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Long-distance dispersal of a subadult male cougar from South Dakota to Connecticut documented with DNA evidence

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We report the long-distance dispersal of a subadult male cougar (*Puma concolor*) from South Dakota to Milford, Connecticut, where it was struck and killed by a vehicle. Genetic samples suggest this animal originated from the Black Hills of South Dakota while isotope analysis and physical inspection revealed no evidence that the animal had been held in captivity. We detected this dispersing individual at 5 locations along its route (Minnesota, 3 times in Wisconsin and New York) with DNA from fecal or hair samples, and with multiple photographs from citizen-run camera traps (3 in Wisconsin and 1 in Michigan). The > 2,450 km straight-line distance (Black Hills of South Dakota to Connecticut) traveled by the cougar is the longest dispersal documented for the species. We propose a likely route of > 2,700 km over 2 years based on topography and our confirmed records. We suggest that this excessive movement was motivated by the absence of female cougars along the route. The documentation of such a rare biological event not only shows the great dispersal potential for male cougars but also highlights our ability to detect these movements with verifiable voucher DNA and photographic records. Evidence collected for this one animal, and complete absence of verifiable data from most anecdotal reports of cougars in the east, further confirms the lack of a breeding population in the region.

Key words: Black Hills, Connecticut, eastern cougar, long-distance dispersal, Minnesota, mountain lion, *Puma concolor*, South Dakota, Wisconsin

Although cougars (*Puma concolor*) were historically distributed across North America, they were eliminated from most of the eastern half of the continent by the late 1800s through a combination of habitat change and direct persecution (Cardoza and Langlois 2002). Cougars persisted in western states and provinces, with many populations making a significant recovery since managed as a game species starting in the 1960s (Sweanor et al. 2000). Modern cougar range covers most of western North America with the easternmost breeding populations occurring in relatively small subpopulations in the Dakotas, Nebraska, the Cypress Hills in Canada, as well as southern Florida (Supporting Information S1).

The last confirmed wild cougar in the eastern United States (excluding Florida) was killed near the Maine/Quebec border in 1938 (Wright 1961; Cardoza and Langlois 2002; Lang et al. 2013). Since then, State and Provincial wildlife agencies and the United States Fish and Wildlife Service (USFWS) have received numerous reports of sightings of cougars throughout the eastern United States and Canada. Over 2,000 sighting reports have been received since 1983 alone (Lang et al. 2013). Other than a few confirmations of individual males in eastern Canada (Lang et al. 2013; Rosatte et al. 2015), most of these reports had no physical evidence needed to confirm the presence of an extirpated species (McKelvey et al. 2008), and no investigations (e.g., camera traps, track investigations) have been able to confirm any wild cougar populations in eastern North America (outside of Florida). Most sightings in the east are presumed to be of other species (Van Dyke and Brocke 1987), other than a few cases of cougars briefly escaping from captivity (Cardoza and Langlois 2002). Given the absence of any physical evidence of a cougar population in the eastern United States, the USFWS officially declared the eastern cougar subspecies extinct (McCollough 2011).

However, the recovery of cougar populations in the west has led to the dispersal of young cougars eastward, with many reaching Midwestern states such as Kansas, Missouri, Illinois, Michigan, and Wisconsin (LaRue et al. 2012). Many of these animals move long distances, through suboptimal habitat. For example, cougars from the Black Hills of South Dakota have been recorded dispersing 1,300 km to Chicago (where one was shot by police—Wiedenhoeft and Wydeven 2009) and 1,067 km to Oklahoma (Thompson and Jenks 2005). Most dispersing cougars are subadult males, which are more likely than females or adults to travel in search of a mate, but cannot establish a new population on their own (LaRue et al. 2012). Here we report the long-distance dispersal of a subadult male cougar that likely originated from the Black Hills of South Dakota, through the Midwest to Milford, Connecticut, and how genetic analysis was used to identify and document this unique event.

