Using Geologgers to Investigate Bimodal Isotope Patterns in Painted Buntings
(Passerina ciris)

Author(s): Andrea Contina, Eli S. Bridge, Nathaniel E. Seavy, Jonah M. Duckles, and Jeffrey F. Kelly
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USING GEOLOGGERS TO INVESTIGATE BIMODAL ISOTOPE PATTERNS IN PAINTED BUNTINGS (PASSERINA CIRIS)

Andrea Contina, Eli S. Bridge, Nathaniel E. Seavy, Jonah M. Duckles, and Jeffrey F. Kelly

1Oklahoma Biological Survey, University of Oklahoma, 111 East Chesapeake Street, SC Building 134, Norman, Oklahoma 73019, USA; 2Department of Biology, University of Oklahoma, 730 Van Vleet Oval, Norman, Oklahoma 73019, USA; 3PRBO Conservation Science, 3820 Cypress Drive, no. 11, Petaluma, California 94954, USA; and 4Center for Spatial Analysis, University of Oklahoma, 301 David L. Boren Boulevard, Norman, Oklahoma 73019, USA

Abstract.—Painted Buntings (Passerina ciris) that breed in Oklahoma and molt in Sinaloa, Mexico, demonstrate a clear bimodal pattern of stable isotope ratios in their flight feathers. Some birds had a C3 carbon signature in primary 1 (P1, the first feather replaced during wing molt) and a C4 carbon signature in primary 9 (P9, the last primary to molt), whereas other sympatric birds evinced a C4-based diet throughout feather molt. The bimodal pattern of stable isotope ratios in flight feathers suggests that some birds may initiate molt immediately upon arrival in northwestern Mexico (and carry a C3 signature with them from the breeding grounds) whereas others may delay molt (and grow feathers solely from C4 plants of Sinaloa). From 2010 to 2012, we used geolocator tags to test whether differences in the timing and route of fall migration movements were related to stable isotope signatures in primary feathers. We analyzed stable isotopes of hydrogen and carbon in P1 and P9 from 25 individuals fitted with geolocator tags in two consecutive years. Of these, 60% changed the diet (C3 vs. C4) that was used to grow P1 between years. We also observed variation among individuals in migration routes, wherein birds from the same breeding population differed greatly in their use of molting and wintering locations. However, we did not find a relationship between isotope signatures and the timing or route of fall migration. We speculate that the bimodal isotope signature we observed represents a carryover effect related to local landscapes (grassland or agriculture vs. shrubland) used during the late breeding season and early molting period, and that these effects diminish as molt progresses. If this is the case, there is the potential for breeding-season diet to directly affect plumage quality in this molt migrant. Received 4 January 2013, accepted 19 March 2013.

Key words: carryover effects, GeoLight, geolocator, geologger, migration, molt, Painted Bunting, Passerina ciris, stable isotope ratio, tripEstimation.

Uso de Geolocalizadores para Investigar Patrones Bimodales de Isótopos en Passerina ciris

