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Stable isotopes identify the natal origins of a generalist brood parasite, the brown-headed cowbird *Molothrus ater*

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Identifying the natal origins of brood parasites is a major challenge that usually requires labor-intensive searching for nests of host species. Stable isotope analysis of feathers and other body tissues of parasitic young could be a possible tool for determining natal origins if tissues reflect the isotopic composition of the diet fed to nestlings. We measured the carbon (^{13}C) and nitrogen (^{15}N) isotope compositions of feathers for two age-classes of brown-headed cowbirds *Molothrus ater* at the Konza Prairie Biological Station near Manhattan, Kansas: nestlings raised by five species of songbird hosts in two different habitats, and juveniles captured after independence. Isotope values from cowbird nestlings did not differ among host species and we were unable to assign juvenile cowbirds to their natal hosts. However, nestlings raised in grassland habitat had feathers that contained significantly higher δ^{13}C values and lower $\delta\ ^{15}$N values than nestlings raised in shrub habitats. In addition, independent juveniles had isotopic signatures that were similar to cowbird nestlings raised on shrub habitats. Although dickcissel *Spiza americana* comprised the majority of samples from shrub habitats, our conclusions reflect the natural pattern of parasitism at the site and should be representative of cowbirds raised at Konza. We conclude that stable isotope analysis of feathers is effective for determining the natal origins of parasitic young if isotope values from nestlings are isotopically distinct among habitats.

Determining the natal origin of brood parasites is a difficult task, especially for generalist species that parasitize many songbird hosts in a range of different habitats (Rothstein 1975, Friedmann et al. 1977). Documenting community-wide patterns of host use is challenging because nest searching is labor-intensive and some hosts within a community may have nests that are hard to locate. Nevertheless, assessing the origin of brood parasites is important because parasitic young often reduce host productivity (Kosciuch and Sandercock 2008) and impact the population viability of songbirds of conservation concern (Kus and Whitfield 2005). Radio-telemetry has provided some insights into the egg-laying behavior of parasitic cowbirds but our understanding of host selection remains incomplete. Tracking of female cowbirds is difficult because they move long distances, lay in the pre-dawn hours, and visit host nests only briefly for egg-laying (Rothstein et al. 1984, Sealy et al. 1995). Host-specific ectoparasites might be used to determine natal origins of cowbird young, although studies of chewing lice collected from cowbird juveniles have failed to provide adequate resolution for determination of host species (Hahn et al. 2000, Clayton and Johnson 2001).

Stable isotopes of body tissues have provided valuable insights into resource use by birds because the isotopic signature of the diet is conserved through trophic food webs and persist at different rates in body tissues (e.g., feathers, muscle; Hobson and Wassenaar 1999, Hobson 2005). In terrestrial ecosystems, carbon isotopes (^{13}C) can indicate the plant functional group (C_3 or C_4) that forms the base of the diet, whereas nitrogen isotopes (^{15}N) can be used to determine the trophic level of the prey consumed (McKechnie 2004). Carbon and nitrogen isotopic composition of animal tissues represent the integration of dietary resources over time and this can lead to distinct isotopic signatures relative to the habitat the individuals occupy (Marra et al. 1998, Evans Ogden et al. 2005). In brood parasites, the isotopic signature of the diet provided by the host parents should be contained in the feathers of both the host and parasitic young (Hobson and Clark 1992a, b, Duxbury et al. 2003).

In this project, we examined whether stable isotope signatures of feathers could be used to determine the natal origins of a generalist brood parasite raised by different songbird hosts in two different habitats in tallgrass prairie. First, we measured the variation in carbon and nitrogen isotope ratios of brown-headed cowbird *Molothrus ater* nestlings from hosts nesting in grassland and shrub habitats. Grassland habitats were burned annually and dominated by native warm season grasses (C_4 plants); whereas shrub habitats were burned at intervals $\geq$4 years and heavily encroached by woody shrubs and broad-leaf forbs (C_3 plants). Second, we used the reference isotope values from nestlings of known origin to determine the natal origin of cowbirds first captured as independent juveniles in our local population.

