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Research Article

Feather Isotope Analysis Reveals Differential Patterns of Habitat and Resource Use in Populations of White-Winged Doves

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ABSTRACT The white-winged dove (*Zenaida asiatica*) serves an important ecological role as a diurnal pollinator of the saguaro cactus in the Sonoran desert and an economic role as a highly sought after game bird in North America. White-winged doves are intimately linked to anthropogenic changes on the landscape and because of this, have experienced dramatic population fluctuations over the last 75 years in response, both positively and negatively, to anthropogenic changes on the landscape. To understand the factors driving population growth and decline of migratory species like the white-winged dove, it is imperative we study resource use on both their breeding and wintering grounds. To understand how populations are distributed on the wintering grounds, we tested an alternative to band recovery approaches by using stable isotope analysis. Before we could use isotope analysis to link breeding and wintering locations for this species, we first needed to determine if hydrogen (δ^2H) and carbon ($\delta^{13}C$) stable isotopes in feather tissue (δ^2H_f and $\delta^{13}C_f$, respectively) could differentiate among populations of white-winged doves across their breeding range in Texas, New Mexico, and Arizona. δ^2H_f and $\delta^{13}C_f$ not only differentiated between populations of white-winged doves that breed in the United States, but δ^2H_f also provided further differentiation in white-winged doves that breed in native Sonoran Desert and agricultural habitats in the western portion of their range. Ecological processes associated with desert resources and anthropogenic influences, specifically saguaro cacti and irrigated crops, largely determined δ^2H_f in some white-winged doves in Arizona whereas δ^2H of precipitation (δ^2H_p) largely determined δ^2H_f of doves in New Mexico and Texas. This study highlights the usefulness of stable isotope analysis to differentiate populations of animals across the landscape and the insight isotopes can provide into habitat and resource use. Published 2015. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS feathers, isotopes, migration, saguaro, white-winged dove, *Zenaida asiatica*.

The white-winged dove (*Zenaida asiatica*) is widely distributed from Texas to southern California with large populations centered in southern Texas, southern New Mexico, and southern Arizona (George et al. 1994, Schwertner et al. 2002). Throughout their breeding range, populations vary in migratory behavior and reliance on the fruit and seeds of native and cultivated plants (Schwertner et al. 2002). In Texas and New Mexico, white-winged doves primarily inhabit agricultural or urban habitats but in Arizona inhabit agricultural, urban, and desert habitats, where, in the latter, they are an important diurnal pollinator of the iconic saguaro cactus (*Carnegiea gigantea*) in the Sonoran Desert (Wolf and Martinez del Rio 2000, Schwertner et al. 2002). In addition to their important ecological role in desert ecosystems, white-winged doves are also a highly sought after game bird species (Raftovich et al. 2014).

White-winged dove populations are intimately connected to anthropogenic changes on the landscape and have experienced dramatic population fluctuations over the last 75 years due to changing land use practices (Cottam and Trefethen 1968, George et. al 1994). Over the last few decades, both resident and migratory populations of white-winged doves have exhibited rapid population increases and range expansion, especially in Texas and New Mexico (Sauer et al. 2007, Butcher et al. 2014). Because white-winged doves are of both ecological and economic importance in the United States, we sought to test an alternative method to band recoveries as a means to link the breeding and wintering locations of doves from Texas to California. To do this effectively, we must be able to differentiate white-winged doves across their breeding range. Using different genetic approaches, previous studies have attempted unsuccessfully to differentiate populations of white-winged doves across their historical breeding distribution (Pruett et al. 2000, 2011; Tanksley 2000). Fortunately, other molecular techni-

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ques, such as stable isotope analysis, may hold promise to defining predictable differences in dove populations across their breeding range.

Linking wintering birds to breeding sites using stable isotopes has been an area of particular interest among avian ecologists. Hobson and Wassenaar (1997) and Chamberlain et al. (1997) were the first to report a relationship between the hydrogen isotope values of feathers (δ^2H_f) and continental patterns of hydrogen isotopes in precipitation (δ^2H_p) and that it could be used to determine the geographical breeding origins of wintering migratory birds. The foundation for their premise is the observation that δ^2H_p varies across North America due to continental patterns of precipitation (Sheppard et al. 1969, Taylor 1974, Bowen and Ravenaugh 2003, Meehan et al. 2004) and that feathers grown within these distinct isoclines reflect the isotopic value of precipitation in that region. Their work has spawned a number of studies over the last 20 years investigating this relationship between δ^2H_f and δ^2H_p and used it to successfully link breeding and wintering populations of migratory birds (Wassenaar and Hobson 2000, Wunder et al. 2005, Langin et al. 2007, Kelly et al. 2008, Wunder 2010).

Whereas δ^2H can be useful in delineating geographic origin, $\delta^{13}C$ can be useful in determining differences in the photosynthetic pathways of plants. This is important because the $\delta^{13}C$ value of plant tissues differ between C_3 and C_4 or Crassulacean acid metabolism (CAM) photosynthetic pathways (Sternberg 1988). The process of carbon fixation is similar in C_4 and CAM plants, thus the $\delta^{13}C$ values of their tissues are similar (Marshal et al. 2007). However, by combining $\delta^{13}C$ and δ^2H analysis, it is possible to further differentiate between plants that use C_4 and CAM photosynthetic pathways because δ^2H becomes more positive in CAM plants due to their ability to store water and control evaporative water loss through time (Sternberg 1988). This is important because Wolf and Martinez del Rio (2000) and Wolf et al. (2004) found that tissues of white-winged doves inhabiting the Sonoran Desert in southwestern Arizona begin to resemble the $\delta^{13}C$ and δ^2H value of saguaro cacti, a CAM photosynthetic plant, they consume soon after their arrival.

