

## Tracking large carnivore dispersal using isotopic clues in claws: an application to cougars across the Great Plains

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### Summary

1. Cougar (*Puma concolor*) populations, like other large carnivores, have increased during recent decades and may be recolonizing their former ranges in Midwestern North America. The dispersal routes taken by these animals from established populations are unknown and insight into these movements would facilitate their conservation and management.

2. We inferred the origin and migration route of four dispersing cougars using stable hydrogen ( $\delta\text{D}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope values along one of their claws. We compared isotopic variations within claws to regional and large-scale isoscapes of  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values in prey species. Using a likelihood-based assignment approach, we predicted the most likely dispersal route of each cougar (among several least-cost dispersal paths to potential source populations) in a chronological sequence dating back from its final location.

3. Our model predicted the origin of a radio-collared short-distance disperser and inferences about the most likely dispersal corridors for two long-distance dispersers matched reported information from re-sighting events and genetic investigations.

4. Insights about the most likely migration corridors may help identify critical areas and guide future conservation efforts of cougars and other large carnivores. We encourage managers to extend regional isoscapes based on sedentary prey species as they prove to be valuable tools in isotopic tracking of long-distance migration.

5. Our isotopic approach may be extended to other metabolically inert tissues that grow continuously, to investigate dispersal paths of species of interest, providing that individuals disperse across known isotopically structured landscapes.

**Key-words:** carbon-13, cougar, deuterium, dispersal, least-cost path, isoscapes, migration route, stable isotope

### Introduction

Large carnivore populations have increased in several parts of North America during recent decades following increases in their prey populations and reduced persecution. As a result, carnivores have recolonized several of their former geographical ranges (Gehring & Potter 2005; Hoffman & Genoways 2005). Cougars (*Puma concolor*), once considered rare or extirpated in many Midwestern states in the United States, are now observed with increasing regularity (Tischendorf & Henderson

1995). Cougar populations, following extermination efforts, were restricted to wilderness areas in the Rocky Mountains. Those populations have increased and new breeding populations have been documented in the Black Hills of South Dakota since the 1970s and southwest North Dakota since the 1990s (Cougar Network 2010). Recent evidence suggests that cougars are now breeding in northwest Nebraska (Wilson, Hoffman, & Genoways 2010), and 8% of the Midwest region is comprised of highly suitable habitat for cougars (Larue 2007).

Wildlife managers are increasingly aware of the public's interest in the long-distance dispersal of cougars into the Midwest (Davenport, Nielsen, & Mangun 2010). Indeed,

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confirmed sightings (photographs, roadkills, or captures) since the early 1900s of individuals extirpated from many states create a frustrating management dilemma, as biologists are uncertain of population numbers and the dispersal corridors used by individuals (Sweaner, Logan, & Hornocker 2000). Most confirmations of cougars in the Midwest have been juvenile males, suggesting that their presence likely results from the dispersal of juveniles from established populations (Nielsen *et al.* 2006; Cougar Network 2010). Dispersing cougars leave the natal area permanently to avoid competition for resources and inbreeding (Logan & Sweaner 2001). Subadult dispersal has recently been enhanced by the strong increase in size of the Black Hills and Rocky Mountain populations (Logan & Sweaner 2001; Thompson & Jenks 2005), and dispersal corridors between these populations and cougar confirmations exist throughout the Midwest (LaRue & Nielsen 2008).

Wildlife biologists lack empirical knowledge of paths of travel, which are needed to develop effective cougar conservation strategies. In the past decade, naturally occurring stable isotopes have proved to be useful to study long-distance wildlife migration and determine the previous origin of wild animals (Hobson & Wassenaar 2008). Naturally occurring stable isotopes provide time-integrated information that can be linked directly to geographical regions (Rubenstein & Hobson 2004). Food web isotopic signatures are reflected in the tissues of individuals, and such signatures can vary spatially based on a variety of biogeochemical processes (Hobson 1999). Isotopic measurements of feathers have provided a powerful approach in avian forensics, especially to determine moult or natal location (Hobson 1999; Hobson *et al.* 2004), document migratory connectivity at continental or regional scales (Norris *et al.* 2007) and study long-distance dispersal (Hobson 2005). Other keratinous tissues that grow continuously such as avian claws (Mazerolle & Hobson 2005; Fraser *et al.* 2008), baleen plates (Schell, Saupe, & Haubenstock 1989; Hobson & Schell 1998), whiskers (Hall-Aspland, Rogers, & Canfield 2005) and hair (Cerling *et al.* 2006) have been used to reconstruct past diets and positions of animals. Isotopic analysis of chitin has also been used to track origins of migratory insects such as monarch butterflies (*Danaus plexippus*; Hobson, Wassenaar, & Taylor 1999; Miller *et al.* 2010). Although biologists have used stable isotopes to reconstruct timing of movements of individuals across isotopically distinct regions (Cerling *et al.* 2006; Hobson, Barnett-Johnson, & Cerling 2010), none have previously used stable isotopes to reconstruct a distinct, spatially explicit migratory or dispersal pathway.

The claws of carnivores have potential as a tissue to store isotope information. The tissue consists primarily of keratin which is metabolically inert after synthesis. Cougar claws grow continuously and thus record a temporal dietary time series. The distal region of the claw is the oldest record of diet from previous habitats, while the proximal region reflects the most recent diet and habitat. We predicted that the claws of dispersing cougars could integrate dietary and habitat information during a suitable temporal scale to allow the cougars' migratory path to be spatially assessed. We obtained claw material

from two individuals road-killed in Nebraska, and two individuals shot in Chicago, Illinois, and Saskatoon, Saskatchewan, Canada.

Isoscapes (i.e., spatial patterns of stable isotopes in food webs or inorganic matrices) are largely continental or regional. Small-scale variation in stable isotope patterns has been more difficult to assess (Powell & Hobson 2006). Recent cougar dispersal in the Midwest USA has occurred over large distances, relative to regional variation in stable carbon and hydrogen isoscapes. Stable hydrogen isotope values ( $\delta\text{D}$ ) in animal tissues vary according to growing-season precipitation patterns ( $\delta\text{D}_p$ ), which have proven especially useful in many studies of animal movement (Hobson 2005). Large-scale isotopic information can be inferred from the  $\delta\text{D}_p$  basemap of Bowen, Wassenaar, & Hobson (2005). Stable carbon isotope values ( $\delta^{13}\text{C}$ ) in animal tissues may be affected by altitude, latitude, climate, land-use practices, and by photosynthetic pathways and water-use efficiencies of predominant plants (Rundel, Ehleringer, & Nagy 1989; Marshall, Brooks, & Lajtha 2007). We also predicted that cougar prey would show regional variation in  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values that could be used to trace movements of cougars across the landscape over short distances.

Our goal was to determine whether stable isotopes could inform our understanding of cougar dispersal. Our objectives were (i) to assess local variation in  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values in deer tissues to determine whether predictable variation exists in prey species across the landscape through which cougars disperse, (ii) to assess variation within cougar claws to determine whether changes in the isotopic signature of local diets are detectable in claw tissue, and (iii) to predict spatially explicit cougar dispersal paths by comparing isotopic variations within dispersing cougars' claws to regional and large-scale isoscapes of  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values in prey species.

## Methods

### TISSUE COLLECTION

#### Deer samples

As part of a state-wide chronic wasting disease surveillance programme, lymph nodes from white-tailed deer (*Odocoileus virginianus*) and mule deer (*O. hemionus*) were collected in 2005 from across Nebraska, and from the Black Hills of South Dakota. This provided an opportunity to describe isoscapes for our region of interest. We reasoned that although patterns of isotopic discrimination in general are not well known for individual tissues, all things being equal, these samples would act as a useful proxy to assess spatial patterns in  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values of cougar prey and ultimately isotopic origins predicted by cougar claws. We used deer tissue samples from 21 harvest check stations in Nebraska and four check stations in South Dakota. Locations were chosen from a list of potential check stations to provide spatial representation of potential dispersal paths across these states. All samples were frozen after collection. We thawed the samples and randomly selected 2–3 individuals from each check station. Lymph nodes for each check station were cut into ca. 1-cm<sup>3</sup> pieces, dried for >24 h at 65°C and ground to a fine powder in a mortar/pestle.

Ackerman, Lindzey, & Hemker (1984) reported that mule deer were 81% of the diet of cougars in Utah, deer are very abundant in Nebraska and South Dakota and we have no reason to expect differences in cougar diet in our study area. We assumed that deer sampled at each check station were representative of the area immediately surrounding the check station. Given that deer home ranges usually cover less than 730 ha (Root, Fritzell, & Giessman 1988), we are confident that deer isotopic variation reflected variation in isotopic values of local diets.