Methods

Research was conducted during the 2005–06 breeding seasons at the Konza Prairie Biological Station in northeastern Kansas (39° 05′N, 96° 35′W). Konza Prairie is subdivided into 60 experimental watersheds that contain different combinations of fire and grazing treatments, and is dominated by habitats consisting primarily of C_4 grasses; shrub-encroached habitats are less common (Hartnett et al. 1996). The avian community of Konza Prairie is comprised primarily of grassland and shrubland breeding species (Zimmerman 1993). The dickcissel *Spiza americana* is at least twice as abundant as other hosts such as eastern meadowlark *Sturnella magna* and is parasitized more frequently than other hosts on the landscape (Powell 2006, J.W. Rivers unpubl. data). Host nests containing cowbirds in grassland and shrub habitats on Konza Prairie were located by using behavioral cues of attending parents and by systematic searching. Thus, our sample of nestlings reflects the host community and does not over represent uncommon hosts. When nestlings were at least 5 days old we collected approximately 5 mm of secondary flight feathers. We also sampled feathers from independent juvenile cowbirds of unknown origin captured in four walk-in cowbird traps placed on the boundary between an annual burned watersheds and watersheds burned at ≥ 4 years (Kosciuch and Sandercock 2008).

Feather samples were cleaned by rinsing with a 2:1 chloroform:methanol solution and allowed to air dry for 48 h. Samples were then analyzed with a continuous-flow Thermo Finnigan Delta Plus Mass Spectrometer coupled to a Carlo Erba elemental analyzer (EA 1110). Isotopic composition (R) is expressed as a ratio of the heavy isotope divided by the light isotope (e.g., $R = {}^{13}C/{}^{12}C$). The international standard for carbon is the Pee Dee Belemnite (PDB) limestone formation whereas the standard for nitrogen is atmospheric nitrogen. Internal standards were analyzed after every nine unknown samples to monitor drift in isotopic measurements. While isotopic and elemental drift are not unusual through the course of a mass spectrometer run (8+ hours), the instrument used for this analysis experienced little drift. An oak leaves standard ($n = 17$) had a mean $\delta^{15}N$ value of $-2.8‰$ (SD $= 0.03‰$), and a mean $\delta^{13}C$ value of $-28.6‰$ (SD $= 0.00‰$) during runs of our samples.

We calculated R compared to standards in parts per thousand by:

$$\delta = [(R_{sample}/R_{standard} - 1] \times 1000$$

We did not measure isotope values for plants at our study site, but instead used published reference values for C_3 and C_4 plants (Farquhar et al. 1989).

All statistical analyses were conducted with SAS version 9.0 (SAS). We used a Kruskal-Wallis test to determine if isotope values differed among species or habitats, the k-nearest neighbor randomization test to assess differences between juveniles and nestlings from grassland and shrub habitats (Rosing et al. 1998), and discriminant function analysis (DFA) to determine the proportion of juveniles classified with nestlings from each habitat. For the DFA we derived the function with data from nestlings and then used cross-validation to classify juveniles.

Results

We sampled feathers from cowbirds raised by four hosts ($n = 21$ nests) in grassland habitats and by five hosts ($n = 16$ nests) in shrub habitats (Fig. 1). Nestling isotope values did not form distinct species clusters, and there was no difference in $\delta^{13}C$ or $\delta^{15}N$ values from different hosts within either grassland (Kruskal-Wallis test, $\delta^{13}C$: $\chi^2 = 0.17$, df $= 3$, $P = 0.98$; $\delta^{15}N$: $\chi^2 = 5.3$, df $= 3$, $P = 0.15$) or shrub habitats ($\delta^{13}C$: $\chi^2 = 7.2$, df $= 4$, $P = 0.13$; $\delta^{15}N$: $\chi^2 = 2.3$, df $= 4$, $P = 0.69$; Fig. 1). Feathers from cowbird nestlings raised in dickcissel nests differed between the two habitats, with grassland-raised nestlings possessing feathers that contained significantly more ^{13}C (grassland median $= -13.9‰$, shrub median $= -21.4‰$, $U = 24.0$, $n = 20$, $P = 0.012$), and less ^{15}N (grassland median $= 3.7‰$, shrub median $= 4.7‰$, $U = 70$, $n = 20$, $P = 0.04$) than those from shrub habitats. Although our modest sample from the eastern meadowlark did not permit statistical analysis, the differences in $\delta^{13}C$ between habitats were not as pronounced as dickcissel (grassland median $= -13.6‰$, $n = 4$, shrub median $= -15.9‰$, $n = 2$). Feathers from host nestlings did not differ from cowbird nestlings within a nest for either $\delta\ ^{13}C$ (Wilcoxon signed rank test: $Z = -1.5$, $P = 0.14$, $n = 6$), or $\delta^{15}N$ ($Z = -5.2$, $P = 0.58$).

