

ORIGINAL ARTICLE

Optimizing Surveillance for South American Origin Influenza A Viruses Along the United States Gulf Coast Through Genomic Characterization of Isolates from Blue-winged Teal (*Anas discors*)

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Summary

Relative to research focused on inter-continental viral exchange between Eurasia and North America, less attention has been directed towards understanding the redistribution of influenza A viruses (IAVs) by wild birds between North America and South America. In this study, we genomically characterized 45 viruses isolated from blue-winged teal (*Anas discors*) along the Texas and Louisiana Gulf Coast during March of 2012 and 2013, coincident with northward migration of this species from Neotropical wintering areas to breeding grounds in the United States and Canada. No evidence of South American lineage genes was detected in IAVs isolated from blue-winged teal supporting restricted viral gene flow between the United States and southern South America. However, it is plausible that blue-winged teal redistribute IAVs between North American breeding grounds and wintering areas throughout the Neotropics, including northern South America, and that viral gene flow is limited by geographical barriers further south (e.g. the Amazon Basin). Surveillance for the introduction of IAVs from Central America and northern South America into the United States may be further optimized through genomic characterization of viruses resulting from coordinated, concurrent sampling efforts targeting blue-winged teal and sympatric species throughout the Neotropics and along the United States Gulf Coast.

Introduction

Considerable research efforts have been directed towards understanding the role of migratory birds in the global redistribution of viruses since the outbreak of a highly pathogenic H5N1 influenza A virus (IAV) in wild birds in China in 2005 (Chen et al., 2005). Prior to the 2005 outbreak, few investigations had identified inter-hemispherically reassorted IAV isolates derived from wild birds

(Makarova et al., 1999; Liu et al., 2004; Wallensten et al., 2005). However, numerous phylogenetic investigations conducted since 2005 collectively demonstrate that evidence for inter-continental exchange of IAVs between Eurasia and North America is limited in migratory birds sampled throughout the contiguous United States and central/western Canada (Krauss et al., 2007; Pearce et al., 2009) and higher in avian species sampled near the continental margins of Alaska (Koehler et al., 2008; Ramey

et al., 2010a,b, 2011; Pearce et al., 2011; Reeves et al., 2013) and eastern Canada (Wille et al., 2010; Hall et al., 2013; Huang et al., 2014). Thus, the likelihood of detecting Eurasian–North American lineage reassortant IAVs may be highest at areas where migratory flyways of birds from different continental landmasses overlap and functionally diluted through distance at locations farther away (Pearce et al., 2009; Lam et al., 2012). Genomic characterization of IAVs derived from wild birds may therefore be useful for informing surveillance programmes to detect foreign origin viruses by identifying areas at which the evidence for inter-continental viral exchange is highest (Pearce et al., 2009).

Relative to research efforts focused on inter-continental viral exchange between Eurasia and North America, considerably less attention has been directed towards understanding the redistribution of IAVs by wild birds between North America and South America. Genomic characterization of isolates derived from wild birds sampled in Argentina supports the evolution of South American IAV lineages (Pareda et al., 2008; Rimondi et al., 2011; Xu et al., 2012); however, isolates derived from wild birds sampled in Barbados ($n = 3$), Columbia ($n = 2$) and Guatemala ($n = 17$) were comprised of genes of North American ancestry (Douglas et al., 2007; González-Reiche et al., 2012a; Karlsson et al., 2013; Ramey et al., 2014b). An isolate from a cinnamon teal (*Anas cyanoptera*) sampled in western Bolivia contained genes from both North American and South American lineages (Spackman et al., 2006). As the genetic characterization of IAVs derived from wild birds throughout Central and South America has generally been limited, it remains unclear: (i) where North American and South American IAV lineages converge (González-Reiche and Perez, 2012b) and (ii) if wild birds transport South American lineage IAVs into the United States through migration.

