A Genetic and Isotopic Characterization of Eastern and Western White-winged Dove Breeding Populations to Determine Wintering Ground Distribution and Population Genetic Structure

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Introduction

White-winged doves are a good example of a species that has responded to anthropogenic impacts by changes in its population density, migratory habits, and geographical distribution. white-winged doves are a species with what can be characterized as a geographic mosaic of traits. The variation in these traits appears to be, to a large extent, a consequence of human activities. In both Texas and Arizona, white-winged dove populations seem to be characterized by both a decline in some populations and an expansion in others. The expansion of white-winged doves seems to be caused by the increased availability of water, food, and nesting sites provided by the expansion of cities and suburbs (Scudday et al. 1980; West 1993). The expansion of white-winged doves into urban areas appears to have been accompanied by changes in the dove's biology. Several urban populations of White-winged Dove have longer breeding seasons and seem to have adopted a non-migratory habit. The changes in white-winged dove populations are dramatically illustrated by Christmas Bird count data (http://www.audubon.org/bird/cbc/bb.htm). In New Mexico and Texas the counts of white-winged doves (standardized by party hours) have increased exponentially since the early 1970s. This population increase is restricted to the non-migratory doves that inhabit cities and suburbs. Curiously, and in contrast, white-winged doves in Arizona remain rare in the winter even in cities. Although Christmas bird count data must be interpreted cautiously (Lepage and Francis 2002), the data are consistent with feeder count data (Project FeederWatch: http://birds.cornell.edu/pfw) and the patterns are strikingly clear.

Because some populations are rapidly expanding while others continue to exhibit declines, the need for a better understanding of white-winged dove biology as it relates to 1) a better understanding of their migratory biology and 2) the genetic relationships between what have been believed to be historically different populations were the drivers behind this project.

Project Objectives

1) Use carbon and deuterium stable isotope analysis to characterize the habitat and geographical area of origin of the white-winged dove (*Zenaida asiatica*) populations that breed in the United States.

2) Use amplified fragment length polymorphism DNA (AFLP) analyses to characterize the genetic structure of white-winged dove (*Zenaida asiatica*) populations that breed in the United States

3) Use genetic and stable isotope characterizations to link breeding and wintering populations of white-winged doves that breed in the United States.

Objective 1

Using stable isotopes to differentiate populations of white-winged doves that breed in the United States

In migrant animal populations, demographic changes can occur as a result of events on the breeding and wintering grounds or in transit between these two sites. A central element needed to answer this question is information about where white-winged doves (*Z. asiatica*) spend the winter, how they are distributed on the wintering grounds, and what are the routes they use to get there. We used a novel technique, the analysis of the carbon (C^{13}/C^{14}) and hydrogen (H^{2}/H^{1}) isotope ratios in feathers, to characterize the breeding populations of white-winged doves in the United States (we will use the letter D to refer to deuterium or ^{2}H). This is an essential first step to investigate the migration of white-winged doves and their distribution in the winter (This technique is described in detail in Hobson 1998, Hobson 1999a, 1999b, Wassenaar and Hobson 2000).

Why can we use stable isotopes to achieve these ambitious objectives? Briefly, the tissues and fruits of plants assume very isotopically distinct carbon signatures depending upon the pathway they utilize for photosynthesis (i.e. C3, C4 or CAM; Bender 1968, 1971, Smith and Epstein 1971, Sternberg et al. 1984, Ehleringer and Rundel 1988). Independent of photosynthetic pathways, the content of hydrogen isotopes in plant tissues as well as in surface waters is largely determined by the isotopic composition of rainwater. The deuterium signature of rainwater in North America has a strong regional and latitudinal gradient (Meehan et al. 2005; Figure 1). It differs between the East and West coast and throughout the continent it becomes increasingly more depleted (negative) in deuterium as you travel north. The isotopic signatures of the resources (food and water) animals ingest reflect this continental gradient and are deposited in tissues that are inert, such as feathers after a molt. The isotopic composition of the resources that characterize a site then become permanently recorded in an animal's tissue(s). This signature remains in the bird until it molts again.

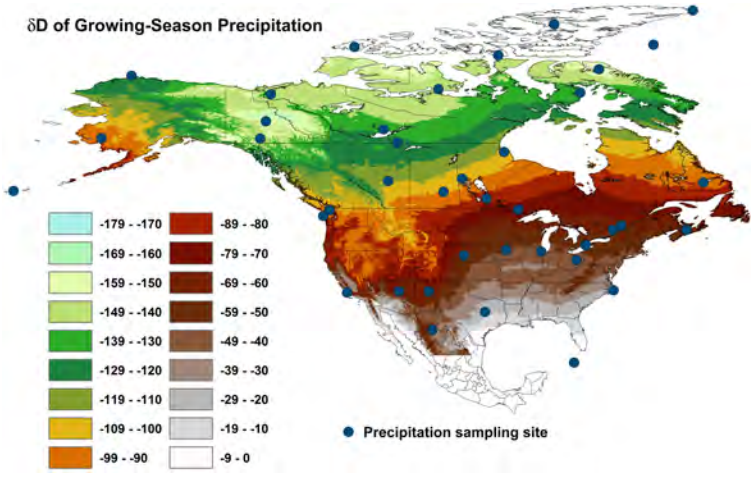

Figure 1. Deuterium values for continental precipitation vary widely across North America and have been shown to be reflected in the molted feathers of breeding birds (from Meehan et al 2005).

Because many bird species undergo a prebasic molt prior to migration, they acquire a label of their breeding location (Kelly et al. 2001, Wassenaar and Hobson 2001, Kelly and Finch 1998, Rubenstein et al. 2002, Hobson and Wassenaar 1997, Chamberlain et al. 1997, Mara et al. 1998, Hobson 1999a, 1999b, Hobson et al. 1999, Hobson et al. 2001). The recognition that we can use

stable isotopes as labels for the location where a bird spent the summer (or winter in the case of birds that undergo a body molt in the wintering grounds) is revolutionary (Webster et al 2002). Migration studies have traditionally relied on recapturing a bird that has been banded. Because only a small fraction of all birds in a population can be banded, and yet a smaller fraction is recaptured (http://www.pwrc.usgs.gov/BBL/homepage/SPEC.HTM#0010), the method can be inefficient. However, all birds have isotopic compositions. Hence using isotopes in a migration study transforms every bird captured into a recapture.

It has also been proposed that the δD gradient in continental precipitation (δD_p) can be used to predict the δD of bird feathers (δD_f) with a minor correction (Wassenaar and Hobson 2001). So, in addition to characterizing breeding ground δD_f signatures, we used white-winged dove feather δD to determine if precipitation δD could be used as a predictor for populations that breed in the United States and provide a tool for migratory game bird managers to use in future studies of bird populations.

Methods

With the cooperation of Texas Parks and Wildlife Department, New Mexico Game and Fish, Arizona Game and Fish, California Game and Fish, and financial support from Texas Parks and Wildlife, Arizona Game and Fish, and the United States Fish and Wildlife Service we collected dove wings from hunters during the opening weekend of dove season from 2004-2008 across their breeding range and from desert and agricultural sites during July in 2007-2009 (Figure 2). A molted primary feather was pulled and analyzed for carbon and hydrogen isotope ratios.