Materials and Methods

On 11 June 2011, a 64-kg male cougar was struck and killed by a vehicle on the Wilbur Cross Parkway in Milford, Connecticut (latitude 41.2500°N, longitude 73.0756°W). We collected the carcass and initiated an extensive biological investigation to determine the animal's origin (Supporting Information S2). We examined the carcass for evidence of past captivity (e.g., neutering, poor body condition, presence of tags or a collar, declawing, tooth wear, pad wear). We obtained full body, orthogonal radiographs to determine the presence of subdermal tags and current or past skeletal injuries. We collected and identified the contents of the gastrointestinal system (including internal parasites by species). We also performed a full necropsy to determine the cause of death and assess the presence of any underlying disease, condition, or abnormality (e.g., gunshot, starvation) (Supporting Information S2). We removed an upper premolar tooth which was cross-sectioned and aged via cementum annuli (Matson 1981). We collected and analyzed samples of hair and rib bone (New York State Museum specimen number zm-16024) for carbon isotope values following the protocols described in Kays and Feranec (2011).

We collected a tissue sample from the tongue, which was extracted using standard protocols and examined at 3 regions of mitochondrial DNA: 16SrRNA, ATPase-8, and NADH-5 (Culver et al. 2000). We subsequently analyzed the DNA using a panel of 20, variable microsatellite loci (nuclear DNA—Juarez et al. 2016) to evaluate the population of origin for this individual using a comparison database that included numerous samples from cougar populations across the western United States (Schwartz et al. 2007; Rosatte et al. 2015). We compared the genetic profile obtained from this individual to a database of various individual dispersing cougars detected through collection of feces and hair samples in Minnesota and western Wisconsin between December 2009 and February 2010 (Wiedenhoeft et al. 2011). We also extracted, analyzed, and compared mitochondrial DNA from a hair sample that was collected from a bedding site in the snow on 17 December 2010 near Lake George, New York (while state officers were investigating a reported cougar sighting) with DNA from the carcass of the Connecticut cougar (Hynes 2011).

Results

The cause of death of this cougar was impact with a motor vehicle, which fractured 5 ribs and caused extensive damage to internal organs. The exam and necropsy revealed no evidence that indicated this animal was ever held in captivity. All claws were present, foot pads were normally calloused, the reproductive system was intact, no subdermal tags, external tags, tattoos, or other markings were present. The animal appeared lean and fit with minimal body fat. Quills from North American porcupine (*Erethizon dorsatum*) were found subcutaneously in the front legs, face, trunk and rump, and in the stomach. A trace amount of hair collected in the stomach was determined (through gross morphological examination) to be that of a cervid (family Cervidae), and from a felid (family Felidae, likely hair ingested by the cougar during grooming). Tapeworms collected from the small intestine were identified as *Taenia omissa*, which is commonly found in North American cougars and for which the white-tailed deer (*Odocoileus virginianus*, a common prey species) is an intermediate host. The estimated age, determined via cementum annuli, was 3 years at the time of death.

Carbon isotope values were −21.3 and −20.6 for the hair and bone samples, respectively. These values are typical for an animal consuming wild prey in the Midwest and northeastern United States, whereas a typical captive diet including corn-based pet foods or grain fed livestock would have had much higher values (Kays and Feranec 2011). The isotope values also indicate that the animal was eating a similar diet over the short term (from the hair sample) and long term (from the bone sample), also suggesting a relatively consistent diet over the life of the animal.

The cougar was found to have mitochondrial DNA consistent with haplotype "M," which is widespread in North American cougars (Culver et al. 2000; Culver and Schwartz 2011). Structure analysis indicated that genetically, this animal was most closely related to the subpopulation of cougars found in the Black Hills of South Dakota (or one of the related nearby subpopulations in Nebraska or North Dakota). Assignment tests showed this animal had a 99.9% chance of originating from the South Dakota cougar population compared to other populations in the database.

The genetic profile matched an individual male cougar in the database (referred to as the "St. Croix Cougar"), which had been detected (via DNA) in 4 different locations in December

2009 and February 2010 from hair and scat samples collected in Minnesota and Wisconsin (Supporting Information S1). The probability that 2 individuals would have matching genetic profiles by random chance was 1.17×10^{-15} (i.e., less than 1 in 854,000,000,000,000). The probability of the 2 individuals having the same genetic profile assuming they were siblings instead of random chance was 1.26×10^{-6} or 1 in 795,544.

The quality of DNA collected from the Lake George, New York sample was marginal; but a partial genetic profile from this hair sample was obtained. Specifically, 8 of 20 loci amplified and matched that of the St. Croix Cougar killed in Connecticut. The probability that 2 individuals with the genetic profile of the New York cougar and the St. Croix cougar would match by random chance was 2.893×10^{-6} (i.e., less than 1 in 345,000). The probability of the 2 individuals sharing the same genetic profile assuming they were siblings instead of random chance was 5.35×10^{-3} or 1 in 187.