In this study, we tested the hypothesis of greater inter-continental exchange of IAVs in avian species sampled near continental margins where migratory flyways of different landmasses overlap through the genetic characterization of viruses isolated from blue-winged teal (*Anas discors*). Many blue-winged teal that breed in North America winter in northern South America (Rohwer et al., 2002), and the distribution of band recoveries for this species extends farther south and is more widespread than any other North American species of waterfowl (Botero and Rusch, 1988). We focused our effort on the genomic characterization of viruses isolated from birds using wetlands at relatively high densities at locations along the United States Gulf Coast in spring, coincident with the northward migration of blue-winged teal from Neotropical wintering areas to breeding locations throughout the United States and Canada (Rohwer et al., 2002). Although the peak of IAV infection in waterfowl is typically during autumn in the northern hemisphere (Hinshaw et al., 1980), the early post-breeding

southward migration of blue-winged teal relative to other North American migratory waterfowl (Rohwer et al., 2002) may result in a disproportionate number of susceptible birds of this species at other times of year (Stallknecht et al., 1990). Thus, through careful consideration of location, timing, migratory patterns of prospective target species and potential of hosts to be infected with IAVs in spring, we attempted to maximize the probability of detection for South American viral lineages in the United States with the goal of identifying an important location/season for surveillance directed at detecting foreign origin IAVs in North America.

Material and Methods

Sample collection and virus isolation were previously reported by Ramey et al. (2014a). Briefly, paired cloacal and oropharyngeal swab samples ($n = 1563$) were collected from blue-winged teal captured at locations along the Texas and Louisiana Gulf Coast during March of 2012 and 2013. Live capture and virus sampling protocols were approved by the U. S. Geological Survey Alaska Science Center Animal Care and Use Committee (Animal Use Permit#: 2012-2) and carried out under the authority of the United States Department of the Interior (Federal Bird Banding Permits #09072 and #23792, Federal Fish and Wildlife Permit #MB779238-2). Pooled swab samples were inoculated into embryonating specific pathogen-free chicken eggs and incubated at 37°C. Allantoic fluid was harvested and screened for IAV by rRT-PCR (Spackman et al., 2002).

RNA was extracted from all IAV positive samples and sequenced using molecular methods reported by Ramey et al. (2010a). Briefly, cDNA fragments were amplified for all eight gene segments using the one-step RT-PCR kit (Qiagen, Inc., Valencia, CA, USA) and previously published primers (Zou, 1997; Hoffmann et al., 2001; Phipps et al., 2004; Bragstad et al., 2005; Obenauer et al., 2006; Li et al., 2007; Pearce et al., 2011). PCR products were either gel purified and extracted using the QIAquick gel extraction kit (Qiagen, Inc.) or treated with ExoSap-IT (USB Inc., Cleveland, OH, USA) without additional purification prior to sequencing. Cycle sequencing was performed with identical primers used for PCR and BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA). Samples were analysed on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems). Contigs were assembled and edited with Sequencher version 5.1 (Gene Codes Corp., Ann Arbor, MI, USA). NCBI GenBank accession numbers for IAV isolates sequenced as part of this study are as follows: KC993202–KC993211 and KJ413388–KJ413735.

Sequence information for 358 (of a possible 360) gene segments from 45 IAVs isolated from blue-winged teal was

compared to homologous genomic information for IAV strain A/blue-winged teal/Texas/AI13-1028/2013(H14N5) (Ramey et al., 2014b) and subsequently subjected to genetic analyses. Sequence data for gene segments was omitted from analyses if multiple peaks were detected at nucleotide positions indicating co-infection ($n = 2$). For each of six internal gene segments (PB2, PB1, PA, NP, M and NS), phylogenetic analysis was conducted to assess whether gene segments were of North American, South American or Eurasian ancestry. Phylogenetic analysis was not conducted for surface glycoprotein genes (HA and NA) as few reference sequences exist for South American IAV lineages for most HA and NA subtypes. Nucleotide sequences for IAV gene segments derived from blue-winged teal sampled in Texas and Louisiana were aligned with sequences for representative IAV lineages of North American, South American and Eurasian ancestry (Table S1) using Sequencher version 5.1 and cropped to a common length as follows (reported in nucleotide positions): PB2 (2,231), PB1 (2,170), PA (2,132), NP (1,411), M (798) and NS (797). Reference sequences were selected to cover wide geographic distributions of collection locations from continents and restricted to samples collected from 2000 to 2012. A consensus neighbour-joining tree was constructed for each internal gene segment with MEGA version 5.1 using the maximum composite likelihood model for nucleotide sequences with 10 000 bootstrap replicates. The continental affiliation for clades in which nucleotide sequences for IAV gene segments derived from blue-winged teal were nested was inferred as the lineage of ancestral origin. In instances in which tree topology did not yield clear evidence for continental affiliation of ancestry for a given nucleotide sequence, data were compared to information on NCBI GenBank using the nucleotide BLAST function (accessed 15–17 January 2014).

