

# **Assessment of virus movement across continents: using Northern Pintails (*Anas acuta*) as a test**

## **A collaborative study between U.S. and Japanese researchers**

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## PROJECT SUMMARY

Through an international collaborative partnership, this study will examine the likelihood that migratory birds will transmit highly pathogenic H5N1 avian influenza from Asia to North America, and to use Northern Pintails (*Anas acuta*) as a model for viral transmission. The various aspects of this project will: (1) evaluate spatial overlap of North American and Asian pintail populations through analysis of band recoveries of birds marked on each continent, (2) deploy satellite transmitters on up to 90 Northern Pintails on wintering areas in Japan to assess movements and summer distribution, and to complement similar research that has occurred in North America, (3) contrast strains of non-H5N1 viruses found in Northern Pintails on Japanese wintering areas and those on North American wintering areas to assess the recent and historic transcontinental transmission of avian viruses, and (4) compare genetic differentiation of Asian and North American pintails to determine the degree to which populations are reproductively isolated. We believe this multi-faceted study will help both Japanese and North American biologists better understand the role of wild migratory bird species in transmission of the H5N1 virus.

## INTRODUCTION

There remains considerable uncertainty regarding the role of wild birds in the spread of highly pathogenic H5N1 across Asia and Europe. Given the occurrence of highly pathogenic H5N1 avian influenza in Asia, the question remains: what is the likelihood that this particular virus will disperse to North America via wild birds? For this transmission to be completed, two things have to occur (1) birds have to cross the inter-continental boundary (one or both ways), and (2) during periods of population overlap, there has to be successful transmission of the virus, either within or among species. The probability of this series of events occurring is a topic of debate. Certain species are known to regularly cross the inter-continental boundary, but it is uncertain what the likelihood of them exchanging viruses with the resident population might be. However, examination of exchange rates of viruses other than H5N1 subtype could be a useful model for predicting the probability of H5N1 dispersal from Asia to North America via wild birds.

Northern Pintails are a good candidate species for consideration of virus exchange rates. Northern Pintails are fairly ubiquitous in both North America and Asia and show generally similar patterns of wintering in temperate areas and breeding in the sub-arctic and arctic zones (Alaska Interagency HPAI working Group 2006; Figure 1). Satellite telemetry data indicate that Northern Pintails wintering in North America regularly migrate to Asia (Miller et al. 2005). It is uncertain if a similar relationship exists for Northern Pintails originating in Asia. Further, Northern Pintails can be captured in large numbers facilitating adequate sample size for characterization of virus populations. Finally, Northern Pintails have been shown to carry