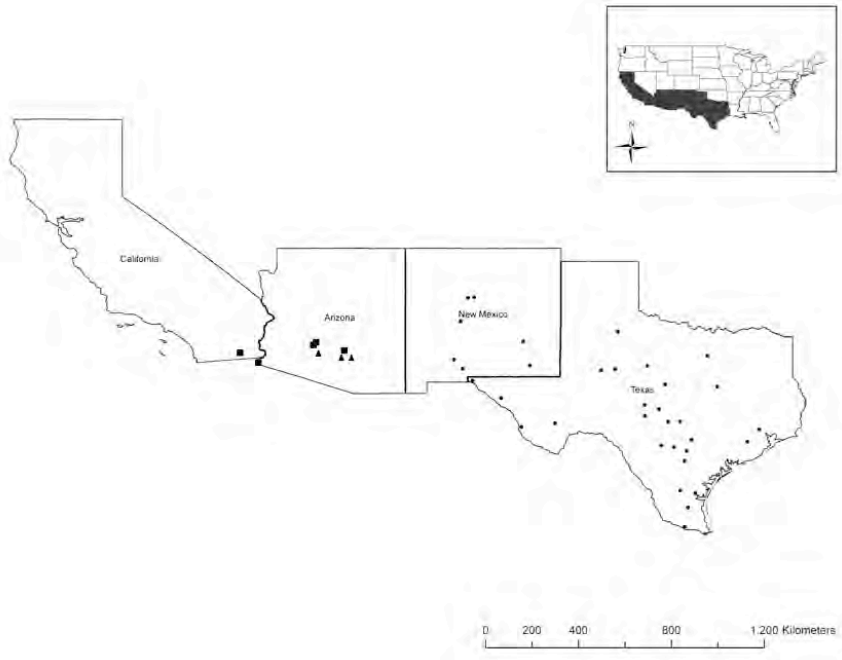

Figure 2. Feather sample locations used for isotope analysis. Desert sites are denoted by triangles (▲) and squares (■) denote agricultural sites in Arizona.

Results

It was important that sampled primary feathers had been molted during the sampling year. An unmolted feather, grown in the previous year, could have been grown in another location. By sampling primary 1, the first feather molted, we can best approximate the breeding ground signature. All individuals had molted primary 1 at all collection sites at the time of collection (Figure 3).

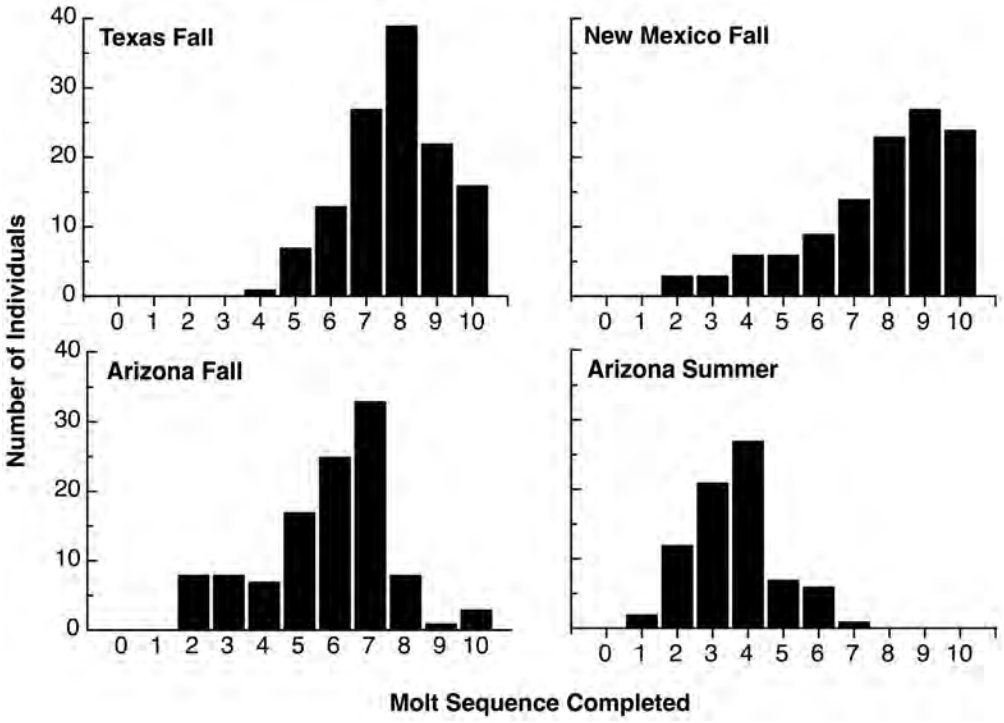

Figure 3. Molt cycles for birds harvested in Texas, New Mexico, and Arizona on September 1st, 2004-2008 and in Arizona on July 15th, 2008-2009 reveal that white-winged doves were well into their molt cycles during the breeding season.

Discriminant analysis correctly distinguished among all populations (Table 1, Figure 4). The lowest discrimination efficiency was between agricultural birds from Arizona/California and birds from New Mexico (Table 1). Curiously, $\delta^{13}C$ and δD correctly discriminated between desert and agricultural birds in Arizona even though collection sites were often separated by relatively short geographical distances (Figure 2 and Figure 4). Desert birds collected in Arizona had significantly more enriched δD_f (δD feather) than birds from the other populations (Figure 4). The $\delta^{13}C$ and δD values of the four different populations differed significantly (MANOVA, Wilke's $\lambda = 0.328$, $F_{6, 2370} = 295.15$, $p = 0.0001$). δD and $\delta^{13}C$ values differed significantly among populations (ANOVA, $F_{3,1186} = 723.83$, $p = 0.0001$ and $F_{3, 1186} = 89.16$, $p = 0.0001$ respectively; Table 2, Figure 4).

Table 1. We used discriminant function analysis between populations of white-winged doves using $\delta^{13}C$ and δD of feather tissue to determine how well doves could be differentiated from other populations. Discriminant function analysis revealed large population differences in isotopic signatures. Interestingly, our analysis was able to place 96% of Arizona desert and agricultural doves in the correct population.