Resumen.—Los individuos de la especie Passerina ciris que se reproducen en Oklahoma y mudan en Sinaloa, Mexico, demuestran claramente un patrón bimodal en los cocientes de isótopos estables en sus plumas de vuelo. Algunas aves tienen señales de carbono C3 en la primaria 1 (P1, la primera pluma reemplazada durante la muda de invierno) y de carbono C4 en la primaria 9 (P9, la última primaria en ser mudada), mientras que otras aves simpátricas presentaron evidencia de una dieta basada en C4 a través de toda la muda de las plumas. El patrón bimodal en el cociente de isótopos estables en las plumas del vuelo sugiere que algunas aves podrían iniciar su muda inmediatamente después de su llegada al noroeste de México (llevando una base C3 desde sus sitios de reproducción), mientras que otras podrían retrasar la muda (produciendo plumas que sólo contienen el C4 de las plantas de Sinaloa). Entre 2010 y 2012, usamos geolocalizadores para probar si las diferencias en la sincronización y ruta de los movimientos migratorios de otoño están relacionadas con señales de presencia de isótopos estables en las plumas primarias. Analizamos isótopos estables de hidrógeno y carbono en P1 y P9 de 25 individuos que fueron marcados con geolocalizadores en dos años consecutivos. De estos, 60% cambiaron la dieta (C3 vs. C4) usada como base para el crecimiento de las P1 entre años. También observamos variación entre individuos en las rutas migratorias, tal que aves de la misma población reproductiva fueron bastante diferentes entre sí en el uso de las localidades de muda e invernada. Sin embargo, no encontramos una relación del contenido de isótopos con el momento ni con la ruta de la migración de otoño. Creemos que el contenido bimodal de isótopos observado representa un efecto remanente relacionado con los paisajes locales (pastizal o agrícola vs. matorral) usados durante la última etapa de la temporada reproductiva y la primera etapa del periodo de muda, y que tales efectos disminuyen a medida que la muda progresa. Si éste es el caso, existe el potencial para que la dieta de la temporada reproductiva afecte directamente la calidad del plumaje en esta ave migrante.

E-mail: andrea.contina@ou.edu

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The Painted Bunting (*Passerina ciris*) is a Neotropical migrant with two geographically disjunct breeding areas in North America that differ markedly in their patterns of migration and molt (Fig. 1; Thompson 1991a). Individuals that breed in the southern Midwest of the United States are molt migrants, which means that they have prolonged stopover periods in late summer associated with feather replacement at locations distant from breeding and wintering areas (Thompson 1991b). These birds depart the breeding grounds in late July and, presumably, move westward to molt in northern Mexico before continuing to winter locations in southern Mexico or northern Central America (Rohwer et al. 2009a, b). Individuals from the eastern population molt on the breeding grounds along the Atlantic coast of the United States (Fig. 1) before migrating to wintering areas in southern Florida and the Caribbean in late September or October (Sykes et al. 2007). The existence of two fundamentally different migration strategies within a single species provides an opportunity to understand the life-history implications of migration and prompted us to examine variation in molt and migration behavior using stable isotope ratios in primary feathers and light-level geolocation tags (hereafter “geologgers”).

One important element of migration and molt strategies is the ability of birds to track seasonal shifts in food availability (Stach et al. 2012; but for other taxa, also see Bischof et al. 2012, 2013). The Painted Bunting migration revealed by geologgers. Breeding and wintering grounds are highlighted in green and yellow, respectively. (A) Unfiltered data points for 12 birds are superimposed to show a clear westward movement and a shift in longitude (~10°) occurring at the end of July 2011. (B) Unfiltered data points for one bird migrating to the Yucatan peninsula in January 2012. (C) Migratory route for the same bird shown in panel B, but modeled for only the first part of the migration until August 2011. (D) Migratory route for one bird that migrated south, overwintered in Mexico, and returned to Oklahoma in spring 2012.
patterns in isotope values indeed arise from timing of molt, rather than period within the documented wintering range. If bimodal molt, followed by a southward movement and subsequent stationing throughout the year (Lowther et al. 1999), we predicted that C4 carbon signatures are common in early migrants that presumably, molt immediately after arrival on the molting grounds. In this case, we would predict that the carbon isotope signature of P1 would be associated with the date of arrival on the molting grounds.

An alternative explanation for the bimodal pattern is that, depending on the timing and duration of migration, stable carbon isotope ratios of P1 can reflect the diet on the breeding ground, which in Oklahoma is dominated by C3 plants, or the diet at the molting location, which is dominated by C4 plants. Although the mechanism behind this relationship could take several forms, the most parsimonious explanation would be that early migrants arrive at the stopover region early and spend a week or more equilibrating with the local trophic web prior to molt, whereas late migrants begin molt immediately after arriving in the molting area, such that they grow their first primary using reserves that reflect isotope ratios from the breeding grounds. In this case, we would predict that the carbon isotope signature of P1 would be associated with the date of arrival on the molting grounds.