Feathers from cowbird nestlings raised in grassland habitats were significantly less depleted in ^{13}C (median $= -14.3\ ‰$, $n = 21$, $U = 400.0$, $P < 0.001$) compared to values for feathers from nestlings reared in shrub habitats

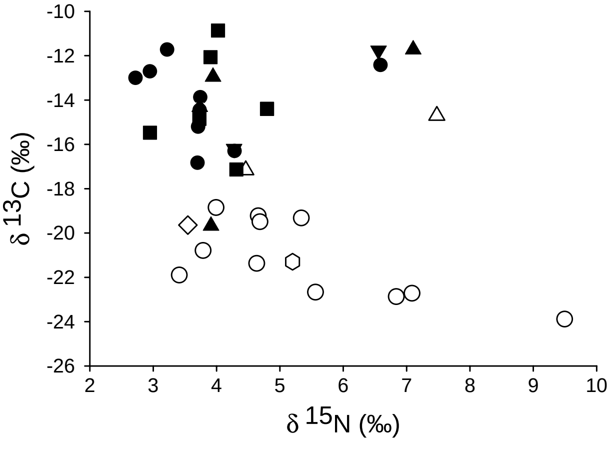

Figure 1. Scatter plot of $\delta^{13}C$ values, and $\delta^{15}N$ values obtained from nestling brown-headed cowbird feathers raised in host nests. Nests were located on shrub (open symbols) or grassland (closed symbols) habitats at Konza Prairie Biological Station, Kansas. Symbols for species are as follows: circle (○,●) dickcissel, triangle (△,▲) eastern meadowlark, upside down triangle (▼), grasshopper sparrow *Ammodramus savannarum*, square (■) red-winged blackbird *Agelaius phoeniceus*, diamond (◇) blue grosbeak *Guiraca caerulea*, and hexagon (⬡) eastern towhee *Pipilo erythrophthalmus*.

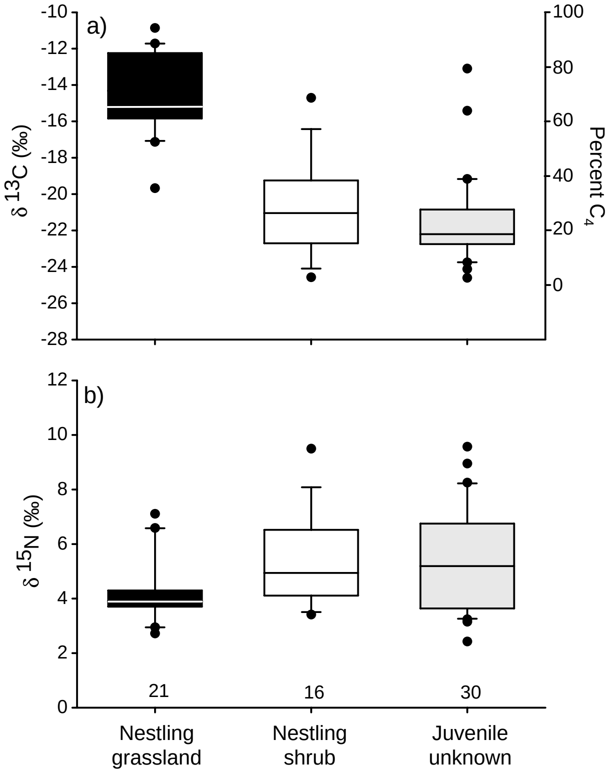

Figure 2. Box and whisker plots (25th and 75th percentiles) for (a) δ^{13}C values, and (b) δ^{15}N values obtained from feather samples from nestling and juvenile brown-headed cowbirds. Nestling cowbirds were raised in host nests in grassland habitats and shrub habitats. Juveniles were trapped at locations surrounded by similar proportions of grassland and shrub-encroached habitats. Sample sizes for each category are listed below the habitats.