Because the isotopic value of an animal's tissue reflects that of its diet (Carleton and Martinez del Rio 2005), combining $\delta^{13}C$ with δ^2H analysis of feathers ($\delta^{13}C_f$ and δ^2H_f, respectively) can potentially identify differences in the plant resources white-winged doves are using if resource use differs across this species' range. To determine if we could differentiate populations of white-winged doves across their breeding ranges in the United States, we used stable isotope analysis to test the hypothesis that white-winged dove feathers vary longitudinally across their breeding range in the United States as would be predicted from interpolated maps of growing season δ^2H_p (Bowen and Ravenaugh 2003).

STUDY AREA

We conducted this study across the breeding range of white-winged doves in the United States from Houston, Texas to Yuma, Arizona and as far north as Dallas in Texas, Albuquerque in New Mexico, and Phoenix in Arizona. We collected wings from hunter-harvested birds at survey checkpoints on the opening weekend of dove season, 1 September from across the entire breeding range to effectively characterize δ^2H_f and $\delta^{13}C_f$ isotopic values (Fig. 1).

METHODS

Feather Collection and Preparation

We collected 1 wing per bird from hunter-harvested white-winged doves during the opening day (1 Sep) of the fall dove hunting season from 2003 to 2008 (Fig. 1). Additionally, we collected samples at 2 desert sites and 2 agricultural sites in Arizona during the summer of 2007 and 2008 to validate desert and agricultural white-winged dove isotope compositions. These collections were performed to determine if Sonoran Desert resources and agriculturally derived resources created contrasting δ^2H_f and $\delta^{13}C_f$ values we observed in feathers collected 1 September at hunter check stations during the first 2 years of this study (Fig. 1). Because our method relied on sampling only flight feathers molted on the breeding grounds, we validated molt status from each wing collected. Additionally, we validated molt patterns of wings collected during the breeding season in 2007 and 2008. Only the first primary feather (P1), typically molted on the breeding grounds, was used for isotope analysis. We did not collect wings in Texas and New Mexico during the breeding season. Latitude and longitude of collection locations and hunter check stations were georeferenced with a hand-held global positioning system (GPS) unit.

We cleaned feathers to remove dirt and/or blood using a toothbrush and deionized water. We then allowed feathers to air dry at room temperature. Feather barbs were cut from the rachis along the entire length of the vane, chopped them into fine pieces, and then stored them dry. Currently, standard procedure is to treat feathers to remove lipids because lipids tend to have higher carbon values than proteinaceous tissues from the same individual (Hobson et al. 2009). However, we did not perform any lipid removing procedures on feather material because there were no standard protocols for sample preparation at the start of this study and we wanted to maintain consistency in sample processing and data analysis.

Water and Food Collection

We sampled water from canals that delivered irrigation water in agriculturally dominated sampling sites in Yuma and Tucson, Arizona from concrete pads (guzzlers) designed to concentrate and collect rainwater for storage at desert sites in the Sonoran Desert National Monument, and from saguaro fruits collected at 2 desert sites near Tucson and the Sonoran Desert National Monument, Arizona. We did not collect water samples at Texas and New Mexico sites due to time and cost constraints. In hindsight, collecting some samples in New Mexico would have helped resolve the more negative values seen in some doves and potentially assisted in identifying them as agriculturally derived or from local