While phylogenetic analysis was not conducted for genetic data for HA and NA genes of IAV isolates from blue-winged teal on account of insufficient reference data, nucleotide sequences for these gene segments were compared to data on NCBI GenBank using the nucleotide BLAST function (accessed 10 January 2014) to infer possible ancestral origins. High nucleotide identity (i.e. >95%) to IAV strains previously isolated from samples collected in North America, South America or Eurasia was inferred as weak evidence for possible ancestral origin of gene segments.

Results

Phylogenetic analysis of six internal gene segments provided no evidence of South American or Eurasian lineage genes in IAVs isolated from samples collected from blue-winged teal in Texas or Louisiana in 2012–2013 (Fig. 1). Nucleotide sequences for internal gene segments were

nested within North American IAV lineages except for the M, NP and NS gene segments of A/blue-winged teal/Louisiana/AI12-1334/2013(H4N2) (Fig. 1). Nucleotide sequences for these gene segments were basal in relation to the North American lineage for IAV genes in tree topology resulting in unclear ancestral continental affiliation; however, top ten NCBI BLAST results for these sequences indicated high identity ($\geq 98\%$) to homologous gene segments of IAVs isolated from waterfowl in North America (Table S2).

Top NCBI BLAST results for nucleotide sequences of HA and NA genes from blue-winged teal isolates revealed high identity ($\geq 98\%$) to homologous genes for IAVs isolated from waterfowl in North America except for the NA gene of isolate A/blue-winged teal/Texas/AI12-614/2012 (H10N3) (Table 1). The top NCBI BLAST result for the NA gene of this isolate indicated that the N3 NA gene of A/blue-winged teal/Texas/AI12-614/2012(H10N3) shared 99% identity with the N3 NA gene of an isolate derived from a poultry worker sampled in Mexico in 2012 during an outbreak of a highly pathogenic H7N3 IAV (Table 1). A more comprehensive review of the top ten NCBI BLAST results provided further inference of possible genetic ancestry for this and other HA and NA genes.

Top ten BLAST results for nucleotide sequences for HA and NA genes from blue-winged teal isolates revealed identity $\geq 95\%$ compared with homologous genes for IAVs isolated from avian samples collected in North America except for the N3 NA gene mentioned previously, the H14 HA gene of isolate A/blue-winged teal/Texas/AI13-1028/2013 (H14N5) and the HA and NA genes of isolate A/blue-winged teal/Louisiana/AI13-1334/2013(H4N2) (Table S3). Top ten BLAST results for the N3 NA gene of isolate A/blue-winged teal/Texas/AI12-614/2012(H10N3) also indicated shared identity of 99% to sequence information for the N3 NA gene of a poultry isolate recovered during the same outbreak of highly pathogenic H7N3 IAV in Mexico reported previously, in addition to 97–98% shared identity with other low pathogenic viruses recovered from migratory waterfowl sampled throughout the United States during 2006–2010. The highest ranking NCBI BLAST results for the H14 HA gene of A/blue-winged teal/Texas/AI13-1028/2013(H14N5) indicated shared identity $\geq 96\%$ to sequence data for other H14 HA genes detected since 2010 in the United States; however, lower identity (88%) to H14 HA genes derived from wild bird samples collected in the former Soviet Union was also identified among the top ten results (Table S3). Highest ranking BLAST results for nucleotide sequences for both the HA and NA genes of isolate A/blue-winged teal/Louisiana/AI13-1334/2013(H4N2) indicated identity $\geq 97\%$ to other samples collected in the United States since 2006; however, lower shared identity values (87–91%) with nucleotide sequences for surface glycoproteins derived from wild bird origin isolates collected