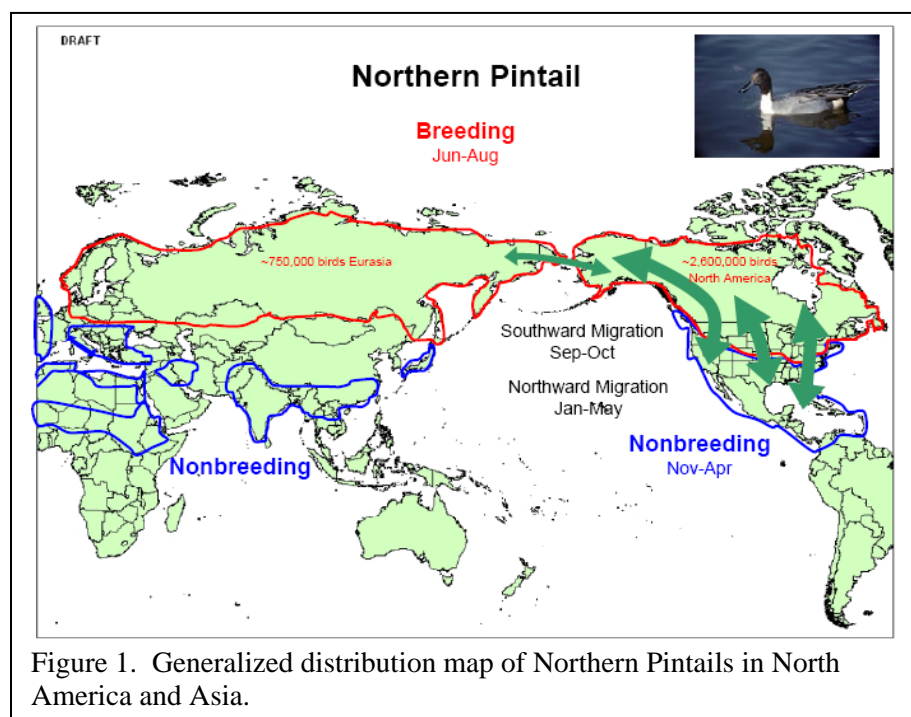


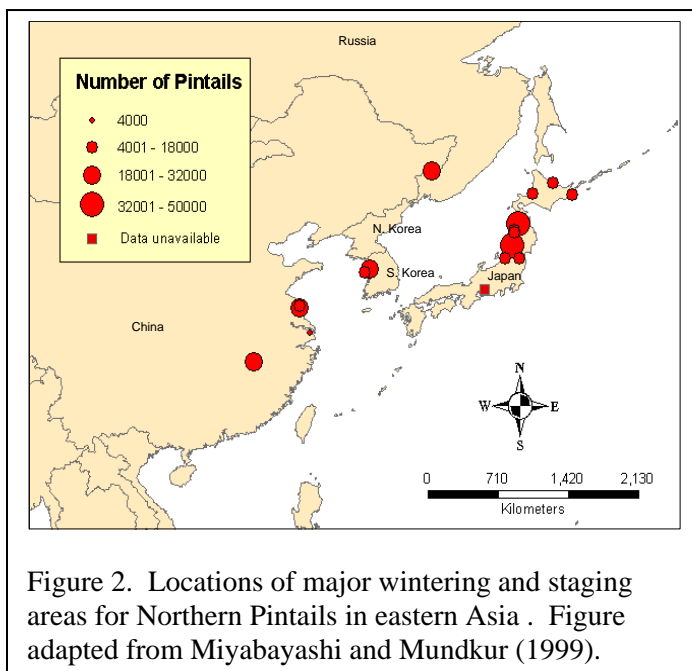
Figure 1. Generalized distribution map of Northern Pintails in North America and Asia.

a wide variety of viruses facilitating comparison of virus populations amongst continental populations of birds.

### Population status

North America – The North American breeding population is currently estimated at approximately 3 million birds. The majority of these birds breed in the prairie pothole region of the U.S. and Canada as well as Alaska (Bellrose 1976). The majority of the North American population winters along the Pacific Flyway with the largest concentration in California.

Japan – Up to 500,000-1,000,000 Northern Pintails winter in eastern and southeastern Asia with the largest known wintering groups occurring in Japan (Figure 2; Perennou et al. 1994, Wild Bird Society of Japan 1992, Rose and Scott 1994, Miyabayashi and Mundkur 1999). Other important east Asian wintering areas exist in China and South Korea (Figure 2; Miyabayashi and Mundkur 1999). The largest winter concentrations in Japan occur on the northwestern portion of Honshu in the Aomori, Akita, and Yamagata prefectures. However, Northern Pintails winter throughout the islands of Honshu, Shikoku, and Kyushu (Perennou et al. 1994).



Band recoveries indicate that most Northern Pintails which winter in Japan originate from nesting areas on the Chukotka and Kamchatka peninsulas in eastern Russia (Yamashina Institute for Ornithology 1985, Bianki and Dobrynina 1997). Twenty-two Northern Pintails that have been marked on breeding or molting areas in North America have also been recovered during winter in Japan (Yamashina Institute for Ornithology 2004, Nicolai et al. 2005). Conversely, there are at least 37 winter recoveries in North America of Northern Pintails that were banded at wintering sites in Japan (Yamashina Institute for Ornithology 2004). Furthermore, some Northern Pintails migrate from wintering areas in North America to nesting and molting areas in eastern Russia (Rienecker 1987, Miller et al. 2005), where they could come into contact with birds that migrate from Asian wintering sites. Thus transcontinental exchange of avian-borne pathogens could occur through (1) mixing of North American and Asian migrants on shared summer habitats in eastern Russia, (2) Northern Pintails that migrate between North American nesting areas and Asian wintering areas, or (3) birds that shift wintering sites from Asia to North America.

### GENERAL OBJECTIVES

Here, we propose a four-faceted collaborative study to examine the movement patterns of Northern Pintails and estimate associated virus transmission rates among continental pintail populations.

- (1) **Examine regions of overlap in summer distributions of Northern Pintails from North American and Japanese wintering areas.** We will analyze band recovery data for birds banded in both North America and Japan to establish zones of overlap in summer distributions.

- (2) **Examine Pintail Movement Patterns in Russia.** A comprehensive satellite telemetry study has already examined movement and dispersal patterns for female Northern Pintails wintering in North America. We will replicate this study for Northern Pintails wintering in Asia. The degree of spatial and temporal overlap in range of North American birds will be estimated.
- (3) **Examine Virus Transmission Rates.** We will collect virus samples on North American wintering areas for direct comparison with samples collected on Japanese wintering areas. We will compare prevalence of viruses among populations as well as compare genetic differentiation among viruses from each continent.
- (4) **Examine Population Genetic Characteristics.** We will compare nuclear and mitochondrial DNA characteristics of Asian and North American wintering populations as an independent assessment of the degree of population interchange.

The combination of these component studies will then allow us to assess the overall risk of specific viruses (i.e., highly pathogenic H5N1 in this case) being carried to North America via wild Northern Pintails. Similarly, these data will allow assessment of the potential for transmission of viruses from North America to Japan.

