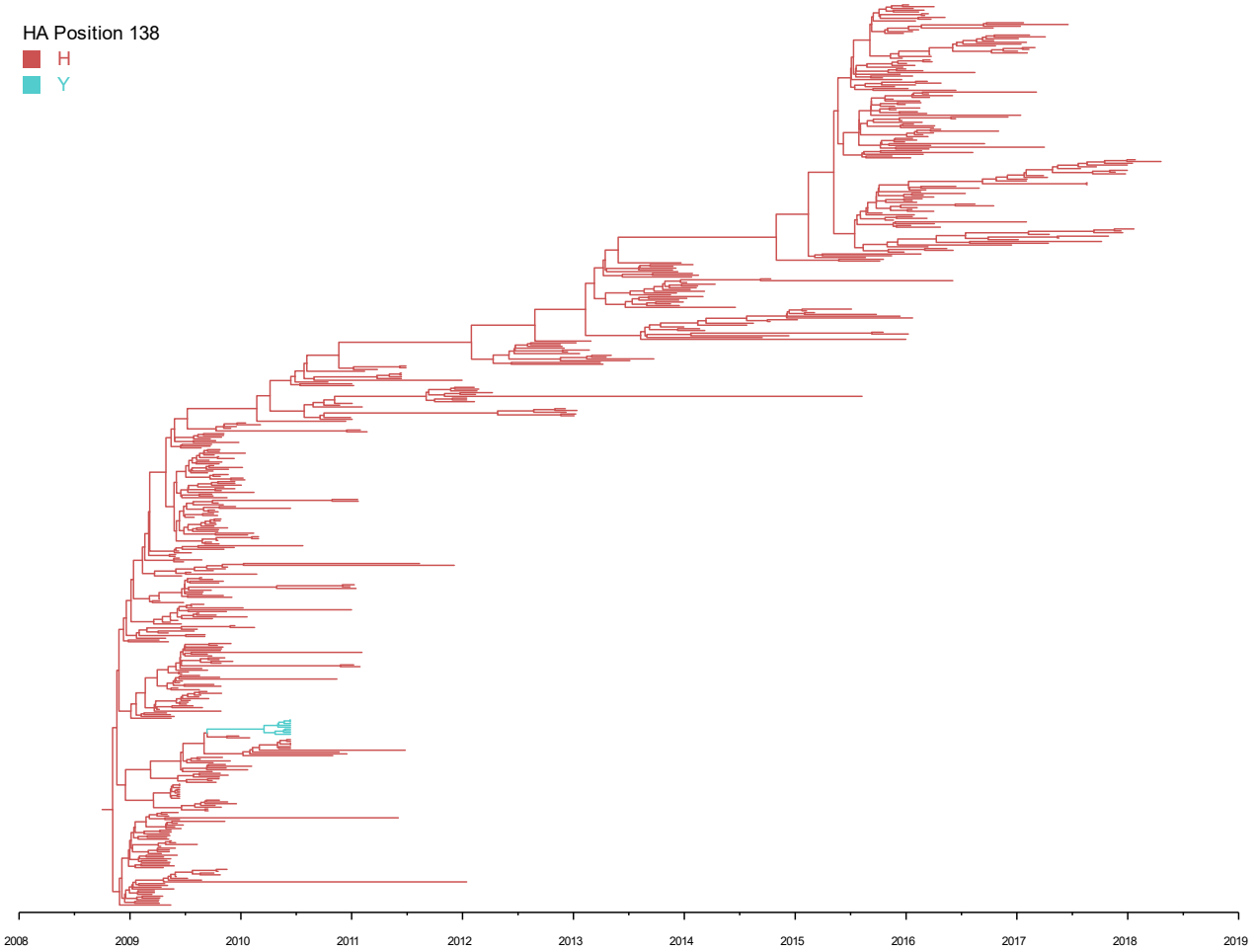

Figure 3-12. Empirical BEAST Tree for HA Position 138.

Annotated based on presence of amino acid at position 138. 138H is colored in red and 138Y is colored in green.

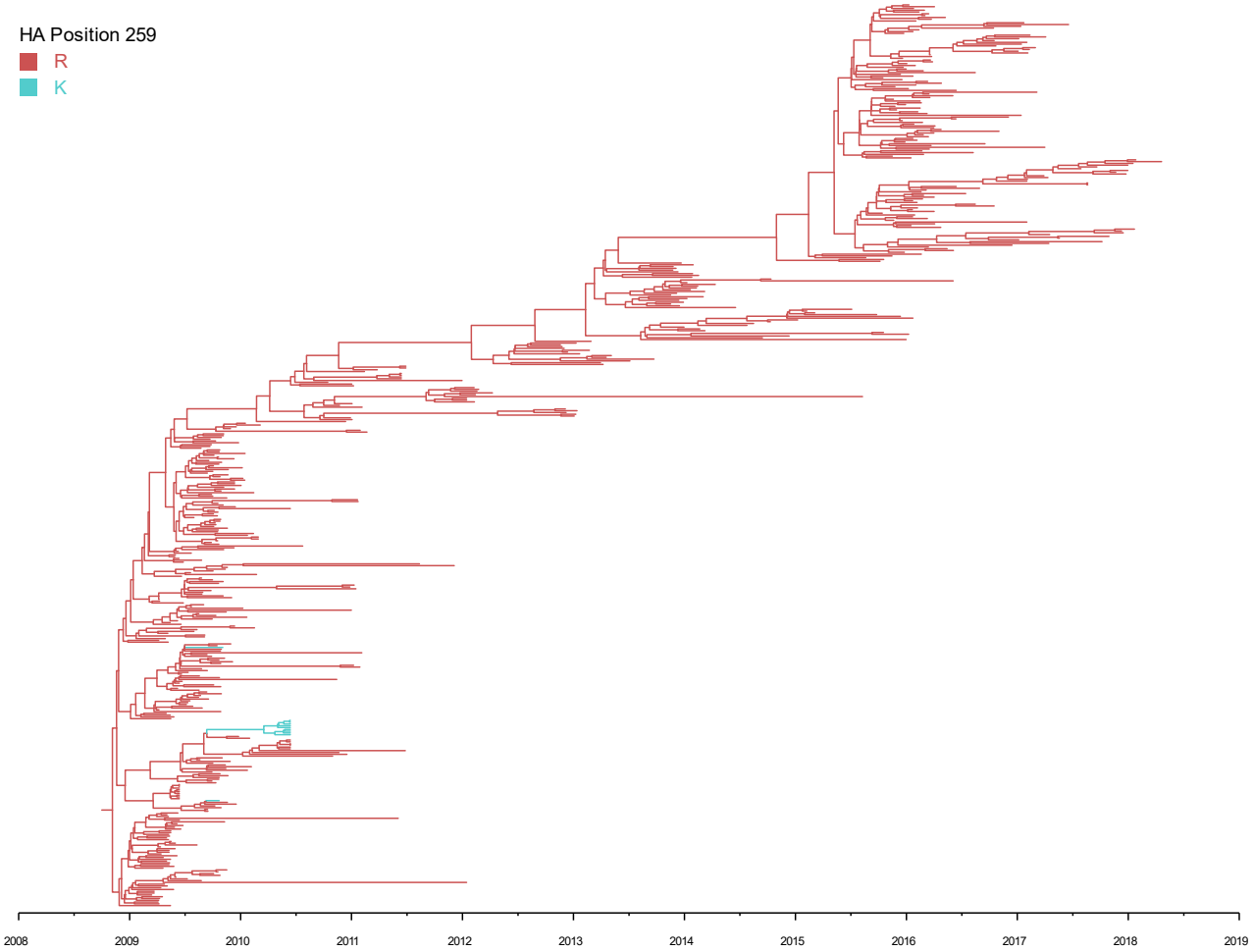

Figure 3-13. Empirical BEAST Tree for HA Position 259.

Annotated based on presence of amino acid at position 259. 259R is colored in red and 259K is colored in green.

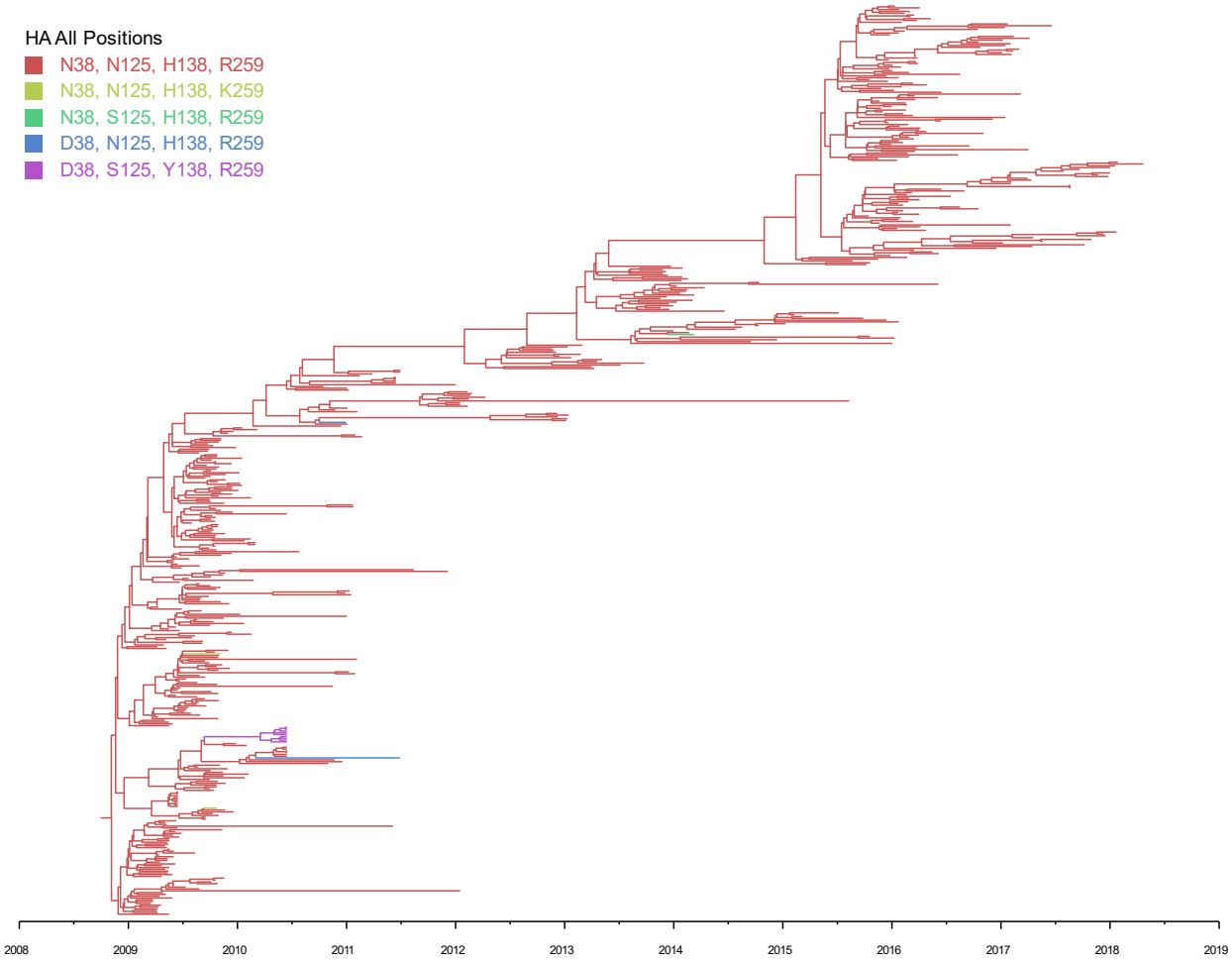

Figure 3-14. Empirical BEAST Tree for All HA Positions.

