Migratory double breeding in Neotropical migrant birds

Sievert Rohwer[a,1], Keith A. Hobson[b], and Vanya G. Rohwer[a,2]

[a]Burke Museum and Department of Biology, University of Washington, Seattle, WA 98195; and [b]Environment Canada, Saskatoon, SK, Canada S7N 0H3

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Neotropical migratory songbirds typically breed in temperate regions and then travel long distances to spend the majority of the annual cycle in tropical wintering areas. Using stable-isotope methodology, we provide quantitative evidence of dual breeding ranges for 5 species of Neotropical migrants. Each is well known to have a Neotropical winter range and a breeding range in the United States and Canada. However, after their first bout of breeding in the north, many individuals migrate hundreds to thousands of kilometers south in midsummer to breed a second time during the same summer in coastal west Mexico or Baja California Sur. They then migrate further south to their final wintering areas in the Neotropics. Our discovery of dual breeding ranges in Neotropical migrants reveals a hitherto unrealized flexibility in life-history strategies for these species and underscores that demographic models and conservation plans must consider dual breeding for these migrants.

dual breeding | isotopes | itinerant breeding | west Mexico

Itinerant breeding, wherein the same individuals breed in different regions in a single season, is established or suspected for just a few species of New and Old World birds (1). Some itinerant breeders exploit unpredictable food supplies, so their breeding areas may not be in the same place from year to year, as in the red-billed quelea (*Quelea quelea*) (2, 3). However, 2 other species, European quail (*Coturnix coturnix*) and dotterel (*Charadrius morinellus*), apparently breed first in southern regions, then move north with the progression of spring to breed again (ref. 4 and Whitfield in ref. 5). We suggest that the term *migratory double breeding* might appropriately distinguish species with dual breeding ranges that are consistent from year to year. Unlike more nomadic itinerant breeders, migratory double breeders move between early and later breeding sites that are used reliably from year to year.

In July and August 2005–2007, we discovered many individuals of 5 Neotropical migrant birds breeding in coastal Sinaloa and Baja California Sur, Mexico. Coastal west Mexico receives most of its annual precipitation during a July–September monsoon (6, 7). These late summer rains stimulate the tropical deciduous forests of this region to leaf out and flower, generating an abundance of insect prey, as well as seeds from grasses and other plants. This seasonal pulse of resources is well known to attract many western Neotropical migrant birds to this region for their postbreeding molt (8).

At least 5 Neotropoical migrants [yellow-billed cuckoo (*Coccyzus americanus*), Cassin's vireo (*Vireo cassinii*), yellow-breasted chat (*Icteria virens*), hooded oriole (*Icterus cucullatus*), and orchard oriole (*Icterus spurius*)] apparently move to west Mexico, not primarily to molt but to breed a second time, after having bred earlier in the north. Several field observations support this claim. First, females of these 5 species that were initiating breeding had regressed and featherless brood patches, demonstrating that they had bred earlier that same summer. Second, during hundreds of hours of netting and observation in July, we found no recently fledged juveniles of these species, suggesting that they had not bred in west Mexico earlier in the same summer. Third, many individuals of the 5 putative double breeders sampled for this study were indisputably breeding when collected in west Mexico: males had fully enlarged testes, and females were laying or incubating [supporting information (SI) Table S1]. Fourth, we found many active nests for orchard orioles and hooded orioles, and males of all 5 species were singing and defending territories or guarding females. Together, these observations suggested to us that the individuals we found breeding in west Mexico had attempted to breed in the United States or Canada and then migrated to breed again in the lowland thorn forests of west Mexico.

We present 3 analyses of stable-isotope data supporting our reasoning. Although our analyses of stable isotopes constitute circumstantial evidence of dual breeding, we emphasize that such quantitative evidence is considerably stronger and more revealing than evidence based solely on physical evidence of earlier breeding in females or mirrored times of departure and arrival in different geographic regions. Unlike population comparisons, data from isotopes give evidence that specific individuals have moved to breed again in a different region, making data on the breeding condition of these individuals especially valuable. Only for the Old World red-billed quelea (2) do we have proof of dual breeding ranges based on numerous marked individuals.

To test whether these species bred earlier in the United States or Canada, we compared their stable-isotope profiles with those of a reference sample of molt migrants (31 individuals of 6 species) and of a reference sample of local resident breeders (56 individuals of 19 species) (Table S1). Molt migrants breed largely in the United States and Canada and migrate to the southwestern United States or to northwestern Mexico to molt. As expected, no molt migrant was in breeding condition when collected in west Mexico (Table S1). Resident breeders are well known to breed in west Mexico, and only species with breeding ranges that do not or scarcely extend into the United States were used as reference samples in this study; many of these resident breeders were in breeding condition when collected (Table S1).

We hypothesized that resident breeders would differ in their stable H (δD), C (δ^{13}C), and N (δ^{15}N) isotope composition compared with molt migrants or migratory double breeders that had recently arrived from the United States or Canada, thereby providing us with an isotopic tag of origin (*SI Text*). We tested for migratory double breeding in 3 ways: (*i*) by discriminant function analysis of isotope data to separate birds by origin, (*ii*) by regressions of δD on day of sampling to identify north–south patterns of movement, and (*iii*) by correlations between δD values in muscle and reproductive tissue to identify those individuals that had initiated breeding in Mexico after arriving there from further north.

Results

Discriminant Analyses. Because molt migrants do not breed in coastal Sinaloa or Baja California Sur (8, 9), we know that recently

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[1]To whom correspondence should be addressed. E-mail: rohwer@u.washington.edu.

[2]Present address: Department of Biology, Queen's University, Kingston, ON, Canada K7L 3N6.

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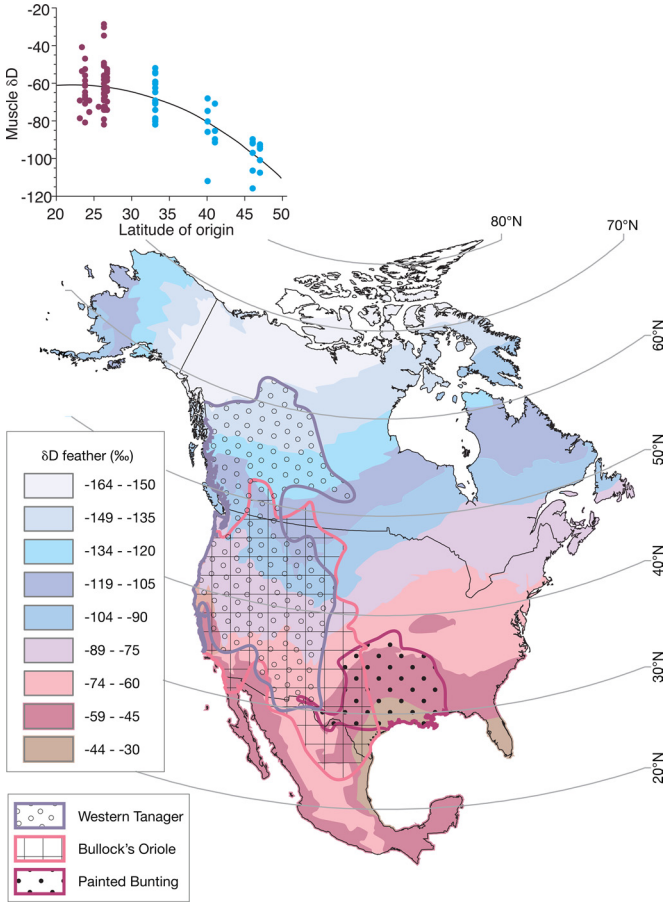

Fig. 1. Feather δD clines for North America based on the regression between feather δD in ref. 35 and the growing-season mean precipitation δD of ref. 36. *Inset*: Relationship between muscle δD and latitude of origin for the birds in the 2 reference samples used for the discriminant analysis: west Mexican resident breeders (maroon) and molt migrants from the United States and Canada (blue). We show the ranges of 3 exemplar molt migrants on the continental map for δD to illustrate how the latitude at which molt migrants breed affects the δD values they will show when collected in west Mexico. The southern-most range is for painted buntings from the midwest, which are molt migrants; painted buntings that breed in the eastern United States are not shown because they molt on their breeding range.