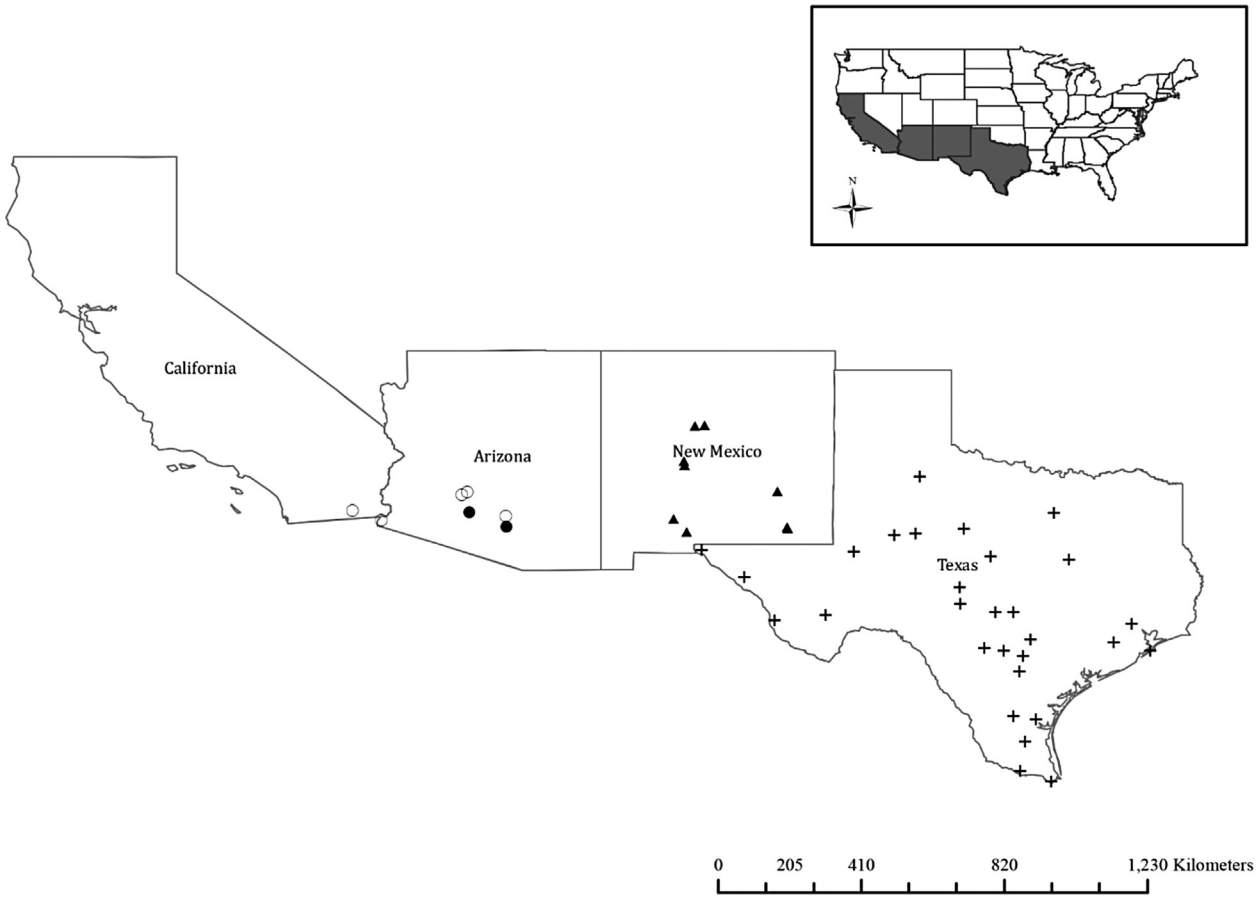

Figure 1. White-winged dove collection sites from 2003–2008 in the United States. Collection sites in Arizona are differentiated by agricultural (○) and desert (●) sites.

precipitation based resources. This is because most of the irrigation water in southern New Mexico is derived from snowmelt runoff or derived from ground water sources and is typically more negative than water derived from precipitation (Doucett et al. 2007, Bowen et al. 2011). In Texas, where most surface water resources are ultimately derived from precipitation (Good et al. 2014, Winnick et al. 2014), it is unknown if additional water samples would have assisted with further clarification between native and agricultural habitats. We filtered water samples from canals and desert tanks through 0.2-μm filters, and collected the water in 50-ml centrifuge tubes; we wrapped tubes in Parafilm® (Bemis, Neenah, WI) and refrigerated them until isotope analysis. Centrifuge tubes were filled to the rim to minimize gas exchange in the headspace of the tube. Saguaro fruits were collected along 2, 500-m transects at each desert site. We collected 1 ripe but closed fruit from each saguaro cactus encountered on each transect. Pulp material was removed from each fruit and placed into 20-ml scintillation vials, sealed the vials with Parafilm®, and flash froze samples in liquid nitrogen until water could be extracted on a vacuum line. Food items collected from dove crops at each of the sites in Arizona were identified to species using a reference collection in the Desert Ecology Laboratory at the University of New Mexico. We also collected seeds from crop plants grown in agricultural fields adjacent to sites where we collected canal water. All seeds were dried in an oven to constant mass, and ground them to a homogeneous powder for isotope analysis.

Isotopic Analysis

For $\delta^{13}C$ analysis, feather and food samples were weighed into individual 3×5-mm tin capsules (approx. 0.5 mg) and analyzed with a Thermo Finnigan DeltaPLUS XP Continuous Flow IRMS (Ringoes, NJ) with samples combusted in a Costech (Valencia, CA) elemental analyzer at the University of Wyoming Stable Isotope Facility. The precision of these analyses was $\pm 0.2‰$ for both isotopes. Our standards were vacuum oil ($\delta^{13}C = -27.5‰$, VPDB) and ANU sucrose ($\delta^{13}C = -10.5‰$, VPDB, NIST 8542). We included standards in every run to correct raw values obtained from the mass spectrometer. Water samples collected from open water sources and water samples extracted from saguaro cacti fruit pulp samples, following West et al. (2006), were analyzed for $\delta^{2}H$ at the University of Wyoming Stable Isotope Facility with a Los Gatos® (Mountain View, CA) laser liquid water. The precision of these analyses was $\pm 0.2‰$. Two internal standards (UWSIF301 and UWSIF302; $\delta^{2}H = -14.39‰$ and $-151.92‰$, VPDB) were included in each run and were used to correct raw

values. Feather samples were analyzed in the Environmental Isotope Laboratory of the University of Arizona using a method based on a modification of the comparative equilibrium method of Wassenaar and Hobson (2000) and incorporating recommendations of Qi and Coplen (2011). Samples and control keratin standards were equilibrated with laboratory air for 2 weeks in open silver capsules. They were then placed in a desiccator with Drierite under low vacuum for at least 6 hours (often overnight) to remove adsorbed water, after which the silver capsules were closed quickly (<10 min exposure to lab air) and placed in the He atmosphere of a Costech zero blank autosampler. Samples and control standards were analyzed at 1,400°C in a high temperature glassy carbon furnace (Finnigan TC/EA) coupled to a mass spectrometer (Finnigan Delta PlusXL). Standardization for the δ^2H measurement of the total hydrogen in samples is based on NBS-30 biotite and IAEA-CH7 polyethylene. Precision was 1.8‰ based on repeated internal standards.