Annotated based on presence of amino acid at all identified positions.

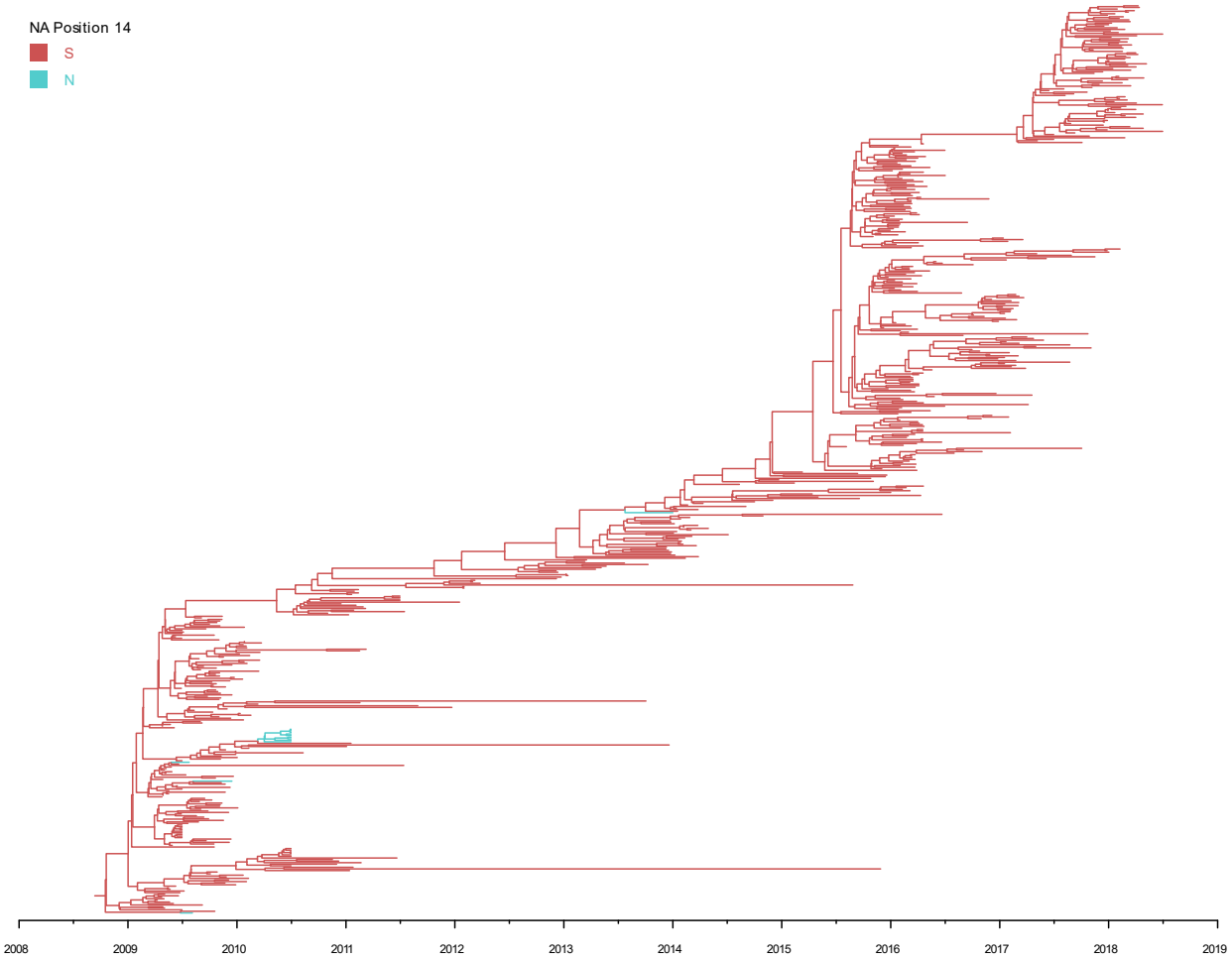

Figure 3-15. Empirical BEAST Tree for NA Position 14.

Annotated based on presence of amino acid at position 14. 14S is colored in red and 14N is colored in green.

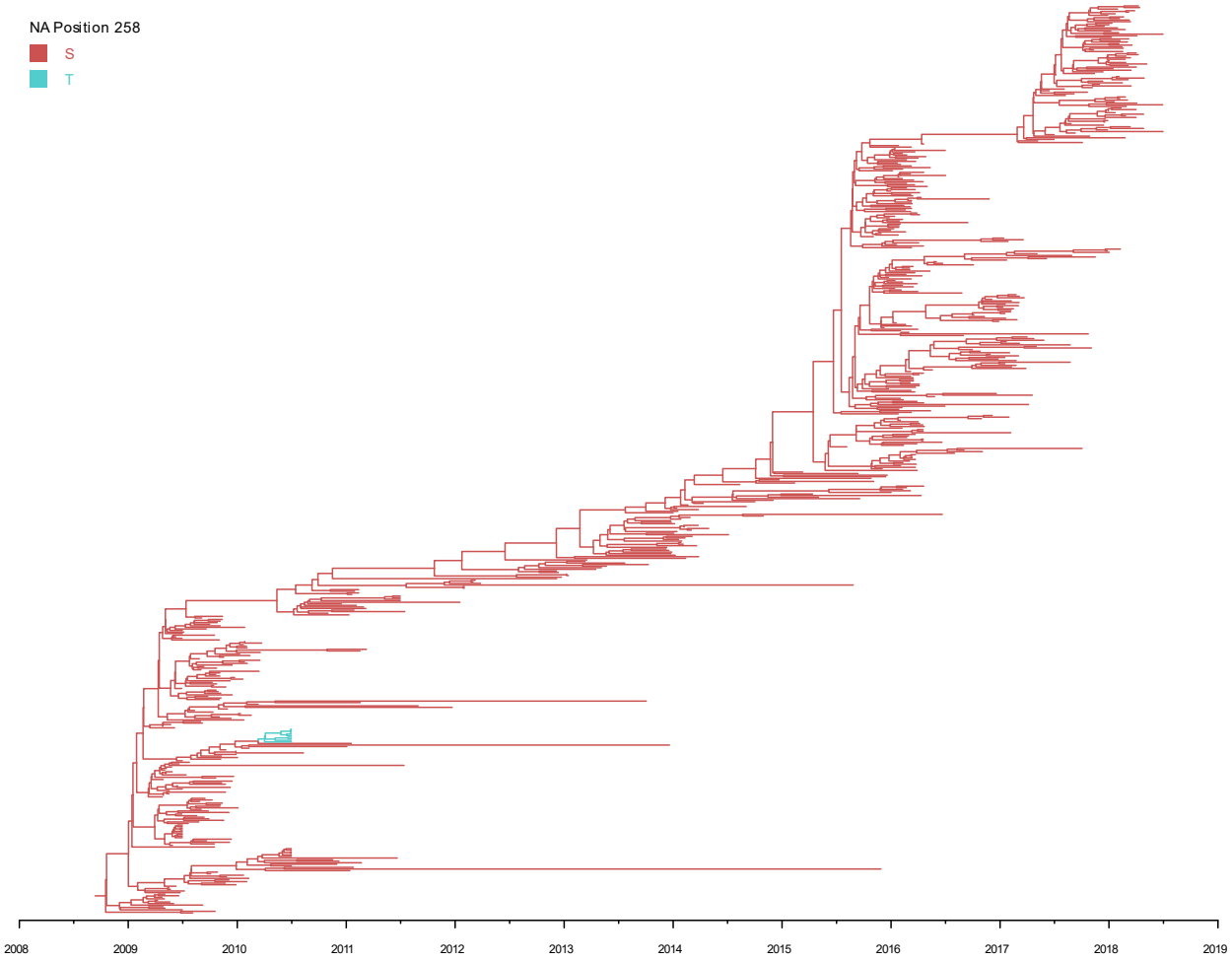

Figure 3-16. Empirical BEAST Tree for NA Position 258.

Annotated based on presence of amino acid at position 258. 258S is colored in red and 258T is colored in green.

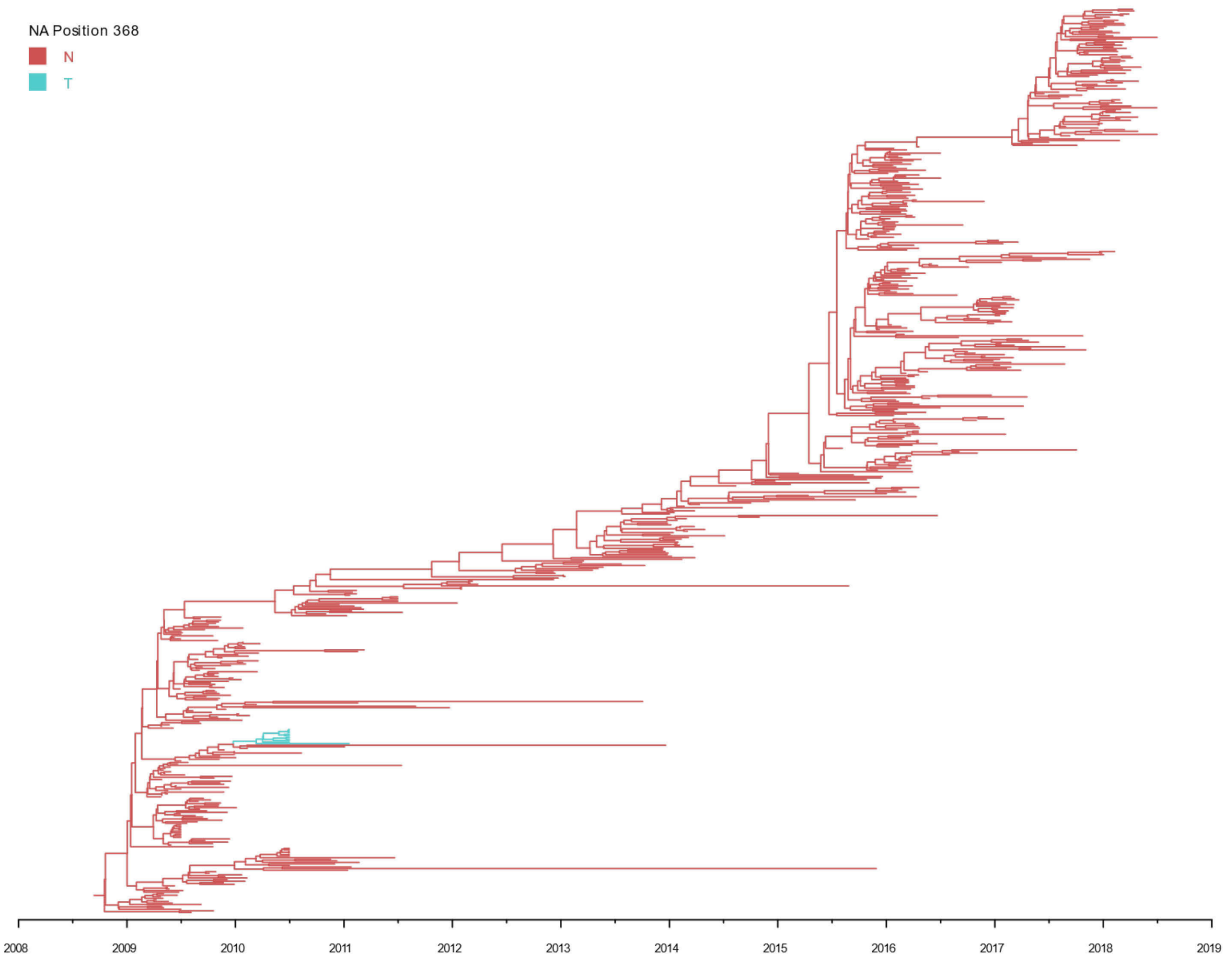

Figure 3-17. Empirical BEAST Tree for NA Position 368.