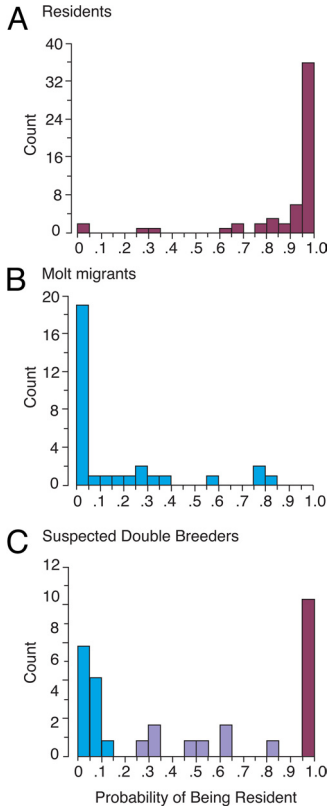

Fig. 2. Assignment probabilities from the discriminant analysis. (*A*) Probability of classifying as resident for the reference sample of resident breeders ($n = 57$). (*B*) Probability of classifying as resident for the reference sample of molt migrants ($n = 37$). (*C*) Assignment probabilities for 31 suspected double breeders (yellow-billed cuckoo, $n = 10$; Cassin's vireo, $n = 8$; hooded oriole, $n = 13$).

arrived molt migrants (individuals not molting or in early stages of the molt) should differ from resident breeders in δD values, owing to well-established continental patterns in foodwebs (Fig. 1), and in $\delta^{15}N$ and $\delta^{13}C$ values, owing to broad influences of climate on plants, including photosynthetic pathways (e.g., ref. 10). For our first analysis, we used a discriminant function to distinguish resident breeders and molt migrants according to 3 isotopes measured in both muscle and claw. True migratory double breeders (i.e., those that had bred previously in the same year in the United States or Canada) should classify with the reference sample of molt migrants, whereas potential double breeders that breed in west Mexico without having moved there from the north should classify with known Mexican residents. As predicted, discriminant analysis readily separated most individuals of our reference samples (molt migrants and resident breeders), with only 4 individuals in each reference group misclassified (Fig. 2 *A* and *B*). Most of this discrimination was due to the strong north–south geographic clines in precipitation δD in North America, but differences in $\delta^{15}N$ between residents and molt migrants also contributed importantly to discriminating the reference samples (*SI Text*).

Group assignment probabilities for 31 individuals of 3 species suspected of double breeding (yellow-billed cuckoo, Cassin's vireo, and hooded oriole) are shown in Fig. 2*C*. Nine of the 13 hooded orioles, 3 of the 8 Cassin's vireos, and 1 of the 10 yellow-billed cuckoos were assigned to the molt migrant reference group with probabilities of .85 or higher, and 2 more Cassin's vireos and 1 more hooded oriole classified with the molt migrants with probabilities between .65 and .75 (Fig. 2*C*). The reproductive condition or behavior of these 13 individuals suggests that most could have moved to western Mexico for a second round of breeding after an earlier breeding attempt in the United States or Canada (Table 1). The single yellow-billed cuckoo was a female collected August 19, 2006 that had just laid a 3-egg clutch [3 collapsed follicles (11)]. Two of the 3 Cassin's vireos were singing persistently from territories when collected, and at least 1 had enlarged testes (testes destroyed when shot for the second); the third was a postbreeding male that had started to molt, and this species is not a molt migrant (12). Five of the 9 hooded orioles were males with breeding testes that had not initiated molt; 2 were incubating females from late August; and 2 were males with regressing testes that had initiated molt (Table 1). Although hooded orioles are thought to be molt migrants (8), the 2 molting males had likely moved to Mexico to breed because their testes were still moderately enlarged (Table 1). All of these 13 individuals were collected at the end of July or in August, giving them plenty of time to have bred earlier north of Mexico.

In contrast, 8 yellow-billed cuckoos, 1 Cassin's vireo, and 2 hooded orioles classified with resident breeders with probabilities of .87 or higher (Fig. 2*C*), suggesting either that they had bred earlier in Mexico or that they carried an isotopic signature from an earlier breeding in the United States that could not be distinguished from the signature of our reference sample of resident breeders. Our

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Table 1. Breeding details for the 23 migratory double breeders identified by the discriminant analysis and by the 2 patterns of tissue contrasts

Species	Number	Date	Breeding data (measurements in mm)
Discriminant analysis			
YBCU	82371	Aug. 19	Female starting incubation (3 collapsed follicles), no molt
CAVI	81189	Aug. 4	Singing, territorial male, no molt; gonads shot
CAVI	82683	Aug. 28	Singing, territorial male, testis 6.5 × 4.5, no molt
CAVI	82690	Aug. 30	Singing, postbreeding male, molting, testis 2.5 × 1.5
HOOR	81191	Aug. 6	Postbreeding male, starting molt, testis 4 × 4
HOOR	81253	July 29	Postbreeding male, starting molt, testis 7 × 4.5
HOOR	82353	Aug. 30	Incubating female, no molt (edematous brood patch)
HOOR	82373	Aug. 20	Incubating female, no molt (edematous brood patch)
HOOR	82386	Aug. 28	Breeding male, starting primary molt, testis 13 × 7
HOOR	82461	Aug. 6	Breeding male, no molt, testis 11 × 7
HOOR	82462	Aug. 6	Breeding male, no molt, testis 8.5 × 6
HOOR	82507	Aug. 6	Breeding male, no molt, testis 10 × 7
HOOR	82623	Aug. 6	Breeding male, no molt, testis 7 × 5
Depleted δD muscle compared with reproductive tissue			
YBCU	84072	July 17	Breeding male, calling when shot, testis 10 × 6, no molt
YBCU	84088	July 24	Breeding male, calling when shot, testis 11 × 5.5, no molt
ORORO	84046	July 7	Breeding male, testis 12 × 9, seminal vesicles 6 × 5, no molt
ORORO	83975	July 7	Breeding male, testis 11.5 × 8, seminal vesicles 5 × 4, no molt
ORORO	84049	July 7	Breeding male, testis 10 × 7.5, seminal vesicles 5 × 4.5, no molt
ORORO	84076	July 19	Laying female, no molt
Enriched δD muscle compared with reproductive tissue			
YBCU	83966	July 24	Laying female, no molt
YBCH	83981	July 13	Female about to lay, oviduct enlarging, ovum 6, old brood patch, no molt
YBCH	84020	July 13	Breeding male, testis 9 × 6, no molt
ORORO	83973	July 7	Laying female, no molt

YBCU, yellow-billed cuckoo; CAVI, Cassin's vireo; HOOR, hooded oriole; ORORO, orchard oriole; YBCH, yellow-breasted chat.

discriminant analysis failed to classify any yellow-breasted chat or orchard oriole with the molt migrant reference group. In retrospect, this makes sense because deuterium (δD) contributed strongly to distinguishing molt migrants and resident breeders (*SI Text*), yet the deuterium isoscape for west Mexico matches that found in southwestern and south central regions of the United States, where these 2 species regularly breed (Fig. 1).

Deuterium and Date of Sampling. Our second analysis sought evidence of double breeding by contrasting the predicted relationships between muscle δD on date of sampling for resident breeders with that for molt migrants and suspected double breeders. We attempted to sample molt migrants and suspected double breeders shortly after they had arrived in west Mexico. If we succeeded in sampling these individuals soon after their arrival in west Mexico, the δD values for both groups should be negatively associated with date of collection; this follows because individuals that bred earlier in southern regions of the United States (areas more enriched with deuterium) should arrive earlier in west Mexico, whereas individuals that bred later in more northern regions of the United States or Canada (areas more depleted in deuterium) should arrive later in west Mexico. In contrast, resident breeders from west Mexico collected during the same time period should show no correlation between δD values and day of collection because we did not expect the local isoscape to change during our July–August sampling period.