Correction for exchangeable hydrogen in the feather samples was based on 1 homogenized control standard, SWAN1, with a non-exchangeable δ^2H value of −89.5‰ VSMOW. Internal variability for control standards was approximately 3‰ (1 SD). This control standard was calibrated using room temperature equilibration with 3 waters with very different δ^2H values (Qi and Coplen, 2011) and assuming a water—exchangeable hydrogen isotope fractionation of 1.120 (based on a fractionation of 1.095 at 114°C; Schimmelmann 1991). Using this fractionation, one can calculate that 9% of the total hydrogen is exchangeable hydrogen in the feather standard (see the discussion of equation 4 in Bowen et al. [2005] and of equation 4 in Wassenaar and Hobson [2000]). Sample δ^2H values for each batch of samples are calculated based on 9% of the sample being exchangeable hydrogen and having the δ^2H value back-calculated for the average SWAN1 control standard. A second control standard was run in most batches; WOOL has 11.3% exchangeable hydrogen and a δ^2H value of −135.5‰ VSMOW. After the samples for this paper were run, a third control standard was developed, SWAN2, with a δ^2H value of −168‰ and 9% exchangeable hydrogen. Although most labs use 2 control standards to normalize and stretch their measured δ^2H values, this lab used only 1, SWAN1. By explicitly calculating the δ^2H of exchangeable hydrogen and correcting control standards exchangeable hydrogen, WOOL and SWAN2 yielded values within 0.2% and 2.3‰ of their calibrated values when treated as unknowns, respectively. This implies that a scale compression or expansion is not needed using this method.

We obtained growing season δ^2H values of precipitation from www.waterisotopes.org using the online isotopes in precipitation calculator to generate predicted isotope values. The estimates from the calculator were modeled precipitation isotope data compiled from the Global Network of Isotopes in Precipitation (GNIP) operated by the International Atomic Energy Agency (International Atomic Energy Agency 2001, Bowen and Revenaugh 2003).

Statistical Analysis

White-winged doves that breed in the United States are broadly divided into 2 nominal subspecies: *Zenaida asiatica mearnsii*, which is found primarily in Arizona and California and *Z. asiatica asiatica*, which is found primarily in Texas and New Mexico. In Arizona, white-winged doves primarily occupy 2 distinct habitats: agricultural and desert. A linear discriminant function analysis using a leave-one-out cross-validation method was used to determine whether δ^2H_f and $\delta^{13}C_f$ values could differentiate among 4 divisions of birds determined a priori (Texas, New Mexico, Arizona Desert, and Arizona Agricultural). We based these a priori divisions first on state political boundaries and secondly as an extension to differentiate contrasting use of desert and agricultural habitats in Arizona. These divisions do not denote actual geographic boundaries separating white-winged doves but instead are a means to simplify analysis of isotope values across the longitudinal range of collection sites. The large, relatively uninhabited areas between New Mexico and Arizona may represent a geographic boundary that could be denoted by a state-specific population designation, whereas between New Mexico and Texas the divide, that likely once existed, has largely disappeared.

We compared the δ^2H_f and $\delta^{13}C_f$ values across geographic divisions and at sites sampled multiple years with analysis of variance (ANOVA). We performed post-hoc tests of differences using Tukey's honest significant difference tests. The relationship between δ^2H_f and δ^2H_p was assessed with ordinary least squares regression. At 2 of the 6 sites we repeated during the study, the differences in δ^2H_f across years was small (<15‰) and determined to be well below the threshold values of 32‰ suggested by Farmer et al. (2008) and 50‰ by Wunder (2007) for differentiating populations. Consequently, we pooled data at these sample locations for our analyses. All statistical analyses were performed using JMP® (SAS Institute Inc., Cary, NC) and R statistical analysis software (R Core Team 2012).

RESULTS

Analyses of molt sequences for doves from each sample site collected on 1 September indicated they were well into their flight feather molt (P5–P10 Texas, P3–P10 New Mexico, and P3–P10 Arizona). Additionally, molt sequences of doves collected 15 July in Arizona had already begun flight feather molt indicating, for Arizona birds at least, flight feather molt did begin early on the breeding grounds (P2–P5). Analysis of crop content revealed that doves collected at 2 agriculturally dominated sites (Yuma and Tucson) were exclusively consuming agriculturally produced food items (100%). Crop contents collected at 2 desert sites, Gila Bend and Silver Bells, indicated doves were consuming a combination of seed and pulp from saguaro cacti (59% and 88%, respectively) and seeds from both native forb and shrub plants (41% and 12%, respectively). At agricultural sites, crop contents included watermelon (*Citrullus lanatus*), wheat (*Triticum* spp.), and black-eyed pea (*Vigna unguiculata*; Table 1). Food items consumed at desert sites included saguaro cactus (*Carnegiea gigantea*), white thorn acacia

Table 1. δ^2H values of source water collected from saguaro fruits and free water sources in Arizona and food items recovered from white-winged dove crops in 2003–2008 from desert to agricultural habitats reveal widely contrasting values between habitat types.