### Cougar samples

We obtained claw material from one- to two-year-old male cougars found near Valentine, Nebraska (42°8'N, 100°5'W), Gretna, Nebraska (41°1'N, 96°1'W), Chicago, Illinois (41°9'N, 87°7'W), and Saskatoon, Saskatchewan, Canada (52°1'N, 106°7'W). The Gretna cougar was found as a roadkill on 6 November 2005, as was the Valentine cougar on 27 June 2006. The Chicago and Saskatoon cougars were shot by law enforcement officials in Chicago, IL, on 15 April 2008, and Saskatoon, SK, on 7 October 2008, respectively. The Valentine and Saskatoon cougars had been previously captured, radio-marked (VHF transmitters) and released in the Black Hills, SD, allowing biologists to follow their movements in their natal area. The Valentine cougar's last observation alive was in Custer State Park, SD, on 13 April 2006, and the Saskatoon cougar was last recorded in Black Hills, across the Wyoming border, in April 2008. Genetic investigations determined that the Chicago cougar was the same animal as observed near Milton, Wisconsin, three months before death (Wisconsin Department of Natural Resources 2008); this animal's location of origin was confirmed to be the Black Hills, SD (Cougar Network 2010). The Gretna cougar had unknown origin.

We removed 1–2 claws from each cougar's distal phalanx bone and cleaned with distilled water. Claw length ranged from 35 to 49 mm. We used a rotating, hand-held grinder to produce successive samples of about 1 mm width along the claw from tip to root. We obtained samples from one claw for each cougar, but we added samples from a second claw for the Saskatoon cougar to assess between-claw variation in isotopic values. Our sample of cougars allowed us to assess  $\delta\text{D}$  and  $\delta^{13}\text{C}$  isotopic variation within claws ( $\delta\text{D}_{\text{claw}}$  and  $\delta^{13}\text{C}_{\text{claw}}$ , respectively) of short- (Valentine), medium- (Gretna) and long-distance (Chicago, Saskatoon) dispersers. We were also able to use the known origins for two cougars (Valentine, Saskatoon) and genetically confirmed observation for the Chicago cougar to check predictions of our proposed dispersal paths.

### Isotopic analyses

We measured  $\delta\text{D}$  in claw and deer lymph node tissues. Stable hydrogen isotope analyses were performed at the National Water Research Institute in Saskatoon, SK. Measurements were performed on  $\text{H}_2$  derived from high-temperature flash pyrolysis of samples and continuous-flow isotope ratio mass spectrometry (CF-IRMS). Pure  $\text{H}_2$  was used as the sample analysis gas and the isotopic reference gas. A Eurovector 3000TM (Milan, Italy) high-temperature elemental analyzer (EA) with autosampler was used to automatically pyrolyse claw samples to a single pulse of  $\text{H}_2$  gas (and  $\text{N}_2$  and  $\text{CO}$  gas). The resolved  $\text{H}_2$  sample pulse was then introduced to the isotope ratio mass spectrometer [Micromass IsoprimeTM (Manchester, England) with electrostatic analyzer] via an open split capillary. Within each analytical run, we also measured replicates of three keratin standards (CFS, BWB-II and CHS) that we used to calibrate our unknown keratins to provide an estimate of  $\delta\text{D}$  of nonexchangeable H (Wassenaar & Hob-

son 2003, 2006). As no comparable calibration standards are available for lymph node material, that tissue was still run in the same manner. Repeated analysis of hydrogen isotope intercomparison material IAEA-CH-7, routinely included as a check, yielded an external repeatability of better than  $\pm 1.5\text{‰}$ .

For  $\delta^{13}\text{C}$  measurements of cougar and deer samples, approximately 1 mg of each powdered sample was loaded into tin cups. Isotopic measurements were made on a Europa Robo Prep combustion system interfaced with a Europa TracerMat continuous-flow isotope ratio mass spectrometer (Europa, Crewe, England). Samples were analysed as five unknowns separated by two albumen standards. Based on within-run replicates of standards, the analytical precision was estimated to be  $\pm 0.1\text{‰}$ .

All stable isotopes values are expressed in the  $\delta$  notation (in units of per mil, ‰):  $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where X denotes  $^{13}\text{C}$  or D, and R the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or D/H. The standards are Vienna Pee Dee Belemnite (VPDB) standard for carbon and Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW–SLAP) for hydrogen. When two–three successive claw samples were combined to obtain the mass needed to make one measurement, the isotopic value was associated with the mean distance of these samples from the claw tip. We were not able to obtain  $\delta^{13}\text{C}$  values for the Chicago cougar and for deer tissue from South Dakota because of small tissue sizes provided from these collections.

## ISOSCAPES

### Local deer isoscapes

We used  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values from deer lymph nodes ( $\delta\text{D}_{\text{node}}$  and  $\delta^{13}\text{C}_{\text{node}}$ , respectively) to construct local, fine-scale isoscapes for Nebraska and the Black Hills, SD. The locations of the 25 deer sampling stations were geo-referenced. The spatial trend of  $\delta\text{D}$  and  $\delta^{13}\text{C}$  in deer lymph nodes was modelled in ArcGIS (ESRI, Redlands, CA) using ordinary kriging (spherical semivariance model), which interpolates isotope values in unsampled areas from data associated with the deer stations. We used these regional isoscapes (as described below) to predict the dispersal paths of the Valentine and Gretna cougars.

### Large-scale $\delta\text{D}$ isoscape in deer tissues

Our local, fine-scale  $\delta\text{D}$  and  $\delta^{13}\text{C}$  isoscapes, created from deer lymph nodes, did not extend to cover the dispersal paths of the Chicago and Saskatoon cougars because we lacked deer samples from across the range of interest. Instead, we used the growing-season precipitation  $\delta\text{D}_p$  basemap of Bowen, Wassenaar, & Hobson (2005) to infer a predicted, large-scale isoscape of  $\delta\text{D}$  in deer tissues. However, we needed a means of associating predicted mean growing-season average precipitation  $\delta\text{D}_p$  values expected at a given location with the corresponding deer isotopic value that would be expected there. Fortunately, Cormie, Schwarcz, & Gray (1994) derived a relationship between  $\delta\text{D}_p$  and deer bone collagen  $\delta\text{D}_{\text{collagen}}$ , as  $\delta\text{D}_{\text{collagen}} = 4 + 1.02 \times \delta\text{D}_p$ . We used this relationship and the precipitation isoscape of Bowen, Wassenaar, & Hobson (2005) to create a  $\delta\text{D}_{\text{collagen}}$  isoscape for North America. This large-scale isoscape is of lower resolution than our local isoscape, because of the greater distance (typically > 300 km) among the long-term precipitation sampling stations forming the basis of the  $\delta\text{D}_p$  data used by Bowen, Wassenaar, & Hobson (2005). We were unable to produce an equivalent  $\delta^{13}\text{C}$  isoscape across the larger region because to our knowledge no large-scale

sampling of animal tissues has been conducted for this isotope. By anchoring claw tissues to expected prey  $\delta D_{\text{collagen}}$  at known sites (e.g., mortality locations), we were able to avoid the need to know claw-diet isotopic discrimination factors *a priori* (see below).

## PREDICTION OF PATHS

### Claw growth rate

Claw tissue grows continuously and therefore records a temporal dietary isotopic record. However, claws wear at the tip, and the oldest dietary information is continuously removed from the tip. We determined the growth rate of claw tissue (i.e. the time integrated per 1 mm of claw) through a combined assessment of our claw isotopic values and the available telemetry records from the radio-marked Valentine cougar. We had the most information regarding this cougar's prior locations, and we could be certain the entire short-distance dispersal was recorded on its claw. We hypothesized that a dispersal event would be indicated by a shift from equilibrium  $\delta D_{\text{claw}}$  and  $\delta^{13}\text{C}_{\text{claw}}$  values at the former location (i.e. representing integrated isotopic conditions at a natal region). To interpret distance reflected in changes in  $\delta D_{\text{claw}}$  and  $\delta^{13}\text{C}_{\text{claw}}$  values between adjacent 1-mm samples of the Valentine cougar, we calculated the straight-line distance between its dispersal initiation and eventual death. We related that dispersal distance to the length of the claw representing claw growth during the dispersal, and by dividing the dispersal distance by the length of claw growth during dispersal, we obtained an approximate, straight-line dispersal distance represented by each 1 mm of claw sample of the Valentine cougar. Because the claws of mid- and long-distance dispersers do not provide information about their natal place, the mean dispersal speed of these individuals could not be directly estimated. Cougars may travel at speeds ranging between 1–8 km day<sup>-1</sup> (Maehr 1997; Thompson & Jenks 2005; Stoner *et al.* 2008), with highest mean daily distances during May–November (Stoner *et al.* 2008). We considered two scenarios of mean straight-line distance travelled per day: 3 km day<sup>-1</sup> and 5 km day<sup>-1</sup> and we took into account the season integrated in the claw to select the best scenario for each cougar.