As predicted, molt migrants and suspected double breeders showed strong negative regressions of muscle δD on day of collection, whereas Mexican residents showed no relationship (Fig. 3 *A–C*). Furthermore, each of our suspected double breeders with samples of 10 or more individuals from west Mexico (yellow-billed cuckoo, orchard oriole, hooded oriole, and yellow-breasted chat) showed significant negative regressions of muscle δD on day of collection (Fig. S1 *A–D*). These negative regressions further support migratory double breeding for hooded oriole and yellow-billed cuckoo and suggest double breeding for orchard oriole and yellow-breasted chat.

Comparing δD in Muscle and Reproductive Tissue. Our third analysis sought to identify double breeders by regressing muscle δD on δD for reproductive tissue (enlarged testes or oviducts; Fig. S2), which we began sampling only in 2007 (Table S1). The logic behind this approach is that migratory birds regress reproductive tissues but augment breast muscles before they migrate (13, 14). Thus, if a bird bred in the north and then migrated to west Mexico to breed again, it should have regressed its reproductive organs and enlarged its breast muscles in the United States or Canada, before it migrated to Mexico (see *SI Text* for exceptions that would not follow this pattern). Once in Mexico this bird would then rebuild its reproductive organs for the second breeding attempt. Thus, there should be no correlation between the δD signatures in reproductive and muscle tissue for our presumed migratory double breeders collected in west Mexico: their reproductive tissue should carry a local δD signature, but their breast muscle should carry a δD signature from their northern breeding site. In contrast, the δD signatures from these 2 tissues should be highly correlated in resident breeders because both their reproductive and muscle tissues were built in west Mexico. Comparing δD values in different tissues from the same individuals has the additional advantage of controlling for much of the interindividual variation in δD values (well illustrated in Figs. 1 and 3 and Fig. S2) that is caused by diet and other factors.

As predicted, the correlation between δD in reproductive and muscle tissue was near zero for suspected double breeders ($n = 16$, $r^2 = 0.03$; Fig. 4*B*), suggesting that most of these individuals had built their muscle and reproductive tissue in different places and were true migratory double breeders. In contrast, this same correlation was high for west Mexican residents ($n = 16$, $r^2 = 0.86$; Fig. 4*A*), presumably because their muscle and reproductive tissues were built in the same location. The strength of this correlation for residents, together with the lack of relationship for suspected double breeders, supports the utility of this approach to better defining movement histories.

We can now combine these regressions for residents (Fig. 4*A*) and suspected double breeders (Fig. 4*B*) to identify individuals that are especially likely to be double breeders. To do this we used the

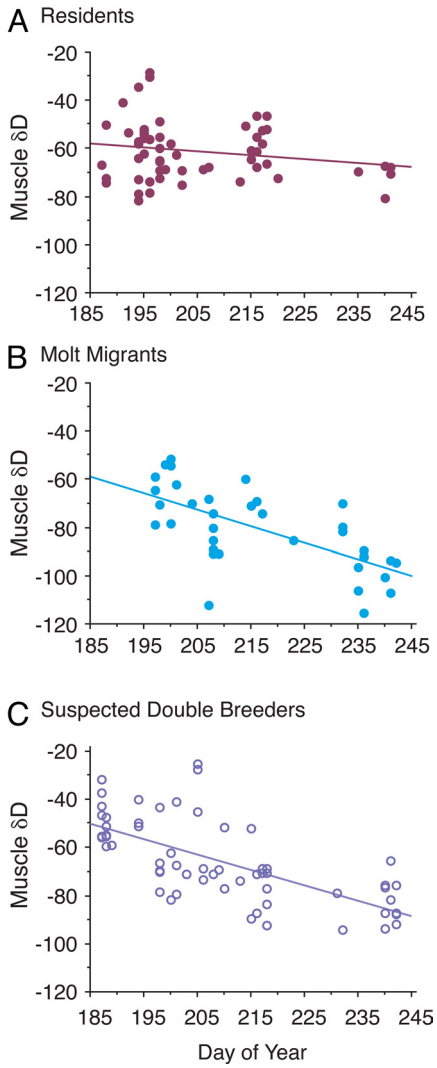

Fig. 3. Regression of muscle δD on day collected in west Mexico for (*A*) residents ($n = 56$; $P = 0.15$; $r^2 = 0.04$), (*B*) molt migrants ($n = 35$; $P < 0.0001$; $r^2 = 0.44$), and (*C*) suspected double breeders ($n = 60$; $P < 0.0001$; $r^2 = 0.46$). Day 185 is July 4. The lack of relationship for residents, together with the strong and similarly negative regressions for molt migrants and for suspected double breeders, suggest that latitude of origin determines δD values in the latter 2 groups. Because we know molt migrants bred earlier in the north, we can infer that the suspected double breeders did the same because their regression statistics (*C*) match those for molt migrants (*B*) and not those for resident breeders from west Mexico (*A*).

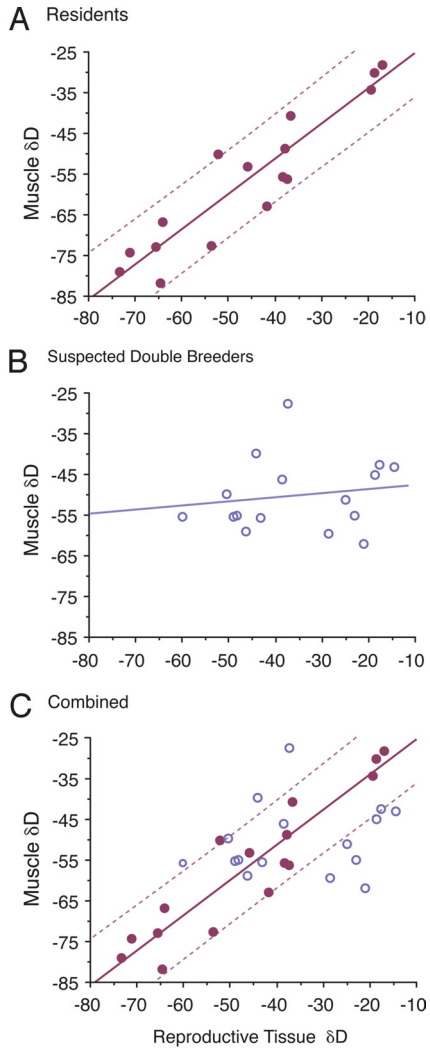

Fig. 4. Regression of muscle δD on δD for reproductive tissue for (*A*) residents and (*B*) suspected double breeders. The close association in residents ($n = 16$; $r^2 = 0.86$; $P < 0.0001$) follows because muscle and reproductive tissues were both generated locally. The lack of association in suspected double breeders ($n = 16$; $r^2 = 0.03$; $P = 0.55$) is expected if their reproductive tissue was generated in west Mexico but their muscle tissue was generated during an earlier breeding attempt in the United States or Canada. (*C*) Combination of graphs in *A* and *B*, identifying migratory double breeders that fall outside of the 85% confidence limits for individuals in the resident regression (*A*).

regressions for resident breeders as our expected relationship for birds that are not migratory double breeders and set confidence intervals for individuals of 85% around this regression line (matching the assignment cutoffs used in the discriminant analysis; Fig. 4*A*). We then superimposed the plot for suspected double breeders (Fig. 4*B*) on the plot for residents and recognized individuals falling outside the 85% confidence intervals around this line as likely migratory double breeders (Fig. 4*C*). Whether suspected double breeders fall above or below the confidence intervals around this line suggests their region of origin in the United States or Canada.