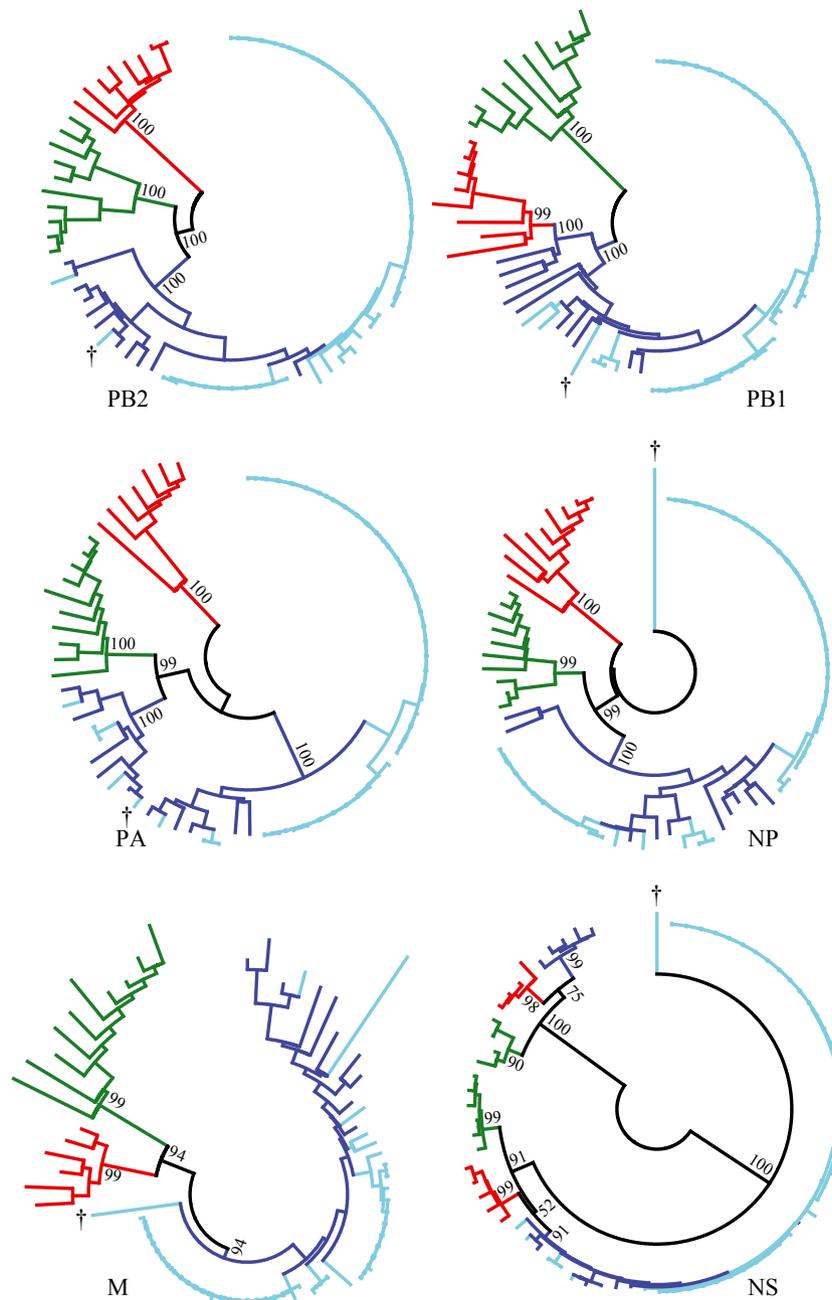


Fig. 1. Phylogenetic trees showing inferred relationship among nucleotide sequences for the internal gene segments for influenza A viruses isolated from blue-winged teal in Texas and Louisiana, 2012–2013 (light blue) and reference sequences derived from viruses isolated from wild birds in North America (dark blue), South America (red) and Eurasia (green). Bootstrap support values for major clades are shown. Nucleotide sequences for isolate A/blue-winged teal/Louisiana/AI13-1334/2013(H4N2) are indicated with a dagger (†).

in the United States during 1975–1990 were also identified in top ten results (Table S3).

