

# Notes

## Cougars, *Puma concolor*, in Ontario: Additional Evidence

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Recent evidence suggests that the Cougar (*Puma concolor*) has returned to New Brunswick, Quebec, Manitoba, and Ontario. An abundance of sightings have been reported for many decades throughout south-central Ontario, but genetic confirmation has been confounded by a lack of carcasses or DNA. In this paper, we identify (1) genetic evidence of a single Cougar in the wild of Ontario, (2) a gene (cytochrome b) and methodology to distinguish the Cougar from other mammals in Ontario using scats, hair follicles, and soft and hard tissue, and (3) a gene that can distinguish individual Cougars from each other and would distinguish populations of subspecies if they exist in Ontario. Potential Cougar scats and other tissue samples were collected from across Ontario, and hair snares baited with catnip and carnivore lure were placed in locations where Cougar sightings were frequent, near Sudbury, Ontario. We analyzed samples for mtDNA, and one scat sample from the Wainfleet Bog Conservation Area, Port Colborne, Ontario, was positive for Cougar. Evidence from archeological data and Cougar sightings suggests that the historical range of Cougars extended in Ontario from the Great Lakes–St. Lawrence Ecotone south, primarily associated with the Eastern Deciduous Forest Biome and the primary prey of the Cougar, the White-tailed Deer (*Odocoileus virginianus*) and the North American Elk (*Cervus elaphus*). The data suggest that Cougar distribution has moved north in recent times due to clear-cutting, along with their primary prey. Further studies of Cougar presence in the province are warranted.

Key Words: Cougar, Puma, Mountain Lion, *Puma concolor*, scats, hair, mtDNA analysis, hair-snares, Ontario.

The present distribution of the Cougar (*Puma concolor*) in North America is restricted mainly to the western mountain ranges and the southeastern United States, primarily Florida. Cougars were extirpated from most of eastern and central North America and still are persecuted in some jurisdictions (Ross et al. 1995; Anderson et al. 2010; Garcia Alaniz et al. 2010a). In 1978, the eastern subspecies of the Cougar was declared endangered by the Committee on the Status of Endangered Wildlife in Canada; however, the status was re-examined in 1998 and the animal was designated data deficient, because there was not enough data to evaluate the status of the animal or its classification as a subspecies. Recent evidence suggests that the Cougar has returned to New Brunswick, Quebec, Manitoba, and Ontario (Nero and Wrigley 1977; Rosatte 2011). Our study provides genetic data confirming the presence of cougar in Ontario.

White-tailed Deer (*Odocoileus virginianus*) and North American Elk (*Cervus elaphus*) have been identified as the primary prey of Cougar in the U.S. and Canada, with Mule Deer (*O. hemionus*) replacing White-tailed Deer in the west (Hornocker 1970; Seidensticker et al. 1973; Dixon 1982; Belden and Maehr 1986). Historically, White-tailed Deer and North American Elk were found in the Eastern Deciduous Forest

Biome in southern Ontario and to a lesser extent in the Great Lakes–St. Lawrence Ecotone (Seton 1909, 1927; Gleason and Cronquist 1964; Rowe 1972; Bryant and Maser 1982; Baker 1984).

The primary objectives of this study were; (1) to identify Cougar in the wild of Ontario; (2) to identify a gene (cytochrome b) and methodology to distinguish Cougar from other mammals in Ontario using scats, hair follicles, and soft and hard tissue; (3) to identify a gene that could distinguish individual Cougars from each other and would distinguish populations of subspecies if they exist in Ontario; and (4) to discuss possible changes in Cougar distribution across the province.

### Study Area

Two transects 2 km in length were located in two sites (one north of Sudbury, Ontario, and one south of Sudbury) (80°W, 45°N), where Cougar sightings were frequent. The location north of Sudbury was primarily abandoned agricultural land composed of old fields and drainage ditches with secondary deciduous forest, such as birch (*Betula*) and poplar (*Populus*) along the edges. The location south of Sudbury was in the French River region and consisted of rock outcrops with relatively mature mixed deciduous and conifer-dominated forest cover.

## Methods and Materials

### Hair morphology

Hair snares baited with catnip and carnivore lure (Wildcat Lure #2, Hawbaker's Wildcat Lures, Hawbaker and Sons, Fort Loudon, Penn.) were placed along each transect (Weaver et al. 2005; Garcia Alaniz et al. 2010b). Hair snares were made from pieces of carpet 12 × 17 cm with two strips of hook and loop fasteners 7 cm in length and five roofing nails that projected through the carpet (Garcia Alaniz et al. 2010b). We placed these snares along game trails at both sites. At the northern site, 23 hair snares were nailed approximately 0.5 m from the ground every 30 m on tree trunks and stumps. Hair snares were put out on 10 September 2006 and retrieved on 14 October 2006. At the southern site, 28 hair snares were similarly put out, on 22 September 2006 and retrieved on 24 October 2006. On retrieval, hair snares were placed in re-sealable plastic bags, labelled and the GPS coordinates were recorded using a Garmin hand-held GPS Unit (Model GPS 72HBUNDLE).