### DISTRIBUTIONAL ANALYSES OF BANDED NORTHERN PINTAILS RECOVERED IN RUSSIA

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Banded Northern Pintails from both North America and Japan are regularly recovered in Russia (Figures 3 and 4). General analyses of these distributions led Bianki and Dobrynina (1997) to hypothesize geographical distributions for both populations in Russia. These hypothesized distributions included

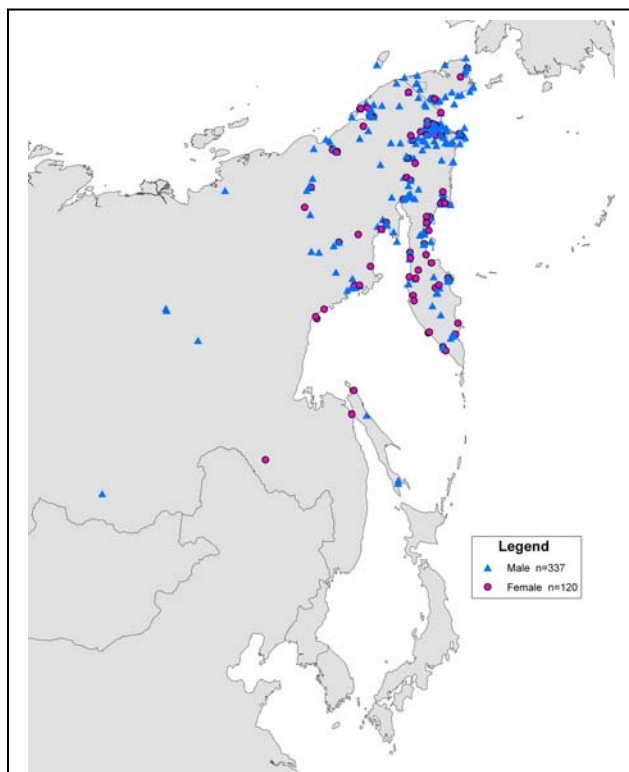


Figure 3. Recovery locations (both direct and indirect) of North American banded Northern Pintails in Russia. Symbol indicates sex of individuals. Data from USGS Bird Banding Lab, Patuxent, Maryland.

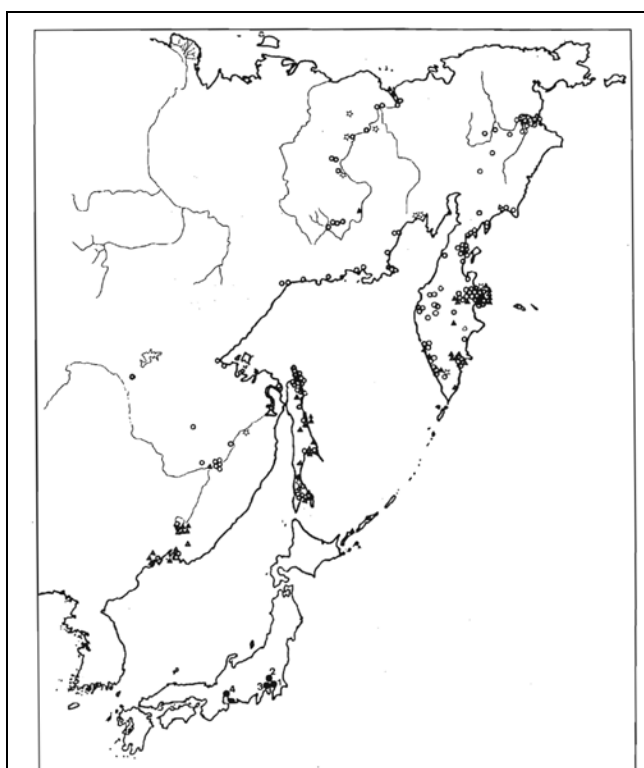


Figure 4. Recovery locations (both direct and indirect) of Japan banded Northern Pintails in Russia. Symbol indicated month of recovery. ★ = August, ○ = September, △ = October, and X = November. Figure adapted from Yamashina Institute for Ornithology (1985).

areas of separation as well as an area of sympatry (Figure 5). Inclusion of more recent data from North American banded Northern Pintails (Figure 3) suggests that the area of sympatry may be substantially larger than proposed by Bianki and Dobrynina (1997) and likely includes the entire Kamchatka Peninsula and areas west to the Kolyma River. We propose to re-analyze recovery distributions for Northern Pintails banded in North America and Japan to establish geographic areas where these distinct wintering populations overlap during summer.

#### Specific Objectives

1. Update databases with most recent banding and band recovery information for both Japanese and North American banding areas.
2. Estimate population ranges in Russia for both wintering populations using kernel home range analyses approaches.