(median = −21.0‰, n = 16; Fig. 2a). Our carbon isotope values from grassland and shrub habitats were close to expected reference values for C_4 plants (mean δ^{13}C value = −12‰) and C_3 plants (mean δ^{13}C value = −27‰; Farquhar et al. 1989). We found a median difference of 6.7‰ for δ^{13}C values for feathers between nestlings from the two habitats, but only a difference of 0.9‰ between nestlings from shrub habitats and juveniles of unknown source. Feathers from nestlings reared in grassland habitats contained significantly less ^{15}N (median = 3.9‰, n = 11) than feathers from nestlings raised in shrub habitats (median = 4.9‰, n = 9; U = 310.5, $P = 0.002$; Fig. 2b).

Using k-nearest neighbor analysis, we found that nestlings reared on shrub habitats differed from nestlings reared on grassland habitats ($P < 0.001$). The isotopic signature of cowbirds first captured as independent juveniles (median = −22.2‰, n = 30) differed from nestlings reared in grassland habitats ($P < 0.001$), but was not different from nestlings reared in shrub habitats ($P = 0.49$). The discriminant function analysis provided sufficient resolution to separate nestlings into grassland and shrub habitat based on δ^{13}C and δ^{15}N values ($\chi^2 = 26.0$, df = 2, $P < 0.001$). The vast majority (93%) of independent juveniles were classified with nestlings from shrub habitats, but the two individuals were outliers with δ^{13}C values nearly identical to nestlings from grassland habitats (Fig. 2a).

Discussion

We found that the carbon isotopic composition of feathers from cowbird nestlings covaried with habitat but not host species and that juvenile cowbirds did not differ isotopically from nestlings raised on shrub habitats. Our sample size was modest for some hosts, which gives us low statistical power to detect differences between habitats within a host. However, our conclusion that juveniles cannot be assigned to hosts should be robust because isotopic values of cowbird young overlapped extensively among hosts. The δ^{13}C values for some species such as eastern meadowlark may not align with habitat as cleanly as other species such as dickcissel. Nevertheless, our conclusion that most juvenile cowbirds originate from shrub habitats, despite the majority of samples being from dickcissel, is valid because our sample reflects the host use patterns of cowbirds on our study site. Nitrogen isotope values provided little additional resolution, presumably because all the songbird hosts that we examined feed at a similar trophic level, and rely on arthropods to provision host and parasitic young. The use of nitrogen values to identify habitats may be limited by landscape features that are not unique to grassland or shrub habitats, including moisture stress and soil type.

Previous work has shown that host parents use similar rules to provision cowbirds and host nestlings (Lichtenstein and Sealy 1998). In this study, we found that cowbird nestlings were isotopically similar to host nest mates, yet cowbirds raised by the same species had different isotopic signatures when raised in different habitats. Taken together, our results suggest that host parents do not discriminate among host and parasitic young during provisioning, but feed the same types of food items to both cowbirds and their own young. Monitoring of nests with video cameras has confirmed that cowbird and host nestlings are offered the similar food items by host parents during feeding bouts (J.W. Rivers unpubl. data).

Shrub expansion in mesic grasslands of North America creates habitats that are favorable for cowbirds. Broad scale fire suppression has facilitated the establishment of woody plants, and mechanical removal may be necessary to restore grasslands to historic plant communities (Briggs et al. 2005). Shrub-encroached grasslands, such as those present on Konza Prairie, are beneficial for cowbirds because breeding and foraging habitat are in close proximity and daily commuting distances are reduced (Rothstein et al. 1984, Goguen and Mathews 2001). While the benefits of shrub habitats to adult cowbirds have been identified, it is unclear why most juvenile cowbirds captured in this study had isotopic values consistent with rearing in a shrub habitat. Habitat-specific variation in host abundance, host susceptibility to parasitism or nest survival rates are possible explanations for this pattern, and a useful avenue of future research would be to examine the proximate causes of habitat-specific variation in cowbird productivity.

Due to the limitations of large-scale nest monitoring projects to estimate habitat- and host-specific patterns of parasitism, indirect methods that utilize natural biological markers are of great value. Although we were unable to assign juvenile cowbirds to specific hosts at our study site, stable isotopes may be more effective in identifying host species if generalist parasites prefer hosts that are habitat specialists (e.g., Kirtland's warbler *Dendroica kirtlandii*), or if specialist parasites use a limited number of hosts. In the future, combining stable isotopes with molecular markers, ectoparasites, or other techniques may lead to better resolution of the natal origins of generalist brood parasites.

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References

Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J. L., Hoch, G. A., Lett, M. S. and McCarron, J. K. 2005. An ecosystem in transition. Causes and consequences of the conversion of mesic grassland to shrubland. – Bioscience 55: 243–254.