Here, we provide the first quantitative descriptions of the migration biology of individual Painted Buntings using data from custom-designed, low-cost geologgers. This is the smallest North American migrant tracked to date and among the first molt-migrant species to be tracked using geologgers. In particular, we used light data collected from individual Painted Buntings to estimate (1) the date of initiation of migration, (2) the number of days between initiation of migration and stopover for molt, (3) the date of arrival at the molting grounds, and (4) routes of migratory movements. We used these data to test the prediction that C3 carbon signatures in P1 occur more often in late migrants that, presumably, molt immediately after arrival on the molting grounds and that C4 carbon signatures are common in early migrants that may remain on the molting grounds for several days or weeks prior to molt. In addition, we measured stable isotope ratios in these birds’ feathers in two consecutive years so that we could examine year-to-year consistency of isotope ratios in feathers, which is presumably related to consistency in diet and molting locations. On the basis of known distributions of Painted Buntings throughout the year (Lowther et al. 1999), we predicted that the migration route of birds from our study population in Oklahoma would involve a westward flight to northwestern Mexico to molt, followed by a southward movement and subsequent stationary period within the documented wintering range. If bimodal patterns in isotope values indeed arise from timing of molt, rather than differences in migratory pathways, we expected that all birds from our breeding population would follow similar migratory trajectories, but that the isotope ratios of the first primary would be related to the timing of migration.

**Methods**

**Field site and tagging effort.**—We studied a population of Painted Buntings that bred at the Wichita Mountains Wildlife Refuge (N34.4°–W98.4°, Oklahoma. This refuge is largely dominated by mixed prairie grasses (mostly C4 species) such as Little Bluestem (Schizachyrium scoparium), Indiangrass (Sorghastrum nutans), and Switchgrass (Panicum virgatum), but Painted Buntings are located primarily in patches of forest characterized by oaks (Quercus spp.) and Eastern Redcedar (Juniperus virginiana) that represent an important source of dietary C3 carbon (Eskew 1938, Martin et al. 1951, Askins 1993, Carter et al. 2008).

We deployed 120 geologgers on free-ranging male Painted Buntings during summer 2010 and 2011 (May–July) and released them shortly after capture. We used solar-powered geologgers of our own design that weighed 0.6 to 0.7 g (including harness material), representing ~4% of the body mass of this species. The geologgers measured light intensity every minute and recorded the average light measurement every 10 min on an arbitrary scale from zero to 127. We mounted the tags using leg-loop harnesses (Rappole and Tipton 1991) made of 0.7-mm elastic silicon beading thread (Stretch Magic brand; Pepperell Braiding, Pepperell, Massachusetts) that were presized (see Naef-Daenzer 2007) and assembled by heat welding (rather than tying) prior to deployment. We also tagged each bird with individually numbered federal bands and plastic color bands. Adult singing males were captured in 12-m mist nets close to nests or active territories using playback calls and decoy mounts.

To study the effect of migration timing on migration route, we deployed 80 additional geologgers (40 in 2010 and another 40 in 2011) on individuals captured in May of each year and assigned to a manipulative experiment, in which birds were held in captivity under different light regimes before being fitted and released with geologgers. This manipulative experiment was designed to accelerate or decelerate the annual cycle of the birds. Half of the birds belonged to an “early” group, which was released on 1 July of each year, and the other half belonged to a “late” group that was released on 1 August (for details, see supplemental material with the online version of this article). Recaptured birds with geologgers from year 1 were fitted with new geologgers in year 2 to enable us to document migratory behavior of single individuals in consecutive years. Thus, we deployed a total of 200 geologgers over the course of the study.