Six suspected double breeders (4 orchard orioles and 2 yellow-billed cuckoos) fell below the 85% confidence intervals for the resident regression (Fig. 4*C*). Their muscle δD was depleted relative to the expected value predicted from their reproductive tissue, suggesting that their muscle δD matched those expected from the northern United States or Canada. Four suspected double breeders (1 orchard oriole, 1 yellow-billed cuckoo, and 2 yellow-breasted chats) carried muscle δD that was enriched relative to that expected from their reproductive δD (Fig. 4*C*), suggesting that these individuals had come from the southern United States. Remarkably, the discriminant analysis classified every one of these 10 suspected double breeders with Mexican residents at probabilities of 0.96 or higher. Only with information from newly recrudesced reproductive tissue were we able to determine that these birds were not residents.

The gonadal data for these 10 specimens leave no doubt that all were breeding when collected in west Mexico (Table 1). Six specimens had δD depleted in their breast muscle compared with their reproductive tissue: 2 were yellow-billed cuckoos that were calling when shot and had fully enlarged testes; 3 were male orchard orioles with fully enlarged testes and seminal vesicles; the sixth was a female orchard oriole that was laying. Four other specimens had δD enriched in their breast muscle compared with their reproductive tissue: 1 was a laying female yellow-billed cuckoo, collected on July 24; 1 was a female yellow-breasted chat that was about to lay

and that had bred earlier that season (indicated by her old brood patch); 1 was a male chat with fully enlarged testes; the last was a laying female orchard oriole. All of these 10 specimens were collected in July, which is late enough for them either to have bred earlier that season north of Mexico.

A third group of 6 suspected double breeders fell within the 85% confidence limits of the regression of muscle δD on reproductive tissue δD for residents (Fig. 4*C*), making it impossible for this analysis to classify them as migratory double breeders. Note, however, that even these 6 individuals could have bred earlier in the United States in regions where the isoscape for muscle δD matches that for west Mexico. The fact that the regression of muscle δD on day of collection was as negative for our sample of suspected double breeders as it was for our sample of molt migrants (Fig. 3 *B* and *C*) suggests that the similarity in west Mexican and midwestern isoscapes is why these individuals fell near the line for west Mexican resident breeders. All were in breeding condition (Table S1).

Discussion

Our discovery of distinct breeding areas separated by hundreds to thousands of kilometers heralds a new chapter in the use of stable isotopes in documenting migratory connectivity (15, 16) and has important conservation and research implications. Although dual breeding in nocturnal migrants has been inferred for the northward migration in species that winter in Africa and breed in Eurasia (ref. 4 and Whitfield in ref. 5), the double breeding we document here is for the Neotropical–Nearctic migration system and occurs on the southward migration. These isotopic data defy a long-held assumption that migrants breed, with almost no exception, in only one place between just 2 bouts of migration. Instead migratory double breeders have added an additional migration and breeding season to their annual cycle, with attendant periods of physiologic transition. This discovery challenges the notion that physiologic mechanisms prevent migrants from incorporating additional life-history stages in their annual cycle, either because of constraints on the time required to move from one physiologic state to another (e.g., migration to breeding), or because of incompatibilities in the endocrine control mechanisms supporting different life-history stages (17, 18). Instead, migratory double breeding shows that entirely new life-history stages can be added to the annual cycle and challenges the notion that physiologic transition times are as important in constraining life history diversification as previously hypothesized.

Because the half-life of deuterium in muscle is just 8 days or so for the range of body masses we examined, we collected our molt migrant reference sample and our potential double breeders shortly after their arrival in west Mexico to minimize the risk of missing a northern signature in both groups. To this end, we excluded molt migrants that were advanced in flight feather molt, and we limited most of our sampling of potential double breeders to July (Table S1), fearing that August specimens might represent birds that had moved to breed in west Mexico in July and that could have been there long enough to have lost their northern signature (half-life hypothesis). Alternatively, however, migrants that breed reasonably far north in the United States or Canada (where δD values are more negative than in west Mexico) breed later than southern conspecifics; thus the opportunity for northern migrants to move to west Mexico to breed again comes later in the summer (opportunity hypothesis). The strong negative regression between day of collection and deuterium levels in muscle suggests that the opportunity hypothesis applies more strongly to our data (Fig. 3 and Fig. S1). There should be no relationship between day of collection and muscle deuterium for residents, and none was found, but the negative regressions for potential double breeders suggest that they first bred further north in North America (Fig. 3 and Fig. S1). These negative relationships suggest that we erred in not collecting more potential double breeders in August, when nests were still being initiated. At least some of these later breeders had likely just arrived in west Mexico from more northern areas and could easily have been distinguished from west Mexican resident breeders by the discriminant analysis.

Contrasts in the development of reproductive tissues between our reference sample of molt migrants and our suspected double breeders help exclude the possibility that double breeders were molt migrants that had not yet started their molt. Most of the suspected double breeders included in our isotope analyses were in full breeding condition with enlarged testes or oviducts (Table S1). Additionally, many of the males were singing and defending territories or guarding females, several of the females were laying or incubating, and we found many active nests of orchard and hooded orioles. Finally, orchard orioles and yellow-billed cuckoos molt on their wintering grounds, well to the south of the region where they breed again in northwest Mexico, whereas yellow-breasted chats and Cassin's vireos are both thought to molt on their breeding grounds; only hooded orioles are thought to be molt migrants (8), and we found them to be molting in west Mexico after breeding there in late summer. In contrast, our reference sample of molt migrants had regressed reproductive structures, and we observed no evidence of breeding in these species during 3 years of fieldwork (Table S1). Many additional females of our 5 species of suspected double breeders had dry brood patches, suggesting that they had bred earlier that same season, presumably in the north.

Two of our tests suggest that many the individuals of the 5 potential double breeders we examined had bred earlier in the north. However, these numbers tell us nothing about the percentage of individuals in northern breeding populations that attempt a second round of breeding in Mexico. Hooded orioles are extraordinarily common as late summer breeders in Baja California Sur, and orchard orioles, yellow-breasted chats, and yellow-billed cuckoos breed abundantly in the coastal lowlands of extreme southern Sonora and much of coastal Sinaloa. Thus, migratory double breeding may be common in at least some North American breeding populations of these species.

Molt migrants, as well as migratory double breeders, depend on habitat preservation in coastal Sinaloa and southern Sonora. The recent conversion of vast regions of coastal thorn forest to industrial agriculture in this region could be responsible for notable population declines in some species of both molt migrants and double breeders. Painted buntings (*Passerina ciris*) from the Midwestern breeding population move to west Mexico to molt (19) and have declined rapidly since the 1970s (20). Similarly, the yellow-billed cuckoo, which seems to be a migratory double breeder, is declining in eastern North America (20) and has disappeared from most of its breeding range in the western United States and Canada, even though substantial areas of riparian breeding habitat still exist in some areas where historically it was a common breeder (21, 22). Yellow-billed cuckoos are abundant late-summer breeders in the deciduous thorn scrub of coastal west Mexico that has not been converted to agriculture (23). If, as we propose, yellow-billed cuckoos are migratory double breeders, the population viability for cuckoos breeding in the United States and Canada may require that some fraction of those birds breed again in west Mexico. This second breeding may have been especially critical to the viability of western yellow-billed cuckoo populations because dry conditions west of the Rocky Mountains arrest primary productivity in midsummer, precisely when food becomes abundant in west Mexico.

Migratory double breeding poses many interesting questions about plasticity in avian life histories. How will spacing and mating systems, clutch sizes, and patterns of parental care and sexual conflict vary between early breeding sessions in the United States and Canada and later sessions in west Mexico? How do offspring from the same parents that are produced in regions separated by thousands of kilometers of longitude orient appropriately for migration? And how do adults that breed after a bout of fall migration to west Mexico schedule their molt appropriately? Although the direction and distance of intercontinental migrations

are known to be inherited in some passerines that migrate long distances at night (24–26), the direction of migration and its scheduling relative to the annual molt must be flexible in migratory double breeders. Orchard orioles and eastern yellow-billed cuckoos are unusual among Neotropical migrants that breed in eastern North America for molting not on their breeding grounds but on their winter range (8). If, as we suggest, some fraction of these eastern-breeding populations breeds a second time in west Mexico before going on to winter in Central and South America, then some adults molt after a single bout of breeding and migration, whereas others molt after 2 bouts of breeding and migration. Finally, as nocturnal migrants, how do the offspring of cuckoos and orioles hatched in eastern North America orient southwest in their migration, whereas offspring hatched from the same parents in west Mexico orient southeast in their migration toward a presumably common winter range?