Hairs were subsequently removed from the hair snares using tweezers and a magnifying glass, and imprints were made by pressing each hair into a thin layer of clear nail polish (Lady Clairol) applied to a clean microscope slide (Garcia Alaniz et al. 2010b). After 2 to 3 minutes, the hair was slowly removed, leaving an imprint of the scale pattern. Hairs were analyzed using a compound microscope at 400× magnification and identified based on scale patterns described in guard hair keys by Adorjan and Kolenosky (1969) and Garcia Alaniz (2009).

### DNA analysis

Potential Cougar scats, hair, and bone were collected from volunteers across southern and central Ontario between 2003 and 2006. Eleven samples were collected. All 11 samples were placed in re-sealable plastic bags, maintained in a conventional freezer (approximately -20°C), and later analyzed for mtDNA. In addition, all hairs identified as Cougar from the Sudbury hair snare study were also sent for mtDNA analysis to the Natural Resources DNA Profiling and Forensic Centre in Peterborough, Ontario.

All samples were analyzed using the QIAamp (Qiagen) DNA extraction protocol. Extracted DNA from each sample was quantified using a fluorometer-based picogreen assay on the BMG FluoStar Galaxy 96-well plate system. A dilution series of DNA from each sample was analyzed using an amplified region of the cytochrome b gene on mitochondrial DNA to assess the quality of each sample. A sub-region of the cytochrome b gene on the mtDNA was subsequently amplified and run on a MegaBACE 1000 DNA analysis system.

The sequences from each hair sample were compared to control sequences from Gene Bank for Canada Lynx (*Lynx canadensis*), Bobcat (*L. rufus*), American Beaver (*Castor canadensis*), Cougar, Red Fox

(*Vulpes vulpes*), White-tailed Deer, and Humans (*Homo sapiens*).

### Historical data, recent Cougar sightings, and distribution of North American Elk and White-tailed Deer

Cougar trapping records from 1919 to 1984, as well as more recent sighting reports for Ontario (Rosette 2011) and adjacent provinces and states, were gleaned from the literature (Wright 1953; Nero and Wrigley 1977; Anonymous 1987; Gerson 1988; Cumberland and Dempsey 1994; Stocck 1995; Swanson and Rusz 2006) and compared to White-tailed Deer and North American Elk distribution across Ontario (Seton 1909, 1927; Bryant and Maser 1982; Baker 1984).

## Results

### Hair morphology

We collected a total of 298 hairs from 51 hair snares; 180 were analyzed. The other 118 hairs were either under-fur or fragments of guard hairs. A total of 8 Cougar hairs from 5 hair snares (3 from the mixed deciduous-conifer site and 2 from the old field site) were identified by their scale pattern. Seventy-six hairs from the old field site, which was associated with residential housing, were identified as Domestic Cat (*Felis catus*) and 21 as Domestic Dog (*Canis familiaris*). Thirty-one Deer Mouse (*Peromyscus maniculatus*), 8 American Black Bear (*Ursus americanus*), 4 Fisher (*Martes pennanti*), 3 Canada Lynx, and 2 Coyote (*C. latrans*) hairs were also identified. Deer Mouse and Fisher hairs were found at both sites. American Black Bear and Lynx hairs were found only at the mixed deciduous-conifer site, and Coyote hair was found only at the old field site. The 8 potential Cougar hairs were subsequently subjected to DNA testing to confirm identity.

### DNA analysis

Sequence comparison and phylogenetic analysis revealed that, of the 11 samples collected from across Ontario, scat sample no. 5 collected in 2004 from the Port Colborne/Wainfleet Bog area (79°W, 43°N) had low sequence divergence from and a statistically significant grouping with the control sequence for Cougar (Figure 1). A sequence comparison and phylogenetic analysis of the 10 other samples collected by volunteers did not reveal Cougar DNA. Scat samples 6, 8, and 10 from across Ontario indicated that the mtDNA originated from canids, the sample labelled no. 1 originated from American Beaver, and sample no. 3 originated from Lynx. Hair samples 2, 4, 7, 9, and 11 did not generate the quality or quantity of mtDNA to allow analysis. Due to a laboratory mix-up, none of the 8 hairs identified morphologically as Cougar from the Sudbury region hair snare study generated the quality of mtDNA required to identify them and unfortunately all were destroyed in the process.