Discussion

Genomic characterization of IAVs isolated from blue-winged teal sampled along the United States Gulf Coast

in spring does not provide evidence for the introduction of South American lineage IAVs into North America. Rather, the lack of detection of South American lineage IAV genes in viruses isolated from blue-winged teal sampled in Texas and Louisiana during spring provides further support for restricted viral gene flow between the United States and southern South America (Pereda et al.,

Table 1. Top NCBI BLAST result for nucleotide sequences of hemagglutinin and neuraminidase genes derived from influenza A viruses isolated from blue-winged teal captured in Louisiana and Texas, 2012–2013. Standard abbreviations for wild bird species and United States have been used in strain names.

Strain	Hemagglutinin gene top BLAST result	Neuraminidase gene top BLAST result
A/BWTE/TX/AI12-433/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-500/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-555/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-568/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-570/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-572/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-590/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-614/2012(H10N3)	A/MALL/IL/10OS4334/2010(H10N7)	A/Mexico/InDREE7218/2012(H7N3)
A/BWTE/TX/AI12-795/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-801/2012(H10N3)	A/AMWI/CA/2930/2011(H10N3)	A/AGWT/MS/11OS250/2011(H7N3)
A/BWTE/TX/AI12-802/2012(H10N3)	A/AMWI/CA/2930/2011(H10N3)	A/AGWT/MS/11OS250/2011(H7N3)
A/BWTE/TX/AI12-864/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-909/2012(H7N1)	A/AGWT/MS/11OS247/2011(mixed)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/TX/AI12-1043/2012(H10N1)	A/MALL/IL/10OS4334/2010(H10N7)	A/STEI/AK/44354-187/2007(H6N1)
A/BWTE/LA/AI13-145/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-178/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-232/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-238/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/TX/AI13-330/2013(H11N9)	A/AGWT/MS/11OS90/2011(H11N9)	A/AGWT/MS/11OS90/2011(H11N9)
A/BWTE/TX/AI13-462/2013(H7N3)	A/ABDU/WI/10OS3949/2010(H7N8)	A/NSHO/MO/10OS4750/2010(H7N3)
A/BWTE/TX/AI13-1009/2013(H11N3)	A/AGWT/MS/11OS90/2011(H11N9)	A/MALL/WI/4236/2009(mixed)
A/BWTE/TX/AI13-1028/2013(H14N5)	A/NSHO/CA/2696/2011(H14N2)	A/NSHO/MS/09OS025/2009(H12N5)
A/BWTE/LA/AI13-1225/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1226/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1334/2013(H4N2)	A/pekin duck/CA/P30/2006(H4N2)	A/MALL/MS/407/2010(mixed)
A/BWTE/LA/AI13-1337/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1338/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1339/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1340/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1341/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1345/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1357/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1381/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1412/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1413/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1414/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1415/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1416/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1420/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1421/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1423/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1424/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1425/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1429/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1435/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)
A/BWTE/LA/AI13-1437/2013(H7N7)	A/AGWT/MS/11OS250/2011(H7N3)	A/MALL/MO/10MO0551/2010(H7N7)

2008; Rimondi et al., 2011; Xu et al., 2012). It is plausible that blue-winged teal redistribute IAVs between North American breeding grounds and wintering areas throughout the Neotropics, including northern South America, and that gene flow is limited within the Americas by geographical barriers further south (e.g. the

Amazon Basin). Further efforts to genomically characterize virus isolates derived from wild birds, including blue-winged teal, throughout the Neotropics would be useful for clarifying the distribution of South American lineage IAVs and understanding viral gene flow in the New World.