The St. Croix cougar was first documented via DNA in Ramsey County, Minnesota on 8 December 2009, a straight-line distance of 1,697 km west of its death site in Connecticut, the longest straight-line distance movement ever documented by a cougar. However, this animal most likely originated from the Black Hills of South Dakota, given the genetic assignment test results and the absence of a breeding population of cougars in Minnesota, suggesting a straight-line distance > 2,450 km (Supporting Information S1).

Discussion

Our analysis indicated that the St. Croix cougar killed in Connecticut was a naturally dispersing animal that likely originated in South Dakota and moved a straight-line distance > 2,450 km in 2 years. This dispersal is ecologically significant because it further demonstrates the dispersal potential of cougars and highlights the importance of verifiable evidence to document these rare movements.

Perhaps the most remarkable aspect of this event was our ability to identify an individual dispersing animal through DNA, 6 times along its path from South Dakota to Connecticut (including the place of death in Connecticut). These DNA samples show the power of modern genetics to not only identify individual animals but also estimate place of origin, related to reference populations (the Black Hills in this case). Genetic matches of feces and hair collected along the route helped to reconstruct the movement of the St. Croix cougar from 1 location in Minnesota, 3 locations in Wisconsin, and 1 location in New York, before being killed in Connecticut (Wiedenhoeft et al. 2011; Supporting Information S1).

Nine other confirmed cougar sightings (tracks or photographs, no DNA collected) in eastern Minnesota, northwestern Wisconsin, northeast Wisconsin, and the eastern Upper Peninsula of Michigan in 2009 and 2010 also may have been the St. Croix cougar traveling eastward (Wiedenhoeft et al. 2011). Four of these confirmed sightings were photographs captured by citizen-run camera traps (Supporting Information S1 and S3). While camera traps cannot typically identify individuals to the same level of certainty as DNA evidence, these 4 photographs (3 in Wisconsin and 1 in Michigan) do fit into the dispersal timeline for the St. Croix cougar and add valuable insight into the likely path of travel for this animal (Supporting Information S1). The documentation of physical evidence through tracks and camera-trap photographs also provides an example of how citizen science can help study rare species on the landscape (McShea et al. 2016).

Despite the unusual abundance of evidence documenting the dispersal of the St. Croix cougar, there is no way to confirm the exact path the animal traveled from South Dakota to Connecticut. We can only attempt to create a likely path by connecting DNA confirmed locations. The largest mystery remains the route traveled from the Midwest to New York. The straight-line distance from the 1st DNA confirmation in Minnesota to the place of death in Connecticut was nearly 1,700 km, and from the likely place of origin in the Black Hills of South Dakota to Connecticut, > 2,450 km (Supporting Information S1). In both cases, these straight-line distances pass through the Great Lakes system. The most likely route based on large-scale landscape features was through the Upper Peninsula of Michigan, east through Ontario, and south into New York (Supporting Information S1). This is also the path that has been suggested for the eastward expansion of coyotes (*Canis latrans*—Kays et al. 2010). If this was in fact the path the animal traveled, then the minimum dispersal distance would have been nearly 2,700 km. We suggest that if an animal such as a cougar traveled south of the Great Lakes, it would have likely been detected multiple times, particularly around the Chicago, Illinois metropolitan area (Wiedenhoeft and Wydeven 2009).

The cougar population in the Black Hills of South Dakota was estimated at about 250 breeding animals in 2009 (Juarez et al. 2016). Jansen and Jenks (2012) found that while cougars in the Black Hills can give birth year round, a significant birth pulse occurs between June and August, and that most male cougars disperse between 13 and 17 months of age. The St. Croix cougar was 3 years old at the time of death in June 2011, which suggested an approximate birth in spring or summer 2008, and dispersal from the natal range (Black Hills) would likely have occurred in fall 2009. Assuming a dispersal distance of 2,700 km, the St. Croix cougar would have traveled a minimum net rate of 2.74 km per day (2,700 km in 983 days), similar to what has been documented for radiocollared dispersing cougars (Stoner et al. 2008). However, it is likely this animal traveled a much greater actual distance to reach its final location. Radiocollared dispersing cougars have been shown to meander greatly, between 4 and 7 times the distance of the straight-line dispersal distance (Stoner et al. 2008; Elbroch et al. 2009).