Annotated based on presence of amino acid at position 368. 368N is colored in red and 368T is colored in green.

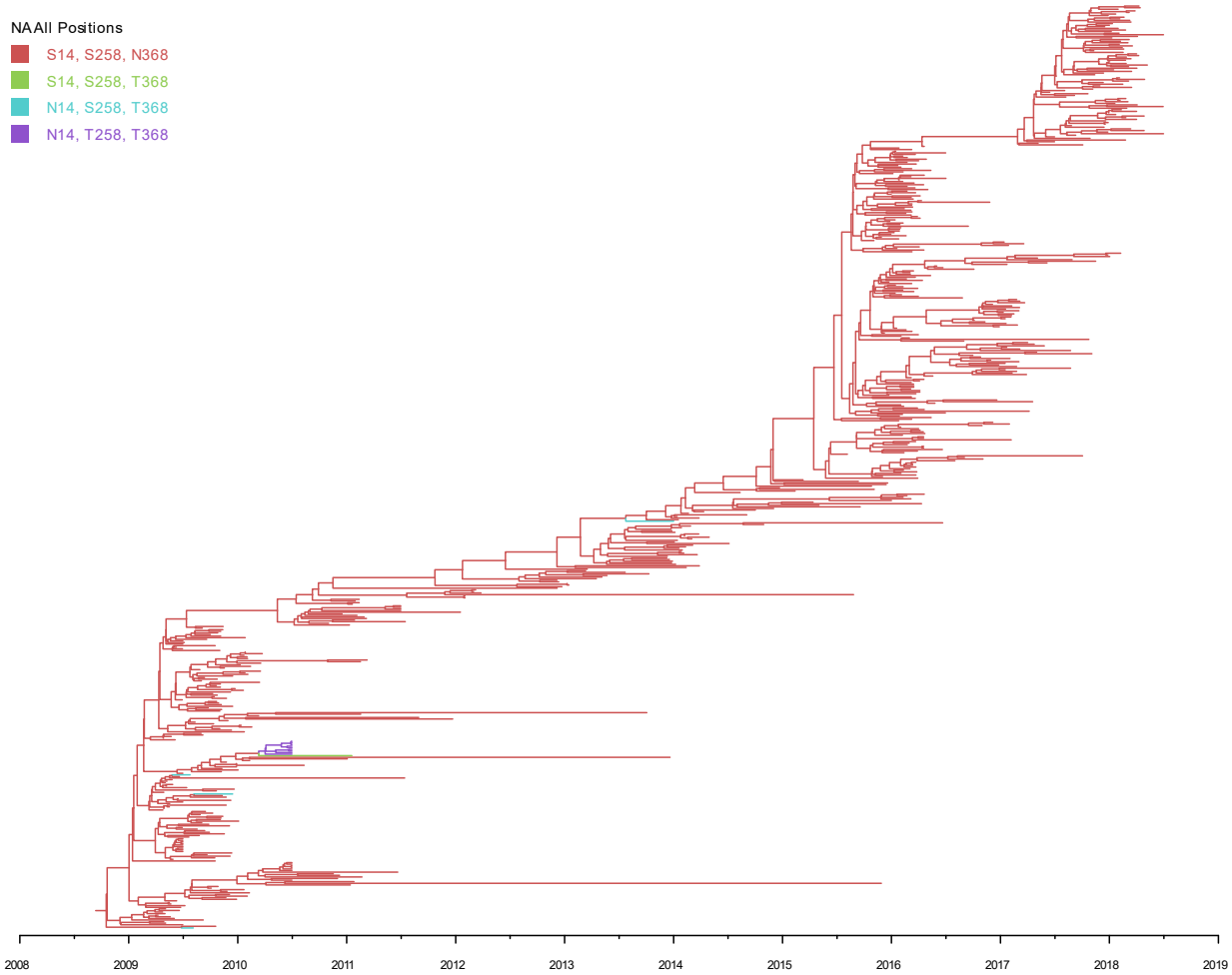

Figure 3-18. Empirical BEAST Tree for All NA Positions.

Annotated based on presence of amino acid at all identified positions.

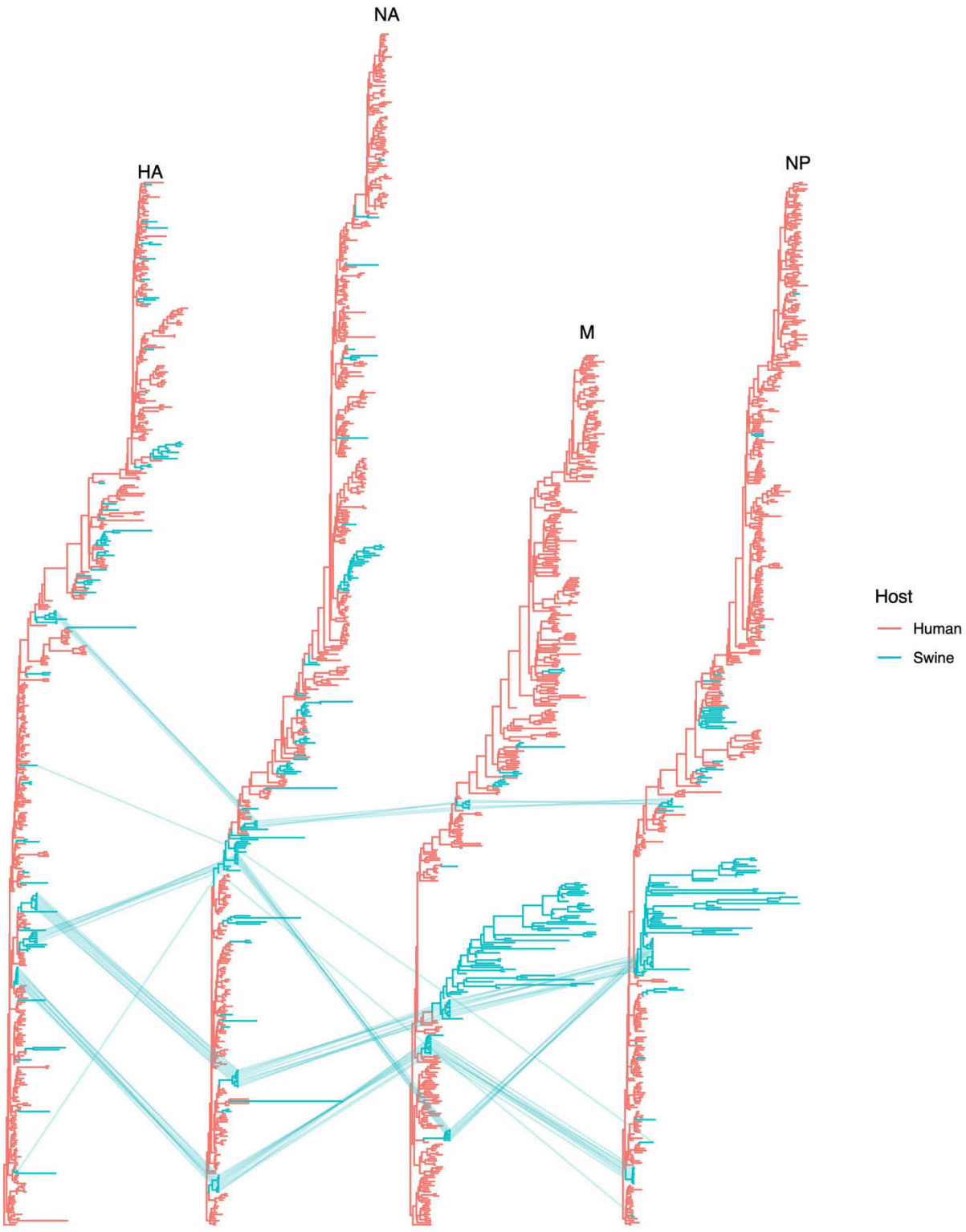

Figure 3-19. HA, NA, M, NP Tanglegram.

Produced via R.[48] Connecting green lines (between trees) indicate where identical swine viruses sort across multiple gene segment phylogenies.

CHAPTER 4. DISCUSSION

Although much work has already been done to elucidate the determinants of host range in influenza viruses,[28-32] much of this work has been done in avian viruses. The number of identified host range markers that differentiate human and swine viruses is few which was a major scientific premise for this thesis. We identified seven specific changes in HA and NA differentiating human and swine influenza viruses. While A/swine/Minnesota/36MN1026/2011 was the only swine virus studied to possess all seven changes, there were several other swine viruses with many of the changes. **Table 4-1** compares the number of changes observed in all the swine viruses tested in this thesis. It is interesting to note that there is no clear pattern as to how many changes are needed to exhibit a low-growth phenotype. However, it does appear necessary to have all seven changes in order to exhibit a high-growth phenotype (at least within the scope of this thesis). I conducted a thorough search to determine if any of these changes occur in critical areas with known host-range properties. Based on my findings, none of these seven residues have been previously linked to receptor binding, host range specificity, or interspecies transmission. Although the exact effect of each change remains unknown, we do feel that they likely play some role in the fitness of these viruses based on the phylogenetic experiments and the growth experiments of this study. The fact that out of thousands of sequences, these specific changes were seen only a handful of times and exclusively present only in swine, indicates they are preferentially selected for after replication in swine and offer no fitness advantage in humans. While future studies are required to test the hypothesis, it is also possible that a subset of these mutations actually come at a fitness cost for replication in humans and are responsible for the poor growth of swine-adapted H1N1pdm09 viruses in human cells that we observed.

The phylogenetics provided by BEAST analysis revealed several reverse zoonosis transmission events where a human virus was introduced back into the swine population (**Figures 3-1** through **3-4**). The empirical BEAST trees give us a glimpse at the population-wide prevalence of these changes in both swine and human viruses. It is important to note that every "low-growth" change we noted in this thesis occurred in swine viruses only; all human viruses possessed the "high-growth" amino acid at that position. With the addition of the growth kinetics data, it appears that when these human-origin viruses are re-introduced back into swine, they lose some replicative fitness for the previous host (human) (**Figures 3-5** through **3-7**). There are likely other genetic and AA changes associated with the growth differences we observed in this thesis. An overview of genome-wide changes can be found in **Appendix A**. This figure is designed to show the total number of nucleotide and amino acid changes on the top rows and the percent identity between the viruses on the bottom rows. The A/swine/MN/36MN1026/2011 virus has more nucleotide and amino acid changes than the other swine viruses. There is the possibility that more than just the changes in HA and NA are responsible for the replication differences we have identified in this thesis. More work must be done to completely elucidate the effect of every change observed in these viruses. It is also likely that many of these changes are stochastic in nature and do not have any bearing on growth phenotype. Because of the infidelity of the polymerase complex of influenza

Table 4-1. Number of Either High- or Low-Growth Associated Changes in Swine Viruses Tested.

Virus	Growth Phenotype	Number of High-Growth AA	Number of Low-Growth AA
A/swine/Illinois/A01047715/2010	Low	5	2
A/swine/Illinois/10-001551-2/2009	Low	5	2
A/swine/Illinois/21IL1207/2009	High	7	0
A/swine/Illinois/35572/2009	Low	5	2
A/swine/Illinois/A01049981/2011	High	7	0
A/swine/Indiana/30IN0428/2010	Low	5	2
A/swine/Iowa/21IA1207/2010	Low	4	3
A/swine/Iowa/44837-1/2009	Low	7	0
A/swine/Iowa/A01049128/2010	Low	4	3
A/swine/Iowa/A01049980/2011	Low	5	2
A/swine/Iowa/A01202854/2011	Low	4	3
A/swine/Minnesota/130A/2009	Low	4	3
A/swine/Minnesota/25618/2011	Low	4	3
A/swine/Minnesota/36MN1026/2011	Low	0	7
A/swine/Minnesota/36MN2142/2012	Low	3	4
A/swine/Minnesota/54354/2010	Low	4	3
A/swine/Minnesota/8762-2/2010	High	7	0
A/swine/Missouri/15534/2010	High	7	0
A/swine/North Carolina/38/2009	Low	6	1
A/swine/North Carolina/A01049174/2010	Low	4	3
A/swine/Oregon/A00700068/2011	Low	4	3
A/swine/Texas/A01202511/2011	Low	4	3

viruses, changes and mutations can arise that have little to no effect on the virus. Alternatively, the genetic diversity that the polymerase complex produces is a key mechanism by which influenza viruses can escape population immunity (antigenic drift mentioned in Chapter 1).