Clayton, D. H. and Johnson, K. P. 2001. Lice as probes-Response. – Trends Ecol. Evol. 16: 433–433.

Duxbury, J. M., Holroyd, G. L. and Muehlenbachs, K. 2003. Changes in hydrogen isotope ratios in sequential plumage stages: an implication for the creation of isotope-base maps for tracking migratory birds. – Isot. Environ. Healt. Sci. 39: 179–189.

Evans Ogden, L. J., Hobson, K. A., Lank, D. B. and Bittman, S. 2005. Stable isotope analysis reveals that agricultural habitat provides an important dietary component for nonbreeding dunlin. – Avian Conserv. and Ecol. – Écologie et conservation des oiseaux 1: http://www.ace-eco.org/vol1/iss1/art3/.

Farquhar, G. D., Ehleringer, J. R. and Hubick, K. T. 1989. Carbon isotope discrimination and photosynthesis. – Annu. Rev. Plant. Physiol. Plant Mol. Biol. 40: 503–537.

Friedmann, H., Kiff, L. F. and Rothstein, S. I. 1977. A further contribution to knowledge of the host relations of the parasitic cowbirds. – Smithson. Contrib. Zool. 235: 1–75.

Goguen, C. B. and Mathews, N. E. 2001. Brown-headed cowbird behavior and movements in relation to livestock grazing. – Ecol. Appl. 11: 1533–1544.

Hahn, D. C., Price, R. D. and Osenton, P. C. 2000. Use of lice to identify cowbird hosts. – Auk 117: 943–951.

Hartnett, D. C., Hickman, K. R., and Fischer Walter, L. E. 1996. Effects of bison grazing, fire, and topography on floristic diversity in tallgrass prairie. – J. Range Manage. 49: 413–420.

Hobson, K. A. 2005. Using stable isotopes to trace long-distance dispersal in birds and other taxa. – Divers. Distrib. 11: 157–164.

Hobson, K. A. and Clark, R. G. 1992a. Assessing avian diets using stable isotopes. 1. Turnover of C-13 in tissues. – Condor 94: 181–188.

Hobson, K. A. and Clark, R. G. 1992b. Assessing avian diets using stable isotopes. 2. Factors influencing diet-tissue fractionation. – Condor 94: 189–197.

Hobson, K. A. and Wassenaar, L. I. 1999. Stable isotope ecology: an introduction. – Oecologia 120: 312–313.

Kosciuch, K. L. and Sandercock B. K. 2008. Experimental removal of brown-headed cowbirds increases productivity of the parasite and their songbird host. – Ecol. Appl. 18: 537–548.

Kus, B. E. and Whitfield, M. J. 2005. Parasitism, productivity, and population growth: response of least Bell's vireo (*Vireo bellii pusillus*) and southwestern willow flycatcher (*Empidonax traillii extimus*) to cowbird (*Molothrus* spp.) control. – Ornithol. Monogr. 57: 16–27.

Lichtenstein, G. and Sealy, S. G. 1998. Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. – Proc. R. Soc. B 265: 249–254.

Marra, P. P., Hobson, K. A. and Holmes, R. T. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. – Science 282: 1884–1886.

McKechnie, A. E. 2004. Stable isotopes: powerful new tools for animal ecologists. – S. Afr. J. Sci. 100: 131–134.

Powell, A. F. L. A. 2006. Effects of prescribed burns and bison (*Bos bison*) grazing on breeding bird abundances in tallgrass prairie. – Auk 123: 183–197.

Rosing, M. N., Ben-David, M. and Barry, R. P. 1998. Analysis of stable isotope data: a K nearest-neighbors randomization test. – J. Wildl. Mgmt. 62: 380–388.

Rothstein, S. I. 1975. An experimental and teleonomic investigation of avian brood parasitism. – Condor 77: 250–271.

Rothstein, S. I., Verner, J. and Stevens, E. 1984. Radio-tracking confirms a unique diurnal pattern of spatial occurrence in the parasitic brown-headed cowbird. – Ecology 65: 77–88.

Sealy, S. G., Neudorf, D. L. and Hill, D. P. 1995. Rapid laying by brown-headed cowbirds *Molothrus ater* and other parasitic birds. – Ibis. 137: 76–84.

Zimmerman, J. L. 1993. The birds of Konza: the avian ecology of the tallgrass prairie. – University Press of Kansas, Lawrence.