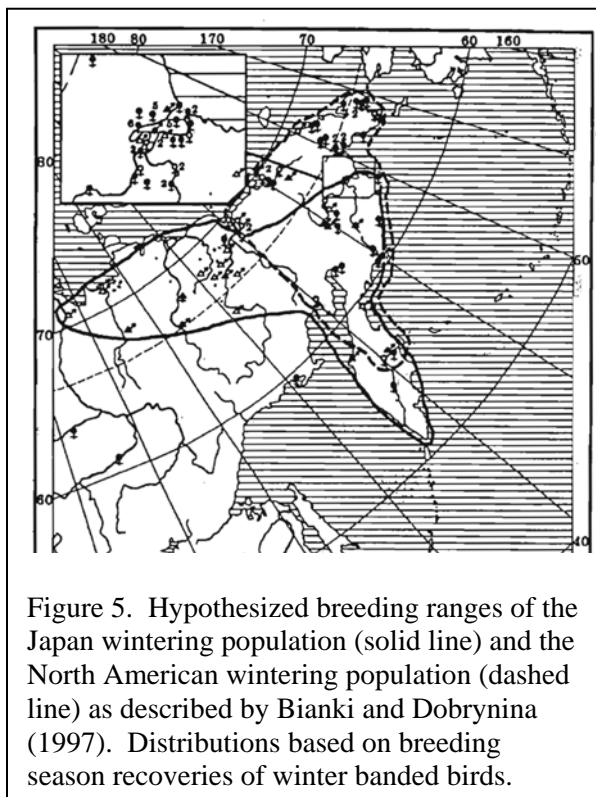


Figure 5. Hypothesized breeding ranges of the Japan wintering population (solid line) and the North American wintering population (dashed line) as described by Bianki and Dobrynina (1997). Distributions based on breeding season recoveries of winter banded birds.

#### Methods

North American banding and band recovery data can be obtained from the U.S. Geological Survey, Bird Banding Laboratory in Patuxent, Maryland. Comparable data from Japanese banded birds will be obtained from the Yamashina Institute for Ornithology, Bird Migration Research Center. We will query Russian banding authorities to ensure all Russian recovered bands have been reported and recorded appropriately. We should consider populations (i.e., North American, Japanese) and sexes separately, since males are more likely to disperse, and ages (hatch year and after hatch year) as an indication of natal origin. Then if we also consider direct and indirect recoveries, we end up with a somewhat complicated data-set. However, at the onset all we need are recovery locations and dates, banding dates, and age and sex at banding.

We will calculate Kernel home-ranges for each “class” of individual (where classes represent age and sex groups). We will then calculate the degree of overlap among these distributions. We will use a bootstrap randomization procedure to reassign individuals to populations (without replacement) and recalculate these distributions and associated overlap. We will repeat this randomization 1000 times, and directly estimate probabilities associated with differences in distributions. Based on the most parsimonious assignment of groups, we will develop maps describing these distributions and estimating overlap in continental populations. Areas defined in this analysis will be used as strata to analyze satellite telemetry data as described below. Defining the area of sympatry will be useful for estimating the degree of overall population overlap between continental populations as it relates to risk of virus transmission.

## SATELLITE TELEMETRY TRACKING OF NORTHERN PINTAILS IN ASIA

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Evaluating exchange of avian-borne pathogens between Asia and North America by migratory birds requires a clear understanding of where birds from each continent are likely to come into contact. Much information on shared distributions of North American and Asian Northern Pintails comes from recoveries of tarsus-banded birds which indicate an area of sympatry in Eastern Russia, and movement between continents (Figure 6). Although band recovery data are useful to understand where North American and Asian Northern Pintails may come into contact, these data can be biased by geographic variation in recapture efforts, hunter harvest, or reporting rates. They indicate where band recoveries have been reported, but may not necessarily reflect true population distributions.

Satellite telemetry provides an unbiased method of identifying areas used by migratory species and for testing whether band recovery data truly measure population distribution. Satellite telemetry is especially useful for assessing distribution of migratory species across remote areas such as eastern Russia where the human population is small and the likelihood of band recoveries is low. Furthermore, satellite telemetry provides a measure of temporal use which is also important for understanding the likelihood of contact between North American and Asian migrants. Discerning temporal associations from band recovery data is difficult. Combining satellite telemetry data with band recovery distributions may more clearly define areas of overlap between Asian and North American migrants.

Miller et al. (2005) used satellite transmitters (also referred to as platform transmitting terminals or PTT's) to document spring migration routes, stopover areas, and breeding and molting distributions for a relatively large sample ( $n = 129$ ) of adult female Northern Pintails marked on wintering areas in the Central Valley of California. Of special interest is their observation that four

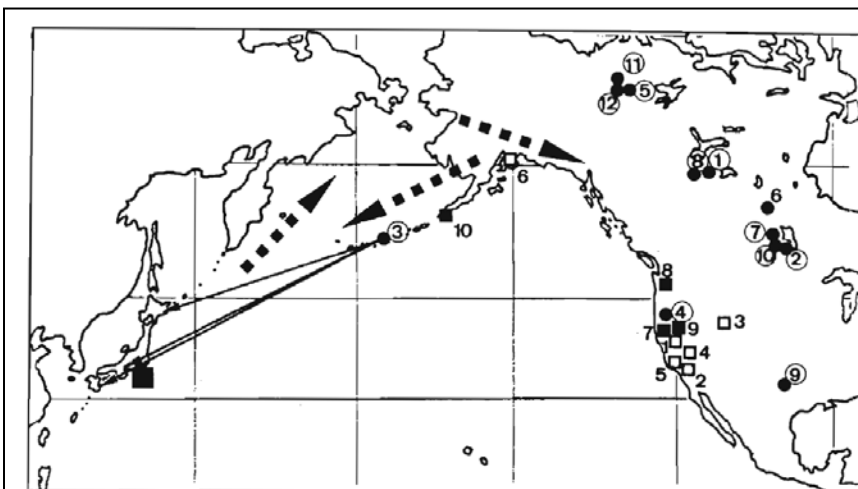


Figure 6. Recovery locations of Northern Pintails banded in Japan and North America. Circles indicate banding locations for birds recovered in Japan. Open squares indicate direct recoveries and closed squares indicate indirect recoveries of Japan banded birds. Figure adapted from (Yamashima Institute for Ornithology 1985).