To further investigate the effect these changes pose for influenza viruses, we propose to utilize a reverse genetics (rg) system whereby each AA change can be added into a human-origin virus or a swine-origin virus. Once these rg viruses have been rescued and grown, further growth kinetics experiments should be performed to determine if any effect on growth phenotype is observed with each AA change. Initially, it will be easier to add all changes at once to either HA or NA and then repeat testing in NHBE cells. If growth phenotype differences are observed, further rg viruses could be generated with each individual change.

In conclusion, the seven amino acid changes identified in this thesis appear to have some effect on replication fitness in both human and swine hosts. More investigation needs to be done to elucidate the effect these changes have on virulence, transmission, and host range. Further, other genetic and AA changes present between the human and swine viruses might also play a role in host range determination and therefore should also be investigated.

"No amount of experimentation can prove me right; a single experiment can prove me wrong." – Albert Einstein

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APPENDIX A. GENOME-WIDE COMPARISON BETWEEN SWINE AND HUMAN VIRUSES

Genome-wide changes:

Nucleotide

		1	2	3	4
A/swine/IL/21IL1207	1		58	91	100
A/swine/IN/30IN0428	2	99.55		95	98
A/swine/MN/36MN1026	3	99.30	99.27		135
A/TN/F2090	4	99.23	99.24	98.96	

Amino Acid

		1	2	3	4
A/swine/IL/21IL1207	1		14	27	41
A/swine/IN/30IN0428	2	99.68		28	39
A/swine/MN/36MN1026	3	99.38	99.35		55
A/TN/F2090	4	99.05	99.10	98.73	

APPENDIX B. DATASET MASTERFILE FOR HA GENE (FASTA SEQUENCE TRUNCATED FOR SPACE)

Accession #	Sample Name	Host Species	Date	FASTA Sequence
FJ966082	A/California/04/2009	Human	2009-04-01	ATGAA…
KF009554	A/California/07/2009	Human	2009-04-09	ATGAA…
GQ117097	A/Indiana/09/2009	Human	2009-04-22	ATGAA…
GQ168644	A/Kansas/03/2009	Human	2009-04-24	ATGAA…
FJ984397	A/Ohio/07/2009	Human	2009-04-24	ATGAA…
GQ168652	A/New-York/11/2009	Human	2009-04-25	ATGAA…
GQ117032	A/Texas/09/2009	Human	2009-04-25	ATGAA…
CY041122	A/New-York/3214/2009	Human	2009-04-25	ATGAA…
CY040838	A/New-York/3262/2009	Human	2009-04-27	ATGAA…
GQ160526	A/Florida/04/2009	Human	2009-04-27	ATGAA…
CY046387	A/Wisconsin/629-D00750/2009	Human	2009-04-30	ATGAA…
CY053103	A/Houston/15H/2009	Human	2009-05-01	ATGAA…
CY046235	A/Wisconsin/629-D01735/2009	Human	2009-05-02	ATGAA…
CY041750	A/New-York/3323/2009	Human	2009-05-06	ATGAA…
CY046571	A/Wisconsin/629-D00905/2009	Human	2009-05-08	ATGAA…
CY046563	A/Wisconsin/629-D01919/2009	Human	2009-05-09	ATGAA…
KC781320	A/Mississippi/01/2009	Human	2009-05-13	ATGAA…
CY043235	A/New-York/3502/2009	Human	2009-05-16	ATGAA…
CY044917	A/New-York/3613/2009	Human	2009-05-19	ATGAA…
CY046731	A/Wisconsin/629-D01521/2009	Human	2009-05-20	ATGAA…
CY053174	A/Brownsville/26OS/2009	Human	2009-05-20	ATGAA…
CY044877	A/New-York/3573/2009	Human	2009-05-21	ATGAA…

CY053214	A/Brownsville/31H/2009	Human	2009-05-22	ATGAA…
CY044957	A/New-York/3629/2009	Human	2009-05-24	ATGAA…
CY044925	A/New-York/3617/2009	Human	2009-05-25	ATGAA…
CY046779	A/Wisconsin/629-D01894/2009	Human	2009-05-26	ATGAA…
CY046955	A/New-York/3654/2009	Human	2009-05-26	ATGAA…
CY046811	A/Wisconsin/629-D00698/2009	Human	2009-05-27	ATGAA…
CY050150	A/Wisconsin/629-D00117/2009	Human	2009-05-28	ATGAA…
CY046907	A/Wisconsin/629-D01083/2009	Human	2009-05-28	ATGAA…
CY050903	A/Wisconsin/629-D01779/2009	Human	2009-05-28	ATGAA…
CY053277	A/Brownsville/39H/2009	Human	2009-05-30	ATGAA…
CY046803	A/Wisconsin/629-D00223/2009	Human	2009-05-30	ATGAA…
CY071039	A/New-York/NHRC0003/2009	Human	2009-06-01	ATGAA…
CY047366	A/New-York/3795/2009	Human	2009-06-01	ATGAA…
CY053301	A/Brownsville/43H/2009	Human	2009-06-02	ATGAA…
CY050983	A/Wisconsin/629-D00592/2009	Human	2009-06-02	ATGAA…
KC781928	A/Virginia/24/2009	Human	2009-06-03	ATGAA…
CY051231	A/Wisconsin/629-D01664/2009	Human	2009-06-03	ATGAA…
CY054683	A/Wisconsin/629-D00589/2009	Human	2009-06-03	ATGAA…
CY051015	A/Wisconsin/629-D00453/2009	Human	2009-06-04	ATGAA…
CY051839	A/Texas/42123701/2009	Human	2009-06-12	ATGAA…
CY064460	A/Boston/96/2009	Human	2009-06-13	ATGAA…
CY050999	A/Wisconsin/629-D00665/2009	Human	2009-06-13	ATGAA…
KC781551	A/Massachusetts/16/2009	Human	2009-06-15	ATGAA…
CY044171	A/Bethesda/SP506/2009	Human	2009-06-16	ATGAA…
CY055447	A/California/VRDL11/2009	Human	2009-06-17	ATGAA…
CY051167	A/Wisconsin/629-D02063/2009	Human	2009-06-18	ATGAA…
CY043118	A/Bethesda/SP508/2009	Human	2009-06-18	ATGAA…

CY064524	A/Boston/118/2009	Human	2009-06-20	ATGAA…
CY051551	A/New-York/4434/2009	Human	2009-06-23	ATGAA…
CY051479	A/Wisconsin/629-S0410/2009	Human	2009-06-23	ATGAA…
CY052154	A/New-York/4401/2009	Human	2009-06-25	ATGAA…
CY064676	A/Boston/141/2009	Human	2009-06-25	ATGAA…
CY064564	A/Boston/124/2009	Human	2009-06-26	ATGAA…
CY054835	A/California/VRDL30/2009	Human	2009-06-28	ATGAA…
CY054795	A/California/VRDL25/2009	Human	2009-06-30	ATGAA…
SJ0001	A/swine/IA/14IA1011	Swine	2009-07-01	ATGAA…
SJ0002	A/swine/IL/21IL1206	Swine	2009-07-01	ATGAA…
SJ0003	A/swine/IL/21IL1207	Swine	2009-07-01	ATGAA…
SJ0004	A/swine/IL/21IL1208	Swine	2009-07-01	ATGAA…
SJ0005	A/swine/IL/21IL1224	Swine	2009-07-01	ATGAA…
SJ0006	A/swine/IL/21IL1225	Swine	2009-07-01	ATGAA…
SJ0007	A/swine/IL/21IL1227	Swine	2009-07-01	ATGAA…
SJ0008	A/swine/IL/21IL1228	Swine	2009-07-01	ATGAA…
SJ0009	A/swine/IL/21IL1230	Swine	2009-07-01	ATGAA…
SJ0010	A/swine/IL/22IL1213	Swine	2009-07-01	ATGAA…
CY054851	A/California/VRDL32/2009	Human	2009-07-01	ATGAA…
CY063550	A/Boston/151/2009	Human	2009-07-06	ATGAA…
CY053158	A/Houston/23H/2009	Human	2009-07-07	ATGAA…
CY051599	A/New-York/4566/2009	Human	2009-07-08	ATGAA…
CY051607	A/New-York/4567/2009	Human	2009-07-08	ATGAA…
CY054939	A/California/VRDL48/2009	Human	2009-07-10	ATGAA…
CY052407	A/Texas/43132503/2009	Human	2009-07-13	ATGAA…
CY051631	A/New-York/4620/2009	Human	2009-07-14	ATGAA…
CY051623	A/New-York/4607/2009	Human	2009-07-14	ATGAA…

CY051655	A/New-York/4728/2009	Human	2009-07-24	ATGAA…
CY052367	A/Texas/43272683/2009	Human	2009-07-27	ATGAA…
CY083224	A/California/WRAIR1507P/2009	Human	2009-07-29	ATGAA…
CY051679	A/New-York/4747/2009	Human	2009-07-30	ATGAA…
CY055035	A/California/VRDL69/2009	Human	2009-08-05	ATGAA…
CY071530	A/California/WR1320P/2009	Human	2009-08-07	ATGAA…
KC781733	A/Oregon/30/2009	Human	2009-08-09	ATGAA…
CY055494	A/California/VRDL74/2009	Human	2009-08-10	ATGAA…
CY051695	A/New-York/4777/2009	Human	2009-08-14	ATGAA…
CY063574	A/Boston/154/2009	Human	2009-08-18	ATGAA…
CY051703	A/New-York/4780/2009	Human	2009-08-18	ATGAA…
KC782315	A/Minnesota/16/2009	Human	2009-08-28	ATGAA…
CY052767	A/Texas/44282651/2009	Human	2009-08-28	ATGAA…
HQ840306	A/swine/Minnesota/130A/2009	Swine	2009-09-01	ATGAA…
CY052775	A/Texas/45021632/2009	Human	2009-09-02	ATGAA…
CY052591	A/Texas/45033774/2009	Human	2009-09-03	ATGAA…
CY052783	A/Texas/45043852/2009	Human	2009-09-04	ATGAA…
CY071834	A/South-Carolina/WRSP520/2009	Human	2009-09-05	ATGAA…
CY057878	A/Wisconsin/629-D01987/2009	Human	2009-09-07	ATGAA…
CY052727	A/Texas/45091405/2009	Human	2009-09-09	ATGAA…
CY052527	A/Texas/45103998/2009	Human	2009-09-10	ATGAA…
KC780106	A/Alaska/38/2009	Human	2009-09-11	ATGAA…
CY057366	A/Wisconsin/629-D00643/2009	Human	2009-09-12	ATGAA…
CY052439	A/Texas/45122538/2009	Human	2009-09-12	ATGAA…
CY052559	A/Texas/45122722/2009	Human	2009-09-12	ATGAA…
CY052575	A/Texas/45131774/2009	Human	2009-09-13	ATGAA…
KC780830	A/Kansas/20/2009	Human	2009-09-14	ATGAA…