### Potential origins and least-cost dispersal paths

Origins of Gretna and Chicago cougars were unknown. We used available information to develop hypothetical, potential sources for our dispersing cougars. Each potential source was located on the eastern border of the established range for cougar in North America and has been identified as a breeding population (LaRue & Nielsen 2008): western North Dakota (ND), Black Hills of South Dakota (SD), southeast Wyoming (WY), central Colorado (CO), the panhandle of Oklahoma (OK) and west Texas (TX). For the Saskatoon cougar, we considered two other potential sources, in western Montana (MT) and on the west of the mortality location at the border between British Columbia and Alberta (AB), to test the extent in which our model discriminate the known origin (SD) and most likely dispersal path of this cougar among several plausible distinct routes. We identified the centroid of each source patch to use as the hypothetical initiation point of dispersal. Two-km-wide dispersal corridors were backward modelled from the mortality locations to each potential source, using a habitat suitability model and least-cost path (LCP) analysis (LaRue & Nielsen 2008). Because the habitat suitability model has not been developed for Canada, we created straight paths from each potential source to the mortality location of the Saskatoon cougar. In addition, taking advantage of the confirmed re-sighting of the Chicago cougar

in Wisconsin, we considered an additional route of dispersal going straight north-west through Wisconsin (WI) that will allow us to check model predictions. We identified each dispersal corridor  $\Omega_r$ , with regard to the associated route from each source ( $r$ : ND, SD, WY, CO, OK, TX, MT, AB, WI). When routes overlapped geographically, we limited our analysis to only one of the routes. We considered each dispersal corridor as a hypothesis to explain a route from a potential site of origin for each cougar.

### Dispersal path prediction

We evaluated the likely origin of each cougar using likelihood-based assignment as described by Royle & Rubenstein (2004). The rationale behind this method is that a migrating animal can be associated with the location most likely to produce the observed tissue isotope value. Royle & Rubenstein (2004) used their assignment method to distinguish whether an individual was a member of  $> 1$  subpopulations, but we extend their method to take advantage of the time series of information available within the cougar claw (i.e.,  $> 1$  samples available for an individual). We used their method to identify the most probable route of a dispersing individual, rather than simply assigning an individual to one of  $> 1$  areas of origin.

As a general introduction to our extension of Royle & Rubenstein's (2004) method, we can consider an individual with an unknown dispersal origin and path, we could hypothesize (based on best available anecdotal data) that the individual may have followed  $R$  potential routes,  $r = 1, 2, \dots, R$  from the hypothetical sources. We can then assume that the expected stable isotope values (for any given isotope  $X$ ) along  $\Omega_r$  have been predicted (e.g., using the Bowen, Wassenaar, & Hobson (2005) calculator) and are defined as  $y_{r,\delta X}^i = \mu_{r,\delta X}^i \pm \sigma_{\delta X}$ , where  $\mu$  and  $\sigma$  represent the mean  $\delta X$  value and standard deviation, respectively, at the approximate distance from the death place integrated in sample  $i$  of the claw. The index  $i$  ranged from 0 to  $n$ , with 0 representing the sample at the root of the claw and  $n$  the sample at the tip of the claw. We used  $y_{\delta X}^i$  to denote the isotopic value measured in sample  $i$  of the claw. To determine the most likely dispersal route of this individual, one can evaluate the likelihood  $f(y_{\delta X}^i | \mu_{r,\delta X}^i, \sigma_{\delta X})$  for each sample  $i$  along a  $\Omega_r$ . The likelihood  $f(y_{\delta X}^i | \mu_{r,\delta X}^i, \sigma_{\delta X})$ , defined as the conditional probability of  $y_{\delta X}^i$  given that the individual travels on  $\Omega_r$  from a potential source, is calculated as:  $f(y_{\delta X}^i | \mu_{r,\delta X}^i, \sigma_{\delta X}) = \frac{1}{\sqrt{2\pi}\sigma_{\delta X}} \exp[-\frac{1}{2\sigma_{\delta X}^2}(y_{\delta X}^i - \mu_{r,\delta X}^i)^2]$ , where

$$\sigma_{\delta X} = \sqrt{\frac{\sum_{i=1}^n (y_{\delta X}^i - \mu_{r,\delta X}^i)^2}{n}}$$

is the standard deviation of the differences between the observed  $y_{\delta X}^i$  and predicted  $\mu_{r,\delta X}^i$  values along all potential paths (e.g., Hobbs & Hilborn 2006). If expected values for two isotopes (e.g.,  $\delta D$  and  $\delta^{13}\text{C}$ ) are known along the dispersal corridor, the framework could be extended to a two-isotope approach. The bivariate normal likelihood corresponding to the set of observations  $y^{i*} = \{y_{\delta D}^{i*}, y_{\delta^{13}\text{C}}^{i*}\}$  would be

$$f(y_{\delta D}^{i*}, y_{\delta^{13}\text{C}}^{i*} | \mu_{r,\delta D}^i, \sigma_{\delta D}, \mu_{r,\delta^{13}\text{C}}^i, \sigma_{\delta^{13}\text{C}}) = \frac{1}{2\pi\sigma_{\delta D}\sigma_{\delta^{13}\text{C}}\sqrt{1-\rho_{\delta D,\delta^{13}\text{C}}^2}} \times \exp \left\{ -\frac{1}{2(1-\rho_{\delta D,\delta^{13}\text{C}}^2)} \left[ \frac{(\delta D - \mu_{r,\delta D}^i)^2}{\sigma_{\delta D}^2} - 2\rho_{\delta D,\delta^{13}\text{C}} \left( \frac{\delta D - \mu_{r,\delta D}^i}{\sigma_{\delta D}} \right) \times \left( \frac{\delta^{13}\text{C} - \mu_{r,\delta^{13}\text{C}}^i}{\sigma_{\delta^{13}\text{C}}} \right) + \frac{(\delta^{13}\text{C} - \mu_{r,\delta^{13}\text{C}}^i)^2}{\sigma_{\delta^{13}\text{C}}^2} \right] \right\}$$



where  $\rho_{\delta D, \delta^{13}C}$  is the correlation coefficient between  $\delta D$  and  $\delta^{13}C$ , estimated from isotopic values at deer check stations.

We applied this general approach to the data available in our study area. When we had data from cougars and expected data from isoscapes for both  $\delta D$  and  $\delta^{13}C$  tissue values, we employed the two-isotope approach to improve predictions (e.g. Royle & Rubenstein 2004). We estimated  $\pi_r^i$ , defined as the probability that the cougar dispersed along  $\Omega_r$  at a distance  $i$  from the death place using a Bayesian approach. Our initial (*a priori*) values of  $\pi_r^0$  were calculated as 1/number of hypothetical routes. We then estimated the probability of travelling along route  $\Omega_r$  at each sample  $i$  sequentially from the root to the tip of the claw, as:

$$\pi_r^i = \frac{\pi_r^{i-1} \times f(y^i * |\mu_r^i, \sigma)}{\sum_r \pi_r^{i-1} \times f(y^i * |\mu_r^i, \sigma)}$$

The probability of origin  $\pi_r^{i-1}$ , for  $i = 1, 2, \dots, n$ , is used as prior information in the Bayesian framework, and the cumulative probability of travelling along a given path gains more weight if the observed isotope values for the claw segments are closer to the expected values for a path, relative to other paths under consideration. Finally, the cumulative probabilities reach their end-point at the last sample (claw tip), where  $i = n$ . The dispersal corridor  $\Omega_r$  with the maximum value of  $\pi$  is the most likely route followed by the cougar.

#### Expected claw isotopic values along dispersal corridors

The method described earlier requires that for each cougar, we established expected claw  $\delta D$  and  $\delta^{13}C$  values along each dispersal corridor. We used ArcGIS to overlay the dispersal corridors  $\Omega_r$  created for the Gretna and Valentine cougars on the regional  $\delta D_{\text{node}}$  and  $\delta^{13}C_{\text{node}}$  isoscapes, and the dispersal corridors for the Saskatoon and Chicago cougars on the large-scale  $\delta D_{\text{collagen}}$  isoscape. Then we extracted isotopic values from these prey isoscapes at successive points of the raster along each route to match the distances represented by our samples of claw tissue.