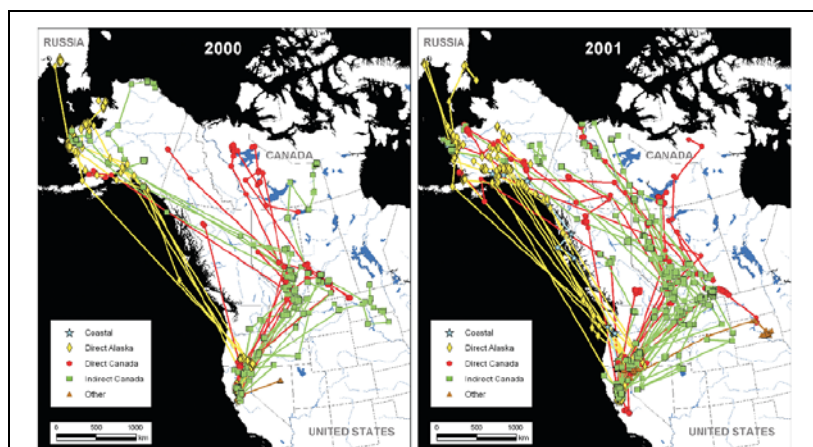


Figure 7. Satellite telemetry tracking documented female Northern Pintails crossing from North America to Asia (Miller et al. 2005).

marked females migrated to summer habitats in Russia via Alaska (Figure 7). This is approximately 6% of the females that migrated from the Central Valley to Alaska, and is likely a conservative estimate as PTT's of some Alaskan migrants may have failed before they migrated to Russia. Northern Pintails that migrated to Russia used the Anadyr Delta or north coast of Chukotka (Miller et al. 2005).

Similar data on summer distribution and transcontinental movement of Northern Pintails marked with satellite transmitters on Asian wintering areas are needed to fully assess their likelihood of coming into contact with Northern Pintails from North America. Importantly, the sample of Asian Northern Pintails marked with satellite transmitters should be sufficiently large so that inference regarding summer distribution can be drawn at the population level. Lindberg and Walker (2006) note that estimates of population distribution based on satellite telemetry may be imprecise unless a sufficiently large sample of individuals is marked. They suggest that a sample of  $\geq 75$  individuals must be marked with satellite transmitters in order to derive useful estimates of a population's distribution among as few as three geographic strata.

Japan is the primary wintering area for Northern Pintails in east Asia (Miyabayashi and Mundkur 1999) and migration studies of Japanese Northern Pintails via satellite telemetry are ongoing (H. Higuchi, personal communication). We seek a partnership with Japanese colleagues to mark up to 90 Northern Pintails in Japan with satellite transmitters over a three-year period beginning in 2007 if possible. We believe this study could benefit both Japanese and North American biologists by providing precise estimates of the summer distribution of Northern Pintails that winter in Japan. Marking Northern Pintails in Japan with satellite transmitters will complement the study by Miller et al. (2005) on the species' primary wintering area in North America. Because large numbers of Northern Pintails have been banded in both regions, satellite telemetry can be used to test whether geographic distributions of band recoveries reflect actual population distributions. This information is critically important to define areas of overlap between North American and Asian Northern Pintails and to estimate the likelihood of contact. Satellite telemetry can also provide a direct measure of the proportion of Japanese pintails that conduct a transcontinental migration to North America.

### Objectives

1. Use satellite telemetry to estimate the likelihood that Northern Pintails which winter in Japan will migrate to areas of Russia where recoveries of tarsus-banded birds suggest overlap between Asian and North American Northern Pintails.
2. Measure temporal occupancy of Japanese pintails in areas of Russia where band recovery data suggest Asian and North American Northern Pintails both occur.
3. Estimate the proportion of Northern Pintails marked in Japan that migrate to North America.

### Methods

We will capture Northern Pintails at Izunuma Lake (38° 43' N, 141° 06' E) beginning in late January of each year of the study. This work will directly compliment ongoing work where 30 Northern Pintails are being marked at Hokkaido, central Honshu, and Kyushu in Japan. Marking in February will allow the birds enough time to adjust to PTT's prior to undertaking spring migration. All captured Northern Pintails will be weighed, marked with tarsus bands, and a cloacal swab obtained to sample for strains of avian influenza. In addition, a feather sample will be obtained from each individual for genetic analyses.

As part of this study, we will mark up to 30 adult Northern Pintails with PTT's in each year over a three year period. An additional 20 birds are being marked in conjunction with other cooperative studies. Adults will be identified on the basis of wing plumage (Duncan 1985), and both males and females would be marked with PTT's. Approximately 55-60% of PTT's would be deployed on males and the remainder

on females so that the marked sample reflects the sex ratio of the population (Bellrose 1976, Austin and Miller 1995). PTT's would only be deployed on adults that are near or above average mass for their respective gender in order to reduce the likelihood of adversely affecting smaller birds (Miller et al. 2005).