CY057430	A/Wisconsin/629-D01935/2009	Human	2009-09-16	ATGAA…
CY055502	A/California/VRDL75/2009	Human	2009-09-19	ATGAA…
CY057470	A/Wisconsin/629-D00402/2009	Human	2009-09-22	ATGAA…
CY057494	A/Wisconsin/629-D00287/2009	Human	2009-09-23	ATGAA…
KC780998	A/Texas/66/2009	Human	2009-09-29	ATGAA…
CY083439	A/Ft.Benning/WRAIR1669P/2009	Human	2009-09-30	ATGAA…
CY063219	A/Wisconsin/629-D01351/2009	Human	2009-10-01	ATGAA…
CY056571	A/New-York/5755/2009	Human	2009-10-01	ATGAA…
CY057542	A/Wisconsin/629-D00853/2009	Human	2009-10-02	ATGAA…
CY057550	A/Wisconsin/629-D02337/2009	Human	2009-10-03	ATGAA…
CY056451	A/New-York/4986/2009	Human	2009-10-05	ATGAA…
CY056435	A/New-York/4984/2009	Human	2009-10-06	ATGAA…
CY057606	A/Wisconsin/629-D00557/2009	Human	2009-10-10	ATGAA…
CY089187	A/Boston/583/2009	Human	2009-10-10	ATGAA…
CY056507	A/New-York/5083/2009	Human	2009-10-13	ATGAA…
CY056012	A/San-Diego/INS11/2009	Human	2009-10-14	ATGAA…
CY057630	A/Wisconsin/629-S1348/2009	Human	2009-10-14	ATGAA…
CY066535	A/San-Diego/INS195/2009	Human	2009-10-15	ATGAA…
CY057238	A/New-York/5158/2009	Human	2009-10-15	ATGAA…
CY092928	A/Maryland/NHRC0003/2009	Human	2009-10-16	ATGAA…
KC780510	A/North-Carolina/46/2009	Human	2009-10-16	ATGAA…
CY063091	A/California/VRDL90/2009	Human	2009-10-17	ATGAA…
CY058054	A/Texas/46181235/2009	Human	2009-10-18	ATGAA…
CY057686	A/Wisconsin/629-S1388/2009	Human	2009-10-19	ATGAA…
CY061243	A/San-Diego/INS103/2009	Human	2009-10-19	ATGAA…
CY057254	A/New-York/5186/2009	Human	2009-10-19	ATGAA…
CY056859	A/San-Diego/INS15/2009	Human	2009-10-20	ATGAA…

CY083669	A/San-Diego/INS62/2009	Human	2009-10-21	ATGAA…
CY057694	A/Wisconsin/629-S1398/2009	Human	2009-10-21	ATGAA…
CY057302	A/New-York/5297/2009	Human	2009-10-22	ATGAA…
CY057286	A/New-York/5271/2009	Human	2009-10-22	ATGAA…
CY089203	A/Boston/594/2009	Human	2009-10-22	ATGAA…
CY056100	A/District-of-Columbia/INS28/2009	Human	2009-10-23	ATGAA…
CY060835	A/Texas/46241654/2009	Human	2009-10-24	ATGAA…
CY056180	A/District-of-Columbia/INS43/2009	Human	2009-10-26	ATGAA…
KC780722	A/Rhode-Island/18/2009	Human	2009-10-27	ATGAA…
CY066815	A/San-Diego/INS218/2009	Human	2009-10-28	ATGAA…
CY063139	A/California/VRDL98/2009	Human	2009-10-29	ATGAA…
CY063131	A/California/VRDL97/2009	Human	2009-10-29	ATGAA…
CY066191	A/California/VRDL94/2009	Human	2009-10-29	ATGAA…
CY075524	A/Boston/606/2009	Human	2009-11-02	ATGAA…
CY089211	A/Boston/618/2009	Human	2009-11-03	ATGAA…
CY066583	A/San-Diego/INS203/2009	Human	2009-11-04	ATGAA…
CY060851	A/Texas/JMS358/2009	Human	2009-11-04	ATGAA…
CY063163	A/California/VRDL101/2009	Human	2009-11-05	ATGAA…
CY083870	A/San-Diego/INS49/2009	Human	2009-11-05	ATGAA…
CY056228	A/San-Diego/INS69/2009	Human	2009-11-05	ATGAA…
KC780748	A/Illinois/15/2009	Human	2009-11-06	ATGAA…
CY060867	A/Texas/JMS361/2009	Human	2009-11-07	ATGAA…
CY089219	A/Boston/630/2009	Human	2009-11-07	ATGAA…
KC782060	A/Wisconsin/55/2009	Human	2009-11-08	ATGAA…
CY057782	A/Wisconsin/629-D01014/2009	Human	2009-11-08	ATGAA…
CY056651	A/New-York/6110/2009	Human	2009-11-08	ATGAA…
CY060883	A/Texas/JMS363/2009	Human	2009-11-08	ATGAA…

HQ424885	A/swine/Iowa/44837-1/2009	Swine	2009-11-08	ATGAA…
KC780381	A/Washington/62/2009	Human	2009-11-09	ATGAA…
CY056611	A/New-York/5976/2009	Human	2009-11-09	ATGAA…
CY056603	A/New-York/5931/2009	Human	2009-11-09	ATGAA…
CY066751	A/Pensacola/INS210/2009	Human	2009-11-10	ATGAA…
CY084438	A/New-York/6064/2009	Human	2009-11-11	ATGAA…
CY061275	A/Pensacola/INS107/2009	Human	2009-11-12	ATGAA…
CY057342	A/San-Diego/INS75/2009	Human	2009-11-12	ATGAA…
CY089259	A/Boston/650/2009	Human	2009-11-12	ATGAA…
CY075548	A/Boston/648/2009	Human	2009-11-12	ATGAA…
CY075572	A/Boston/658/2009	Human	2009-11-13	ATGAA…
CY060923	A/Texas/JMS371/2009	Human	2009-11-14	ATGAA…
CY057822	A/Wisconsin/629-D00965/2009	Human	2009-11-14	ATGAA…
CY060915	A/Texas/JMS370/2009	Human	2009-11-14	ATGAA…
CY089267	A/Boston/657/2009	Human	2009-11-14	ATGAA…
CY066239	A/California/VRDL108/2009	Human	2009-11-15	ATGAA…
CY057846	A/Wisconsin/629-D02060/2009	Human	2009-11-15	ATGAA…
CY058332	A/Wisconsin/629-D01347/2009	Human	2009-11-16	ATGAA…
CY089307	A/Boston/673/2009	Human	2009-11-16	ATGAA…
CY075580	A/Boston/663/2009	Human	2009-11-16	ATGAA…
CY061283	A/Pensacola/INS108/2009	Human	2009-11-17	ATGAA…
CY063307	A/Wisconsin/629-D01572/2009	Human	2009-11-19	ATGAA…
CY060939	A/Texas/JMS373/2009	Human	2009-11-21	ATGAA…
CY084454	A/New-York/6418/2009	Human	2009-11-22	ATGAA…
CY057894	A/Wisconsin/629-D00908/2009	Human	2009-11-22	ATGAA…
CY056723	A/New-York/6473/2009	Human	2009-11-22	ATGAA…
CY075620	A/Boston/698/2009	Human	2009-11-23	ATGAA…

CY057934	A/Wisconsin/629-D00968/2009	Human	2009-11-24	ATGAA…
CY056779	A/New-York/6675/2009	Human	2009-11-24	ATGAA…
CY066271	A/California/VRDL112/2009	Human	2009-11-24	ATGAA…
CY057958	A/Wisconsin/629-D00970/2009	Human	2009-11-27	ATGAA…
CY060955	A/Texas/JMS380/2009	Human	2009-11-27	ATGAA…
CY088593	A/Boston/702/2009	Human	2009-11-29	ATGAA…
CY057974	A/Wisconsin/629-D01434/2009	Human	2009-11-29	ATGAA…
CY057982	A/Wisconsin/629-D01412/2009	Human	2009-12-01	ATGAA…
CY075636	A/Boston/703/2009	Human	2009-12-02	ATGAA…
CY066303	A/California/VRDL116/2009	Human	2009-12-04	ATGAA…
CY066295	A/California/VRDL115/2009	Human	2009-12-04	ATGAA…
CY061003	A/Texas/JMS386/2009	Human	2009-12-06	ATGAA…
CY084446	A/New-York/6902/2009	Human	2009-12-06	ATGAA…
KC780599	A/Arizona/20/2009	Human	2009-12-06	ATGAA…
CY065099	A/New-York/7426/2009	Human	2009-12-08	ATGAA…
CY058380	A/Wisconsin/629-D00780/2009	Human	2009-12-08	ATGAA…
CY058388	A/Wisconsin/629-D00147/2009	Human	2009-12-09	ATGAA…
CY066343	A/California/VRDL121/2009	Human	2009-12-15	ATGAA…
CY061027	A/Texas/JMS389/2009	Human	2009-12-16	ATGAA…
GU984390	A/swine/Illinois/35572/2009	Swine	2009-12-16	ATGAA…
CY072318	A/New-York/INS317/2009	Human	2009-12-17	ATGAA…
CY061035	A/Texas/JMS390/2009	Human	2009-12-20	ATGAA…
GU984403	A/swine/Illinois/10-001551-2/2009	Swine	2009-12-20	ATGAA…
CY061043	A/Texas/JMS391/2009	Human	2009-12-23	ATGAA…
CY158257	A/swine/Arkansas/SG1321/2009	Swine	2009-12-28	ATGAA…
KC780322	A/Georgia/25/2009	Human	2009-12-28	ATGAA…
CY066431	A/California/VRDL132/2009	Human	2009-12-30	ATGAA…

CY066415	A/California/VRDL130/2009	Human	2009-12-30	ATGAA…
CY062058	A/New-York/0461/2009	Human	2009-12-30	ATGAA…
CY066439	A/California/VRDL133/2009	Human	2009-12-30	ATGAA…
KC780547	A/New-Jersey/01/2010	Human	2010-01-01	ATGAA…
CY061107	A/Texas/JMS402/2010	Human	2010-01-04	ATGAA…
KC781903	A/Alabama/01/2010	Human	2010-01-04	ATGAA…
CY158993	A/swine/Minnesota/02976/2010	Swine	2010-01-12	ATGAA…
KC780888	A/Nevada/01/2010	Human	2010-01-12	ATGAA…
CY061155	A/Texas/JMS409/2010	Human	2010-01-20	ATGAA…
KC780464	A/Wisconsin/01/2010	Human	2010-01-24	ATGAA…
CY158441	A/swine/Illinois/02957/2010	Swine	2010-01-26	ATGAA…
CY062138	A/New-York/2960/2010	Human	2010-01-26	ATGAA…
CY064995	A/New-York/3681/2010	Human	2010-02-01	ATGAA…
KC780537	A/Florida/02/2010	Human	2010-02-02	ATGAA…
KC781452	A/Louisiana/01/2010	Human	2010-02-03	ATGAA…
CY066471	A/California/VRDL4/2010	Human	2010-02-08	ATGAA…
JQ023770	A/swine/Minnesota/0432/2010	Swine	2010-02-10	ATGAA…
KC780502	A/Utah/02/2010	Human	2010-02-10	ATGAA…
GU984417	A/swine/Minnesota/8762-2/2010	Swine	2010-02-16	ATGAA…
CY099183	A/swine/Minnesota/02979/2010	Swine	2010-02-17	ATGAA…
CY096594	A/District-of-Columbia/INS527/2010	Human	2010-02-23	ATGAA…
CY167388	A/Tennessee/F1071/2010	Human	2010-03-02	ATGAA…
KC781355	A/Iowa/04/2010	Human	2010-03-03	ATGAA…
CY071367	A/Newark/INS429/2010	Human	2010-03-05	ATGAA…
CY159991	A/swine/Oklahoma/02989/2010	Swine	2010-03-12	ATGAA…
KR859558	A/swine/Illinois/A00970254/2010	Swine	2010-03-18	ATGAA…
KR859639	A/swine/Illinois/A00970252/2010	Swine	2010-03-18	ATGAA…