	Habitat	δ^2H (‰)	$\delta^{13}C$ (‰)
Water			
Saguaro fruit-Gila Bend	Desert	77.5 ± 8.2	
Saguaro fruit-Silver Bells	Desert	86.8 ± 14.2	
Guzzler-Gila Bend[a]	Desert	−34.5	
Irrigation canal-Yuma	Agricultural	−94.4 ± 1.6	
Colorado River-Yuma	Agricultural	−93.5 ± 1.4	
Irrigation canal-Tucson	Agricultural	−79.3 ± 2.9	
Food			
Saguaro pulp-Silver Bells	Desert	34.5 ± 8.3	−13.03 ± 0.5
Saguaro pulp-Gila Bend	Desert	19.5 ± 20.7	−13.2 ± 0.3
Acacia constricta	Desert	−82.6 ± 9.3	−22.8 ± 0.9
Fouquieria splendens	Desert	−71.3 ± 6.3	−21.5 ± 0.5
Caliandra eriophylla	Desert	−65 ± 21.3	−24.3 ± 0.7
Lupinus sp.	Desert	−78 ± 3.6	−23.8 ± 1
Citrullus linatus[a]	Agricultural	−117	−24.6 ± 1
Triticum sp.	Agricultural	−86.3 ± 5.6	−25.1 ± 0.5
Vigna unguiculata[a]	Agricultural	−96	−26.5

[a] We ran only 1 sample.

(*Acacia constricta*), *Lupinus* spp., ocotillo (*Fouquieria splendens*), and fairy duster (*Calliandra eriophylla*; Table 1). Water collected from saguaro cacti fruit at 2 desert sites had δ^2H values that contrasted widely with water sampled at a guzzler tank in the desert near the Gila Bend site and agricultural canals in Yuma and Tucson, Arizona (Table 1). δ^2H values from irrigation canals in Yuma and Tucson (-94 ± 1.6‰ and -79.3 ± 2.9‰, mean ± SD) closely matched water from the Colorado River (-93.5 ± 1.4‰) where it originates, whereas water from the desert guzzler (−34.5‰) more closely resembled predicted δ^2H_p (−51‰) for that geographic location (Table 1 and www.waterisotopes.org, respectively).

Both hydrogen and carbon contributed to discrimination among populations (Table 2; Wilks $\lambda = 0.409$, $P < 0.001$). The classification accuracy of assigning doves to the correct population based on cross-validation was 63% for Arizona agricultural, 99% for Arizona desert, 66% for New Mexico, and 75% for Texas; overall accuracy was 70%. Doves collected at desert sites in Arizona had higher δ^2H_f than birds from the other populations (Table 3, Fig. 2). The $\delta^{13}C_f$ and δ^2H_f values of the 4 different populations differed (ANOVA, F_3, $1{,}439 = 631.96$, $P \leq 0.001$ and F_3, $1{,}439 = 102.29$, $P \leq 0.001$, respectively; Table 3). Linear regression of δ^2H_f and δ^2H_p values across all collection sites showed a weak relationship ($r^2 = 0.03$, $P = 0.24$; Fig. 3A). When analyzed without data from Arizona, the regression fit for New Mexico and Texas improved ($r^2 = 0.26$, $P = 0.001$; Fig. 3B). When analyzed without Texas and New Mexico feather data, the δ^2H_f values from Arizona showed a negative relationship with the δ^2H_p ($r^2 = 0.74$, $P = 0.006$; Fig. 3C).

Table 2. Cross-validated classification results (horizontal rows) of a linear discriminant function analysis for classifying white-winged doves collected 2003–2008 to their breeding location based on hydrogen and carbon stable isotope analysis of feathers. For example, out of 114 feathers sampled in the Arizona desert, 102 (90%) were correctly assigned to the Arizona desert location where they were sampled.

	Assigned population			
Collection population	Arizona agricultural	Arizona desert	New Mexico	Texas
Arizona agricultural	366	35	134	49
Arizona desert	2	102	4	6
New Mexico	25	0	76	15
Texas	28	12	120	470

DISCUSSION

In the United States, white-winged doves generally have 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). Band recovery data traditionally has been used to differentiate these populations and until now, an alternative method for differentiating these populations did not exist. Our analysis revealed that white-winged doves could be differentiated across the breeding grounds using a combination of δ^2H_f and $\delta^{13}C_f$ (Table 3, Fig. 2). The lower discrimination rates observed between Arizona agricultural and New Mexico birds and New Mexico and Texas birds is most likely a result of a mixing of birds in New Mexico that breed in native Chihuahuan Desert habitat and birds that breed in agricultural habitats (Table 2). Some birds from New Mexico appear to have negative δ^2H_f values similar to what we observed for agricultural birds in Arizona (Fig. 4). This is most likely due to the use of irrigated water from rivers

Table 3. White-winged dove populations differed significantly (Tukey's honest significant difference) in both δ^2H_f and $\delta^{13}C_f$ (‰) at all sites for doves collected 2003–2008.