Materials and Methods

The value of using naturally occurring stable isotope ratios to trace the origin of migratory wildlife is well established (27). The underlying principle is that tissues of organisms come into isotopic equilibrium with local foodwebs and that these foodwebs vary geographically in their isotopic signatures. These differences in isotopic patterns, or *isoscapes*, derive from biogeochemical processes related to climate, topography, and weather patterns, from differences in photosynthetic pathways, and from variation in geologic substrates (28). For organisms that move between isoscapes, information on previous origins can be inferred, provided the elemental turnover rate in tissue retains enough of the original signature of origin to be traced. Elemental turnover rates for C and N in avian muscle, liver, and blood have been determined (29–31) and in general follow allometric relationships related to body mass (32). For a bird of 40 g, typical of the species examined here, the half-life of C and N in muscle tissue is on the order of 10 days. Source isotope signatures are typically considered to be traceable within 2 half-lives or 20 days. For metabolically inactive tissues like feathers and claws, source isotopic signatures are essentially locked in and represent the location and food where they were grown.

Less information is available for the turnover rates of H in avian tissues, but we anticipate that in general those values established for C and N apply. Use of this isotope is further complicated by the fact that H bonded to N and O in tissues is capable of exchange with ambient body water. For example, controlled laboratory studies on captive birds have established that approximately 20% of H is exchangeable with drinking water (33). For metabolically inactive tissues like feathers and claws, our H isotope measurements correspond to the nonexchangeable fraction of H (34). Stable H isotope values in muscle tissue may take up a local signal faster than for C and N, owing to exchange with drinking water, but because the bulk (i.e., 80%) of that tissue does not exchange water, a half-life of 8 days is not unreasonable. To our knowledge, no elemental turnover information is available for reproductive tissues, but unless solely stored reserves are used to generate these tissues, they are almost certainly formed from elements derived from the breeding grounds and so can be considered local.

Our samples in Sinaloa and Baja California Sur were collected in July and August of 2005–2007 (Table S1). Molt migrants were collected from July 16 (the earliest date we encountered them) to August 30. To ensure that the molt migrants included in our analyses had recently arrived in west Mexico and would give us northern isotopic signatures, we used individuals that either had not initiated molt or were in early stages of the primary molt. Residents were collected between July 6 and August 29. Potential double breeders were collected from July 6 to August 30; only 12 of the 57 potential double breeders were collected in the latter part of August. If these birds had moved to west Mexico to breed some weeks earlier, they may have been sampled late enough to have acquired a local isotopic signature, biasing results against the discovery of migratory double breeding.

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Supporting Information

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SI Text

Character Contributions to the Discriminate Analysis and δD Isoscapes. For the discrimination of residents and molt migrants we measured stable isotope levels for C, N, and H in muscle and claw (6 characters). Claw is metabolically inactive and so should reflect a northern signature when collected in Mexico. However, this assumption faltered because we could not limit our samples to just the outer, nonmetabolizing, horny part of the claw; thus, our claw samples included variable fractions of metabolizing tissue.

For our discriminate analysis, we used the quadratic method because the within-group covariance matrices were different for the reference samples of molt migrants and resident breeders. When these matrices differ, using a quadratic model achieves better discrimination between the reference samples but complicates estimation of the contribution of each character to discriminating the reference groups. For this reason we can only offer changes in log likelihood ratios that compare the full model using all 6 characters with that achieved by single characters. These likelihood ratios showed that muscle δD and δ^{15}N muscle contributed most strongly to discriminating the reference samples. For the full model the −2 log likelihood score was 21.38; the score for muscle δD alone was 11.6, and the score for muscle δ^{15}N alone was 8.0. These 2 characters accounted for most of the discrimination between the reference samples. Muscle δD was our most valuable character, but samples of just the outer, nonmetabolizing layer of claw should be even more valuable if they could be obtained in sufficient quantity.

The reason δD values contributed strongly to the discrimination of molt migrants and resident breeders is that 4 of the 6 molt migrants in our reference sample have breeding ranges that extend into Canada with lower δD values (Fig. 1). However, several suspected double breeders (yellow-billed cuckoo, yellow-bellied chat, and orchard oriole) whose northern breeding ranges include southwestern and south central regions of the United States mostly classified with the resident breeders from west Mexico. They did so because feather δD levels for west Mexico are similar to those found in southwestern and south central states of the United States (Fig. 1). Thus potential double breeders from the southwest and south central United States cannot classify with the molt migrant reference sample using δD values alone, even though δD muscle contributed most strongly to distinguishing the reference samples.

We illustrate this problem by regressing muscle deuterium on latitude of origin for the combined sample of Mexican residents and the 6 species of molt migrants collected in west Mexico (Fig. 1, *Inset*). For residents, we recorded each individual's latitude of collection; for the 6 species of molt migrants we assigned latitude of origin as the mid-latitude of their breeding range in the United States and Canada. This regression shows that muscle deuterium fails to distinguish Mexican residents from Lucy's warblers and painted buntings, molt migrants from the southwest and central United States (the single cluster of points nearest the Mexican residents; Fig. 1, *Inset*), but that muscle deuterium can distinguish molt migrants that breed further north in the United States and Canada (Bullock's oriole, black-headed grosbeak, western warbling vireo, and western tanager) from west Mexican residents.

Stable Isotope Analyses. Claw samples were cleansed of surface oils in a 2:1 chloroform/methanol solvent rinse before stable isotope analysis. Muscle and reproductive tissues were first freeze-dried and then delipidized and air-dried. Stable-hydrogen isotope analyses of tissues were conducted using the comparative equilibration method (1) through the use of calibrated keratin hydrogen-isotope reference materials. This provided an estimate of the nonexchangeable H δD for our samples. Hydrogen isotope measurements were performed on H_2 derived from high-temperature (1,400 °C) flash pyrolysis of 350 ± 10 μg subsamples using continuous-flow isotope ratio mass spectrometry. Measurement of 3 keratin laboratory reference materials (cow hoof, chicken feather, and bowhead whale baleen), corrected for linear instrumental drift, were both accurate and precise, with typical mean δD ± SD values of −147.4‰ ± 0.8‰ (n = 5), −187.0‰ ± 0.6‰ (n = 5), and −108.0‰ ± 0.3‰ (n = 5) per autorun, respectively. A control keratin reference yielded a 6-month SD of ±3.3‰ (n = 76). All results are for nonexchangeable δD expressed in the typical delta (δ) notation, in units of per mil (‰), and normalized on the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation standard scale.

Stable C and N isotope analyses of tissues were performed using a 20:20 continuous-flow Europa isotope ratio mass spectrometer interfaced with a Robo-Prep elemental analyzer (Europa Instrument). Results are reported in typical δ notation relative to Atmospheric AIR (δ^{15}N) and the Pee Dee Belmenite (δ^{13}C) standards. On the basis of within-run replicates of bowhead whale and egg albumen laboratory standards, we estimate measurement error to be ±0.3‰ and ±0.1‰, for δ^{15}N and δ^{13}C measurements, respectively.

Estimating the Frequency of Migratory Double Breeding. We can estimate the percentage of suspected double breeders that likely bred earlier in the same summer in the United States or Canada from 2 of our 3 analyses. In the discriminate analysis, 52.4% of the sample of 8 Cassin's vireos and 13 hooded orioles classify with the molt migrants, suggesting that they were migratory double breeders. Because we were unable to collect reproductive tissues for either of these 2 species in 2007, this is the only measure we can use to estimate the frequency of migratory double breeding for these 2 species. We excluded 10 yellow-breasted chats, 16 orchard orioles, and 11 yellow-billed cuckoos from this frequency estimate because our west Mexico tissues for these birds were collected in July. If these individuals were actual double breeders, they could only have gotten to west Mexico early enough to breed there in July by having first bred in the southern United States in late April or early May, and our discriminate analysis could not distinguish the isoscape of the southern United States from that of west Mexico (see Fig. 1).