CY167452	A/Tennessee/F1089/2010	Human	2010-03-24	ATGAA…
HM219624	A/swine/Missouri/15534/2010	Swine	2010-03-24	ATGAA…
SJ0011	A/swine/IN/29IN1001	Swine	2010-07-01	ATGAA…
SJ0012	A/swine/IN/29IN1002	Swine	2010-07-01	ATGAA…
SJ0013	A/swine/IN/29IN1015	Swine	2010-07-01	ATGAA…
SJ0014	A/swine/IN/29IN1016	Swine	2010-07-01	ATGAA…
SJ0015	A/swine/IN/29IN1022	Swine	2010-07-01	ATGAA…
SJ0016	A/swine/IN/29IN1024	Swine	2010-07-01	ATGAA…
SJ0017	A/swine/IN/30IN0428	Swine	2010-07-01	ATGAA…
SJ0022	A/swine/MN/36MN0601	Swine	2010-07-01	ATGAA…
SJ0023	A/swine/MN/36MN0607	Swine	2010-07-01	ATGAA…
SJ0024	A/swine/MN/36MN0609	Swine	2010-07-01	ATGAA…
SJ0025	A/swine/MN/36MN0610	Swine	2010-07-01	ATGAA…
SJ0026	A/swine/MN/36MN1005	Swine	2010-07-01	ATGAA…
SJ0027	A/swine/MN/36MN1008	Swine	2010-07-01	ATGAA…
SJ0028	A/swine/MN/36MN1012	Swine	2010-07-01	ATGAA…
SJ0029	A/swine/MN/36MN1020	Swine	2010-07-01	ATGAA…
SJ0030	A/swine/MN/36MN1026	Swine	2011-07-01	ATGAA…
CY159457	A/swine/Arkansas/SG1499/2010	Swine	2010-08-11	ATGAA…
HQ622586	A/swine/Minnesota/54354/2010	Swine	2010-10-27	ATGAA…
KC881830	A/Kentucky/09/2010	Human	2010-11-01	ATGAA…
JF812280	A/swine/Nebraska/A01049048/2010	Swine	2010-11-17	ATGAA…
JF833337	A/swine/Iowa/A01049128/2010	Swine	2010-11-22	ATGAA…
JF833344	A/swine/North Carolina/A01049174/2010	Swine	2010-11-30	ATGAA…
JX080620	A/swine/Iowa/A01049195/2010	Swine	2010-12-01	ATGAA…
JN162047	A/swine/Iowa/A01049239/2010	Swine	2010-12-08	ATGAA…
CY167468	A/Tennessee/F2005A/2010	Human	2010-12-10	ATGAA…

CY114669	A/swine/Illinois/A01047715/2010	Swine	2010-12-14	ATGAA…
CY097837	A/District-of-Columbia/WRAIR0309/2010	Human	2010-12-30	ATGAA…
JN162057	A/swine/Iowa/A01049379/2011	Swine	2011-01-03	ATGAA…
CY134465	A/Boston/DOA08/2011	Human	2011-01-13	ATGAA…
KC881643	A/Wisconsin/16/2011	Human	2011-01-15	ATGAA…
JN162058	A/swine/Minnesota/A01049428/2011	Swine	2011-01-18	ATGAA…
KC882343	A/Indiana/04/2011	Human	2011-01-19	ATGAA…
KC881716	A/North-Carolina/09/2011	Human	2011-01-20	ATGAA…
KC881705	A/North-Carolina/06/2011	Human	2011-01-20	ATGAA…
CY092888	A/South-Carolina/NHRC0001/2011	Human	2011-01-25	ATGAA…
CY092417	A/Missouri/NHRC0001/2011	Human	2011-01-25	ATGAA…
CY134473	A/Boston/DOA28/2011	Human	2011-01-27	ATGAA…
KC882018	A/Maryland/04/2011	Human	2011-02-02	ATGAA…
KC881912	A/New-Mexico/04/2011	Human	2011-02-07	ATGAA…
KC881943	A/New-Mexico/05/2011	Human	2011-02-09	ATGAA…
JN193422	A/swine/Minnesota/25618/2011	Swine	2011-02-10	ATGAA…
KC882257	A/California/17/2011	Human	2011-02-15	ATGAA…
JN652409	A/swine/Illinois/A01049574/2011	Swine	2011-02-16	ATGAA…
JF916682	A/swine/OH/9838/2011	Swine	2011-02-21	ATGAA…
KC882336	A/Maryland/06/2011	Human	2011-02-22	ATGAA…
KC882395	A/Maryland/08/2011	Human	2011-03-02	ATGAA…
JN652417	A/swine/Illinois/A01049673/2011	Swine	2011-03-10	ATGAA…
CY167724	A/Tennessee/F2083C/2011	Human	2011-04-13	ATGAA…
JX045997	A/swine/Illinois/A01049981/2011	Swine	2011-05-17	ATGAA…
JN863540	A/swine/Iowa/A01049980/2011	Swine	2011-05-17	ATGAA…
JN193425	A/swine/Oregon/A00700068/2011	Swine	2011-05-18	ATGAA…
JX092275	A/swine/Iowa/A01202099/2011	Swine	2011-06-21	ATGAA…

SJ0018	A/swine/IN/30IN0801	Swine	2011-07-01	ATGAA…
SJ0019	A/swine/IN/30IN0816	Swine	2011-07-01	ATGAA…
SJ0020	A/swine/IN/30IN0824	Swine	2011-07-01	ATGAA…
SJ0021	A/swine/IN/30IN1017	Swine	2011-07-01	ATGAA…
JX092286	A/swine/North-Carolina/A01202450/2011	Swine	2011-07-14	ATGAA…
JN673250	A/swine/Texas/A01104003/2011	Swine	2011-07-16	ATGAA…
JN673258	A/swine/Texas/A01104004/2011	Swine	2011-07-16	ATGAA…
JX092296	A/swine/Texas/A01202511/2011	Swine	2011-08-11	ATGAA…
JX092299	A/swine/Iowa/A01202554/2011	Swine	2011-08-30	ATGAA…
JX092451	A/swine/Iowa/A01202854/2011	Swine	2011-11-15	ATGAA…
JX092551	A/swine/Colorado/A01203099/2011	Swine	2011-12-22	ATGAA…
JX092560	A/swine/Missouri/A01203163/2012	Swine	2012-01-17	ATGAA…
CY147971	A/Georgia/M5081/2012	Human	2012-02-01	ATGAA…
CY148003	A/Georgia/M5081/2012	Human	2012-02-01	ATGAA…
CY148067	A/Georgia/M5081/2012	Human	2012-02-01	ATGAA…
KC891093	A/Texas/22/2012	Human	2012-02-26	ATGAA…
JX905426	A/Florida/06/2012	Human	2012-02-27	ATGAA…
KC891408	A/North-Carolina/09/2012	Human	2012-03-01	ATGAA…
CY176690	A/Bronx/INS3-673/2012	Human	2012-03-06	ATGAA…
CY176714	A/Dayton/INS3-676/2012	Human	2012-03-12	ATGAA…
KC891216	A/North-Carolina/18/2012	Human	2012-04-26	ATGAA…
CY135108	A/Texas/JMM-52/2012	Human	2012-12-06	ATGAA…
CY182713	A/Houston/JMM-64/2012	Human	2012-12-12	ATGAA…
CY168535	A/Boston/YGA-01002/2012	Human	2012-12-19	ATGAA…
CY148316	A/Boston/DOA2-099/2012	Human	2012-12-23	ATGAA…
CY168807	A/Boston/YGA-01041/2012	Human	2012-12-25	ATGAA…
CY171543	A/Chicago/YGA-04123/2012	Human	2012-12-30	ATGAA…

CY169863	A/Boston/YGA-01185/2013	Human	2013-01-21	ATGAA…
CY170927	A/Santa-Clara/YGA-03065/2013	Human	2013-01-26	ATGAA…
CY186187	A/Houston/JMM-171/2013	Human	2013-01-27	ATGAA…
CY168423	A/Boston/YGA-00087/2013	Human	2013-01-30	ATGAA…
CY186099	A/Houston/JMM-159/2013	Human	2013-02-08	ATGAA…
KC871058	A/swine/Ohio/A01432602/2013	Swine	2013-03-12	ATGAA…
CY170079	A/Boston/YGA-01217/2013	Human	2013-03-17	ATGAA…
KF013677	A/swine/Ohio/A01349978/2013	Swine	2013-04-17	ATGAA…
CY170095	A/Boston/YGA-01220/2013	Human	2013-04-26	ATGAA…
CY194605	A/swine/Arkansas/D0386/2013	Swine	2013-05-06	ATGAA…
KF251047	A/swine/Minnesota/A01381276/2013	Swine	2013-05-23	ATGAA…
KF537364	A/swine/Illinois/A01398316/2013	Swine	2013-07-23	ATGAA…
KF772961	A/swine/Minnesota/A01392911/2013	Swine	2013-10-10	ATGAA…
CY188841	A/New-York/WC-LVD-13-004/2013	Human	2013-12-04	ATGAA…
KJ645761	A/Gainesville/08/2013	Human	2013-12-04	ATGAA…
CY188897	A/New-York/WC-LVD-13-011/2013	Human	2013-12-11	ATGAA…
CY188913	A/New-York/WC-LVD-13-013/2013	Human	2013-12-12	ATGAA…
KM409069	A/Rhode-Island/09/2013	Human	2013-12-22	ATGAA…
CY188977	A/New-York/WC-LVD-13-021/2013	Human	2013-12-24	ATGAA…
CY189033	A/New-York/WC-LVD-13-028/2013	Human	2013-12-28	ATGAA…
CY189041	A/New-York/WC-LVD-13-030/2013	Human	2013-12-31	ATGAA…
CY189049	A/New-York/WC-LVD-14-001/2014	Human	2014-01-02	ATGAA…
KJ206094	A/swine/Illinois/A01490609/2014	Swine	2014-01-08	ATGAA…
KJ645769	A/Gainesville/05/2014	Human	2014-01-15	ATGAA…
KJ206223	A/swine/Nebraska/A01366774/2014	Swine	2014-01-17	ATGAA…
KJ417899	A/swine/Minnesota/A01491447/2014	Swine	2014-01-27	ATGAA…
KJ417890	A/swine/Nebraska/A01491300/2014	Swine	2014-01-27	ATGAA…