Control Sample
GTGGCTATGACCCGAAATAGTAGTATGATTCCAATATTTTCATGTTCCGAGAAGGT <b>ATAGGAGCCATAATATATTC</b> CTCGTCCCACGTGCATGTATAGGCAGATAAAGAATA
Wild Sample from Port Colborne/Wainfleet Bog
GTGGCTATGACCCGAAATAGTAGTATGATTCCAATATTTTCATGTTCCGAGAAGAT <b>GTAGATGCCATAATATATTC</b> CTCGTCCCACGTGCATGTATAGGCAGATAAAGAATA

FIGURE 1. Sequence comparison and phylogenetic analysis revealed that sample no. 5 collected in 2004 from the Port Colborne/Wainfleet Bog area (79°W, 43°N) had low sequence divergence from and a statistically significant grouping with the control sequence for Cougar (differences in the base sequence between the control and wild sample are in bold).

## Discussion

The mtDNA finding confirms that at least one Cougar was present in the wild in southern Ontario in association with the Niagara Escarpment, which may have been the epicentre of the original range of Cougar in Ontario. The potential Cougar hair samples identified by scale pattern further suggest that Cougar occur in the Sudbury region. Both these results from this study have been previously cited by Rosatte (2011) and further support his data on tracks, scats and photographs that Cougars reside in Ontario.

Although it was thought that an eastern and western subspecies of the Cougar existed (Seton 1909, 1927), recent genetic work by Culver et al. (2000) has determined that only a single subspecies, *P. concolor cougar*, existed north of Mexico. We do not know the origin (e.g., wild resident, disperser, or released captive) of the Cougar identified from sample no. 5 or if it is of this subspecies. However, if there is a breeding population of Cougars in Ontario from whatever genetic source, we need to understand their demographics and ecological impact.

Trapping records between 1919 and 1984 indicated that not a single Cougar pelt was sold in Ontario or the rest of eastern Canada, with the exception of Quebec, which recorded 8 animals sold in 1919-1920 (Anonymous 1987). Similarly, no Cougars were reported in the trapping records of Manitoba, Michigan, New York, Pennsylvania, Ohio, and Minnesota (all adjacent to Ontario) during this same period (Anonymous 1987). In contrast, in the west, 7 animals were recorded from the Northwest Territories, 61 animals were recorded from Alberta, and 3,690 Cougar skins were sold from British Columbia during the same period (Anonymous 1987).

Cougars were largely absent and likely extirpated from Ontario and eastern Canada by the early 1900s. The last Cougar shot in Ontario was in 1884 near Creemore (43°N, 79°W), approximately 20 km west of Barrie, Ontario, and just east of the Niagara Escarpment (Joe Belanger, personal communication).

Historically, the primary prey of the Cougar, White-tailed Deer and North American Elk, were confined to southern Ontario and did not occur north of the Great

Lakes–St. Lawrence Ecotone (Baker 1984). Numerous records and archeological sites in southern Ontario contained artifacts made from White-tailed Deer and North American Elk (Taber et al. 1982; Patrick Julig, personal communication). Evidence of North American Elk has not been found in any northern Ontario sites, and White-tailed Deer appeared only in recent historical times, according to Laurentian University anthropologists, Patrick Julig (personal communication) and biologists (Peterson 1966; Taber et al. 1982).

Prior to extirpation, North American Elk ranged throughout south-central Ontario and Quebec (Peterson 1966); however, by the late 1800s, agriculture and hunting had significantly reduced North American Elk populations (Smith 1901; Seton 1927; Ranta 1979). Between 1893 and 1910, only a few kills and sightings of North American Elk were recorded from North Bay, Ontario (Seton 1927); Elk Lake, Ontario (Bosveld 1996); and Grand Lac Victoria, Quebec (Seton 1927). Gerson (1988) recorded recent Cougar sightings as far north as the Hudson Bay lowlands, including most of the Precambrian Shield area.

Although North American Elk were extirpated from Ontario in the late 1800s, White-tailed Deer moved north in association with clear-cutting and homesteading, and the data suggest that Cougar have recently expanded their range in association with their primary prey, as has been suggested in Manitoba (Nero and Wrigley 1977).

A review of more recent data indicates that Cougars have been confirmed in New Brunswick (Wright 1953; Cumberland and Dempsey 1994; Anonymous 1987), Manitoba (Nero and Wrigley 1977), Ontario (Rosatte 2011) and adjacent eastern states (Wright 1953; Nero and Wrigley 1977; Cumberland and Dempsey 1994; Stoeck 1995; Swanson and Rusz 2006). These data indicate that Cougars may be returning to eastern Canada, although the sources of these animals remain unclear. Nevertheless, sightings of Cougars with kittens and reports of young animals suggest that a breeding population exists in Ontario and adjacent provinces (Wright 1953; Nero and Wrigley 1977; Gerson 1988; Rosette 2011).

The findings from this study (1) confirm the presence of a Cougar occurring in the wild in Ontario, (2)

provide circumstantial evidence from Cougar sightings (Gerson 1988; Rosatte 2011) and hair morphology that a breeding population of Cougar exists in Ontario, and (3) support the hypothesis that the distribution of the Cougar has moved northward in recent times, approximating the line of continuous clear-cut, as have their primary prey, the White-tailed Deer. Further studies of Cougar presence in the province are warranted.

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