Population	Arizona agricultural	Arizona desert	New Mexico	Texas
$\delta^{13}C_f \pm SE$	−18.7 ± 0.2	−12.3 ± 0.2	−15.9 ± 0.2	−17.3 ± 0.2
n	584	112	115	630
$\delta^2H_f \pm SE$	−95.1 ± 0.8	−34.6 ± 1.7	−87.4 ± 0.8	−73.3 ± 0.4
n	584	112	115	630

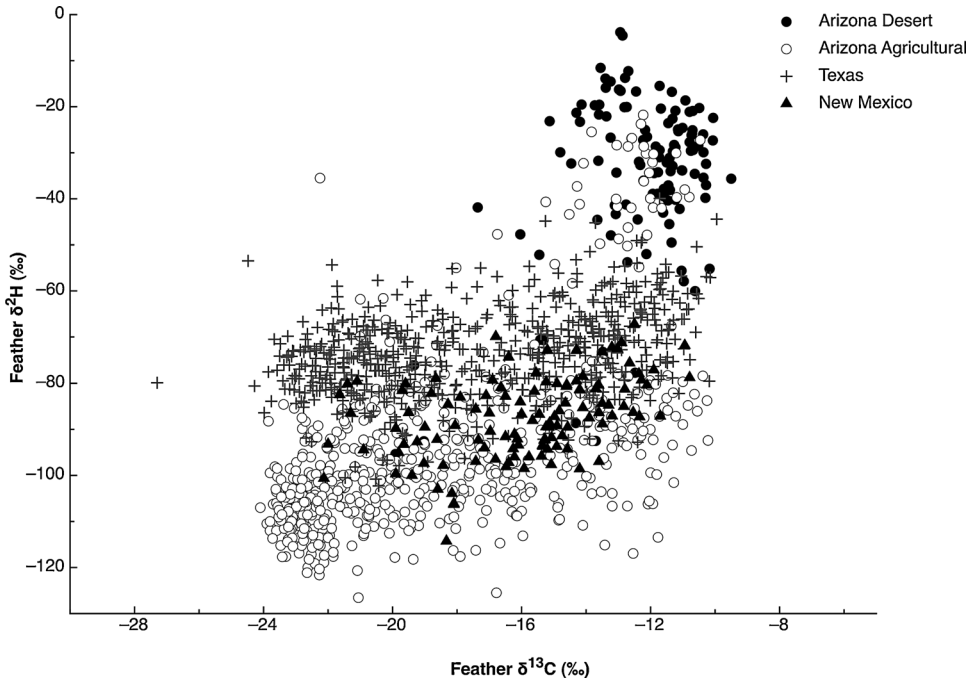

Figure 2. Analysis of δ^2H and $\delta^{13}C$ of feather tissues reveal clear differentiation between Texas (+), Arizona agricultural (○), and Arizona desert (●) white-winged doves collected in 2003–2008. New Mexico (▲) doves are intermediate between Texas and Arizona agricultural. Note that some fall-collected Arizona agricultural birds (○) are more similar to summer collected Arizona desert birds (●) indicating a movement of desert birds into agricultural habitats after the breeding season.

coming from the southern Rocky Mountains and water pumped from ground wells, similar to Arizona agricultural habitats, which have more negative δ^2H values (Doucett et al. 2007), whereas other New Mexico doves have more positive δ^2H values reflecting a diet of native plants reliant on precipitation. Thus, it appears that New Mexico doves are a mix of birds with agricultural signatures reflective of agricultural doves from Arizona and doves using native habitats that are more reflective of birds from Texas, which are more reliant on plants dependent on precipitation. Sampling of surface waters and food plants at New Mexico and Texas sampling sites could provide better isotopic characterization between doves using agricultural sites and those using native habitats and could possibly increase differentiation rates using δ^2H_f in future studies across their breeding range.

The discrimination we did observe among birds from Texas, New Mexico, and Arizona was the result of a clear and rather steep longitudinal gradient in δ^2H_f (Fig. 4). The gradient described above is not completely explained by a corresponding gradient in the deuterium content of growing season precipitation (Fig. 4). Our results suggest that the δ^2H_f values of birds from New Mexico and Texas more closely resembled that of growing season precipitation (Figs. 3B and 4). Wassenaar and Hobson (2000) were the first to observe a discrimination factor between δ^2H_f and δ^2H_p of approximately −27.4‰ using intercept values from linear regression on red-winged blackbird (*Agelaius phoeniceus*) feathers collected from Louisiana to Iowa, USA. When we considered all collection sites in this study, the discrimination factor between δ^2H_f and δ^2H_p was almost double (−49.59‰) that reported by Wassenaar and Hobson (2000). However, when we considered doves only from New Mexico and Texas, the discrimination between δ^2H_f and δ^2H_p was much closer (−30.52) indicating that the δ^2H_f of these birds was most likely influenced by δ^2H_p. In contrast, the δ^2H_f values of agricultural and desert birds in Arizona had distinctly low and high values that appear disconnected from δ^2H_p (Fig. 3C).

Why were the δ^2H_f values of Arizona's desert and agricultural birds different from those predicted by the online isotopes in precipitation calculator (Fig. 3C)? Agricultural complexes in southern Arizona depend on irrigation, which has 2 sources: groundwater and the Colorado River. Both are distinctly deuterium depleted (more negative values; Doucett et al. 2007; Table 1). Crop analysis of agricultural doves in Arizona revealed that 100% of doves sampled were feeding on agricultural seeds grown in irrigated fields and that food derived from these fields had values very close to those measured in irrigation water (Table 1). These observations suggest that the isotopic composition of agricultural birds in Arizona was strongly influenced not by the composition of rainwater but by the water used to irrigate the crops that these birds depend on.