To link cougar claw isotopic values to the extracted  $\delta D$  and  $\delta^{13}C$  values from prey isoscapes along each dispersal corridor, we established 'claw-prey' discrimination factors ( $\Delta\delta D_{\text{claw-prey}}$  and  $\Delta\delta^{13}C_{\text{claw-prey}}$ , respectively). For each cougar, we obtained  $\Delta\delta D_{\text{claw-prey}}$  by subtracting the mean prey  $\delta D$  value (based on predicted deer collagen,  $\delta D_{\text{collagen}}$  or lymph node tissues,  $\delta D_{\text{node}}$ ) around the mortality location (i.e. within a 50-km-diameter circle) from the mean  $\delta D_{\text{claw}}$  value in the proximal 15% of the claw (i.e. most recently synthesized part of the claw). Considering the proximal 15% of the claw provided conservative  $\Delta\delta D_{\text{claw-prey}}$  values (i.e., not influenced by outlier isotopic measurements). The mean prey  $\delta D$  value around the mortality location derived from the regional  $\delta D_{\text{node}}$  isoscape for the Gretna and Valentine cougars and from the large-scale  $\delta D_{\text{collagen}}$  isoscape for the Saskatoon and Chicago cougars. For each cougar, we determined the expected  $\delta D_{\text{claw}}$  ( $\mu_{r, \delta D}$ ) along each  $\Omega_r$ , by adding  $\Delta\delta D_{\text{claw-prey}}$  to extracted prey tissue ( $\delta D_{\text{node}}$  or  $\delta D_{\text{collagen}}$ ) values. We used the same approach to estimate  $\Delta\delta^{13}C_{\text{claw-prey}}$  from the regional  $\delta^{13}C_{\text{node}}$  isoscape for the Gretna and Valentine cougars.

When the modelled route ( $\Omega$ ) between a potential source and the mortality location was shorter than the maximum distance that may be integrated in the claw, we assumed that the origin and dispersal route of the cougar were represented in the distal and proximal parts of the claw, respectively; claw samples representing time before dispersal were assigned the expected claw isotopic values at the source.

## Results

### SPATIAL VARIATION IN DEER TISSUE

Deer lymph nodes produced a southeast–northwest  $\delta D$  gradient consistent with our predictions ranging from  $-148.6\text{‰}$  in western South Dakota (Black Hills) to  $-101.7\text{‰}$  in southeast Nebraska (Fig. 1). Values of  $\delta D_{\text{collagen}}$  reflected the known north–south enrichment pattern expected for mean annual precipitation  $\delta D_p$  for North America (Bowen, Wassenaar, & Hobson 2005). The carbon isotopic gradient in deer revealed an east–west gradient of  $\delta^{13}C$  in Nebraska (Fig. 1) with high  $\delta^{13}C$  values in eastern Nebraska ( $-14.9\text{‰}$ ) and at the border with Wyoming ( $-15.9\text{‰}$ ) and low  $\delta^{13}C$  values in the Great Plains ( $-24.8\text{‰}$ ). We estimated a correlation coefficient  $\rho_{\delta D, \delta^{13}C} = 0.26$  between  $\delta D$  and  $\delta^{13}C$  values from deer stations in Nebraska ( $t_{10} = 0.85, n = 12, P = 0.41$ ).

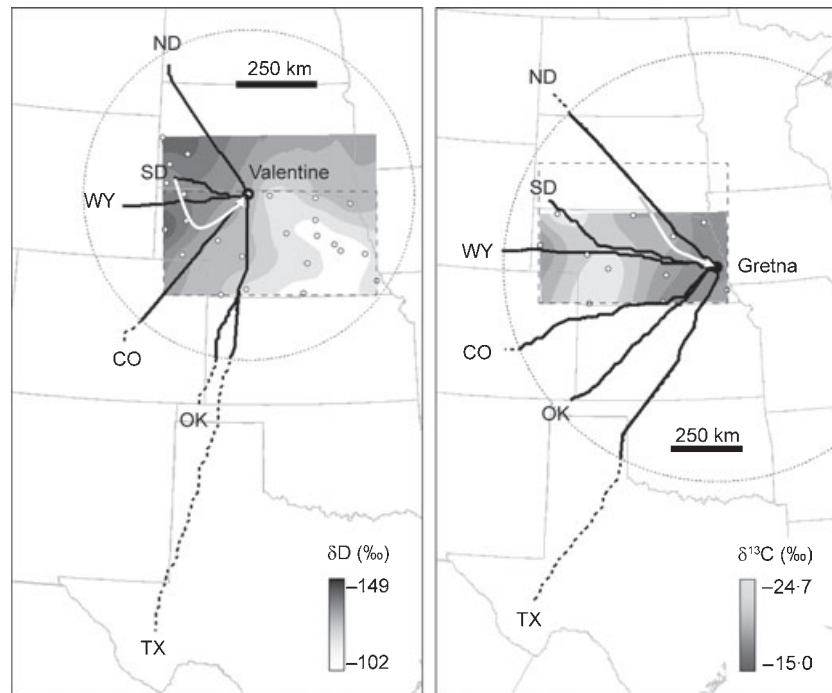
### ISOTOPIC VARIATION ALONG COUGAR CLAWS

Plotting  $\delta D_{\text{claw}}$  and  $\delta^{13}C_{\text{claw}}$  values against the proportion of claw length showed that the isotopic patterns in the two claws from the Saskatoon cougar overlapped, indicating that the claws integrated dietary information over the same period of time (Fig. S1). But, because the claws were different lengths, we decided to use proportion of claw length rather than section length, as the standard to compare between claws. We report our proportions from the tip ( $i = 0.0$ ) to the root ( $i = 1.0$ ) of the claw.

Each cougar claw showed continuous change in  $\delta D$  and  $\delta^{13}C$  values from proximal samples consistent with our predictions (Fig. 2). We found a declining trend in  $\delta D_{\text{claw}}$  values for the Saskatoon cougar (corresponding to northern movement) and increasing  $\delta D_{\text{claw}}$  values for the other three cougars. For the Chicago cougar, the very low  $\delta D_{\text{claw}}$  values ( $> 19\text{‰}$  difference with the closest measurement) observed in the tip and root samples were not expected based on known landscape variation of  $\delta D$  in cougar prey and were therefore ignored in further analyses.  $\delta^{13}C_{\text{claw}}$  values declined for the Saskatoon and Valentine cougars towards their mortality location, whereas we observed an overall increasing pattern in  $\delta^{13}C_{\text{claw}}$  values for the Gretna cougar (Fig. 2). We did not perform  $\delta^{13}C$  analyses for the Chicago cougar because of sample size restrictions.

### DETERMINING RATES OF GROWTH

The Valentine cougar travelled about 250 km in ca. 75 days, which represented a mean speed of  $3 \text{ km day}^{-1}$ . The graphical combined analysis of  $\delta D$  and  $\delta^{13}C$  results from this cougar showed that its dispersal trip was integrated in the last 20 mm or last 49% of the claw (Fig. 2). Therefore, we estimated that 1% of claw represented 1.5 days, which means that a cougar claw integrated dietary information over approximately five months. More specifically, for the Valentine cougar, 1% of claw represented about 5 km. The Chicago cougar was killed in mid-April which means its claw represented winter months. Therefore, we used the scenario assuming a mean speed of



**Fig. 1.** Least-cost dispersal paths for the Valentine and Gretna cougars from each potential source. The white dots indicate the location of the deer sampling stations. Dotted-line circles indicate the estimated maximum distance from the death place integrated in an entire claw, based on a mean speed of 3 km/day (which corresponds to a mean distance of 5 km integrated per 1% of claw) for the Valentine cougar, and 5 km/day (7.5 km/% of claw) for the Gretna cougar. The solid part of each dispersal corridor ( $\Omega$ ) represents the portion predicted to be integrated in the claw, and the dashed part represents the initial part of the dispersal trip. The background pattern represents the regional stable hydrogen (left) and carbon (right) isotopic basemaps. The dashed-line rectangles represent the surface covered by the stable carbon (left) and hydrogen (right) isotopic basemaps. We stress that the least-cost dispersal paths from OK and TX for the Gretna cougar were not considered in the model given that they were only partially covered by the regional  $\delta D$  and  $\delta^{13}C$  isoscapes. The white arrows indicate our interpretation of the most likely dispersal route based on predictions by the model.