Population	Arizona/California Agricultural	Arizona Desert	New Mexico	Texas
Arizona/California Agricultural	--	96%	71%	89%
Arizona Desert	--	--	99%	97%
New Mexico	--	--	--	85%
Texas	--	--	--	--

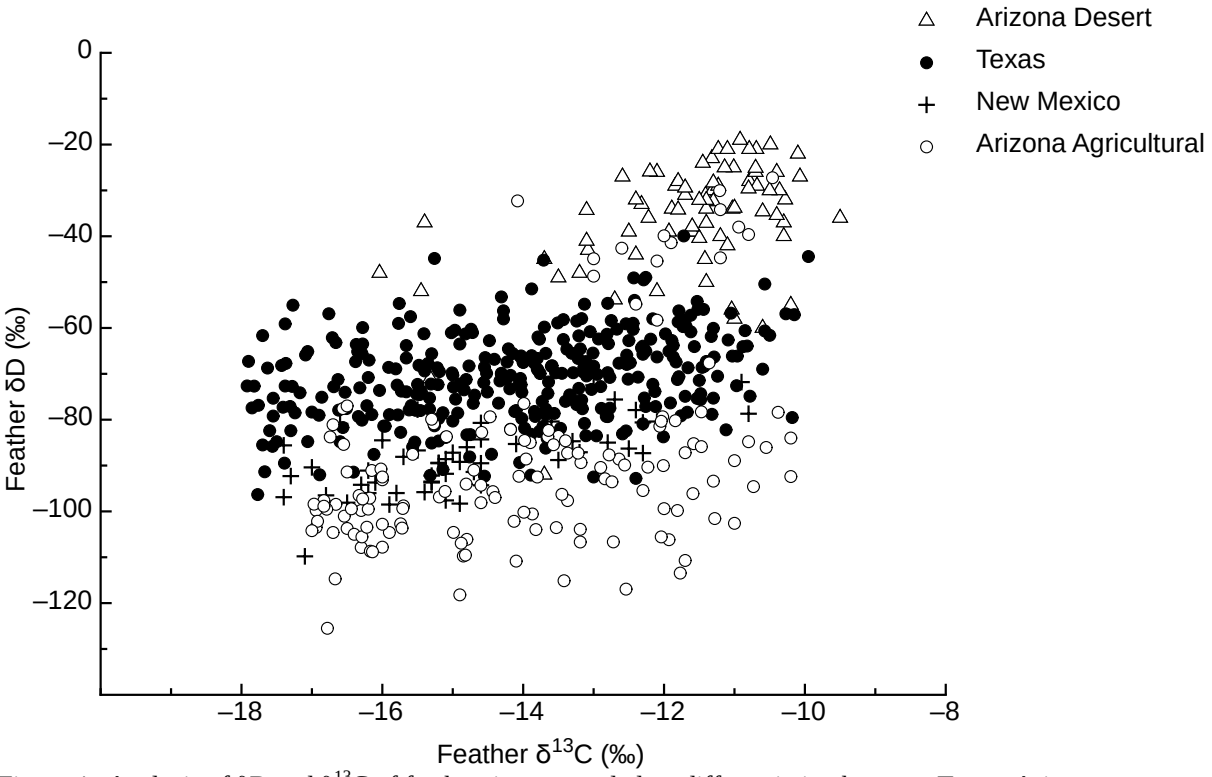

Figure 4. Analysis of δD and $\delta^{13}C$ of feather tissue reveal clear differentiation between Texas, Arizona Agricultural, and Arizona Desert white-winged dove populations. New Mexico doves (+) are intermediate between Texas (•) and Arizona Agricultural (o). Only desert doves (Δ) were collected during the breeding season. All other doves were collected during the opening weekend of dove season on September 1st. Interestingly, doves with desert signatures were collected in agricultural areas by hunters in Arizona indicating movement from desert habitats to agricultural habitats prior to migration.

Table 2. ANOVA revealed that White-winged dove populations differed significantly in both δD and $\delta^{13}C$. Means with a different letter, both δD and $\delta^{13}C$, differed significantly after Tukey's HSD comparisons.

Population	**Arizona/California**	**Arizona Desert**	**New Mexico**	**Texas**
$\delta^{13}C \pm SD$	-18.7 ± 3.8	-11.6 ± 1.3	-15.8 ± 2.4	-17.4 ± 3.8
(N)	416	78	67	629
δD ± SD	-97.9 ± 15.7	-35.9 ± 12.4	-89.7 ± 8.3	-73.6 ± 9.3
(N)	416	78	67	629
Tukey's HSD	A	B	C	D

δD_f values decreased roughly linearly along an east to west gradient ($r^2 = 0.06$, $p = 0.09$, Figure 5A). However, when Arizona desert birds were removed, the relationship between δD_f and longitude became significant ($r^2 = 0.59$, $p = 0.0001$, Figure 5B). δD_f values and δD_p (δD precipitation; Figure 6A, 6B) were poorly correlated ($r^2 = 0.03$, $p = 0.24$). When analyzed without data from Arizona birds (Figure 6C) and after the -25‰ correction was applied (the -25‰ correction is applied because of a physiological fractionation that occurs between drinking water/food and tissue formation; Figure 6D), the regression fit the data better ($r^2 = 0.26$, $p = 0.0014$). The relationship had a y-intercept (-5.5 ± 13.06 ‰) that, as expected, did not differ significantly from 0 ($t=0.42$, $p = 0.67$) and a slope that did not differ significantly from 1 (0.97 ± 0.028, $t = 0.09$, $p = 0.93$). When analyzed without Texas and New Mexico, the δD_f values from Arizona were significantly negatively correlated with the δD_p (Figure 6E and 6F, $r^2 = 0.74$, $p = 0.0060$).

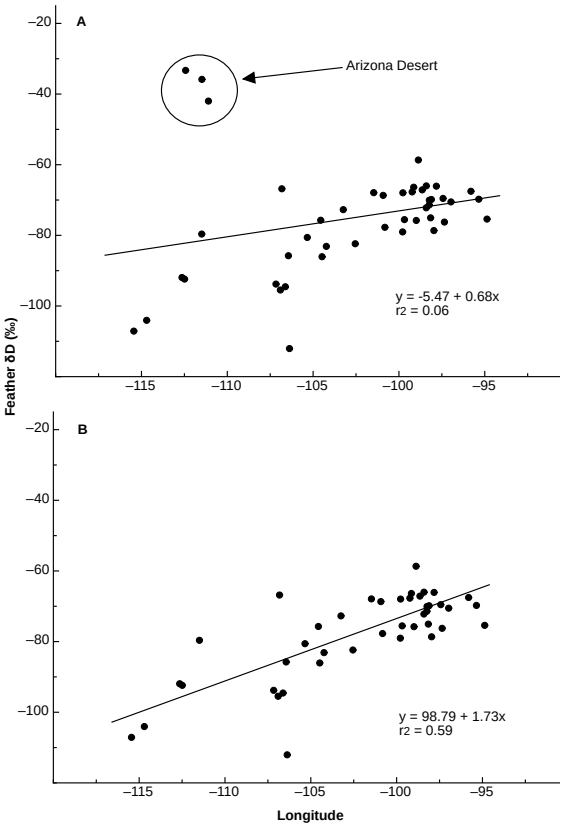

Figure 5. Regressions of longitude and feather δD reveal a strong gradient in feather isotope values as you move from east to west. Removal of Arizona desert birds greatly improved the relationship indicating that desert dwelling doves derive water and nutrients from a source different from other doves.