Birds will be marked with solar powered PTT's manufactured by Microwave Telemetry Incorporated (Columbia, Maryland, USA) to study Northern Pintail migration and distribution. The PTT's will be programmed to transmit for 10 hours during each 2 day interval. Transmitters will be mounted dorsally between the wings via a Teflon ribbon harness similar to that used by Petrie et al. (1996) and Miller et al. (2005).

#### Data Analysis

Data will be received monthly through the Argos Data Collection and Location System (Largo, Maryland, USA), and copies provided to both Japanese and North American collaborators; all data will be jointly held by all collaborators. However, biologists in Japan or the United States can download data at any time via the Internet to query specific PTT's. We will use the ARGOS multi-satellite tracking service to increase the frequency of received locations. Quality of each location will be assigned following Harris et al. (1990). We will filter the Argos data via a computer program that removes unlikely locations based on the rate of movement, distance, and angle between locations (Douglas 2006). The program creates ASCII files that can be imported into standard spreadsheet or statistical programs for analysis. When we receive multiple locations for an individual within a single transmission cycle, we will select the highest quality location to represent the bird's position.

Our primary interest is the distribution of Northern Pintails marked with PTT's among three geographic strata in eastern Russia that will be defined by band recovery data. The strata are: (1) regions where Northern Pintails banded in Japan have been recovered, but where recoveries of North American bands are lacking, (2) areas where both Northern Pintails banded in Japan and pintails banded in North America have been recovered, and (3) areas where North American Northern Pintails have been recovered, but recoveries of Japanese Northern Pintails are lacking. Bianki and Dobrynina (1997) suggest that such strata exist and that Northern Pintails in the eastern Chukotka Peninsula originate from North America, Northern Pintails west of the Omolon River originate from Japan, and that an area of overlap occurs between these regions and includes the northern Kamchatka Peninsula. We will define boundaries of the strata by plotting band recoveries of Japanese versus North American Northern Pintails in eastern Russia and using a kernel analysis (Worton 1995) to plot a 95% probability distribution around each. The potential area of contact between Asian and North American Northern Pintails will be the region where the respective probability distributions overlap.

We will note presence or absence of each marked bird in each geographic stratum during 10-day intervals starting with departure of Northern Pintails from Japanese wintering areas in spring through the summer period until birds initiate return migration in autumn. Within each period the stratum in which a bird was observed will be separately recorded for each marked individual. We will use multi-strata models in program MARK (White and Burnham 1999) to analyze these data because it will allow us to estimate the likelihood that a marked pintail will occur in a stratum within a single time period, given that a bird or its PTT have survived to that period. It is also useful for estimation in the event that locations of some PTT's are not determined during a 10-day period due to failure to receive a transmission (equivalent to lack of a recapture within an encounter period). Program MARK will allow us to examine competing models that include different parameters under an information-theoretic framework (Burnham and Anderson 2002). Thus we can contrast models that include various effects of gender, years, or time periods (e.g. migration versus nesting) on distribution of Northern Pintails among strata, and compute the likelihood that birds will move among strata. The result is parameter estimation based on the most



parsimonious model (Burnham and Anderson 2002). For this study the parameter of interest is the likelihood that marked Northern Pintails occurred in areas where band recovery data suggest overlap between North American and Asian pintails. We will also contrast distribution as determined by satellite telemetry with recovery distribution of Northern Pintails banded in Japan to determine if the latter should be adjusted to account for geographic differences in recovery likelihood.