The more powerful analysis contrasting δD in muscle and reproductive tissue categorized 62.5% of the 16 suspected double breeders as having bred earlier in the north. The 16 birds in this sample (11 orchard orioles, 2 yellow-breasted chats, and 3 yellow-billed cuckoos) represent all of the suspected double breeders for which we were able to collect both muscle and reproductive tissue in 2007. Although we considered potential double breeders with assignment probabilities as low as 85% to be considered actual double breeders, both the discriminate and the regression analyses probably underestimate the percentage of potential double breeders that are recognized as actual double breeders because the isoscapes for residents and suspected double breeders overlap in both analyses, although in different ways.

Previous Status of These Species in West Mexico. Here we briefly summarize what was previously known of the west Mexican breeding status for our 5 migratory double breeders. Yellow-billed cuckoos were known to breed in Baja California Sur, Sonora (August 6), and Sinaloa (June 24–September 10) (2, 3). Cassin's vireos were recorded to breed in the mountains at the southern tip of Baja California, but no dates are given (3). Yellow-breasted chats were recognized as spring breeders at moderate elevations on the Pacific side of the Sierra Madre Occidental (3) and recorded by Short (4) as apparently breeding at Los Alamos, Sonora in late July and early August. Orchard orioles are listed as breeding in Sinaloa (June 17) and Jalisco (June 29, July 30), with additional records from Nayarit (July and August), Oaxaca (July 30), and Chiapas (August 16); the dates for these records suggest specimens in breeding condition, although details are not given (3). Hooded orioles were considered resident breeders in Baja California Sur and as migrant breeders in Sonora and Sinaloa; breeding dates were not given for these records, presumably because none was very late (3). Short (4) recorded nest construction by a hooded oriole in a palm in the town plaza of Los Alamos, Sonora in late July or early August.

This short summary shows that all 5 of our migratory double breeders were previously known to breed in coastal west Mexico. However, special notation in the Mexican checklist of late summer breeding dates shows that the authors considered these records unusual. When the checklist was published, neither the importance of the late summer monsoon in stimulating breeding by residents (4) nor the possibility of double breeding by migrants was appreciated. Finally, we note that the late summer orchard orioles collected in other southern states of Mexico (3) suggest that it may also breed a second time south of Sonora and Sinaloa, where we found it to be a common breeder in July and August.

The Value of Reproductive Tissue. Because there have been no previous uses of reproductive tissues in isotopic analyses of connectivity, we compared δD values from enlarged testes and from enlarged oviducts to confirm that male and female reproductive tissues could be pooled in the same analyses (Fig. S1). For 11 males and 21 females of various species of residents and potential double breeders, we found no difference in the distributions of δD values, suggesting that male and female reproductive tissues are comparable ($P = 0.17$, t test).

The contrast we predict between deuterium levels in reproductive tissues and muscle presumes that gonads are regressed before migrations and rebuilt locally. This assumption has not been addressed for the species we analyze. In the itinerate breeding red-billed quelea of Africa, males do not regress their testes before moving to new breeding areas, and females develop ova that are ready to ovulate just before fledging young and abandoning a colony to move elsewhere to breed (5). Female birds, however, always regress their oviducts immediately after laying, so the assumption that reproductive tissues are built locally and may contrast in their isotopic signatures from muscle should apply to laying females of most species. Exceptions would be females that lay using stored protein and lipids, as is well documented for arctic nesting waterfowl (6), and female quelea that prepare to lay during the end of parental care in their current breeding site before moving to their next breeding site.

Voucher Specimens. The specimens for this study were collected on joint expeditions by the University of Washington Burke Museum (UWBM) and the Museo de Zoología, Facultad de Ciencias collection at the Universidad Nacional Autónoma de México. Half of the specimens were deposited at each institution, but duplicate samples of tissues were collected; thus all of the voucher numbers listed in Table S1 are UWBM numbers, even though the 2 collections have contributed about equally to the samples used in these analyses.

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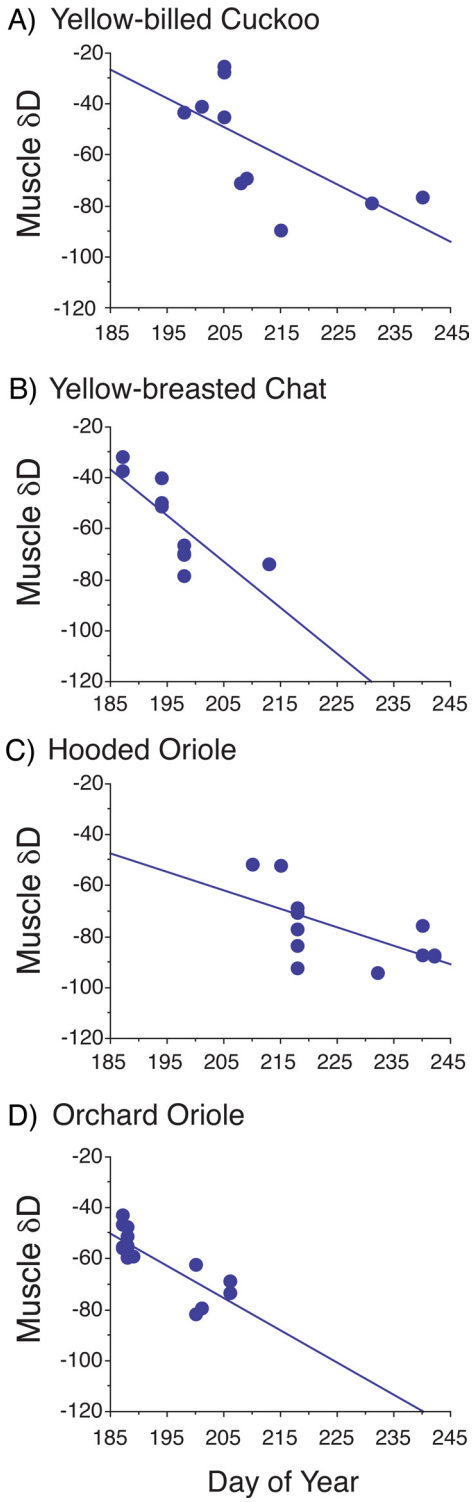

Fig. S1. Regression of muscle δD on day collected for 4 suspected double breeders: (*A*) yellow-billed cuckoo ($n = 10$; $P < 0.04$; $r^2 = 0.44$), (*B*) yellow-breasted chat ($n = 10$; $P = 0.008$; $r^2 = 0.61$), (*C*) hooded oriole ($n = 12$; $P = 0.02$; $r^2 = 0.39$), and (*D*) orchard oriole ($n = 16$; $P < 0.0001$; $r^2 = 0.68$). Day 185 is July 4. These negative regressions suggest that the latitude at which those individuals first bred in the United States or Canada largely determined the muscle δD values they carried when collected in west Mexico.

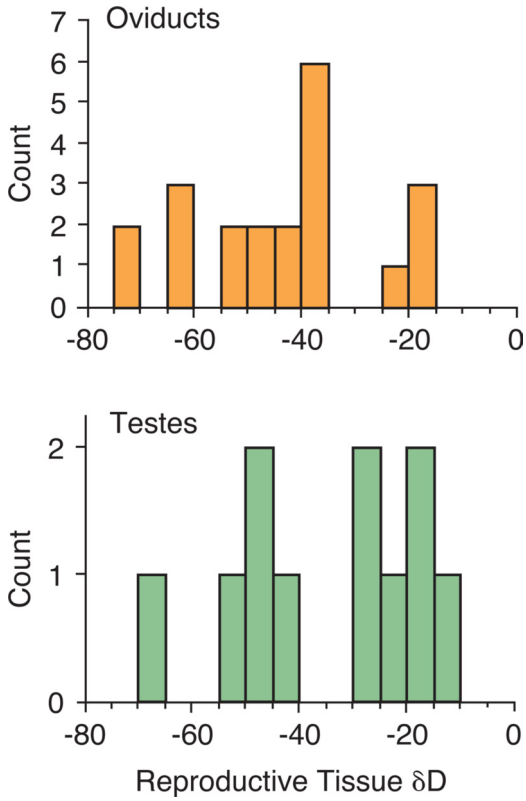

Fig. S2. Comparison of δD values from 11 males (enlarges tested) and 21 females (enlarged oviducts). The distributions were not different ($P = 0.17$; t test).