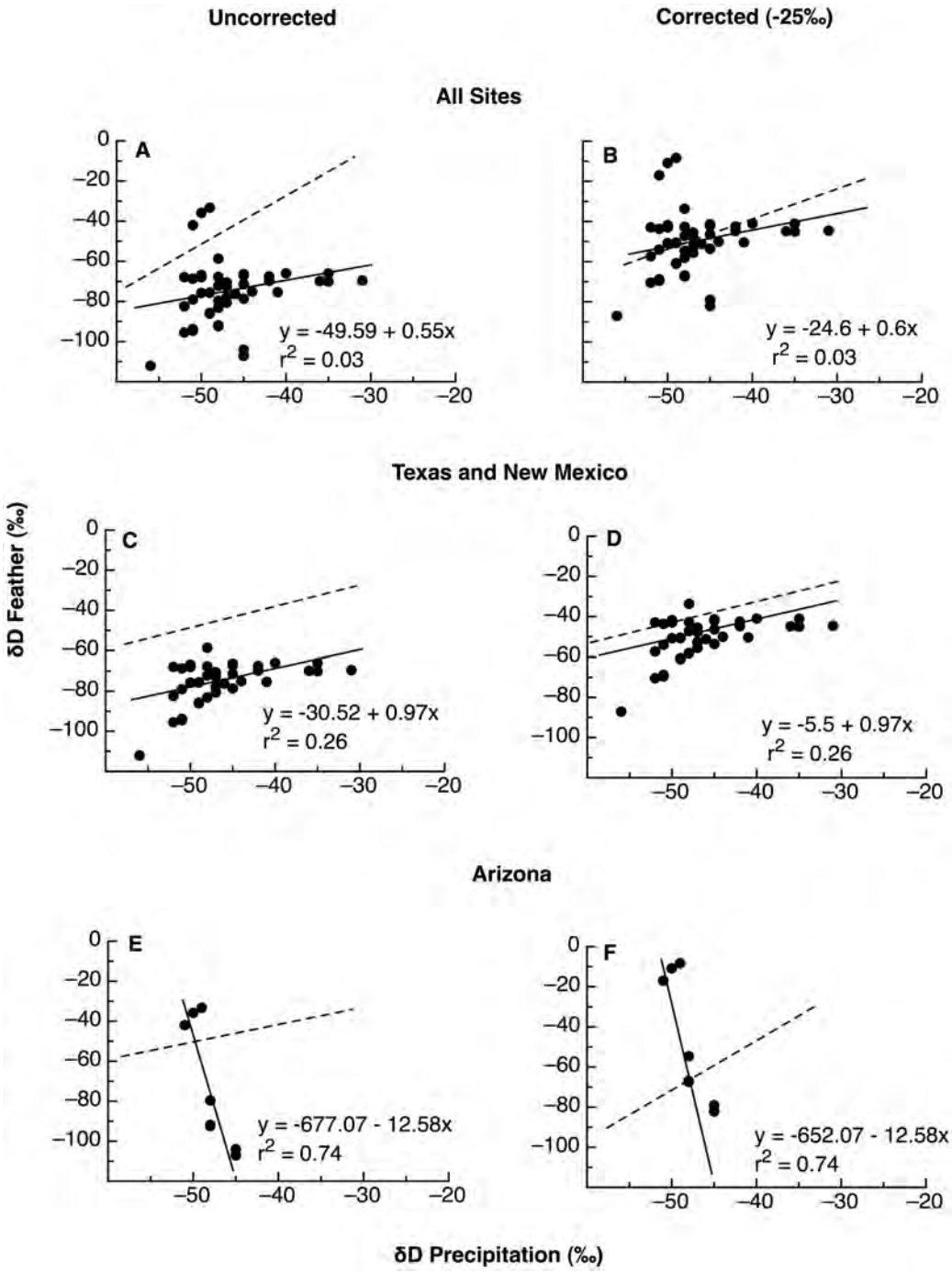

Figure 6. Linear regressions of uncorrected and -25‰ corrected δD_f and δD_p for all sites studied (A and B). Regressions with Texas and New Mexico (C and D) separate from Arizona (E and F) show that Texas and New Mexico birds more closely resemble precipitation after the correction factor is applied with the intercept nearing zero and a slope (solid line) approaching and similar to the one-to-one line (dashed). However, δD_f values from Arizona are completed disconnected from δD_p values indicating both desert and agricultural dove feather signatures are not tied to growing season precipitation.

Discussion

δD_f showed a strong longitudinal gradient across the breeding range. δD and $\delta^{13}C$ were not only useful in differentiating eastern and western populations of white-winged doves that breed in the United States, they were also useful in differentiating desert and agricultural doves within the western population. Although δD_f differentiated the populations of interest, we found that δD_p was a poor predictor of δD_f. In this discussion we will consider two related questions concerning the application of δD_f to differentiate populations of white-winged doves and the use of δD_p to predict δD_f .

1) What are the factors that allow using δD to discriminate even among populations that share the same geographical area but utilize different habitats.

2) Why is the relationship between δD_f and longitude different from that expected from the δD_p isoscape?

In answering these questions, we will suggest that for some species the scale of resolution of δD isoscapes does not reveal the complexity of factors that determine the δD of feathers and in these cases a finer scale analysis is necessary.

Differentiating populations of white-winged doves

In the United States, white-winged doves have generally been classified into an eastern (*Zenaida asiatica asiatica*) and a western (*Zenaida asiatica mearnsii*) subspecies based on subtle differences in morphology (George et al. 1994,Schwertner et al. 2002, Martinez del Rio et al. 2004). Until now, a reliable method for differentiating these populations did not exist. My analysis revealed that populations of white-winged doves can be differentiated across the breeding grounds using a combination of δD_f and $\delta^{13}C_f$ (Table 1, Figure 4). The discrimination among birds from Texas, New Mexico, and agricultural birds from Arizona, was the result of a clear and rather steep longitudinal gradient in δD_f (Figure 5 and Figure 7). This gradient, however, was not explained by a gradient in the deuterium content of rainwater (Figure 7).

Figure 6 suggests that the δD_f values of birds from New Mexico and Texas were relatively close to those of precipitation (after performing Hobson's et al.'s 2004 -25‰ correction), whereas the δD_f values of agricultural and desert birds in Arizona had distinctly depleted and enriched values, respectively (Figure 4 and Figure 6F). Why were the δD_f values of these birds different from those predicted by the δD_p isoscape (Figure 7)? Answering this question not only holds the key to understand why δD_f values differentiated clearly among birds from Arizona, New Mexico and Texas, but also why we could distinguish between agricultural and desert birds in Arizona.