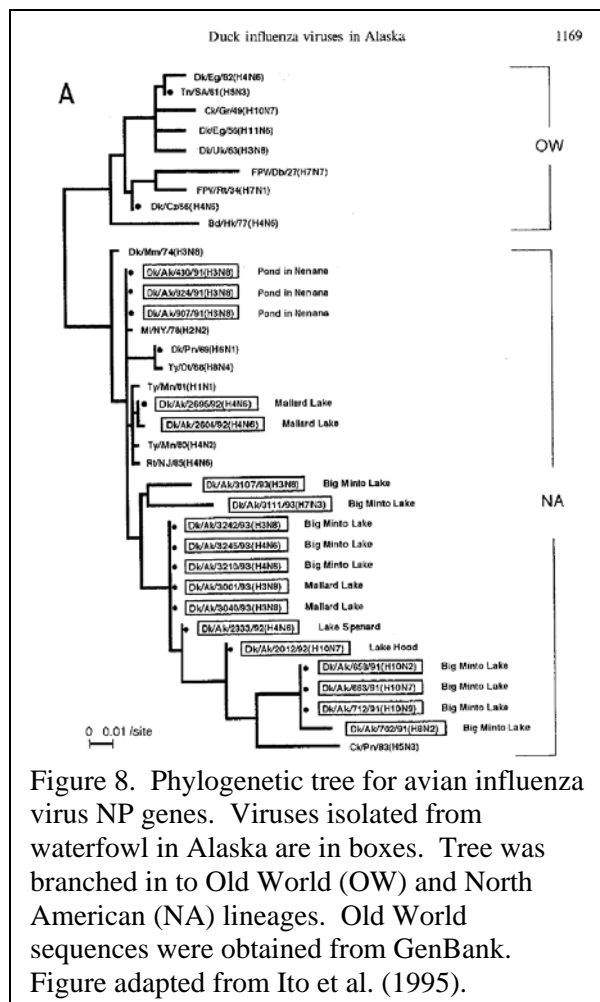
## COMPARISON OF VIRUSES AMONG WINTERING POPULATIONS

Primary Contacts: J. Chris Franson, Hon Ip, National Wildlife Health Center; Japanese cooperators to be named.

Northern Pintails sampled in North America and Japan have been found to carry a wide variety of influenza viruses (Ito et al. 1995, Shengong et al. 2002). During a three-year study in Canada, influenza viruses were isolated from 256 of 745 (34%) Northern Pintails sampled (Hinshaw et al. 1980). Ito et al. (1995) found 8 different subtypes of avian influenza viruses as well as an undetermined number of paramyxoviruses in waterfowl, including Northern Pintails, sampled across Alaska. In the current North American avian influenza surveillance effort, 13% of Northern Pintails sampled in Alaska in 2005 were actively shedding avian influenza viruses. Comparison of general virus populations across continental populations of Northern Pintails can be used to assess origin of viruses and rates of virus exchange among Northern Pintail populations (Ito et al. 1995). Further, when similar viruses are identified in each population, genetic sequencing of the viruses can be used to estimate the degree of genetic divergence which should equate to rates of exchange (Ito et al. 1995). Closely related viruses would imply frequent and/or recent exchange, whereas distantly related viruses would imply infrequent or historic exchange. For example, Ito et al. (1995) reports extensive genetic divergence between samples of H10N7 found in Alaska and in Asian samples (Figure 8). Estimating these rates of exchange is essential for assessing the probability of a particular virus (i.e., highly pathogenic H5N1 in this case) moving into North America via wild pintail populations. A comparison of influenza subtype co-infections between Northern Pintails in North American and Japan will be useful, as previous studies suggest that co-infections, and thus virus re-assortments, are nonrandom (Sharp et al. 1997).

### Specific Objectives

1. Describe the suites of viruses carried by wintering populations in Japan and North America
2. Compare virus populations across continents using multivariate approaches.
3. Use genetic sequencing approaches to describe the degree of relatedness when similar viruses are found in both wintering populations.



## Methods

Northern Pintails will be captured in both Japan and North America using ongoing/established procedures. These may include walk- or swim-in traps, and rocket nets. Captured Northern Pintails will be identified to age and sex and virus samples will be obtained via cloacal swabs. Cloacal swabs will be placed in viral transport medium (Docherty and Slota 1988), stored on ice in the field, and placed in ultra-low temperature storage (-80C or colder) at the end of the day. Alternatively, samples are held on ice or at 4C if they will reach the laboratory for testing within 72 hours of collection. Polymerase chain reaction methods are according to the procedures detailed in Attachment 12 of: An Early Detection System for Asian H5N1 Highly Pathogenic Avian Influenza in Wild Migratory Birds, U.S. Interagency Strategic Plan ([http://www.nwhc.usgs.gov/publications/other/Final\\_Wild\\_Bird\\_Strategic\\_Plan\\_0322.pdf](http://www.nwhc.usgs.gov/publications/other/Final_Wild_Bird_Strategic_Plan_0322.pdf)). Samples will also be inoculated into embryonating chicken eggs (Senne 1998) and possible avian influenza viruses identified by the hemagglutination test followed by subtyping using reference antisera and/or by nucleotide sequencing. When similar virus are found in both wintering populations, they will be further identified by genetic sequencing allowing a more direct determination of relatedness (Ito et al. 1995, Widjaja et al. 2004).

## POPULATION GENETICS

**Primary Contacts:** John Pearce and Sandy Talbot, *Alaska Science Center*, Jeff Peters and Kevin McCracken, *University of Alaska Fairbanks*

The Northern Pintail is considered a monotypic species with no geographic variation in genetic characteristics among North American breeding areas or migratory flyways (Austin and Miller 1995). No subspecies are recognized in North America. The only large-scale survey of genetic characteristics of the Northern Pintail found sampling areas in Alaska, prairie Canada, and Montana to be largely homogenous based on mitochondrial (mt) DNA restriction enzyme digest profiles (Cronin et al. 1996). In a smaller-scale survey of wintering birds, genetic variation was found to be higher than for other dabbling-duck species tested in western Texas (Rhodes et al. 1991), a predictable result for a wide-ranging species.

Since the initial surveys of population genetic characteristics of the northern pintail by Rhodes et al. (1991) and Cronin et al. (1995), numerous advances have been made in the field of population genetics.