**Stable isotopes.**—The stable isotope signatures of primary feathers reflect nutrient deposition into keratin tissue at the beginning and end of the molting period. Thus, we measured stable isotope ratios in the innermost primary (P1, first to be molted) and the outermost primary (P9, last to be molted) collected from the right wing upon each recapture event in summer 2011 and 2012. We analyzed the 13C/12C ratio (δ13C) and deuterium/hydrogen ratio (δD) at the University of Oklahoma following the laboratory procedures detailed in previous publications (Kelly et al. 2009, Paritte and Kelly 2009, Bridge et al. 2011). We report our results in delta notation in relation to PeeDee Belemnite for δ13C and in relation to Standard Mean Ocean Water for δD (Craig 1957, 1961). We used generalized linear models to test for correlation between variables and, in particular, the association between δ13C signature in P1 molted in 2011 and three explanatory variables: (1) departure dates from the breeding ground in Oklahoma,
(2) arrival dates at the molting sites in northwestern Mexico, and
(3) duration of migration from Oklahoma to the molting grounds
in days. All statistical analyses were performed using XLSTAT. We
considered feathers with δ¹³C values greater than –18‰ to be
primarily C4 in origin, based on Bridge et al. (2011), and feathers
with δD values greater than –65‰ to be of potential Mexican origin,
based on our interpretation of δD distribution proposed by Hobson
et al. (2009).

Light-level geolocation.—To derive location estimates from raw
light-level data, we employed the threshold method implemented in
the R package GeoLight (R Development Core Team 2005, Lisovski
and Hahn 2012). For purposes of illustration, we also conducted an
exploratory analysis to estimate migratory routes using the curve-
fitting method implemented in the R package tripEstimation (Sum-

Detection of twilight events is a crucial step in defining the
correct location of birds with geologgers. With the threshold
method, we determined twilight events for the GeoLight package
using a threshold value of 6.5 on the scale of zero to 127, which
corresponded to a sun angle of –3 to –4.5 (depending on the
individual tag), based on an individual calibration period that cor-
responded to a minimum of 3 days after deployment while the
birds were still resighted on the breeding grounds. The first 24 h
after deployment were regarded as a behavioral equilibration
period, and we did not use these data for tracking or calibration.
To compute migration departure and arrival dates, we then plot-
ted the coordinates for each data point in ARCGIS and visualized
the longitudinal and latitudinal movements associated with each
day of the year. A consistent longitudinal movement (e.g., ~5
consecutive data points with longitudinal shift ≥5°W) was con-
cidered a real movement and not an artifact of shading. We did
not consider latitude when computing departure and arrival dates
because of the high degree of error in latitude estimates.

We implemented tripEstimation by following the general
steps described in Seavy et al. (2012). We began by truncating the
0–127 raw light measurements to measurements from 6 to 20 that
captured the twilight period. We discarded all twilight transi-
tions with substantial shading, and designated tag-specific cali-
bbrations using the same periods from the threshold method. We
constrained possible locations with a land mask, the known lo-
cations of release day, and the spatial boundaries beyond which
we considered locations unrealistic (values outside of latitude 0
to 40 or longitude –120 to –40). We disregarded latitudinal esti-
mates during the equinox period (15 days on either side of the true
equinoxes). For the light parameters, we used variance in light
data = 2.5, variance in light attenuation = 6, an Ekstrom range of
~20 to 40, and variance outside this range = 7. For our movement
model, we used a log normal distribution with a mean of 2.6 km
h⁻¹ and variance of 1.3. We set up the Markov-chain Monte Carlo
(MCMC) to start by drawing 10,000 samples for burn-in and tun-
ing of the proposal distribution. We then repeated an MCMC
from the end of the burn-in by drawing 10,000 samples and made
sure that the model parameters were tuned in for each tag and
that the MCMC had converged by assessing minimal migration
trajectory shift from the burn-in period and the additional run
of 10,000 simulations. Finally, we set up a new MCMC by draw-
ing 10,000 simulations and then generating a last draw of 10,000
samples to describe the posterior distribution. We used the mean
of the posterior distribution coordinates to plot our estimate of
the most likely routes for each individual. Results are presented
as means ± SD.