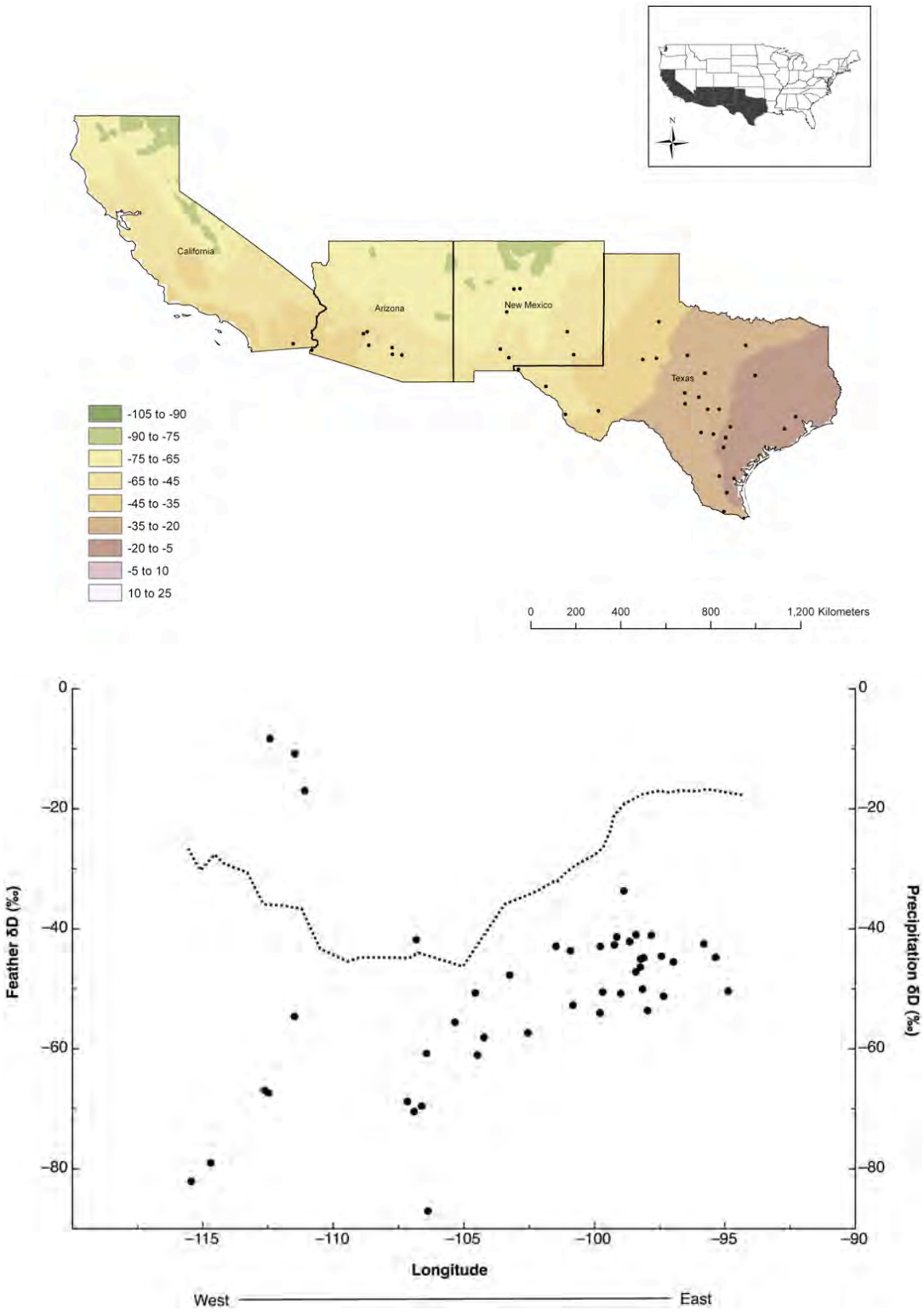

Figure 7. Predicted δD isoscape for precipitation with sampling locations (upper panel) reveals east to west gradient in isotope composition (from Bowen et al 2005). Legend in upper panel represents δD values. However, feather δD (closed circles lower panel) do not match that of predicted precipitation (dashed line). Dashed line represents δD values taken from isoscape layer where sampling locations occurred.

Agricultural complexes in southern Arizona depend on irrigation. Irrigation water in these complexes has two sources, groundwater and water from the Colorado River which is distinctly deuterium depleted (Doucett et al. 2007). Crop analysis of agricultural doves revealed that 100% of doves sampled were feeding on wheat, barley, and sorghum grown in irrigated fields (Table 4). The irrigation water from the agricultural sites where doves were collected had negative δD values and water from saguaro fruits had incredibly positive δD values (Table 5). These observations suggest that the isotopic composition of the feathers of agricultural birds in Arizona was strongly influenced not by the composition of rainwater, but from that of the water used to irrigate the crops that these birds depend on.

Table 4. Crop contents of white-winged doves from desert and agricultural habitats indicates that doves use either desert or agricultural habitats even when they are separated by only a few kilometers.

Location	Habitat Type	% Saguaro	% Agricultural	% Other
Javelina Mountain	Desert	59	0	41
Silver Bells	Desert	88	0	12
Tucson	Agricultural	0	100	0

Table 5. δD values of source water for desert and agricultural habitats in Arizona reveal large differences that explain differences observed in feather tissues between habitats.

Location	Habitat Type	δD
Javelina Mountain Water Tank, AZ	Desert	-32.3 ± 6.1
Saguaro Fruit Javelina Mountain, AZ	Desert	77.5 ± 8.2
Saguaro Fruit Silver Bells, AZ	Desert	86.8 ± 14.2
Yuma, AZ	Agricultural	-94.4 ± 1.6
Tucson, AZ	Agricultural	-80.3 ± 9.
Marana, AZ	Agricultural	-79.3 ± 2.9

Whereas the feathers of agricultural birds seemed to reflect the δD of irrigation water, the isotopic composition of those of desert birds reflected the isotopic composition of saguaro cacti (Table 5). Wolf et al. (2004) using isotopic evidence, found that desert-dwelling white winged doves in Arizona depend heavily on the nectar and fruit of saguaro cacti (*Carnegeia gigantea*). These authors proposed that these birds are saguaro specialists (Martinez del Rio et al. 2004). Because saguaros have CAM photosynthesis, their tissues are enriched in both $\delta^{13}C$ and δD (Lajtha and Marshall 1994). The feathers of Arizona desert birds support Wolf et al's (2004) notion of a group of white-winged doves in Arizona that rely heavily on saguaro. The feathers of these birds were significantly enriched in both $\delta^{13}C$ and δD as expected from birds that rely on a CAM plant for food (Fig. 4 and Table 2). In contrast, all other populations had a much wider range of $\delta^{13}C_f$ and more depleted δD_f values indicating reliance on both C3 and C4 plants (Table 2, Fig. 4).

The distinctive isotopic composition of Arizona's desert white winged doves leads to a question and an observation. The question is how faithful are desert and agricultural white-winged doves to their respective habitats? Note that in Arizona, the feathers of a small number of agricultural birds have δD_f and $\delta^{13}C_f$ values that overlap with those of desert birds (open circles Figure 4). Are these birds that have moved from desert habitats after breeding and were shot by hunters or do some birds occupy both desert and agricultural habitats? The temporal habitat faithfulness of the two Arizona populations can be investigated relatively simply by comparing the isotopic composition of recently molted feathers and feathers deposited on the previous breeding season. The observation that stems from the seemingly CAM isotopic composition of desert white-winged dove feathers is that we should not expect the feathers of all birds to track the isoscape of precipitation. Most of the hydrogen in feathers seems to be derived from food with a small amount (10-20%) from drinking water (Hobson et al. 1999). If the δD content of food differs from that of rainwater, as is the case in CAM plants then species that rely on these foods will have an isotopic composition that is different from and perhaps even independent of that of precipitation.