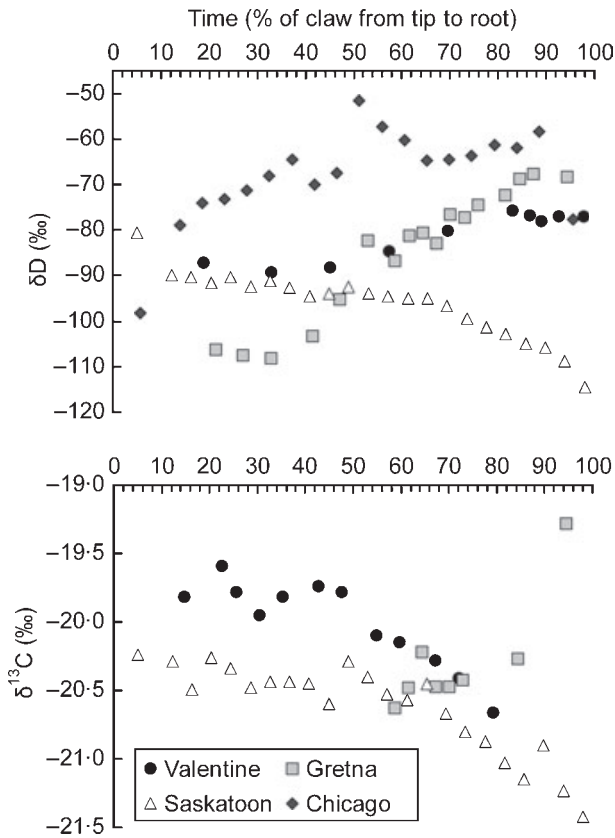
3 km day<sup>-1</sup> to predict its most likely route of dispersal (Fig. S2). For the Gretna and Saskatoon cougars, whose claws represented the summer months, we considered the scenario assuming a mean speed of 5 km day<sup>-1</sup> (which means that 1% of claw represented about 7.5 km; Fig. S2).

#### PREDICTIONS OF DISPERSAL ROUTES

Least-cost dispersal paths, as predicted by habitat models and additional dispersal corridors, are shown in Fig. 1 for the Valentine and Gretna cougars, and Fig. 3 for the Chicago and Saskatoon cougars. In each figure, the solid part of each dispersal corridor  $\Omega$  represents the distance considered to be integrated in the claw.

Based on the regional  $\delta D_{\text{node}}$  and  $\delta^{13}C_{\text{node}}$  isoscapes, we obtained a standard deviation  $\sigma_{\delta D} = 6.23\text{‰}$  and  $\sigma_{\delta^{13}C} = 1.36\text{‰}$  for the Valentine cougar and  $\sigma_{\delta D} = 4.59\text{‰}$  and  $\sigma_{\delta^{13}C} = 1.87\text{‰}$  for the Gretna cougar. We found  $\Delta\delta D_{\text{claw-prey}} = 48.8\text{‰}$  and  $\Delta\delta^{13}C = 0.22\text{‰}$  for the Valentine cougar and  $\Delta\delta D = 38.5\text{‰}$  and  $\Delta\delta^{13}C = -3.9\text{‰}$  for the Gretna cougar. We took advantage of the information provided by the stable hydrogen and carbon isoscapes and used the two-isotope likelihood-based assignment approach to predict the most likely route of dispersal and origin of these two cougars. For the Valentine cougar, we conducted a first analysis considering  $\Omega_{WY}$ ,  $\Omega_{CO}$  and  $\Omega_{OK/TX}$ , the only three routes covered com-

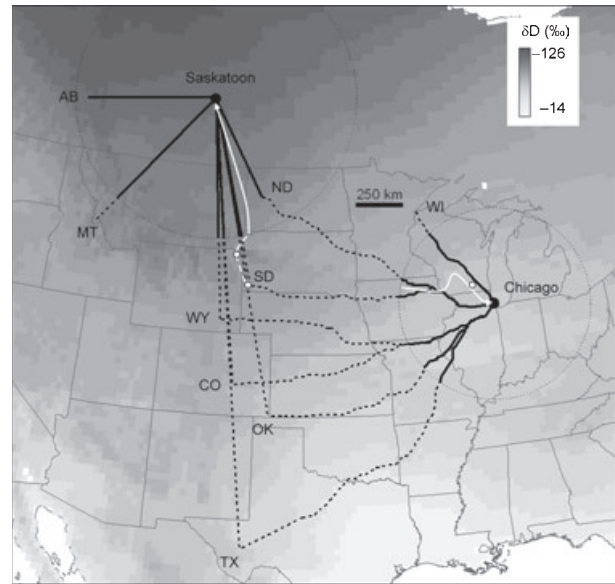
pletely by the two isoscapes;  $\Omega_{SD}$  was not considered because of the absence of coverage of this path by the carbon iscape in South Dakota (Fig. 1). Among these three routes, our model predicted  $\Omega_{CO}$  as the most probable route, with a probability of origin  $\pi_{CO} = 1.0$  (at  $i = 0.55$ ; Fig. 4a). We performed a second analysis where we compared all routes using the regional  $\delta D_{\text{node}}$  iscape only; the number of routes ( $\Omega$ ) covered by the iscape (and thus considered in this analysis) decreased as we went back to old samples (i.e. towards claw tip). This second analysis predicted  $\Omega_{CO}$  as the most probable route (among the routes considered) within 350–400 km of the death place, but predicted that the cougar likely travelled along  $\Omega_{SD}$  earlier ( $\pi_{SD} = 0.8$  and  $\pi_{CO} = 0.2$  at  $i = 0.3$ ; Fig. 4b). This pattern confirms the known origin of the Valentine cougar and suggests that it likely travelled south from its origin in the Black Hills, SD, and then north-east along  $\Omega_{CO}$  before it was killed near Valentine, NE. For the Gretna cougar, to discriminate among  $\Omega_{ND}$ ,  $\Omega_{SD}$  and  $\Omega_{WY}$  (Fig. 1), we used the two-isotope assignment approach for samples between 100% and 70% of the claw (i.e. proximal part; Fig. 2) and continued the analysis with  $\delta D_{\text{claw}}$  measurements for samples between 70% and 47% of the claw, using predictions at  $i = 0.70$  from the dual-isotope approach as priors. Going backward from its place of mortality (i.e., claw root –  $i = 1.0$ ) to its source (i.e., claw tip), our model predicted similar probabilities for the three routes within  $\sim 150$  km of the mortality location, but



**Fig. 2.** Stable hydrogen ( $\delta D$ ) and carbon ( $\delta^{13}C$ ) isotope values along the cougar claws. We used proportion of claw length as the standard to compare between claws and reported isotopic values from the tip (0%) to the root (100%) of the claw. We were not able to obtain  $\delta^{13}C$  values for the Chicago cougar due to small tissue sample sizes.

predicted  $\Omega_{ND}$  as the most likely route of dispersal when the cougar was within  $\sim 400$  km to  $\sim 150$  km-distant from Gretna, NE ( $\pi_{ND} = 1.0$  at  $i = 0.5$ ; Fig. 4c). Although we cannot infer precisely the source of this cougar (probably from populations breeding in ND, or in the Black Hills, SD; Fig. 1), these results suggest that the Gretna cougar likely approached Gretna via north-eastern Nebraska; the riparian areas along the Missouri and/or Elkhorn rivers may provide important movement corridors (Dickson, Jenness, & Beier 2005). To evaluate the accuracy of the dual-isotope approach, we conducted the same analysis using each regional isoscape independently. In both cases, the model predicted  $\Omega_{ND}$  as the most likely route of dispersal (Fig. 4d); however, predictions within  $\sim 150$  km of the place of mortality are slightly different: the cougar was predicted to have approached Gretna following the  $\Omega_{ND}$  path with the  $\delta D_{node}$  isoscape, whereas the  $\Omega_{WY}$  or  $\Omega_{SD}$  paths were more likely with the  $\delta^{13}C_{node}$  isoscape.