Results
In 2011, we retrieved 13 of the 100 geologger tags deployed in 2010,
but only 2 geologgers lasted to the following year of recapture
to provide usable data (see online supplemental material). Dur-
ing summer 2012 we retrieved 32 of 100 geologger tags deployed
in 2011. Of these, 24 provided usable data through fall migration
(e.g., November).

Overall, of the 26 tags with usable data, 23 came from free-
ranging birds tagged in 2011, 2 from birds used in our photoperiod
manipulations trial carried out in 2010, and 1 from our photope-
riod manipulations carried out in 2011. One individual was recap-
tured in consecutive years (2011, 2012).

Migratory geography.—Of the 25 birds with geologger data,
23 migrated from the breeding ground in southwestern Oklah-
oma toward the molting site in northwestern Mexico (Fig. 1A
and supplemental material). The “noisy” light measurements
yielded only approximate estimates of geographic position be-
cause, as is typical of current technology, poor weather conditions
(e.g., clouds), feather shading of the light sensor, and behavior all
influenced light levels recorded by the geologger. Nevertheless,
the overall westward migratory pattern from Oklahoma toward
Mexico was clear. There were two notable exceptions to this pat-
tern: one unmanipulated bird migrated east to northern Louisi-
ana (ID 251112862 in supplemental material, Fig. S1). His location
after that was unknown because the geologger failed in mid-
September. Another bird, a member of the “late” experimental
in 2010, migrated immediately south along the eastern edge
of the Gulf of Mexico and spent the winter at or near the Isth-
mus of Tehuantepec before returning to the breeding ground in
the spring along the same route (ID 222150719 in Fig. 1D). Some
individuals appeared to stop over in southwest Texas (about a
week, possibly along the Rio Grande) during their migration to the
Sinaloa–Sonora region (see supplemental material).

Most geologgers stopped collecting data while birds were still
in the Sinaloa–Sonora region. However, we recovered breeding-
season-to-breeding-season tracks from 2 individuals used in light
manipulation trials in 2010 and a track until late January from
1 wild bird tagged in 2011. As described above, 1 bird from the
“late” light manipulation treatment migrated immediately south
to wintering grounds in southern Mexico and then returned to
Oklahoma in spring (ID 222150719 in Fig. 1D). The other bird
from the “late” light manipulation treatment and the unmanipu-
lated bird both followed similar loop migration paths, beginning
with southwestward movement to northwest Mexico (Sonora
and Sinaloa regions) in late summer, followed by less rapid south-
ward movement in late fall, and an eastward shift in early winter
toward southeastern Mexico (ID 222150714 in supplemental material
and ID 222150755 in Fig. 1B, C, and supplemental material, re-
spectively). There were also variations in movements associated
with later stages of their migration. In particular, the bird from
the 2010 light manipulation group showed an eastward movement
to the Isthmus of Tehuantepec (or thereabouts) and a direct north-
ward spring migration in early May to return to the breeding site,
whereas the wild individual tagged in 2011 showed a longer eastward movement and wintered primarily in the Yucatan Peninsula.

_Migratory timing._—Painted Buntings departed the Oklahoma breeding site from mid-July through mid-August (Table S1). In 2010, one bird migrated to the molting site in 4 days (the other migrated eastward). In 2011, the duration of migration ranged from a few days to as long as 29 days and averaged 11.4 days (Tables S1 and S2).