Phylogenetic and BLAST analyses provided support for North American ancestry of all gene segments derived from United States Gulf Coast blue-winged teal isolates although results for seven gene segments warrant further explanation. Five gene segments (HA, NP, NA, M and NS) for IAV isolate A/blue-winged teal/Louisiana/AI13-1334/2013 (H4N2) had weaker phylogenetic support for North American ancestry and/or lower shared identity with homologous IAV genes derived from samples collected in the United States. The reason for this finding remains unclear, but may be a function of taxonomic, temporal or spatial biases of data used as reference for North American lineages in our analyses and/or available on GenBank. Top ten BLAST results for the H14 HA gene of isolate A/blue-winged teal/Texas/AI13-1028/2013(H14N5) included several Eurasian-origin isolates; however, this can be explained by the limited genetic data available for this HA subtype (Ramey et al., 2014b). Top ten BLAST results for the N3 NA gene of A/blue-winged teal/Texas/AI12-614/2012(H10N3) provide evidence that this gene has shared ancestry with a highly pathogenic H7N3 poultry-origin IAV detected in Mexico and other viruses isolated from migratory waterfowl in the United States. These results are not unexpected considering that Mexico supports large numbers of wintering waterfowl from the United States and Canada, including blue-winged teal (Botero and Rusch, 1988).

A lack of detection of South American lineage genes in IAVs isolated from blue-winged teal along the United States Gulf Coast may be the result of limitations of spring sampling efforts. Limitations may include lack of viral diversity at spring sampling sites, low viral prevalence among blue-winged teal during this season and/or geographic isolation of the United States Gulf Coast from areas at which South American IAV lineages are maintained. The diversity of viruses recovered from blue-winged teal samples collected along the Gulf Coast in 2012 and 2013 was relatively low with many isolates sharing viral subtypes and having identical or nearly identical nucleotide sequences for gene segments, particularly when comparing viruses originating from the same location and year. This is suggestive of intra-species transmission and clonal expansion of viruses. This result suggests that spring surveillance sampling might be further optimized to maximize the recovery of viral diversity and/or minimize sampling effort. Potential additive modifications to the sampling strategy used in the current study include the addition of spatially distant sampling sites and/or sampling for a longer temporal period. Alternatively, it may be possible to decrease sampling effort and still detect a similar level of viral diversity; however, reductions in sampling effort could also inhibit the detection of rare viral diversity.

The isolation rate of IAVs from blue-winged teal paired swab samples obtained in Texas and Louisiana during

March of 2012 and 2013 was relatively low (0.9–5.6% by location/year; Ramey et al., 2014a) which may be a function of population immunity of blue-winged teal to IAVs of particular antigenic subtypes at locations along the United States Gulf Coast in spring. Thus, it is possible that blue-winged teal arrive in the United States while infected with IAVs containing South American lineage genes, but that these relatively rare viruses do not proliferate in an immune population. Such a scenario would suggest that increased sampling effort may be required to identify IAVs with South American lineage genes in the United States.

Perhaps the most parsimonious explanation for a lack of detection of South American lineage genes in IAVs isolated from blue-winged teal sampled during spring may be the presence of geographic barriers restricting viral gene flow between the United States Gulf Coast and areas at which South American IAV lineages are maintained. The lack of South American lineage IAV genes in viruses isolated from blue-winged teal in this study is similar to results for viruses isolated from this species in Guatemala and Barbados (Douglas et al., 2007; González-Reiche et al., 2012a; Ramey et al., 2014b). Limited sequence information is currently available for viruses isolated from waterfowl in northern South America, and sampling of species that move between North America and South America, such as blue-winged teal, has been limited in this region. In South America, IAVs have been isolated from cinnamon teal in Bolivia (Spackman et al., 2006), white-faced (*Dendrocygna viduata*) and black-bellied (*Dendrocygna autumnalis*) whistling ducks in Columbia (Karlsson et al., 2013), cinnamon teal and white-cheeked pintail (*Anas bahamensis*) in Peru (Gherzi et al., 2009) and cinnamon teal, rosy-billed pochards (*Netta peposaca*) and silver teal (*Anas versicolor*) in Argentina (Rimondi et al., 2011). These waterfowl species therefore may be important in the maintenance of IAVs in South America. The distributions of these species are as follows: (i) south of the Amazon Basin (rosy-billed pochard and silver teal), (ii) disjunct with distinct populations (subspecies) north/west and south of the Amazon Basin (cinnamon teal and white-cheeked pintail) or (iii) resident throughout the Neotropics (white-faced and black-bellied whistling ducks; Madge and Burn, 1988; Fig. 2). In contrast, the range of blue-winged teal is generally north/west of the Amazon Basin (Botero and Rusch, 1988; Madge and Burn, 1988; Rohwer et al., 2002; Fig. 2). Thus, it is plausible that extensive forest habitat throughout the Amazon Basin could be a geographical barrier that limits waterfowl migration and/or provides suboptimal habitat for maintaining populations of dabbling ducks which therefore restricts viral gene flow.