Whereas the feathers of agricultural doves seemed to reflect the δ^2H of irrigation water, the isotopic composition of feathers from desert doves more closely reflected the isotopic composition of the saguaro cacti. Using isotopic evidence, Wolf et al. (2004) found that during the breeding season, desert-dwelling white-winged doves in Arizona depend heavily on the nectar and fruit of saguaro cacti. The authors proposed that white-winged doves are saguaro specialists

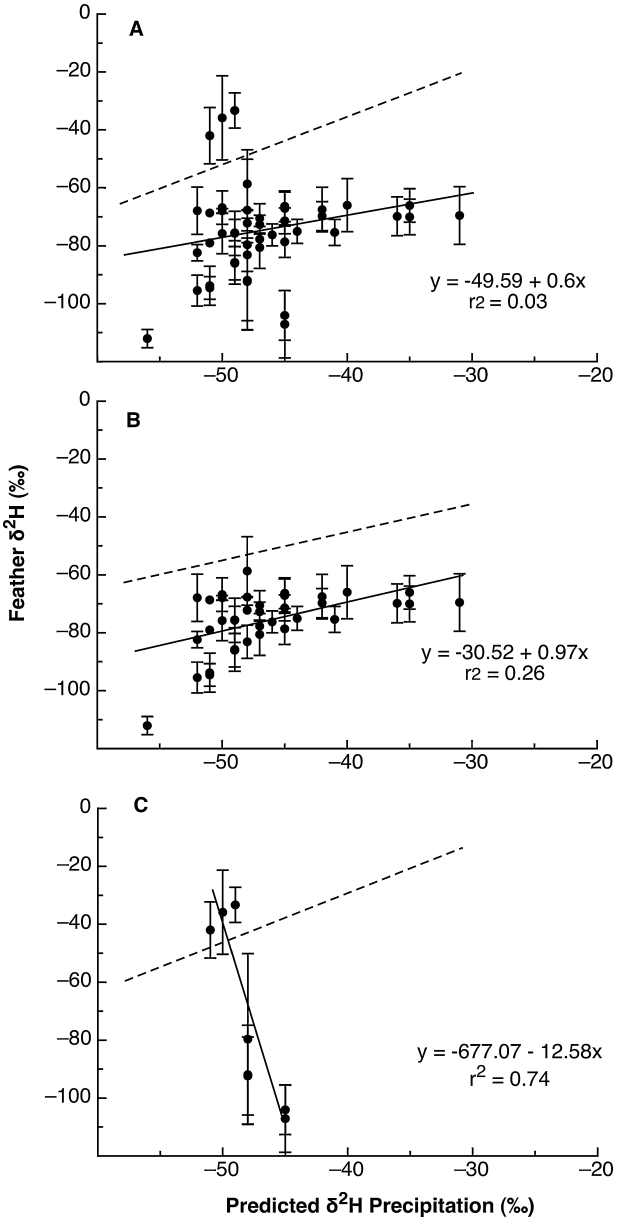

Figure 3. δ^2H_p was not a good predictor of δ^2H_f values when considered for all white-winged doves at all collection locations (A) during 2003–2008 and for doves collected only at sites in Arizona (C). However, when we analyzed δ^2H_f for only doves collected at sites in New Mexico and Texas (B), δ^2H_p was a better predictor of δ^2H_f (as indicated by the 1:1 relationship; dashed line) and suggests that these birds used food and water more dependent on growing season precipitation. Doves from Arizona, however, appear to depend on food and water sources not dependent on seasonal precipitation. Values for δ^2H_f represent mean (± SD) from each location.

(Martinez del Rio et al. 2004). Because saguaros use the CAM photosynthetic pathway, their tissues are enriched in both $\delta^{13}C$ and δ^2H (Lajtha and Marshall 2004). The isotopic composition of feathers of doves collected from the Arizona desert support the notion of Wolf et al. (2004) that a group of white-winged doves in Arizona rely heavily on saguaro. The feathers of these birds were significantly enriched in both $\delta^{13}C$ and δ^2H, as expected from birds that rely on a CAM plant for food (Table 3, Fig. 2). In contrast, all other populations had a much wider range of $\delta^{13}C_f$ indicating reliance on both C_3 and C_4 plants and more depleted δ^2H_f values indicating plants grown in irrigated landscapes (Table 3, Fig. 2).

How faithful are desert and agricultural white-winged doves in Arizona to their respective habitats? Note that in Arizona, the feathers of a small number of agricultural birds had δ^2H_f and $\delta^{13}C_f$ values that overlapped with those of desert birds (Fig. 2). These birds were either stray desert doves that were shot by hunters as they flew over agricultural land or some birds in Arizona occupy both desert and agricultural habitats. The temporal faithfulness of white-winged doves using the 2 different habitats can be investigated relatively simply by comparing the isotopic composition of recently molted feathers and feathers deposited during the previous breeding season. The observation that δ^2H_f and $\delta^{13}C_f$ values of desert white-winged doves reflect CAM plant δ^2H and $\delta^{13}C$ values reveals that we should not expect the feathers of all birds to track the δ^2H 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 δ^2H content of food differs from that of rainwater, as is the case in CAM and flood-irrigated 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.

The purpose of this study was to use δ^2H_f and $\delta^{13}C_f$ collected across the breeding range of white-winged doves to determine if we could differentiate the eastern and western populations. We found that δ^2H_f values differed across the breeding range and that δ^2H_p of growing season precipitation incorrectly predicted δ^2H_f values in the western portion of the breeding range of white-winged doves (Fig. 3C, 4). Although we found a clear longitudinal gradient in δ^2H_f, we argue this gradient was driven by an anthropogenic factor; the use of crops dependent on δ^2H-depleted irrigation water in the western portion of the breeding range and not precipitation. Furthermore, we found that doves occupying geographically similar areas, such as desert and agricultural birds in Arizona, can differ significantly in δ^2H_f if they depend on food and water that has an isotopic composition that differs from that of precipitation. We recommend that researchers planning to use δ^2H_f to predict the breeding origins of migratory birds first characterize the isotopic value of feathers for the population or species of interest at breeding sites prior to collecting feathers on wintering locations. Researchers can then link feather isotope values using approaches that allow for quantitative geographical assignment of individuals back to breeding locations (Kelly et al. 2002, Wunder et al. 2005, Wunder 2010).