Table S1. Important details for the specimens included in our analyses

Species	UWBM number	Date collected	Latitude collected (° north)	Longitude collected (° west)	Sex	Largest testis or ovum (mm)	Reproductive tissue (if sampled)
Molt migrants							
Icterus bullockii	81152	26-July-2005	26.303	108.698	M	4 × 2	
Icterus bullockii	81155	27-July-2005	26.303	108.698	M	4 × 2	
Icterus bullockii	81162	27-July-2005	26.303	108.698	F	<1	
Icterus bullockii	81237	26-July-2005	26.303	108.698	M	2 × 1.5	
*Icterus bullockii**	81399	11-Aug.-2005	26.303	108.698	M	2.5 × 2	
Passerina ciris	81129	16-July-2005	23.025	105.752	M	2 × 1	
Passerina ciris	81134	17-July-2005	23.025	105.752	M	2 × 1	
Passerina ciris	81336	23-July-2005	24.303	106.763	M	1.5 × 1.5	
Passerina	82457	4-Aug.-2006	26.303	108.698	F	<1	
Passerina	82574	20-Aug.-2006	26.303	108.698	M	3 × 1.5	
Passerina	82575	20-Aug.-2006	26.303	108.698	F	<1	
Passerina	82621	5-Aug.-2006	26.303	108.698	M	1.5 × 1	
Passerina	82666	20-Aug.-2006	26.303	108.698	M	2 × 1	
Pheucticus melanocephalus	81265	3-Aug.-2005	23.753	109.975	M	1.5 × 1	
Pheucticus	81165	28-July-2005	26.303	108.698	M	4 × 3	
Pheucticus	81239	27-July-2005	26.303	108.698	M	2.5 × 2	
Pheucticus	81240	27-July-2005	26.303	108.698	M	2 × 1.5	
Piranga ludoviciana	82352	30-Aug.-2006	23.748	109.978	F	<1	
*Piranga ludoviciana**	82384	28-Aug.-2006	23.748	109.978	M	1.5 × 1	
Piranga ludoviciana	82389	29-Aug.-2006	23.748	109.978	M	3.2 × 2.9	
Piranga ludoviciana	82586	28-Aug.-2006	23.748	109.978	M	1.5 × 1	
Piranga ludoviciana	82591	29-Aug.-2006	23.748	109.978	F	<1	
Piranga ludoviciana	82581	24-Aug.-2006	26.653	108.390	M	1.5 × 1	
Vermivora luciae	82413	16-July-2006	26.275	108.795	?	–	
*Vermivora luciae**	82562	16-July-2006	26.275	108.795	M	0.5 × 0.5	
*Vermivora luciae**	82607	2-Aug.-2006	26.275	108.795	M	1 × 1	
Vermivora luciae	82725	18-July-2006	26.275	108.795	F	<1	
Vermivora luciae	82726	19-July-2006	26.275	108.795	M	0.5 × 0.5	
Vermivora luciae	82729	19-July-2006	26.275	108.795	M	1.5 × 1.5	
*Vermivora luciae**	82733	19-July-2006	26.275	108.795	F	<1	
Vermivora luciae	82738	20-July-2006	26.275	108.795	F	<1	
Vireo gilvus	81350	27-July-2005	26.303	108.698	F	<1	
Vireo gilvus	82381	24-Aug.-2006	26.653	108.390	F	<1	
Vireo gilvus	82382	24-Aug.-2006	26.653	108.390	F	<1	
Vireo gilvus	82577	23-Aug.-2006	26.653	108.390	M	1.25 × 1	
*Vireo gilvus**	82578	23-Aug.-2006	26.653	108.390	M	1.5 × 1	
Vireo gilvus	82582	24-Aug.-2006	26.653	108.390	M	1 × 0.75	
Residents							
Aphelocoma californica	82385	28-Aug.-2006	23.748	109.978	F	1	
Aphelocoma californica	82687	29-Aug.-2006	23.748	109.978	F	1	
Aphelocoma californica	81196	8-Aug.-2005	25.587	111.662	F	<1	
Auriparus flaviceps	84061	13-July-2007	26.275	108.795	F	8, laying	Oviduct
Callipepla douglasii	84052	7-July-2007	26.321	108.763	F	22, laying	Oviduct
Campylorhynchus brunneicapillus	81183	4-Aug.-2005	23.753	109.975	F	3	
Campylorhynchus brunneicapillus	81370	4-Aug.-2005	23.753	109.975	M	5 × 4	
Campylorhynchus brunneicapillus	82613	2-Aug.-2006	26.275	108.795	F	Incubating	
Cardinalis cardinalis	82684	28-Aug.-2006	23.748	109.978	F	Incubating	
Cardinalis cardinalis	81480	4-Aug.-2005	23.753	109.975	M	7.5 × 4.5	
Cardinalis cardinalis	82414	17-July-2006	26.275	108.795	M	8.7 × 4.3	
Cardinalis cardinalis	82417	17-July-2006	26.275	108.795	M	9.6 × 6.3	
Cardinalis cardinalis	82421	17-July-2006	26.275	108.795	M	9.8 × 6.3	
*Cardinalis cardinalis**	82720	17-July-2006	26.275	108.795	M	9 × 6	
Cardinalis cardinalis	82721	17-July-2006	26.275	108.795	F	3	
Cardinalis cardinalis	83990	17-July-2006	26.275	108.795	F	Incubating	Oviduct
Cardinalis cardinalis	84009	7-July-2007	26.275	108.795	F	Incubating	Oviduct
Cardinalis cardinalis	82433	25-July-2006	26.300	108.770	F	—	
Columbina talpacoti	84017	13-July-2007	26.275	108.795	F	5	Oviduct
Columbina talpacoti	84059	13-July-2007	26.275	108.795	M	10.5 × 6	Testes
Columbina talpacoti	83977	7-July-2007	26.321	108.763	F	6	Oviduct
Crotophaga sulcirostris	84023	15-July-2007	26.275	108.795	F	14, laying	Oviduct
Icterus parisorum	82349	29-Aug.-2006	23.748	109.978	M	10 × 7	
Icterus parisorum	81391	6-Aug.-2005	23.753	109.975	M	9 × 6	