Considerations for the use of δD in differentiating bird populations

The purpose of this study was to use δD_f collected across the breeding range of white-winged doves to determine if we could differentiate the eastern and western populations. In addition, we were interested in determining if maps of δD_p, commonly used in bird movement studies, could be used to predict δD_f values of white-winged doves. We found that δD_f values differed across the breeding range but more importantly discovered that if used as a substitute, δD_p maps incorrectly predicted δD_f values in the western portion of the breeding range of white-winged doves. If we were interested in using these δD_p maps to predict the breeding location of wintering birds we would incorrectly assign most of these birds to the wrong breeding locations. Although, we found a clear longitudinal gradient in δD, this gradient was driven by an anthropogenic factor: the use of birds of crops dependent on Deuterium-depleted irrigation water. Furthermore, we found that even closely related populations, such as desert and agricultural birds in Arizona, differ significantly in δD if they depend on food that has an isotopic composition that differs from that of precipitation. This study should serve as a cautionary note for anyone intending to describe populations of birds, track their movements, or

link wintering birds to their breeding origins only using δD_p maps. It is imperative for future studies using this technique to first characterize the breeding ground δD_f of the species of interest. Many bird species that depend on food webs with native, non-irrigated plants at their base, probably conform to precipitation models. However, given the importance of irrigation and the amount of irrigated agricultural lands that wild birds use, it might be unwise to assume that δD_p isoscapes can be used to estimate the feather composition of all bird species. The many bird species that inhabit irrigated agricultural lands (this study), mountainous terrains (Wunder et al. 2005), and coastal regions subsidized by marine ecosystems (Lott et al. 2003, Rocque et al. 2006) may deviate significantly from δD_p values.

Management Implications

Stable isotope analysis has incredible potential in wildlife management. This study highlights the differences that exist in populations across their breeding range that can be detected in a molted flight feather that can be collected at check stations, during hunter checks by wardens and wildlife biologists, or through a hunter wing survey program. When populations of birds can be differentiated across the landscape, isotope analysis has huge potential to track seasonal movements and even reveal where birds were the previous breeding season. For white-winged doves, this study found a large gradient in feather isotope signatures from the eastern to the western extent of their breeding range. Had we been able to collect feather samples across this species entire wintering range and describe the isotope signatures of resident doves in southern Mexico, description of the wintering ground distributions would be possible. Because of this limitation, samples were small and only covered a small geographic area that encompassed more of the western populations wintering range and revealed a number of doves that had signatures represented by desert and agricultural doves. We are continuing to analyze the data set and are collaborating with Keith Hobson with the Canadian Wildlife Service using a data set on house sparrow feathers from Mexico as a surrogate for resident dove signatures.

Probably the most contrasting signatures were found between doves in southern Arizona. The distinct signatures in feathers between desert and agricultural habitats allow managers to differentiate desert or agriculture origin doves and determine the proportion of desert and agriculturally produced birds in fall harvests. While these results will likely not change how desert/agricultural doves are managed, i.e. as two separate populations, they do provide a tool that managers can use in the future should the need arise to differentiate habitat/resource use and differences in vital rates between populations. In addition, climate change models predict that habitats at the extremes, cold and hot, will be affected most. If this prediction is indeed true, populations of doves that utilize Sonoran desert habitat may be at greater risk due to habitat loss and reduced availability of resources during the hottest time of the year. Isotope analysis can and would be a valuable tool to identify doves utilizing these very isotopically and physically contrasting habitats.

Objective 2

Use amplified fragment length polymorphism DNA (AFLP) analyses to characterize the genetic structure of White-winged Dove (*Zenaida asiatica*) populations that breed in the United States

White-winged Doves are widely distributed in the Southwestern United States (construed broadly to include Arizona, New Mexico and Texas). Using morphological characteristics, Saunders (1968) differentiated 4 subspecies of white-winged doves in the United States. Browning (1990) and, more recently Pruett et al. (2000), used additional evidence (including molecular data) to conclude that there are only 2 subspecies in the United States: *Zenaida asiatica mearnsii* in Arizona, New Mexico, and Texas West of the Pecos River, and *Z. asiatica asiatica* in Texas East of the Pecos River. This simple picture appears to have been complicated by recent events. Pruett et al. (2000) documented a "melting pot" area in Texas, where Z. a. mearnsii and Z. a. asiatica seem to be exchanging genes (Pruett et al. 2000). This melting pot appears to be the result of the recent westward range expansion of Z. asiatica mearnsii that has placed it in contact with Z .a. asiatica (Pruett et al. 2000). Pruett et al. 2000, however, recommended verification of these results using nuclear DNA. Unpublished work by Tanksley et al (2000), used nuclear DNA (microsatellites) to investigate the genetic relationships between white-winged dove populations, but did not find substantial genetic differences. It appears that in the past there were two distinct allopatric populations of white-winged dove in the United States (an Eastern and a Western), but that recent range expansions may have placed these two populations in contact and diluted any detectable genetic differences.

In an attempt to resolve this issue we were interested in applying a recently developed genetic analysis, amplified fragment length polymorphism DNA (AFLP), in the hopes that it would resolve genetic relationships between dove populations and/or confirm the results previously discovered. We chose AFLP analysis because it has been useful in detecting population level differences where other markers have failed (Bensch et al. 2002). The objectives or our research were simple, to investigate the use of AFLP analysis to differentiate populations of white-winged doves that breed in the United States.

Methods

White wing doves were sampled from across their breeding range (Figure 1). DNA was extracted from 25 mg of muscle tissue using Qiagen DNeasy blood and tissue kits (Qiagen, Inc.). DNA was checked for quality on 1.5% agarose gels, and was quantified using a NanoDrop spectrophotometer (Thermo Scientific , Inc.).