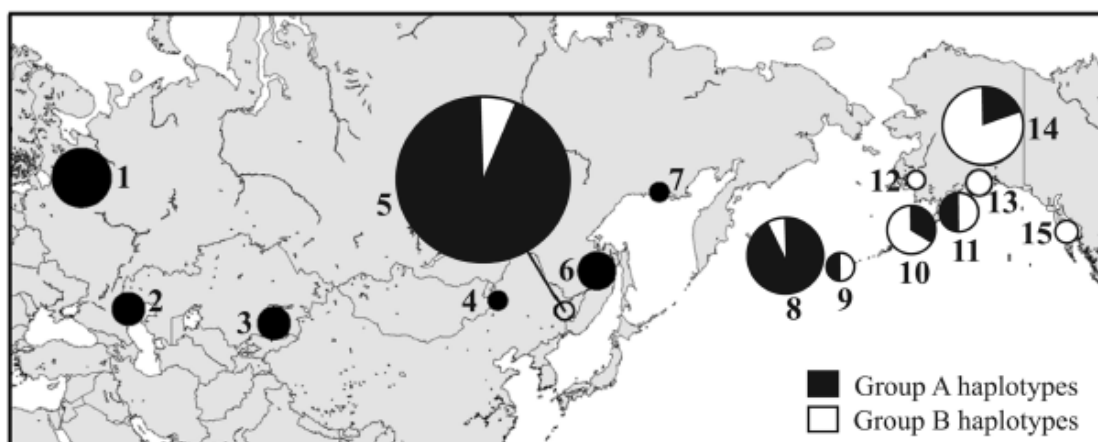


Figure 9. Localities in Asia, Russia, and Alaska where Mallards were collected for the study of Kulikova et al. (2006) and the frequencies of group A (black) and B (white) mtDNA control region haplotypes at each locality. The size of each circle is proportional to the number of individuals sampled, and the pie diagrams show the proportional of individuals with group A and B haplotypes.

These advances now allow rapid access to hundreds of polymerase chain reaction (PCR) primers for obtaining genetic information from neutral mitochondrial and nuclear genes. Analytical advances have also been made, permitting assessments of cryptic population structure (Pritchard et al. 2000) and historical demography (Emerson et al. 2001). Thus, even for species with largely homogenous genetic characteristics across much of their range, valuable information can be extracted and provide information on patterns of gene flow, demography, and species evolution (reviewed in Pearce and Talbot 2006). For example, a recent survey of mitochondrial (mt) DNA variation in the Mallard (*Anas platyrhynchos*) by Kulikova et al. (2006) observed limited genetic structure among Old World (Asia and Russia) and New World (North America) sampling locales, but greater levels of differentiation between Old and New World continental areas (Figure 9). Kulikova et al. (2006) conclude that if gene flow across the Bering Sea is ongoing, the predominant direction of female dispersal across the region has been west-to-east (Old World to New). Therefore, a reassessment of population genetic and phylogeographic characteristics of the Northern Pintail—especially between North America and Asia—is needed and will add valuable information to our study of movement and thus possible directions of virus transmission within and between continents.

#### Specific Objectives:

1. Obtain blood and feather samples for DNA from Northern Pintail wintering areas in both Japan and North America where birds are being sampled for avian influenza viruses.
2. Examine genetic characteristics of these samples by quantifying molecular variation within both nuclear and mtDNA genomes.
3. Estimate levels of gene flow and thus, population connectivity between birds wintering in Japan and North America. Information from satellite telemetry and banding data will assist our understanding of which breeding areas are represented in these disparate wintering areas.

#### Methods

Northern Pintails captured on wintering locations in Japan and in the United States will be sampled for DNA through a collection of blood and body feathers. DNA will be extracted and amplified via the polymerase chain reaction (PCR) using standard procedures for these kinds of materials (Pearce et al. 2004). We propose to amplify neutral genes within both the nuclear and mitochondrial genomes. Specifically, we will obtain mtDNA sequence information (~500 nucleotides) from the hypervariable control region, nuclear sequence information (~500 nucleotides) from up to five intron segments, and genotype information from 8–10 nuclear microsatellite loci. This combination of mitochondrial and nuclear DNA analysis allows a more clear understanding of both male- and female-biased levels of gene flow over time. Additionally, the faster mutation rate of microsatellite loci is useful for understanding more recent population events compared to nuclear intron sequence data. Protocols for obtaining mtDNA and nuclear introns sequence information have already been established (J. Peters, unpublished data). Four microsatellite PCR primers are known to be variable in Northern Pintails (Buchholz et al. 1998) and PCR primers for an additional 50 microsatellite loci (developed in waterfowl species; Fields and Scribner 1997, Maak et al. 2003, Paulus and Tiedemann 2003, Denk et al. 2004) are available at the Alaska Science Center's Molecular Ecology Laboratory for testing. Genetic data will be used to examine models of population differentiation between sampling areas in Japan and North America. Specifically, we will

use estimates of  $F_{ST}$  (Wright 1965) for both mtDNA and nuclear DNA for understanding levels of gene flow. Data will also be used to assess past changes to effective population size and understand how such historical demographic events may have influenced current genetic characteristics of the species across its Pacific distribution (Emerson et al. 2001).

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