Using the North American  $\delta D_{collagen}$  isoscape, we obtained a standard deviation  $\sigma_{\delta D}$  of  $7.02\text{‰}$  for the Saskatoon cougar and  $7.18\text{‰}$  for the Chicago cougars. We estimated a  $\Delta\delta D_{claw-prey}$  discrimination factor of  $-3.8\text{‰}$  for the Saskatoon cougar and  $-19.3\text{‰}$  for the Chicago cougar, by subtracting the mean  $\delta D_{collagen}$  value around the mortality location from the  $\delta D_{claw}$  value in the proximal part of the claw. We used these

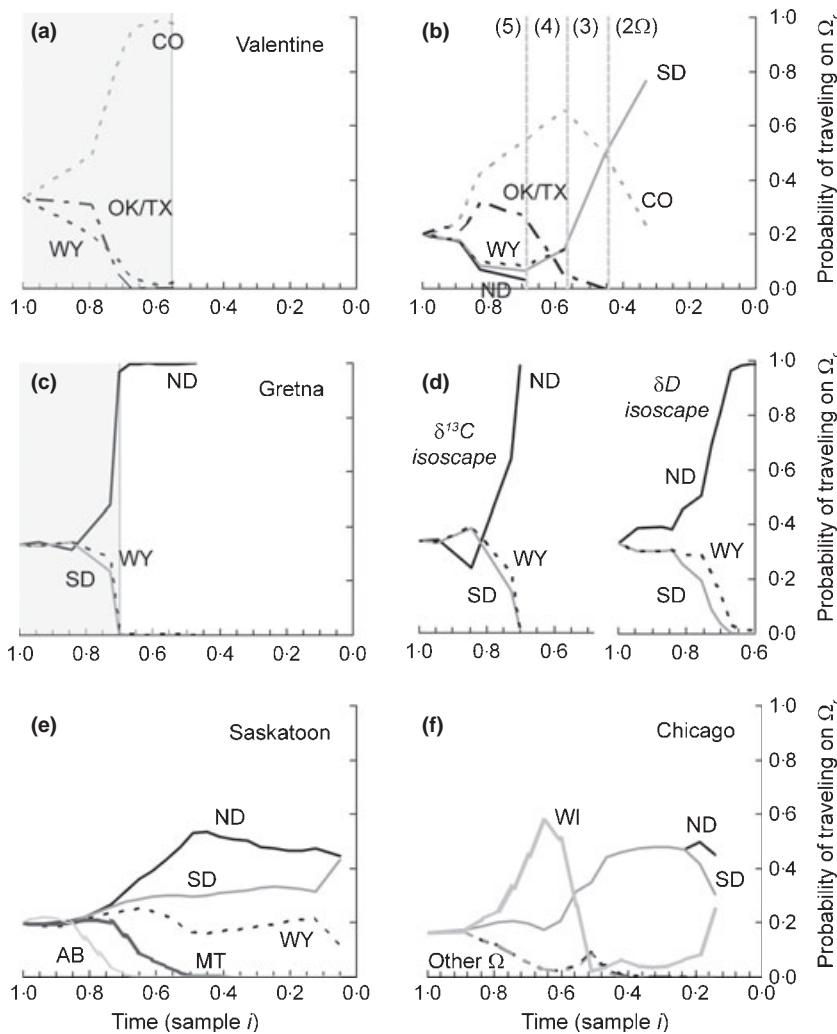


**Fig. 3.** Dispersal corridors for the Chicago and Saskatoon cougars from each potential source. Circles indicate the estimated maximum distance from the death place integrated in an entire claw, based on a mean speed of 3 km/day (which corresponds to a mean distance of 5 km integrated per 1% of claw) for the Chicago cougar, and 5 km/day ( $7.5\text{ km}/\%$  of claw) for the Saskatoon cougar. The solid part of each dispersal corridor ( $\Omega$ ) represents the portion predicted to be integrated in the claw, and the dashed part represents the initial part of the dispersal trip. We stress that the dispersal corridors from OK, CO, and TX for the Saskatoon cougar were not considered in the analysis given that they overlapped the dispersal corridors from SD or WY; the dispersal corridor from CO for the Chicago cougar was not considered in the analysis given that it overlapped the dispersal corridor from WY. The white-filled dots specify the origin or/and resighting location of each cougar and the arrows indicate our interpretation of the most likely dispersal route based on predictions by the model.

discrimination values to predict the expected  $\delta D_{claw}$  values along the hypothesized  $\Omega$  for the Saskatoon and Chicago cougars, respectively. We were not able to predict the source of these cougars because wear at the tip of the claw resulted in the loss of dietary information from their natal place. Of the five routes (ND, SD, WY, MT and AB) considered for the Saskatoon cougar (collared in SD), our model clearly identified that this cougar originated from southern territories and not from western areas (represented by  $\Omega_{MT}$  or  $\Omega_{AB}$ ) and indicated that it likely travelled between  $\Omega_{SD}$  and  $\Omega_{ND}$  ( $\pi_{ND} = \pi_{SD} = 0.4$ ,  $\pi_{WY} = 0.1$  at  $i = 0.05$ ), within  $\sim 750$  km of the location of death (Fig. 4e). Our approach predicted that the Chicago cougar travelled through the Midwest likely between  $\Omega_{ND}$  and  $\Omega_{SD}$  ( $\pi_{ND} = 0.5$ ,  $\pi_{SD} = 0.3$ ,  $\pi_{WI} = 0.2$  at  $i = 0.1$ ), and travelled north to  $\Omega_{WI}$  before going south towards Chicago ( $\pi_{WI} = 0.6$  at  $i = 0.7$ ; Fig. 4f).

## Discussion

Our analysis included three cougars known to originate from the Black Hills because they were radio-collared in this area before dispersal (Valentine and Saskatoon cougars) or from



**Fig. 4.** Bayesian probabilities of traveling along each dispersal corridor. Time on the x-axis is shown as a function of the portion of the claw from which the sample was taken (e.g., 0.0 is the oldest tissue at the tip of claw; 1.0 is the newest tissue from the root and the last tissue created before death). Predictions for the Valentine cougar were based on the regional  $\delta D$  and  $\delta^{13}C$  isoscapes (a) or on the  $\delta D$  isoscape only (b); the number of routes  $\Omega$  (indicated between parentheses) covered by the isoscape decreased as we went back to old samples. Predictions for the Gretna cougar were based on the regional  $\delta D$  and  $\delta^{13}C$  isoscapes using a dual-isotope approach (c) or one-isotope analyses (d;  $\delta^{13}C$  isoscape on the left and  $\delta D$  isoscape on the right). Predictions for the Chicago (e) and Saskatoon (f) cougars were based on the large-scale hydrogen isoscape. A grey background indicates predictions based on a two isotope likelihood-based assignment approach.

genetic analyses (Chicago cougar). Our model confirmed the origin of the Valentine cougar; however, all potential routes could not be fully considered (Fig. 2b) because of limitations associated with the resolution and coverage of the regional isoscapes. Our approach correctly identified the cardinal direction of the Saskatoon and Chicago cougars' origin; we were not able to confirm the exact source of these two long-distance dispersers because isotopic information from their natal place was not conserved in their claw. Nevertheless, our method successfully predicted the changes in direction and travels of the Chicago cougar in Wisconsin, where it was observed three months before being killed. In addition, our approach provided important information about the most probable route of recent dispersal (within five months preceding death) of the four cougars (as indicated by arrows in Fig. 1 and 3), highlighting the potential to learn about the preferred routes of dispersal of short- and long-distance dispersing cougars through isotopic analyses of sequential samples along the claw axis.

This sort of forensic investigation into carnivore movements requires appropriate isoscapes to be established from more sedentary prey animals such as white-tailed or mule deer or another common prey species. We created actual regional  $\delta D_{\text{node}}$  and  $\delta^{13}C_{\text{node}}$  isoscapes for Nebraska and the Black

Hills, SD, based on analyses of deer lymph node tissues. In Nebraska, we found a southeast-northwest  $\delta D$  gradient in deer and cougar tissues, which matched the groundwater pattern derived for this region (Harvey 2005). Measurements of  $\delta^{13}C$  in deer tissues showed an east-west pattern within Nebraska which reflected the sources of agricultural corn ( $C_4$  plant) in an otherwise largely  $C_3$  environment (Weckerly & Nelson 1990). Our approach has thus the potential to provide high-resolution spatially explicit basemaps for any stable isotopes or trace elements of interest providing they are geographically structured. At larger scales, the data set from Bowen, Wassenaar, & Hobson (2005) provided a useable and predictable precipitation  $\delta D$  isoscape that was then linked to deer collagen and cougar claws using the relationship derived by Cormie, Schwarcz, & Gray (1994). However, this approach is susceptible to shorter-term deviations in precipitation  $\delta D$  patterns from the long-term (primarily GNIP, Global Network of Isotopes in Precipitation) mean average values used for the large-scale isoscape resulting in biases in model predictions (e.g. Brewster 2009). In future, year-specific tissue isoscapes can be constructed from models that can correct for these changes (Brewster 2009) or on web-based tools currently being developed (i.e. IsoMAP; G. Bowen, pers. comm.). Also, managers throughout the U.S.



and Canada sample deer tissues for chronic wasting disease monitoring; these samples provide the opportunity to broaden our tissue isotope base maps to wider geographical areas and for specific years of interest.