KJ605091	A/swine/Kansas/A01410327/2014	Swine	2014-02-07	ATGAA…
KJ528259	A/swine/Illinois/A01492501/2014	Swine	2014-02-12	ATGAA…
CY189257	A/New-York/WC-LVD-14-027/2014	Human	2014-02-14	ATGAA…
KT274458	A/North-Carolina/04/2014	Human	2014-02-16	ATGAA…
KJ588390	A/swine/Nebraska/A01492657/2014	Swine	2014-02-27	ATGAA…
KJ701853	A/swine/Iowa/A01410472/2014	Swine	2014-03-03	ATGAA…
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CY189433	A/New-York/WC-LVD-14-050/2014	Human	2014-03-10	ATGAA…
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KJ701784	A/swine/Illinois/A01493472/2014	Swine	2014-03-26	ATGAA…
CY189481	A/New-York/WC-LVD-14-056/2014	Human	2014-03-27	ATGAA…
KJ907733	A/swine/Kansas/A01377299/2014	Swine	2014-04-30	ATGAA…
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KM821600	A/swine/Oklahoma/A01476227/2014	Swine	2014-08-12	ATGAA…
KU592859	A/Alaska/38/2014	Human	2014-09-03	ATGAA…
KP036967	A/swine/Minnesota/A01483170/2014	Swine	2014-10-02	ATGAA…
KT880151	A/Florida/62/2014	Human	2014-10-28	ATGAA…
KP164555	A/swine/Nebraska/A01566172/2014	Swine	2014-10-30	ATGAA…
KT836870	A/California/56/2014	Human	2014-12-29	ATGAA…
KT836895	A/California/49/2015	Human	2015-01-26	ATGAA…
KT836859	A/Hawaii/25/2015	Human	2015-02-25	ATGAA…
KT836762	A/Washington/20/2015	Human	2015-03-23	ATGAA…
KU509695	A/Indiana/15/2015	Human	2015-07-23	ATGAA…
KT965349	A/swine/Indiana/A01260972/2015	Swine	2015-08-27	ATGAA…
KU933493	A/Michigan/45/2015	Human	2015-09-07	ATGAA…
KX004130	A/Connecticut/05/2015	Human	2015-10-13	ATGAA…
KU509625	A/Arizona/26/2015	Human	2015-10-24	ATGAA…

KU509879	A/Illinois/17/2015	Human	2015-10-31	ATGAA…
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KX949386	A/Iowa/53/2015	Human	2015-11-04	ATGAA…
KU509799	A/Arkansas/10/2015	Human	2015-11-05	ATGAA…
KU589402	A/Pennsylvania/49/2015	Human	2015-12-02	ATGAA…
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KX004746	A/Arizona/38/2015	Human	2015-12-07	ATGAA…
KX004754	A/Arizona/39/2015	Human	2015-12-08	ATGAA…
KX004249	A/Maryland/21/2015	Human	2015-12-12	ATGAA…
KX408123	A/New-Mexico/29/2015	Human	2015-12-12	ATGAA…
KX004217	A/Nevada/41/2015	Human	2015-12-17	ATGAA…
KX408235	A/New-Jersey/54/2015	Human	2015-12-30	ATGAA…
KX408203	A/New-York/72/2015	Human	2015-12-30	ATGAA…
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KY044962	A/Alaska/01/2016	Human	2016-01-06	ATGAA…
KX406227	A/Connecticut/02/2016	Human	2016-01-08	ATGAA…
KY487698	A/Baltimore/0008/2016	Human	2016-01-10	ATGAA…
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KX408643	A/New-Jersey/04/2016	Human	2016-01-14	ATGAA…
KX406587	A/Washington/25/2016	Human	2016-01-16	ATGAA…
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KX406475	A/California/29/2016	Human	2016-01-18	ATGAA…
KX408339	A/Pennsylvania/04/2016	Human	2016-01-20	ATGAA…
KX406363	A/Idaho/04/2016	Human	2016-01-25	ATGAA…
KX406659	A/North-Carolina/10/2016	Human	2016-01-26	ATGAA…
KX005618	A/New-York/06/2016	Human	2016-01-27	ATGAA…

KY044724	A/Maine/01/2016	Human	2016-02-03	ATGAA…
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KX919364	A/Texas/140/2016	Human	2016-02-07	ATGAA…
KX408603	A/Georgia/13/2016	Human	2016-02-08	ATGAA…
KX006314	A/Wisconsin/24/2016	Human	2016-02-08	ATGAA…
KX411203	A/Illinois/36/2016	Human	2016-02-09	ATGAA…
KY045022	A/Colorado/10/2016	Human	2016-02-09	ATGAA…
KX406915	A/Texas/31/2016	Human	2016-02-09	ATGAA…
KX409035	A/Nevada/09/2016	Human	2016-02-09	ATGAA…
KX406571	A/Michigan/26/2016	Human	2016-02-11	ATGAA…
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KX407251	A/Nevada/16/2016	Human	2016-02-17	ATGAA…
KX407227	A/Washington/27/2016	Human	2016-02-19	ATGAA…
CY258951	A/New-York/A-WC-LVD-16-072/2016	Human	2016-02-19	ATGAA…
KY044820	A/Georgia/25/2016	Human	2016-02-23	ATGAA…
CY259751	A/New-York/A-WC-LVD-16-053/2016	Human	2016-02-23	ATGAA…
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KX918428	A/Pennsylvania/24/2016	Human	2016-02-25	ATGAA…
KX410411	A/Louisiana/08/2016	Human	2016-02-29	ATGAA…
KX919020	A/Texas/106/2016	Human	2016-02-29	ATGAA…
KY044767	A/Tennessee/10/2016	Human	2016-03-04	ATGAA…
KY615388	A/Baltimore/0096/2016	Human	2016-03-04	ATGAA…
KX915004	A/Utah/25/2016	Human	2016-03-07	ATGAA…
KX410203	A/Washington/42/2016	Human	2016-03-08	ATGAA…
KX410363	A/New-York/39/2016	Human	2016-03-08	ATGAA…
KX410715	A/Michigan/63/2016	Human	2016-03-10	ATGAA…

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KX410067	A/Nevada/26/2016	Human	2016-03-14	ATGAA…
KX411163	A/Illinois/29/2016	Human	2016-03-15	ATGAA…
KX410811	A/Pennsylvania/42/2016	Human	2016-03-15	ATGAA…
KX410523	A/Oregon/11/2016	Human	2016-03-18	ATGAA…
KX411147	A/Illinois/27/2016	Human	2016-03-20	ATGAA…
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KX409291	A/Virginia/29/2016	Human	2016-03-27	ATGAA…
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KX150713	A/swine/Ohio/A01894414/2016	Swine	2016-03-29	ATGAA…
KX411379	A/Pennsylvania/61/2016	Human	2016-03-31	ATGAA…
KX915420	A/Montana/41/2016	Human	2016-03-31	ATGAA…
KY045290	A/Illinois/40/2016	Human	2016-04-01	ATGAA…
KX915428	A/Montana/42/2016	Human	2016-04-02	ATGAA…
KX411675	A/South-Dakota/16/2016	Human	2016-04-03	ATGAA…
KX411587	A/Vermont/14/2016	Human	2016-04-05	ATGAA…
KX411483	A/Virginia/51/2016	Human	2016-04-06	ATGAA…
KY003261	A/Pennsylvania/80/2016	Human	2016-04-07	ATGAA…
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KX915148	A/Indiana/39/2016	Human	2016-04-10	ATGAA…
KX915228	A/Washington/67/2016	Human	2016-04-11	ATGAA…
KY003244	A/Pennsylvania/81/2016	Human	2016-04-14	ATGAA…
KX915772	A/New-Mexico/46/2016	Human	2016-04-18	ATGAA…
KX915308	A/New-Jersey/21/2016	Human	2016-04-18	ATGAA…
KX411595	A/Arkansas/12/2016	Human	2016-04-19	ATGAA…

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KX915468	A/New-York/72/2016	Human	2016-04-20	ATGAA…
KX915668	A/Idaho/26/2016	Human	2016-04-21	ATGAA…
KY045320	A/Florida/62/2016	Human	2016-04-22	ATGAA…
KX358892	A/swine/Missouri/A01775109/2016	Swine	2016-05-11	ATGAA…
KX916028	A/North-Carolina/49/2016	Human	2016-05-12	ATGAA…
KX915988	A/Alaska/21/2016	Human	2016-05-13	ATGAA…
KX433140	A/swine/Illinois/A01775937/2016	Swine	2016-05-26	ATGAA…
KX433143	A/swine/Illinois/A01776206/2016	Swine	2016-06-01	ATGAA…
KX518675	A/swine/Pennsylvania/A01776820/2016	Swine	2016-06-20	ATGAA…
KX518676	A/swine/Nebraska/A01776855/2016	Swine	2016-06-21	ATGAA…
KX618892	A/swine/Illinois/A01777039/2016	Swine	2016-06-22	ATGAA…
KY041973	A/swine/Indiana/A01812242/2016	Swine	2016-06-30	ATGAA…
KX006213	A/California/15/2016	Human	2016-07-01	ATGAA…
KX408579	A/Georgia/10/2016	Human	2016-07-01	ATGAA…
CY242952	A/swine/Indiana/16TOSU4933/2016	Swine	2016-08-01	ATGAA…
KX908023	A/swine/Illinois/A01778882/2016	Swine	2016-08-26	ATGAA…
KX908021	A/swine/Iowa/A01781047/2016	Swine	2016-09-02	ATGAA…
KY003323	A/Hawaii/66/2016	Human	2016-09-02	ATGAA…
KY116814	A/Hawaii/72/2016	Human	2016-09-16	ATGAA…
KY115593	A/swine/Iowa/A01782230/2016	Swine	2016-10-04	ATGAA…
CY213330	A/California/153/2016	Human	2016-11-01	ATGAA…
KY284544	A/swine/Nebraska/A01783006/2016	Swine	2016-11-03	ATGAA…
CY211074	A/California/159/2016	Human	2016-11-03	ATGAA…
CY211026	A/Pennsylvania/94/2016	Human	2016-11-09	ATGAA…
CY211098	A/Maryland/22/2016	Human	2016-11-19	ATGAA…
KY486465	A/swine/Indiana/A01671620/2016	Swine	2016-12-20	ATGAA…

CY217017	A/South-Dakota/01/2017	Human	2017-01-01	ATGAA…
CY218897	A/Idaho/08/2017	Human	2017-01-21	ATGAA…
KY653730	A/swine/Iowa/A01672518/2017	Swine	2017-01-23	ATGAA…
CY220903	A/Wisconsin/21/2017	Human	2017-01-23	ATGAA…
CY220943	A/Minnesota/09/2017	Human	2017-01-30	ATGAA…
CY223333	A/Maryland/09/2017	Human	2017-01-31	ATGAA…
CY223381	A/Florida/09/2017	Human	2017-02-01	ATGAA…
CY238322	A/Texas/86/2017	Human	2017-02-09	ATGAA…
CY225670	A/South-Dakota/14/2017	Human	2017-02-19	ATGAA…
CY225574	A/New-Hampshire/11/2017	Human	2017-02-19	ATGAA…
CY223549	A/Arizona/10/2017	Human	2017-02-19	ATGAA…
CY223557	A/New-Mexico/08/2017	Human	2017-02-21	ATGAA…
CY224238	A/Idaho/11/2017	Human	2017-02-22	ATGAA…
CY223501	A/North-Dakota/10/2017	Human	2017-02-27	ATGAA…
CY225630	A/South-Dakota/17/2017	Human	2017-03-01	ATGAA…
CY229045	A/North-Dakota/15/2017	Human	2017-03-19	ATGAA…
CY229125	A/Connecticut/16/2017	Human	2017-03-24	ATGAA…
CY236146	A/Washington/39/2017	Human	2017-04-16	ATGAA…
MF116358	A/swine/Kansas/A01378027/2017	Swine	2017-04-19	ATGAA…
CY236243	A/Virginia/29/2017	Human	2017-04-22	ATGAA…
CY236307	A/California/56/2017	Human	2017-04-29	ATGAA…
MF144722	A/swine/Iowa/A02215038/2017	Swine	2017-05-02	ATGAA…
MF159347	A/swine/Iowa/A02215202/2017	Swine	2017-05-05	ATGAA…
MF582510	A/swine/Nebraska/A02216645/2017	Swine	2017-06-06	ATGAA…
CY242462	A/Washington/293/2017	Human	2017-07-06	ATGAA…
CY245510	A/California/69/2017	Human	2017-08-16	ATGAA…
CY257501	A/Kentucky/26/2017	Human	2017-09-07	ATGAA…