Many bird species that depend on food webs with native, non-irrigated plants as their primary resources probably have isotopic composition that conforms to models that predict δ^2H_p. However, given the importance of irrigation and the amount of irrigated agricultural lands that wild birds use, it might be naive to assume that isoscapes based on δ^2H_p can be used to estimate the feather composition of all bird species. The many bird species that inhabit irrigated agricultural and arid landscapes (this study), mountainous terrains (Wunder et al. 2005), and coastal regions subsidized by marine

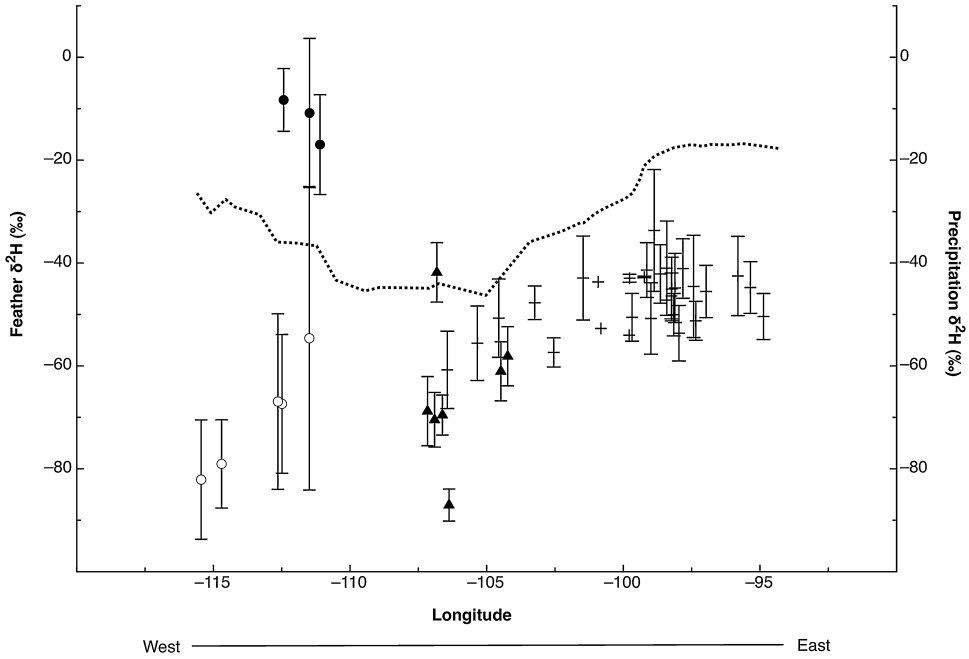

Figure 4. δ^2H_f values for Arizona agricultural (○), New Mexico (▲), and Texas (+) locations appear to increase with decreasing longitude (west to east) for white-winged doves collected from 2003–2008. When compared with values obtained from the online isotopes in precipitation calculator (dashed line; www.waterisotopes.org), doves from Texas and New Mexico track δ^2H_p values, whereas doves from Arizona agricultural sites (○) have much more negative δ^2H_f values indicating that the steep gradient is not completely driven by δ^2H_p but instead by irrigation water pumped into agricultural habitats. Additionally, Arizona desert doves (●) have δ^2H_f values that are much more positive than predicted δ^2H_p values indicating that feather isotope values for desert birds are not derived directly from resources driven by growing season precipitation. Values for δ^2H_f are mean (± SD).

ecosystems (Lott et al. 2003, Rocque et al. 2006) may deviate significantly from δ^2H_p values.

MANAGEMENT IMPLICATIONS

The ability to differentiate populations on the landscape and track their seasonal movements is a powerful tool for wildlife and land managers. Once unique isotope values are identified in populations of animals, every animal in that population becomes a marked individual carrying with it a record of its diet or in the case of this study, breeding origins. When successfully applied in the field, natural isotopic markers far surpass the numbers of individuals that could be marked using traditional methods thereby increasing the level of inference we can make at the population level. Information provided from isotopic analysis can facilitate focused conservation and management efforts on 1) origins of birds at breeding, stopover, and wintering sites; 2) variability in resource use within specific subpopulations; and 3) providing valuable information to link breeding and wintering populations of migratory birds. For white-winged dove conservation and management, biologists can couple banding data and isotopic analysis to better understand the breeding origins of wintering doves in Mexico and Central America, identify variability in resource selection locally and at broader geographic scales, and even determine the breeding origins of harvested birds during hunting seasons. In Arizona, for example, differences in isotopic values between native desert and agricultural resources could be used to understand the susceptibility of doves that use these different habitats to harvest, as most public and private hunt areas are nested within agricultural habitats, and sampling of the previous year and newly molted feathers at breeding sites could provide inference into how faithful these doves are to the respective habitat types temporally. The results of this study are a stepping stone to greater inferences we can make about white-winged dove ecology to inform both management and conservation.

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