All claws provided a continuous gradient of  $\delta D$  and  $\delta^{13}C$  values demonstrating that changes in isotopic signatures of local diets are detectable in claw tissue. Large differences in  $\delta D_{\text{claw}}$  values in the root and tip samples of the claw of the Chicago cougar were not expected based on known landscape variation of  $\delta D$  in cougar prey. The nonexchangeable hydrogen portion of a tissue is controlled by both diet and drinking water (Hobson, Atwell, & Wassenaar 1999). The sudden enrichment in deuterium (i.e. less negative  $\delta D$  value) in the middle of the claw of the Chicago cougar (Fig. 2) was followed by a gradual return to the previous level, suggesting a sudden change in  $\delta D$  of diet and/or drinking water followed by a slower recovery to previous conditions. The claw of this cougar (killed in mid-April) represented its winter diet. Snow typically exhibits depleted  $\delta D$  values compared to rain at a given location (Gat 1996; Oberhänsli, Weise, & Stanichny 2009). This variation may be further altered by spatial variability and vegetation conditions (Koeniger *et al.* 2008). Therefore, a transition in winter–spring diet or the short-term use of isotopically enriched drinking water (potentially derived from north-flowing rivers) may have caused the marked variation in  $\delta D$  values in the middle of the claw of the Chicago cougar. Given that isotopic patterns in an individual's claws integrate dietary information over the same period of time (e.g., Saskatoon cougar; Fig. S1), analysing several claws may help to deal with potential outlier isotopic measurements.

Matching the observed isotopic values in claw with the isoscapes is a critical step in our forensic approach. We corrected the isotopic prey basemaps using the proximal portion of the claw assuming that this correction factor could be applied to all other regions where the cougar was prior to its death. We note that for the Valentine cougar, we found a discrimination factor  $\Delta\delta D_{\text{claw-prey}} = 54.0\text{‰}$  at its origin (based on mean  $\delta D_{\text{claw}}$  within the distal half of the claw and mean  $\delta D_{\text{node}}$  in the Black Hills, SD) versus  $48.8\text{‰}$  at its mortality location, indicating that isotopic discrimination may slightly change over time because of seasonal changes in prey distribution and diet or source of drinking water. Analysing divergences from baseline isotopic values in claws of resident cougars would clarify the extent to which seasonal changes in diet or climatic conditions may alter isotopic basemaps. Such analyses would provide an estimate of uncertainty in discrimination factor that could be incorporated in further analyses. Further variations because of the change in daily dispersal distance over time (e.g., Stoner *et al.* 2008) were minimized by considering a mean dispersal speed adjusted to the season during which the cougar dispersed over the five months preceding its death. Our work shows the value of using local isotope information to provide more localized and year-specific isoscapes than those that are averaged over larger temporal and spatial scales (e.g. Bowen, Wassenaar, & Hobson 2005). The intelligent use of both local (ground-truthed) and large-scale (predicted) isoscapes is likely to yield the most benefit in future forensic studies of this nature.

We believe ours is an innovative approach to reconstruct the most likely dispersal route (and, if possible, origin) of individual carnivores with unknown history. However, we have no information about changes in dispersal speed and direction, or variations in diet of studied individuals, although constant isotopic measurements in the claw would suggest the use of a consistent home range (Beier 1995). Many of the dispersal corridors considered in our analysis are the shortest least-cost routes of dispersal across the Midwest considering habitat factors and ecological requirements for cougars (LaRue & Nielsen 2008). However, a previous satellite tracking of a female cougar showed several changes in directions during dispersal (Stoner *et al.* 2008). Our approach also showed that the studied cougars did not follow the shortest least-cost path between their known origin and mortality locations. In the case of long-distance dispersers, combining our approach with genetic analyses may permit identification of the source of origin and narrow the set of potential routes. Furthermore, considering additional routes to suitable patches within hundreds of km of the mortality location (e.g.,  $\Omega_{WI}$  for the Chicago cougar) may help identify dispersal corridors in habitats apparently unsuitable for cougars and provide basic information on their dispersal strategies. Moreover, by integrating information from the two isoscapes, the dual-isotope approach may enhance the delineation of the most likely dispersal route around the location of mortality (Fig 4c and d; e.g., Royle & Rubenstein 2004). We stress that modelling cumulative probabilities of travelling along each path is expected to reduce the impact of unknown movements.

World-wide, accelerating human development and spread over the landscape lead to the degradation and fragmentation of habitat and dispersal corridors of large carnivores with, as consequences, increased human-caused mortality and poor dispersal success (Schwartz & Franzmann 1992; Smith 1993; Wydeven *et al.* 1998; Sweanor, Logan, & Hornocker 2000). Identifying and protecting habitat corridors between favourable patches is essential to improve dispersal success and increase connectivity among subpopulations. Our method could be applied to investigations of dispersal paths of other large carnivores, and we encourage managers to consider the use of stable isotope techniques to develop integrated, adaptive conservation strategies for carnivores. Because the claw tissue is metabolically inert after synthesis, claws collected several years ago from road-killed or hunted large carnivores are still useful for isotopic analyses, and have the potential to determine the origin of short-distance dispersers and delimit preferred dispersal routes of any reported dead individual. We believe our approach is applicable to other metabolically inert, continuously growing, keratinous tissues (e.g., hair, horn, baleen) of other species of interest, providing that individuals move across known isotopically structured landscapes. Insights about the most likely migration corridors may help identify critical geographical areas for monitoring and guide future conservation efforts. We recommend wildlife biologists develop large-scale isoscapes based on sedentary prey species and primary consumed tissues such as muscle as they prove to be valuable tools in isotopic tracking of long-distance

migration for large carnivores. In addition, the small-scale precision of our regional isotopic basemaps offers a significant opportunity in wildlife forensics to investigate the source of illegally taken game. Finally, the investigation of tissues of captive animals raised on known isotopic diets will assist with refining the diet-tissue isotopic discrimination values so valuable to these sorts of movement reconstructions.