Male cougars are well-known long-distance dispersers, although never to the extent documented here. Thompson and Jenks (2005) reported a 1,067 km straight-line dispersal movement of a male cougar from the Black Hills to Oklahoma in 2004. Morrison et al. (2015) documented a male cougar in Canada that moved 749 km in 100 days. Logan and Sweanor (2000) documented a dispersal movement of a male cougar from the Bighorn Mountains in Wyoming, 483 km south to the Denver, Colorado area. Maehr (1997) documented a male Florida panther (*Puma concolor coryi*) that dispersed 208 km north into Georgia. The maximum dispersal distance reported by Anderson et al. (1992) in Colorado was 274 km. The straight-line distance moved by the St. Croix cougar surpasses the previous record by almost 1,400 km.

The St. Croix cougar also provides an example of how dispersing cougars can move great distances through fragmented landscapes, giving them the potential not only to colonize new areas, but also to maintain gene flow of existing isolated populations. Morrison et al. (2015) found that dispersing subadult male cougars in Canada moved mostly at night through open and fragmented habitat when vehicle traffic and human presence were minimal, allowing them to safely traverse unfamiliar landscapes. While suitable habitat and prey are necessary for cougar survival, they are not the driving force behind long-distance dispersal. Morrison et al. (2015) described cougar dispersal as a 3-stage process including emigration, transience, and settlement. The 1st stage of cougar dispersal, emigration, is likely density dependent. Young cougars likely leave their natal range to reduce competition for mates and resources (Morrison et al. 2015). Thompson and Jenks (2010) suggested that the driving force during the transience stage is the instinct to locate an available mate. Cougars will use less than suitable habitat during dispersal and will even continue moving through areas with adequate habitat and prey until they locate a mate. The St. Croix cougar likely passed through an abundance of suitable habitat with high prey biomass (white-tailed deer) as it traveled eastward.

Cougar dispersal is strongly sex biased, with subadult males moving farther in search of breeding opportunities, while females need only find suitable hunting grounds (Stoner et al. 2008). Of the 38 known-sex dispersing cougars killed in the Midwest from 1990 through 2008, 76% were male (LaRue et al. 2012). Most of these young male dispersers presumably moved such incredible distances because they were unable to locate a female mate, despite the otherwise suitable habitat and abundant prey likely encountered along the way (Thompson and Jenks 2010). The St. Croix cougar obviously never reached the 3rd stage of dispersal, settlement, which occurs when an available mate is located (Morrison et al. 2015).

The St. Croix cougar was the first documented wild cougar in Connecticut in nearly 200 years. The documentation of a single male cougar dispersing from South Dakota to Connecticut, however, does not suggest that cougars will recolonize the eastern United States in the near future. Establishing a cougar population in the eastern United States would require that both male and female cougars disperse eastward from established range. Unlike males, female cougar dispersal distance from the natal home range is usually less than 15 km, if dispersal occurs at all (Sweanor et al. 2000). Approximately 50% of female cougars remain philopatric and do not disperse. Exceptions do occur however, and long-distance dispersing female cougars have been documented (LaRue and Nielsen 2016). Stoner et al. (2008) documented a GPS-collared subadult female cougar that moved an actual distance of 1,341 km from Utah to Colorado over a time period of 1 year. The straight-line distance moved, however, was 357 km, still an extraordinary distance for a female cougar to move. Nevertheless, only one female cougar has been detected east of the Missouri River in Minnesota, and no females have been detected east of the Mississippi River. In order to establish a breeding population in the eastern United States, both male and female cougars would need to successfully disperse thousands of kilometers through highly developed and agricultural habitats. In fact, it required nearly 20 years of dispersal through relatively undeveloped habitat for a breeding population of cougars to establish a population in the Pine Ridge area of Nebraska from the Black Hills of South Dakota, which is less than 160 km to the southeast (LaRue and Nielsen 2016).

LaRue et al. (2012) suggested that cougars fit the stepping stone dispersal model because females tend to establish home ranges adjacent to, or even overlapping their mother's home range. Given this model, expanding South Dakota cougars would need to establish breeding populations progressively eastward throughout the Midwest, well before reaching the northeast. Also, recent harvest protocols in South Dakota and Nebraska are limiting the number of cougars dispersing eastward, thus slowing the process of establishing populations in patches of suitable habitat east of current established cougar range (LaRue and Nielsen 2016). However, even considering cougar harvests in the easternmost populations in South Dakota and Nebraska, LaRue and Nielsen (2016) suggest a small breeding population of cougars is likely to establish suitable habitat in Midwestern states such as Minnesota and Wisconsin within the next 25 years. The presence of small, isolated populations in the Midwest, however, still does not suggest cougars will reestablish in the northeastern United States anytime soon.