Species	UWBM number	Date collected	Latitude collected (° north)	Longitude collected (° west)	Sex	Largest testis or ovum (mm)	Reproductive tissue (if sampled)
Icterus parisorum	81479	3-Aug.-2005	23.753	109.975	F	—	
Icterus pustulatus	81201	15-July-2005	23.025	105.752	M	12 × 9	
Icterus pustulatus	82449	1-Aug.-2006	26.275	108.795	M	7.1 × 3.6	
Icterus pustulatus	81461	26-July-2005	26.303	108.698	M	4 × 4	
Icterus pustulatus	82403	14-July-2006	26.653	108.390	M	11.2 × 8.2	
Icterus pustulatus	82404	14-July-2006	26.653	108.390	F	Incubating	
Icterus pustulatus	82405	14-July-2006	26.653	108.390	M	12.2 × 7.7	
Icterus pustulatus	82556	14-July-2006	26.653	108.390	M	11.5 × 7	
Icterus pustulatus	82709	14-July-2006	26.653	108.390	M	13 × 9	
Leptotila verreauxi	84064	14-July-2007	26.274	108.795	F	15, laying	Oviduct
Melanerpes chrysogenys	83979	10-July-2007	23.363	106.305	F	9, laying	Oviduct
Molothrus aeneus	84081	20-July-2007	26.311	108.810	F	12, laying	Oviduct
Molothrus aeneus	83970	6-July-2007	26.321	108.763	F	10, laying	Oviduct
Myiarchus tyrannulus	82398	13-July-2006	26.653	108.390	M	12 × 4.5	
Myiarchus tyrannulus	82399	13-July-2006	26.653	108.390	M	11 × 5.3	
Myiarchus tyrannulus	82553	13-July-2006	26.653	108.390	M	8 × 2.5	
Myiarchus tyrannulus	82560	15-July-2006	26.653	108.390	F	<1	
Myiozetetes similis	81314	17-July-2005	23.025	105.752	M	8 × 4	
Myiozetetes similis	82425	18-July-2006	26.275	108.795	M	8.3 × 4.1	
Pachyramphus aglaiae	82427	19-July-2006	26.275	108.795	M	10.5 × 4.5	
Pachyramphus aglaiae	82338	23-Aug.-2006	26.653	108.390	M	5 × 2	
Passerina versicolor	84070	15-July-2007	26.227	108.810	F	9, laying	Oviduct
Saltator coerulescens	84021	13-July-2007	26.275	108.795	F	2	Oviduct
Toxostoma cinereum	81192	6-Aug.-2005	23.753	109.975	M	3 × 1	
Toxostoma cinereum	81280	5-Aug.-2005	23.753	109.975	M	12 × 8	
Toxostoma cinereum	81289	6-Aug.-2005	23.753	109.975	M	11 × 6	
Toxostoma cinereum	81477	3-Aug.-2005	23.753	109.975	F	<1	
Toxostoma cinereum	81481	4-Aug.-2005	23.753	109.975	M	13 × 7.5	
Toxostoma cinereum	81484	5-Aug.-2005	23.753	109.975	F	<1	
Toxostoma curvirostre	84067	15-July-2007	26.227	108.810	F	8, laying	Oviduct
Trogon citreolus	84056	11-July-2007	23.363	106.305	M	7 × 6	Testes
Vireo flavoviridis	81219	21-July-2005	24.303	106.763	M	10.5 × 5.5	
Vireo flavoviridis	81220	21-July-2005	24.303	106.763	F	Incubating	
Potential double breeders							
Coccyzus americanus	82680	28-Aug.-2006	23.748	109.978	M	11 × 5	
Coccyzus americanus	81368	3-Aug.-2005	23.753	109.975	M	10 × 5	
Coccyzus americanus	84000	24-July-2007	26.227	108.810	M	8 × 4	
Coccyzus americanus	84072	17-July-2007	26.274	108.795	M	10 × 6	Testes
Coccyzus americanus	84088	24-July-2007	26.274	108.795	M	11 × 5.5	Testes
Coccyzus americanus	81156	27-July-2005	26.303	108.698	M	8 × 7	
Coccyzus americanus	81470	28-July-2005	26.303	108.698	M	10 × 5.5	
Coccyzus americanus	82371	19-Aug.-2006	26.303	108.698	F	Laying	
Coccyzus americanus	83966	24-July-2007	26.311	108.810	F	13, laying	Oviduct
Coccyzus americanus	84080	20-July-2007	26.311	108.810	M	—	
Icteria virens	83981	13-July-2007	26.274	108.795	F	6, laying	Oviduct
Icteria virens	82415	17-July-2006	26.275	108.795	M	8.6 × 6.3	
Icteria virens	82419	17-July-2006	26.275	108.795	F	3.2	
Icteria virens	82448	1-Aug.-2006	26.275	108.795	F	1.8	
Icteria virens	82717	17-July-2006	26.275	108.795	F	8, laying	
Icteria virens	82718	17-July-2006	26.275	108.795	M	13 × 8	
Icteria virens	83935	6-July-2007	26.275	108.795	M	9 × 7	
Icteria virens	83936	6-July-2007	26.275	108.795	M	11 × 6	
Icteria virens	84018	13-July-2007	26.275	108.795	M	10 × 6	
Icteria virens	84020	13-July-2007	26.275	108.795	M	9 × 6	Testes
Icterus cucullatus	82353	30-Aug.-2006	23.748	109.978	F	Incubating	
Icterus cucullatus	82386	28-Aug.-2006	23.748	109.978	M	13 × 7	
Icterus cucullatus	82596	30-Aug.-2006	23.748	109.978	F	Incubating	
Icterus cucullatus	82682	28-Aug.-2006	23.748	109.978	M	11 × 8	
Icterus cucullatus	81191	6-Aug.-2005	23.753	109.975	M	4 × 4	
Icterus cucullatus	81369	3-Aug.-2005	23.753	109.975	M	10 × 8	
Icterus cucullatus	81253	29-July-2005	26.303	108.698	M	7 × 4.5	
Icterus cucullatus	82373	20-Aug.-2006	26.303	108.698	F	1.5	
Icterus cucullatus	82461	6-Aug.-2006	26.303	108.698	M	11 × 7	
Icterus cucullatus	82462	6-Aug.-2006	26.303	108.698	M	8.5 × 6	

Species	UWBM number	Date collected	Latitude collected (° north)	Longitude collected (° west)	Sex	Largest testis or ovum (mm)	Reproductive tissue (if sampled)
Icterus cucullatus	82507	6-Aug.-2006	26.303	108.698	M	10 × 7	
Icterus cucullatus	82622	6-Aug.-2006	26.303	108.698	M	11 × 7	
Icterus cucullatus	82623	6-Aug.-2006	26.303	108.698	M	7 × 5	
Icterus spurius	82727	19-July-2006	26.275	108.795	M	13 × 7	
Icterus spurius	82736	20-July-2006	26.275	108.795	F	9, laying	
Icterus spurius	82472	25-July-2006	26.300	108.770	M	10 × 7	
Icterus spurius	82742	25-July-2006	26.300	108.770	F	Incubating	
Icterus spurius	84076	19-July-2007	26.311	108.810	F	9.5, laying	Oviduct
Icterus spurius	83969	6-July-2007	26.321	108.763	F	3.8, building	Oviduct
Icterus spurius	83972	7-July-2007	26.321	108.763	M	8 × 6	
Icterus spurius	83973	6-July-2007	26.321	108.763	F	9, laying	Oviduct
Icterus spurius	83974	6-July-2007	26.321	108.763	M	10 × 8	Testes
Icterus spurius	83975	6-July-2007	26.321	108.763	M	11.5 × 8	Testes
Icterus spurius	83976	6-July-2007	26.321	108.763	M	10 × 6	Testes
Icterus spurius	84046	7-July-2007	26.321	108.763	M	12 × 9	Testes
Icterus spurius	84047	7-July-2007	26.321	108.763	M	10 × 7	Testes
Icterus spurius	84048	7-July-2007	26.321	108.763	M	12 × 10	Testes
Icterus spurius	84049	7-July-2007	26.321	108.763	M	10 × 7.5	Testes
Icterus spurius	84050	8-July-2007	26.321	108.763	F	2	Oviduct
Vireo cassinii	82590	29-Aug.-2006	23.748	109.978	M	6 × 4.5	
Vireo cassinii	82593	30-Aug.-2006	23.748	109.978	M	3.5 × 2	
Vireo cassinii	82683	28-Aug.-2006	23.748	109.978	M	6.5 × 4.5	
Vireo cassinii	82685	29-Aug.-2006	23.748	109.978	F	<1	
Vireo cassinii	82690	30-Aug.-2006	23.748	109.978	M	2.5 × 1.5	
Vireo cassinii	81189	4-Aug.-2005	23.753	109.975	M	—	
Vireo cassinii	81275	5-Aug.-2005	23.753	109.975	M	6 × 4	
Vireo cassinii	81276	5-Aug.-2005	23.753	109.975	F	3.25, laying	

M, male; F, female. *, Not included in the discriminant analysis because of missing isotope values.