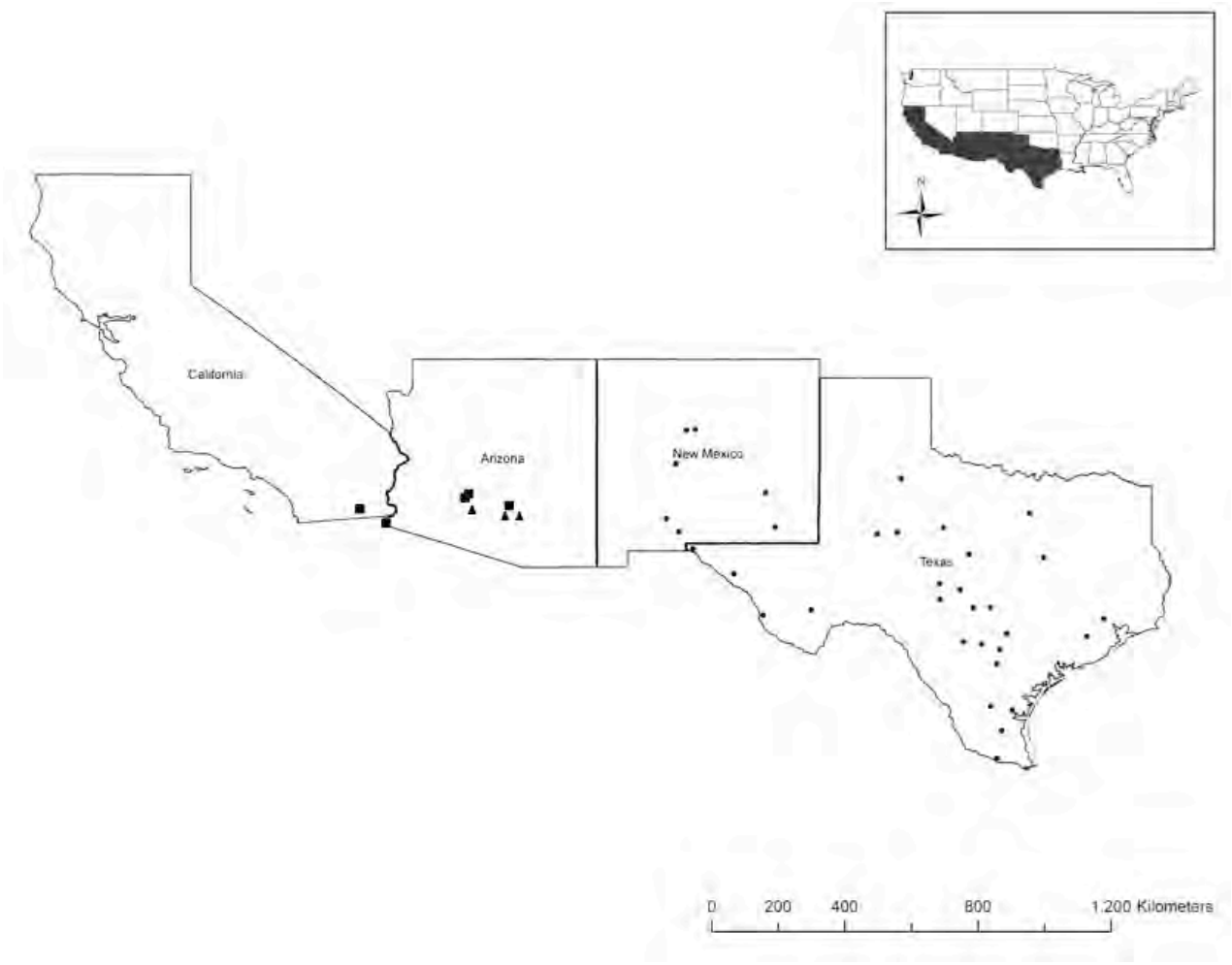

Figure 1. Muscle sample locations used for genetic analysis. Desert sites are denoted by triangles (▲) and squares (■) denote agricultural sites in Arizona.

Genetic Markers

We generated a large dominant marker data set using the AFLP procedure as in Vos et al. (1995) with slight modifications. Restriction digestion and adaptor-ligation were carried out simultaneously on 0.5 µg of genomic DNA using the restriction endonucleases EcoRI and MseI (NEB, Inc.). AFLP adaptor pairs were attached to digested fragments using T4 DNA ligase (NEB, Inc.). Restriction and ligation reactions were performed simultaneously in 11 µL volumes and incubated for 18 hr at 38ºC. After incubation, these reactions were diluted with 170 µL 0.1X TE buffer. Preselective and selective primers were based on primer core sequences EcoRI 5'-GACTGCGTACCAATTC-3' and MseI 5'-GATGAGTCCTGAGTAA-3' (EcoRI and MseI hereafter). Preselective amplifications were run with 4 µL of the diluted restriction-ligation products, 15 µL PCR core mix (385 µl H_2O, 68 µL Promega Inc. 10X reaction buffer, 41 µL $MgCl_2$, and 6.8 µL 40 mM dNTPs for a total volume of 500 µL), and 1 µL pre-selective primers, which each consisting of the adaptor primer sequences with one additional nucleotide at the 3´ ends (EcoRI-A and MseI-C). Preselective PCR conditions were 20 × (94ºC 30 s, 56ºC 1 min, 72ºC 2 min) and a final extension at 60ºC for 30 min. Ten µL of the preselective amplification products were checked on 1.5% agarose gels and the remainder was diluted with 170 µl 0.1X TE.

Selective amplifications were run with 3 µL of diluted preselective amplification product, 15 µL AFLP core mix, 1 µL of selective MseI primer, and 1 µL of the fluorescently labeled EcoRI selective primer. Both EcoRI and MseI selective amplification primers had three extra nucleotides at the 3´ ends in order to reduce the number of fragments amplified to a manageable number. We used three selective primer combinations (EcoRI-ACT MseI- CTA; EcoRI-AAC MseI-CTT; EcoRI-AAT MseI-CAT) to generate AFLP fragments. One µL of each selective amplification product was run with 8.75 µL formamide and 0.45 µL GeneScan 500 ROX labeled size standard (ABI, Inc.) on an ABI 3130 capillary sequencer.

Data analysis

AFLP fragment presence or absence in each lane file was analyzed using the program Genemapper (ABI, Inc.). We considered unambiguously discernible fragment sizes generated by each selective primer combination as dominant marker loci with two states, present (1) or absent (0). We limited analyses to fragment sizes between 70 and 400 bp, which resulted in a total of 252 unambiguously scoreable loci. The percentage of polymorphic loci, heterozygosity, and pairwise estimates of F_{ST} and Nei's genetic distance (D) among different species and/or subspecies were obtained using AFLP-SURV 1.0 (Vekemens 2002) (Table 2). We created 1000 bootstrapped matrices of pairwise estimates of Nei's D for the AFLP data set using AFLP-SURV. Neighbor-joining trees were then constructed separately based on the 1000 bootstrapped distance matrices using the neighbor routine in PHYLIP v 3.6 (Felsenstein 2005). PHYLIP was then used to construct consensus trees based on the 1000 trees generated above, and the number of trees out of 1000 sharing nodes were used as bootstrap support values.

We used the program structure v 2.2 (Pritchard et al. 2000, Falush et al. 2003, Falush et al. 2007) to assess whether the sampled genotypes were consistent with a single or multiple (K) populations, each in Hardy-Weinberg equilibrium. For these analyses we used the combined data

set of nine SSR and 235 AFLP loci. Log-likelihoods from Monte Carlo Markov Chain (MCMC) sampling provide the basis for evaluating the number of clusters that best fit the data. We also used structure to assign genotypic proportions of each individual to the K clusters based on admixture coefficients. We used the admixture model and ran simulations for 12 replicates for each value of K ranging from one to 20. The number of populations (K) that was most likely given the data was determined following Pritchard et al. (2000). We also calculated the change in model likelihoods between successive values of K (ΔK) and used the method of Evanno et al. (2005) to infer K.

Results

Overall (F_{ST} = 0.029) and pair-wise (F_{ST} = 0.01-0.05) estimates of genetic differentiation between populations sampled in different geographic locales were low (Table 1). F_{ST} measures the proportion of genetic diversity due to allele frequency differences among populations. F_{ST} values range from 0 to 1. A zero value implies that the populations are interbreeding freely. A value of one would imply the two populations are completely separate. Although these estimates were statistically distinguishable from zero, they indicate minor levels of genetic differentiation between populations of white-wing doves in the United States. Some geographically proximal populations were less differentiated from each other, than from more distant populations, but the neighbor joining tree did not reveal a clear pattern in which genetic distance was matched to geographical distance (Figure 2).