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## References

- Ackerman, B.B., Lindzey, F.G. & Hemker, T.P. (1984) Cougar Food Habits in Southern Utah. *Journal of Wildlife Management*, **48**, 147–155.
- Beier, P. (1995) Dispersal of juvenile cougars in fragmented habitat. *Journal of Wildlife Management*, **59**, 228–237.
- Bowen, G.J., Wassenaar, L.I. & Hobson, K.A. (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, **143**, 337–348.
- Brewster, K.R. (2009) Role of landscape composition and geographical location on breeding philopatry in grassland passerines, a stable isotope approach, MS thesis. University of Saskatchewan, Saskatoon, Canada.
- Cerling, T.E., Wittenmyer, G., Rasmussen, H.B., Vollrath, F., Cerling, C.E., Robinson, T.J. & Douglas-Hamilton, I. (2006) Stable isotopes in elephant hair document migration patterns and diet changes. *Proceedings of the National Academy of Sciences*, **103**, 371–373.
- Cormie, A.B., Schwarcz, H.P. & Gray, J. (1994) Relation between hydrogen isotope ratios of bone collagen and rain. *Geochimica et Cosmochimica Acta*, **58**, 377–391.
- Cougar Network (2010) Confirmed cougar occurrences recorded by the Cougar Network. <http://www.cougarnet.org/bigpicture.html> (accessed January 2010).
- Davenport, M., Nielsen, C.K. & Mangun, J. (2010) Attitudes toward mountain lion management in the Midwest: implications for a potentially recolonizing large predator. *Human Dimensions of Wildlife*, **15**, 373–388.
- Dickson, B.G., Jenness, J.S. & Beier, P. (2005) Influence of vegetation, topography, and roads on cougar movement in southern California. *Journal of Wildlife Management*, **69**, 264–276.
- Fraser, K.C., Kyser, T.K., Robertson, R.J. & Ratcliff, L.M. (2008) Seasonal patterns on hydrogen isotopes from claws from breeding wood warblers (Parulidae): Utility for estimating migratory origins. *Avian Conservation and Ecology*, **3**, 2.
- Gat, J.R. (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences*, **24**, 225–262.
- Gehring, T.M. & Potter, B.A. (2005) Wolf habitat analysis in Michigan: an example of the need for proactive land management for carnivore species. *Wildlife Society Bulletin*, **33**, 1237–1244.
- Hall-Aspland, S.A., Rogers, T.L. & Canfield, R.B. (2005) Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. *Marine Ecology Progress Series*, **305**, 249–259.
- Harvey, F.E. (2005) Stable Hydrogen and Oxygen Isotope Composition of Precipitation in Northeastern Colorado. *Journal of the American Water Resources Association*, **41**, 447–459.
- Hobbs, N.T. & Hilborn, R. (2006) Alternatives to statistical hypotheses testing in ecology: a guide to self teaching. *Ecological Applications*, **16**, 5–19.
- Hobson, K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314–326.
- Hobson, K.A. (2005) Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. *Auk*, **122**, 1037–1048.
- Hobson, K.A., Atwell, L. & Wassenaar, L.I. (1999) Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Sciences*, **96**, 8003–8006.
- Hobson, K.A., Barnett-Johnson, R. & Cerling, T. (2010) Using isoscapes to track animal migration. *Isoscapes: Isotope Mapping and its Applications* (eds G.J. Bowen, J. West, K. Tu & T. Dawson), pp. 273–298. Springer-Verlag, New York.
- Hobson, K.A. & Schell, D.M. (1998) Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 2601–2607.
- Hobson, K.A. & Wassenaar, L.I. (2008) *Tracking Animal Migration Using Stable Isotopes*. Terrestrial Ecology Series, Vol. 2. Academic Press, London, UK.
- Hobson, K.A., Wassenaar, L.I. & Taylor, O.R. (1999) Stable isotopes ( $\delta\text{D}$  and  $\delta^{13}\text{C}$ ) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia*, **120**, 397–404.
- Hobson, K.A., Bowen, G.J., Wassenaar, L.I., Ferrand, Y. & Lormee, H. (2004) Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia*, **141**, 477–488.
- Hoffman, J.D. & Genoways, H.H. (2005) Recent Records of Formerly Extirpated Carnivores in Nebraska. *The Prairie Naturalist*, **37**, 225–245.
- Koeniger, P., Hubbard, J.A., Link, T. & Marshall, J.D. (2008) Isotopic variation of snow cover and streamflow in response to changes in canopy structure in a snow-dominated mountain catchment. *Hydrological Processes*, **22**, 557–566.
- Larue, M.L. (2007) Predicting potential habitat and dispersal corridors for cougars in midwestern North America, PhD thesis. Southern Illinois University, Carbondale, Illinois, USA.
- LaRue, M.A. & Nielsen, C.K. (2008) Modelling potential dispersal corridors for cougars in midwestern North America using least-cost path methods. *Ecological Modelling*, **212**, 372–381.
- Logan, K.A. & Sweanor, L.L. (2001) *Desert Puma: Evolutionary Ecology and Conservation of an Enduring Carnivore*. Island Press, Washington, District of Columbia, USA.
- Maehr, D.S. (1997) *Florida Panther, Life and Death of a Vanishing Carnivore*. Island Press, Washington, District of Columbia, USA.
- Marshall, J.D., Brooks, J.R. & Lajtha, K. (2007) Sources of variation in the stable isotopic composition of plants. *Stable Isotopes in Ecology and Environmental Science*, Second Edition. (eds R. Michener & K. Lajtha), pp. 22–60. Blackwell, London.
- Mazerolle, D. & Hobson, K.A. (2005) Estimating origins of short-distance migrant songbirds in North America: Contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *Condor*, **107**, 280–288.
- Miller, N.G., Wassenaar, L.I., Hobson, K.A. & Norris, R.D. (2011) Monarch butterflies cross the Appalachians from the west to re-colonize the east coast of North America. *Biology Letters*, **7**, 43–46.
- Nielsen, C.K., Dowling, M., Miller, K. & Wilson, B. (2006) The Cougar Network: using science to assess the status of cougars in eastern North America. *Proceedings of the eastern cougar conference 2004*, pp. 82–86. West Virginia.
- Norris, D.R., Arcese, P., Preikshot, D., Bertram, D.F. & Kyser, T.K. (2007) Diet reconstruction and historic population dynamics in a threatened seabird. *Journal of Applied Ecology*, **44**, 875–884.
- Oberhänsli, H., Weise, S.M. & Stanichny, S. (2009) Oxygen and hydrogen isotopic water characteristics of the Aral Sea, Central Asia. *J Marine Systems*, **76**, 310–321.
- Powell, L.A. & Hobson, K.A. (2006) Enriched feather hydrogen isotope values for Wood Thrushes sampled in Georgia, USA, during the breeding season: implications for quantifying dispersal. *Canadian Journal of Zoology*, **84**, 1331–1338.
- Root, B.G., Fritzell, E.K. & Giessman, N.F. (1988) Effects of Intensive Hunting on White-Tailed Deer Movement. *Wildlife Society Bulletin*, **16**, 145–151.
- Royle, J.A. & Rubenstein, D.R. (2004) The role of species abundance in determining breeding origins of migratory birds with stable isotopes. *Ecological Applications*, **14**, 1780–1788.
- Rubenstein, D.R. & Hobson, K.A. (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology & Evolution*, **19**, 256–263.
- Rundel, P.W., Ehleringer, J.R. & Nagy, K.A. (1989) *Stable Isotopes in Ecological Research*. Springer Verlag, New York.
- Schell, D.M., Saupe, S.M. & Haubenstock, N. (1989) Natural isotope abundances in baleen: markers of ageing and habitat usage. *Ecological Studies*, **68**, 260–269.

- Schwartz, C.C. & Franzmann, A.W. (1992) Dispersal and Survival of Subadult Black Bears from the Kenai Peninsula, Alaska. *Journal of Wildlife Management*, **56**, 426–431.
- Smith, J.L.D. (1993) The role of dispersal in structuring the Chitwan tiger population. *Behavior*, **124**, 165–195.
- Stoner, D.C., Rieth, W.R., Wolfe, M.L., Mecham, M.B. & Neville, A. (2008) Long-Distance Dispersal of a Female Cougar in a Basin and Range Landscape. *Journal of Wildlife Management*, **72**, 933–939.
- Sweanor, L.L., Logan, K.A. & Hornocker, M.G. (2000) Cougar Dispersal Patterns, Metapopulation Dynamics, and Conservation. *Conservation Biology*, **14**, 798–808.
- Thompson, D.J. & Jenks, J.A. (2005) Long-distance dispersal by a subadult male cougar from the black hills, South Dakota. *Journal of Wildlife Management*, **69**, 818–820.
- Tischendorf, J.W. & Henderson, F.R. (1995) The puma in the central mountains and Great Plains. *Twelfth Great Plains Wildlife. Damage Control Workshop Proceedings* (eds R.E. Masters & J.G. Huggins), pp. 99–102. Published by Noble Foundation, Ardmore, OK.
- Wassenaar, L.I. & Hobson, K.A. (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for animal migration studies. *Isotopes in Environmental and Health Studies*, **39**, 1–7.
- Wassenaar, L.I. & Hobson, K.A. (2006) Stable hydrogen isotope heterogeneity in biological tissues: Isotope-Ratio Mass Spectrometry and migratory wildlife sampling strategies. *Rapid Communications in Mass Spectrometry*, **20**, 2505–2510.
- Weckerly, F.W. & Nelson Jr, J.P. (1990) Age and Sex Differences of White-Tailed Deer Diet Composition, Quality, and Calcium. *Journal of Wildlife Management*, **54**, 532–538.
- Wilson, S., Hoffman, J.D. & Genoways, H.H. (2010) Observations of reproduction in mountain lions from Nebraska. *Western North American Naturalist*, **70**, 238–240.
- Wisconsin Department of Natural Resources, April 29, 2008. *Milton Cougar was the same as Chicago Cougar*. [http://dnr.wi.gov/org/land/er/mammals/cougar/milton\\_cougar\\_timeline.htm](http://dnr.wi.gov/org/land/er/mammals/cougar/milton_cougar_timeline.htm) (accessed May 2010).
- Wydeven, A.P., Fuller, T.K., Weber, W. & MacDonals, K. (1998) The potential for wolf recovery in the northeastern United States via dispersal from southeastern Canada. *Wildlife Society Bulletin*, **26**, 776–784.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** Stable hydrogen ( $\delta\text{D}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotopic values along two claws of the Saskatoon cougar – 41 mm long (white) and 49 mm long (black).

**Fig. S2.** Stable hydrogen and carbon isotopic values measured in claw samples and expected along each dispersal corridor. For each cougar, we determined the expected stable isotopic values along each corridor  $\Omega_r$  (lines) by adding the cougar-specific discrimination factor to isotopic values extracted from the regional  $\delta\text{D}$  and  $\delta^{13}\text{C}$  isoscapes (for the Gretna and Valentine cougars) or large-scale  $\delta\text{D}$  isoscape (for the Chicago and Saskatoon cougars). We considered a mean speed of 3 km/day (which corresponds to a mean distance of 5 km integrated per 1% of claw) for the Valentine and Chicago cougars, and 5 km/day (7.5 km/% of claw) for the Gretna and Saskatoon cougars. For each cougar,  $\delta\text{D}_{\text{claw}}$  at  $i = 1.0$  corresponds to the mean  $\delta\text{D}_{\text{claw}}$  in samples  $i \geq 0.85$  (proximal part of the claw). The unexpectedly low measurements in the tip and the root samples of the Chicago cougar were not considered in the analysis and are not shown on this graph (see text for details). Note variations in y-axis values among graphs.

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