The level of documentation of the dispersal of the St. Croix cougar is in stark contrast to the complete lack of verifiable physical data from any of the numerous reported cougar sightings across the eastern United States over the last few decades (Cardoza and Langlois 2002; McCollough 2011). This finding highlights the futility of using citizen sighting reports that lack physical evidence (tracks, photographs, or DNA) as occurrence data for rare or elusive species, and the importance of voucher material that can be examined by multiple scientists, using multiple tools, as part of a scientific evaluation (McKelvey et al. 2008). Thus, the St. Croix cougar is relevant to the extensive public debate over whether a population of cougars exists in the eastern United States (outside of Florida) by showing how unlikely it is for a large terrestrial predator to move undetected though the region (see also Moriarty et al. 2009 for similar circumstances with a wolverine [*Gulo gulo*] and CDFW [California Department of Fish and Wildlife 2015] for wolves [*Canis lupus*] dispersing long distances and being detected).

While potentially expensive and time consuming, genetic identification of individual wild animals from hair, scat, or blood can provide valuable information for species of management or conservation concern. In certain situations, these techniques also can provide information on range-expansion, recolonization, and dispersal behavior (Juarez et al. 2016). Diagnostic assays that use molecular genetic markers allow for the identification of individual animals, and in some cases geographic origin (Schwartz et al. 2007). In the case of the St. Croix cougar, it allowed for the identification of the individual animal, the likely path of dispersal and the subpopulation of origin. This also highlights the need for state and provincial wildlife agencies to establish rare species monitoring protocols. While most reports of cougars in eastern North America turn out to be a case of mistaken identity, if wildlife officials do not investigate the more reliable reports, opportunities to collect valuable evidence could be lost (Hamilton 2006).

The appearance of the St. Croix cougar in Connecticut offers a contrasting set of perspectives on eastern cougar populations. On one hand, it demonstrates that wild cougars can naturally disperse into the region. On the other hand, it shows that even single animals moving through remote areas will still likely be detected by multiple verifiable monitoring protocols and, therefore, that the existence of any relict breeding populations of cougars existing in the region is highly unlikely. Furthermore, the geographic, social, and biological barriers that exist indicate the possibility of a new breeding population in the region is many decades or centuries away. Nonetheless, the St. Croix cougar is a symbol of hope for wildlife conservationists who would like to see the return of large predators to their historic range and serves as new motivation to monitor local mammal populations through fecal (and other DNA evidence) collection and camera trapping with the hope of documenting future dispersals of large predators from their current established range.

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Supporting Information

The Supporting Information document is linked to this manuscript and is available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The material consists of data provided by the author that is published to benefit the reader. The posted material is not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—White dots represent locations of St. Croix cougar confirmed through DNA analysis. White triangles represent probable locations of St. Croix cougar based on photographs taken at the sites. The white line represents probable travel route. The orange shaded area represents current cougar range in North America, including the easternmost breeding populations in the Black Hills of South Dakota, the Badlands of North Dakota, the Pine Ridge area of Nebraska, and Florida. (1) DNA extracted from scat collected on 12/8/09 in Ramsey County, MN. (2) DNA extracted from hair collected on 12/17/09 in St. Croix County, WI. (3) DNA extracted from hair collected on 12/19/09 in Dunn County, MN. (4) Probable trail camera photo of St. Croix cougar taken on 1/18/10 in Clark County, WI. (5) Probable trail camera photo of St. Croix cougar taken on 1/20/10 in Clark County, WI. (6) DNA extracted from scat collected on 2/15/10 in Bayfield County, WI. (7) Probable trail camera photo of St. Croix cougar taken on 5/20/10 in Oconto County, WI. (8) Probable trail camera photo of St. Croix cougar taken on 5/26/10 in Menominee County, MI. (9) DNA extracted from hair collected on 12/16/10 in Warren County, NY. (10) DNA extracted from vehicle-killed carcass collected on 6/11/11 in New Haven County, CT.

Supporting Information S2.—Necropsy being performed on the St. Croix cougar in Connecticut.

Supporting Information S3.—Probable photo of St. Croix cougar taken by a trail camera on 1/18/10 in Clark County WI (Lue Vang, WI).

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