CY257493	A/Kentucky/26/2017	Human	2017-09-07	ATGAA…
MH083432	A/Connecticut/31/2017	Human	2017-10-07	ATGAA…
MG650676	A/swine/South-Dakota/A02134997/2017	Swine	2017-10-24	ATGAA…
MG662640	A/swine/Iowa/A01104104/2017	Swine	2017-11-16	ATGAA…
MH083324	A/Alaska/80/2017	Human	2017-12-08	ATGAA…
MG870284	A/swine/Iowa/A02139244/2017	Swine	2017-12-27	ATGAA…
MG870266	A/swine/Utah/A02139205/2018	Swine	2018-01-02	ATGAA…
MH083805	A/Montana/03/2018	Human	2018-01-11	ATGAA…
MH183281	A/Virginia/03/2018	Human	2018-01-17	ATGAA…
MH125898	A/Iowa/05/2018	Human	2018-01-17	ATGAA…
MH183597	A/Idaho/05/2018	Human	2018-02-03	ATGAA…
MH156829	A/swine/Iowa/A02142548/2018	Swine	2018-02-08	ATGAA…
MH245997	A/Idaho/07/2018	Human	2018-02-12	ATGAA…
MH600280	A/Iowa/56/2018	Human	2018-05-08	ATGAA…

APPENDIX C. PROGRAM CODE USED WITHIN SCOPE OF THESIS

Changes Made to BEAST XML Code to Calculate Empirical Trees

Blue colored lines of code indicate that line was excluded in the tree calculation while yellow and red lines indicate additions to allow calculation based on amino acid residues present at specified sites.

```
1.    <!-- A prior assumption that the population size has remained constant       -->
2.    <!-- throughout the time spanned by the genealogy.                           -->
3.    <!--      <constantSize id="constant" units="years">
4.              <populationSize>
5.              <parameter id="constant.popSize" value="1.0" lower="0.0"/>
6.              </populationSize>
7.              </constantSize>
8.  -->
9.
10.   <!-- Generate a random starting tree under the coalescent process            -->
11.   <!--      <coalescentSimulator id="startingTree">
12.             <taxa idref="taxa"/>
13.             <constantSize idref="constant"/>
14.             </coalescentSimulator>
15. -->
16.   <empiricalTreeDistributionModel id="treeModel"
    fileName="BEAST_HA_Combined_Discrete.trees">
17.             <taxa idref="taxa"/>
18.             <nodeTraits rootNode="false" internalNodes="false" leafNodes="true"
    traitDimension="4" name="">
19.             <parameter id="leafTraits"/>
20.             </nodeTraits>
21.   </empiricalTreeDistributionModel>
22.
23.
24.   <!-- Generate a tree model                                                   -->
25.   <!--      <treeModel id="treeModel">
26.             <coalescentTree idref="startingTree"/>
27.             <rootHeight>
28.             <parameter id="treeModel.rootHeight"/>
29.             </rootHeight>
30.             <nodeHeights internalNodes="true">
31.             <parameter id="treeModel.internalNodeHeights"/>
32.             </nodeHeights>
33.             <nodeHeights internalNodes="true" rootNode="true">
34.             <parameter id="treeModel.allInternalNodeHeights"/>
35.             </nodeHeights>
36.             </treeModel>
37. -->
38.   <!-- Statistic for sum of the branch lengths of the tree (tree length)       -->
39.             <treeLengthStatistic id="treeLength">
40.             <treeModel idref="treeModel"/>
41.             </treeLengthStatistic>
42.
43.   <!-- Statistic for time of most recent common ancestor of tree               -->
44.             <tmrcaStatistic id="age(root)" absolute="true">
45.             <treeModel idref="treeModel"/>
46.             </tmrcaStatistic>
47.
48.
49.   <!-- Generate a coalescent likelihood                                        -->
50.   <!--      <coalescentLikelihood id="coalescent">
51.             <model>
52.             <constantSize idref="constant"/>
53.             </model>
54.             <populationTree>
55.             <treeModel idref="treeModel"/>
```

```
            </populationTree>
            </coalescentLikelihood>
-->

  <!-- The strict clock (Uniform rates across branches)                        -->
  <!--      <strictClockBranchRates id="default.branchRates">
            <rate>
            <parameter id="default.clock.rate" value="1.0" lower="0.0"/>
            </rate>
            </strictClockBranchRates>

            <rateStatistic id="default.meanRate" name="default.meanRate"
  mode="mean" internal="true" external="true">
            <treeModel idref="treeModel"/>
            <strictClockBranchRates idref="default.branchRates"/>
            </rateStatistic>
-->

  <!-- The strict clock (Uniform rates across branches)                        -->
  <strictClockBranchRates id="allPos.branchRates">
            <rate>
            <parameter id="allPos.clock.rate" value="1.0" lower="0.0"/>
            </rate>
  </strictClockBranchRates>

  <rateStatistic id="allPos.meanRate" name="allPos.meanRate" mode="mean"
  internal="true" external="true">
            <treeModel idref="treeModel"/>
            <strictClockBranchRates idref="allPos.branchRates"/>
  </rateStatistic>


  <!-- The strict clock (Uniform rates across branches)                        -->
  <strictClockBranchRates id="Pos38.branchRates">
            <rate>
            <parameter id="Pos38.clock.rate" value="1.0" lower="0.0"/>
            </rate>
  </strictClockBranchRates>

  <rateStatistic id="Pos38.meanRate" name="Pos38.meanRate" mode="mean"
  internal="true" external="true">
            <treeModel idref="treeModel"/>
            <strictClockBranchRates idref="Pos38.branchRates"/>
  </rateStatistic>


  <!-- The strict clock (Uniform rates across branches)                        -->
            <strictClockBranchRates id="Pos125.branchRates">
                     <rate>
                     <parameter id="Pos125.clock.rate" value="1.0" lower="0.0"/>
                     </rate>
            </strictClockBranchRates>

            <rateStatistic id="Pos125.meanRate" name="Pos125.meanRate" mode="mean"
  internal="true" external="true">
                     <treeModel idref="treeModel"/>
                     <strictClockBranchRates idref="Pos125.branchRates"/>
            </rateStatistic>


            <!-- The strict clock (Uniform rates across branches)             -->
            <strictClockBranchRates id="Pos138.branchRates">
                     <rate>
                     <parameter id="Pos138.clock.rate" value="1.0" lower="0.0"/>
                     </rate>
            </strictClockBranchRates>

            <rateStatistic id="Pos138.meanRate" name="Pos138.meanRate" mode="mean"
  internal="true" external="true">
                     <treeModel idref="treeModel"/>
                     <strictClockBranchRates idref="Pos138.branchRates"/>
```

```
122.            </rateStatistic>
123.
124.
125.            <!-- The strict clock (Uniform rates across branches)              -->
126.            <strictClockBranchRates id="Pos259.branchRates">
127.                    <rate>
128.                    <parameter id="Pos259.clock.rate" value="1.0" lower="0.0"/>
129.                    </rate>
130.            </strictClockBranchRates>
131.
132.            <rateStatistic id="Pos259.meanRate" name="Pos259.meanRate" mode="mean"
    internal="true" external="true">
133.                    <treeModel idref="treeModel"/>
134.                    <strictClockBranchRates idref="Pos259.branchRates"/>
135.            </rateStatistic>
136.
137.
138.            <!-- The HKY substitution model (Hasegawa, Kishino & Yano, 1985)   -->
139.            <!--      <HKYModel id="hky">
140.                    <frequencies>
141.                    <frequencyModel dataType="nucleotide">
142.                            <frequencies>
143.                            <parameter id="frequencies" value="0.25 0.25 0.25
    0.25"/>
144.                            </frequencies>
145.                    </frequencyModel>
146.                    </frequencies>
147.                    <kappa>
148.                    <parameter id="kappa" value="2.0" lower="0.0"/>
149.                    </kappa>
150.            </HKYModel>
151.  -->
152.            <!-- site model                                                    -->
153.            <!--      <siteModel id="siteModel">
154.                    <substitutionModel>
155.                    <HKYModel idref="hky"/>
156.                    </substitutionModel>
157.                    <relativeRate>
158.                    <parameter id="mu" value="1.0" lower="0.0"/>
159.                    </relativeRate>
160.            </siteModel>
161.
162.  -->
163.
164.            <!-- Likelihood for tree given sequence data                       -->
165.  <!--      <treeDataLikelihood id="default.treeLikelihood"
    useAmbiguities="false">
166.                    <partition>
167.                            <patterns idref="patterns"/>
168.                            <siteModel idref="siteModel"/>
169.                    </partition>
170.                    <treeModel idref="treeModel"/>
171.                    <strictClockBranchRates idref="default.branchRates"/>
172.            </treeDataLikelihood>
173.  -->
174.   <!-- START Discrete Traits Model
```

Tanglegram Generation in R

Creation of the linked phylogenetic tree map (tanglegram) was performed in R.[48] The following libraries were used: ape, ggplot2, tidyverse, and ggtree.[49,50] Code snippet included:

```
R version 3.5.3 (2019-03-11) -- "Great Truth"
Copyright (C) 2019 The R Foundation for Statistical Computing
Platform: x86_64-apple-darwin15.6.0 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

  Natural language support but running in an English locale

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[R.app GUI 1.70 (7632) x86_64-apple-darwin15.6.0]

[Workspace restored from /Users/daniel/Rdir/.RData]
[History restored from /Users/daniel/Rdir/.Rapp.history]

> library(ape)
> library(ggplot2)
> library(tidyverse)
> library(ggtree)
ggtree v1.14.6
Attaching package: ‘ggtree’
> tree1 <- read.beast("BEAST_HA_MCC.tree")
> tree2 <- read.beast("BEAST_NA_MCC.tree")
> tree3 <- read.beast("BEAST_M_MCC.tree")
> tree4 <- read.beast("BEAST_NP_MCC.tree")
> p1 <- ggtree(tree1)
> p2 <- ggtree(tree2)
> p3 <- ggtree(tree3)
> p4 <- ggtree(tree4)
> d1 <- p1$data
> d2 <- p2$data
> d3 <- p3$data
> d4 <- p4$data
> d2$x <- d2$x + max(d1$x) + 1
> d3$x <- d3$x + max(d2$x) + 1
> d4$x <- d4$x + max(d3$x) + 1
> pp <- p1 + geom_tree(data = d2) + geom_tree(data = d3) + geom_tree(data = d4)
> dd = bind_rows(d1, d2, d3, d4) %>%
+   filter(!is.na(label))
> p1 <- ggtree(tree1, aes(color=Host)) + theme(legend.position="right")
> p2 <- ggtree(tree2, aes(color=Host)) + theme(legend.position="right")
> p3 <- ggtree(tree3, aes(color=Host)) + theme(legend.position="right")
> p4 <- ggtree(tree4, aes(color=Host)) + theme(legend.position="right")
> pp <- p1 + geom_tree(data = d2) + geom_tree(data = d3) + geom_tree(data = d4)
> pp + geom_line(aes(x, y, group=label), data=dd, alpha=.3)
```

VITA

Daniel Darnell was born in Memphis, TN in 1986. He graduated from Houston High School in 2005 and Christian Brothers University in 2009. After graduating with a Bachelor of Science (B.S.) in Biology he began working as a research technologist in the lab of Dr. Richard Webby at St. Jude Children's Research Hospital. In 2011 Daniel decided to resign his current position to pursue a graduate degree with the University of Tennessee Health Science Center. He performed his graduate research work in Dr. Webby's lab at SJCRH. In December 2019 he received his Master of Science (M.S.) in Biomedical Sciences with a concentration in Microbiology, Immunology, and Biochemistry. Daniel has accepted a new position in the Hartwell Center for Bioinformatics and Biotechnology at SJCRH and is excited to further his academic and research career. He has also begun an online program to obtain a degree in Computer Science as his future research goals lie in data science and bioinformatics.

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