_Migration timing and diet shifts._—The bimodal pattern in carbon isotope ratios in the first primary feathers previously observed by Bridge et al. (2011) was evident in samples from 2011 and 2012 (Figs. 2 and 3). We found no significant association between δ¹³C signatures in primary feathers (P1) molted in 2011 and timing of migration variables when considered together in a single model or independently. The full model describing variation in δ¹³C signature in P1 molted in 2011 as a function of departure dates from the breeding ground in Oklahoma, arrival dates at the molting sites in northwestern Mexico, and duration of this migration in days was not significant (R² = 0.14, ANOVA; F = 0.98, df = 3 and 17, P = 0.421). Similarly, δ¹³C values in P9 molted in 2011 had no relationship with departure dates from Oklahoma, arrival dates at the molting sites in Mexico, or duration of fall migration (R² = 0.16, ANOVA; F = 1.12, df = 3 and 19, P = 0.316). We also tested each of these relationships in a univariate regression, and none was significant (P = 0.714, n = 22). The results suggest that the bimodal pattern in carbon stable isotope in P1 is not associated with departure dates from the breeding ground or pace of migration. Interestingly, comparisons of feathers taken from the same bird in consecutive years revealed that 60% of the birds switched “pattern” between years as reflected in the carbon isotope ratios of P1. For P1s molted in 2010, 50% of the birds had a carbon signature compatible with a diet based on C3 carbon sources typical of mesic plants (i.e., < δ¹³C = –18‰), whereas only 15% of the birds had a carbon signature in P9 compatible with a diet based on primarily C3 food intake. The mean δ¹³C value for P1 molted in 2010 was –14.2 ± 2.9‰ (range: –22.1 to –10.5‰). However, our examination of δ¹³C signature in primary feathers molted in the same birds in 2011 showed that 80% of the birds had a carbon signature in P1 compatible with a diet based on C3 carbon sources, and only 5% had a carbon signature in P9 compatible with a diet based on C3 carbon sources. The mean δ¹³C value for P1 molted in 2011 was –19.1 ± 5.1‰ (range: –22.6 to –10.8‰), and that for P9 molted in 2011 was 13.9 ± 3.9‰ (range: –23.0 to –11.4‰).

The hydrogen ratio values of both P1 (mean = –67.1 ± 10.5‰, range: –84.8 to –52.4‰) and P9 (mean = –65.2 ± 11‰, range: –88.6 to –50.2‰) showed that whereas the wild individual tagged in 2011 showed a longer eastward movement and wintered primarily in the Yucatan Peninsula.

_Fig. 2._ Distribution of (A, B) carbon and (C, D) hydrogen stable isotope ratios in primary feathers for individual Painted Buntings sampled in two consecutive years at the breeding site in Oklahoma (Wichita Mountains Wildlife Refuge). Feathers collected in summer 2011 (white bars) were molted in 2010, and feathers collected in 2012 (black bars) were molted in 2011.

_Fig. 3._ Histograms indicating carbon stable isotope ratios in P1 associated with the length of migration and departure dates from the breeding ground of Painted Buntings at Wichita Mountains Wildlife Refuge in Oklahoma (OK). Black bars represent free-ranging birds, white bars represent carbon stable isotope ratios in P1, and green bar indicates east migration (Louisiana); the photoperiod-manipulation late group (migration onset delayed) is represented by red bars, and the photoperiod-manipulation early group and south migration (migration onset accelerated) are represented by blue bar. For a description of photoperiod manipulations (delayed and accelerated treatments), see details in the text.
to $-36.8\%$) grown by 60% of birds in 2010 generally indicated latitudes associated with the central United States (less than $-65\%$ δD; Bowen and Revenaugh 2003). However, δD values in primary feathers molted in the same birds during the following year all exceeded $-65\%$ δD (P1: mean = $-37.9 \pm 17.7\%$, range: $-64.14$ to $-5.9\%$; P9: mean = $-49.6 \pm 10.5\%$, range: $-62.26$ to $-28.5\%$).

Mean δ¹³C values of P1 and P9 did not differ between years (P1: $t = -0.7$, df = 19, $P = 0.43$; P9: $t = -0.4$, df = 19, $P = 0.68$). By contrast, mean δD in P1 and P9 differed significantly between years (P1: $t = -6.7$, df = 19, $P = 0.000002$; P9: $t = -4.8$, df = 19, $P = 0.00001$). High annual variation in hydrogen isotope ratios among years in the arid west has been demonstrated previously (Wunder 2005).
For example, the degree to which plumage quality is a useful proxy in selection of breeding partners may depend on the information it contains about the ability of individuals to garner resources on the breeding ground in the previous year rather than at stopover sites encountered during migration (Keyser and Hill 2000).

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