Previous phylogenetic analyses conducted on IAV isolates derived from wild bird samples collected in South America support such a scenario. All IAVs collected north

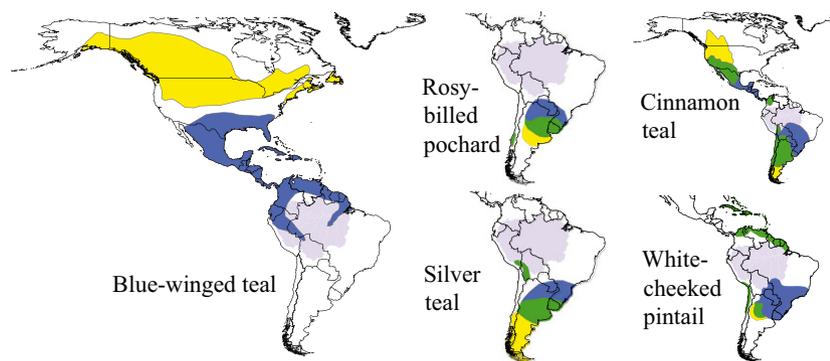


Fig. 2. Approximate extent of the Amazon Basin (grey) and breeding (yellow), wintering (blue) and year-round (green) distributions of migratory waterfowl that may be important in Influenza A ecology in South America and the Neotropics. Ranges of migratory waterfowl can generally be described as follows: north/northwest of the Amazon Basin (blue-winged teal), south of the Amazon Basin (rosy-billed pochard and silver teal) or disjunct with distinct populations (subspecies) north/northwest and south of the Amazon Basin (cinnamon teal and white-cheeked pintail). Ranges for resident waterfowl discussed in the text (white-faced and black-bellied whistling ducks) are not shown. Figure adapted from Madge and Burn (1988).

of the Amazon Basin have been genomically characterized as being of North American ancestry (Karlsson et al., 2013) in contrast to those south of the Amazon Basin (Pereda et al., 2008; Rimondi et al., 2011; Xu et al., 2012). The identification of a North–South American lineage reassortant virus originating from a sample collected from western South America on Lake Titicaca (Spackman et al., 2006) may indicate a region where such viruses are more likely to be detected. Further testing of waterfowl for IAVs in South America at areas both north and south of the Amazon Basin on opposite sides of the Andes Cordillera, another potential geographical barrier, and in the region where inter-continental reassortment has previously been detected (i.e. Eastern Peru and Western Bolivia) may help to resolve patterns of IAV gene flow in the Americas.

For reasons discussed previously, the isolation of North American lineage IAVs in blue-winged teal along the United States Gulf Coast does not rule out the possibility that this migratory species is important in the transport of IAVs between North America and the Neotropics. Additional sampling of this abundant species throughout its range in Mexico, Central America and northern South America may provide a critical link to unravelling the role of this host in the maintenance and spread of IAVs in the New World. Through genetic comparisons among IAV isolates obtained from blue-winged teal and sympatric species sampled throughout the Neotropics in winter and at locations along the United Gulf Coast in spring, it may be possible to elucidate the extent of viral redistribution by migratory birds between these regions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. GenBank accession numbers for reference sequences used in phylogenetic analyses of influenza A viruses isolated from blue-winged teal captured in Louisiana and Texas, 2012–2013.

Table S2. Top ten NCBI BLAST results for nucleotide sequences for internal genes derived from influenza A virus isolate A/blue-winged teal/Louisiana/AI12-1334/2013 (H4N2).

Table S3. Top ten NCBI BLAST results for nucleotide sequences for hemagglutinin and neuraminidase genes derived from influenza A viruses isolated from blue-winged teal captured in Louisiana and Texas, 2012–2013.