Table 1. Number of loci polymorphic (P), percentage of loci polymorphic (%P), and F_{ST} for populations of white-winged doves across their breeding range and from different habitat types.

Location	N	P	%P	F_{st}
Imperial Valley, CA	8	232	92	0.297
Yuma, AZ (agricultural)	11	215	85	0.248
Gila Bend, AZ (desert)	16	246	98	0.381
Phoenix, AZ (agricultural)	12	233	93	0.303
Silver Bells, AZ (desert)	19	216	86	0.239
Tucson, AZ (agricultural)	9	221	88	0.269
Las Cruces, NM	15	211	84	0.293
El Paso, TX	14	214	85	0.248
Presidio, TX	14	208	83	0.281
Fort Bend, TX	17	228	91	0.286
Cameron County, TX	16	206	82	0.236

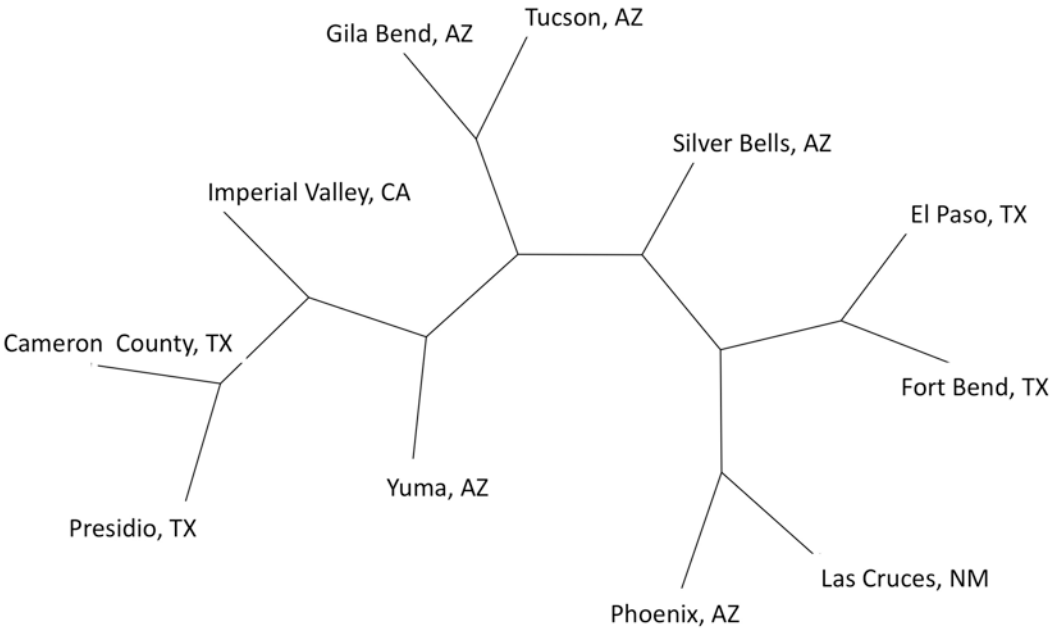

Figure 1. AFLP analysis showed weak population level differences as reflected in a neighbor joining tree. This tree suggests

Discussion

Advances in population genetic analysis has resulted in the reclassification of species level relationships once based on morphological characteristics. Using morphological characteristics, Saunders (1968) differentiated 4 subspecies of White-winged Doves in the United States. This classification has been a challenge for wildlife biologists charged with managing white-winged dove populations. Subspecies classifications indicated a set of unique traits or characteristics that could be important for conserving genetic differences across population and can be important for managing game bird populations. With the emergence of new genetic analyses, managers in the southwestern United States have been keenly interested in resolving genetic relationships in white-winged dove populations so that a comprehensive management plan can be designed for this species.

Using mitochondrial DNA, Pruett et al. (2000) found weak support for two populations of white-winged doves in the United States. They further suggested that analysis of nuclear DNA would help to further resolve white-winged dove genetic relationships. Using microsatellite (nuclear) DNA, Tankersley (2000) investigated population level differentiation in white-winged doves and concluded that classification of subspecies level differences was unwarranted based on genetic analysis. In this study, we were interested in exploring markers that assisted in differentiating populations of white-winged doves that breed in the United States. A recently developed genetic approach, AFLP DNA analysis, showed promise in resolving genetic relationships in populations

where other markers have failed (Bensch et al. 2002). AFLP genetic analysis again failed to produce population level genetic relationships that indicate subspecies classification for populations of white-winged doves that breed in the United States.

The results of this and previous studies cast doubt on whether white-winged doves have ever exhibited a large degree of genetic differentiation beyond what would be expected for a species that encompasses a large geographic range. We assume that prior to European settlement and the introduction of agriculture to western Texas, New Mexico, and southern Arizona that rivers like the Rio Grande did not act as corridors for dove movement and the exchange of genetic information. However, riparian corridors may have always acted as highways exchanging genetic material across the landscape. Alternatively, this does not discount the hypothesis that anthropogenic changes on the landscape could have increased the recent dilution of unique population level differences that might have existed. The results of repeated genetic analysis on white-winged dove populations reveal that any genetic differences that might have existed no longer are detectable across the range of this species.

Management Implications

AFLP analyses results agree with those found using microsatellite DNA (Tankersley 2000)). The results of this study indicate minor genetic differences that would be expected for a species that inhabits a large geographic range with populations more closely located to each other having a higher degree of similarity than populations more distantly associated. For migratory game bird managers, the results of this study indicate that management of white-winged doves as two distinct sub-units is not warranted and that white-winged doves can be managed as a single population exhibiting typical genetic relationships for a widely distributed species across their geographical range.

Objective 3

Use genetic and stable isotope characterizations to link breeding and wintering populations of White-winged Doves that breed in the United States

Our success using isotopes and genetics to differentiate white-winged doves across their breeding range was encouraging. Efforts to collect wings in southern Mexico began quite fruitfully. In 2007, with the assistance of our Mexican collaborators, we were able to collect wings from hunt clubs in 4 Mexican states in southwestern Mexico. In 2008, increasing conflicts between the government and drug traffickers made travel along migratory corridors and in wintering areas too dangerous. Because of this, our overall sample sizes were to small to allow for meaningful comparisons between breeding ground and wintering ground signatures using genetics and isotopes. In addition, the presence of resident white-winged doves further complicated our ability to differentiate resident from migratory dove signatures. We are currently trying to use the existing isotope and genetic data in collaboration with Keith Hobson with the Canadian Wildlife Service to use a data set on the isotopic composition of house sparrows from Mexico to describe resident white-winged dove signatures so that they can be differentiated from migratory doves. Our hope is that this collaboration results in the completion of this third objective within the coming year and that we can submit a final report on this portion. We regret that we could not complete this final objective but hope that the contribution of the first two objectives will assist in a further understanding of white-winged dove biology that can be used by managers in Texas, New Mexico, Arizona, and California.

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