Recent Occurrences of Wild-origin Wolves (*Canis* spp.) in Canada South of the St. Lawrence River Revealed by Stable Isotope and Genetic Analysis

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A free-ranging canid killed near Caraquet, New Brunswick, Canada, in 2012 exhibited a mitochondrial DNA sequence of Gray Wolf (*Canis lupus*) origin and a Y-chromosome haplotype of Eastern Wolf (*C. lycaon*) origin. The animal, which is the first wolf recorded in New Brunswick since 1862, was identified as a Gray–Eastern Wolf hybrid (*C. lupus* × *C. lycaon*) based on analysis of its autosomal microsatellite genotype. Stable carbon isotope values ($\delta^{13}C$) suggest that the Caraquet wolf was of wild origin. Likewise, $\delta^{13}C$ analysis suggests that a wolf–coyote hybrid killed in Quebec south of the St. Lawrence River in 2002 was also of wild origin. However, $\delta^{13}C$ values for a wolf from the same region in 2006 suggest that this animal spent most of its life feeding predominantly on non-wild-source food items. Recent occurrences of wild-origin animals south of the St. Lawrence River demonstrate that wolves are capable of dispersal to formerly occupied areas in southeastern Canada and the United States. However, limited natural dispersal alone will likely not be sufficient to re-establish wolves in northeastern North America.

Key Words: Gray Wolf; *Canis lupus*; Eastern Wolf; *Canis lycaon*; Coyote; *Canis latrans*; conservation; stable isotopes; genetic analysis; New Brunswick; Quebec; St. Lawrence River

Introduction

Information concerning the history of the wolf (*Canis* spp.) in Maritime Canada (New Brunswick, Nova Scotia, and Prince Edward Island) is sparse. The scant literature suggests that wolves were rare in the region at the time of European settlement in the early 1600s (Ganong 1908; Scott and Hebda 2004; Sobey 2007). Reports of wolves increased in New Brunswick starting in 1774 and peaked during 1840–1860 (Ganong 1908; Parker 1995). Reports of occurrence in Nova Scotia are likewise limited, with the last known wolf in Nova Scotia killed for bounty in 1845–1847 (Ganong 1908; Scott and Hebda 2004). A bounty on wolves was introduced in New Brunswick in 1792, apparently in response to the loss of domestic sheep; a further New Brunswick bounty on wolves was in effect from 1858 to 1870, with the last bounties paid for three wolves killed in 1862 (Ganong 1908). Historic information on the status of wolves on Prince Edward Island is anecdotal and limited, but suggests that animals may have moved onto the Island over the ice from adjacent New Brunswick and Nova Scotia (Sobey 2007). In 1900, Thaddeus Thurber collected mammals across parts of northern New Brunswick and southern Quebec, reporting at that time that wolves were rare throughout the region, with only a few still present in Quebec south of the St. Lawrence River outside the Gaspé (Elliot 1901). Although wolves may have continued to occur in New Brunswick until 1921 (Lohr and Ballard 1996), Ganong (1908) considered the wolf nearly extirpated in the province by 1867.

Wolves were, therefore, never common in Maritime Canada, and Lohr and Ballard (1996) concluded that there was insufficient historical information to ascertain whether New Brunswick ever supported a minimum viable wolf population. By the early 1970s, following an eastward range expansion, Coyote (*Canis latrans*) were abundant in New Brunswick. Recent evidence suggests that Coyotes now inhabiting eastern Canada are hybrid, having interbred with wolves as they moved east (Kays *et al.* 2010). Regardless, it is now more than a century since any canid deemed to be a wolf has been confirmed to be free-ranging in New Brunswick. Thus, it is significant that in the late winter of 2012 a large wolf-like canid was shot and killed in northern New Brunswick.

Over the past several decades, the few free-ranging wolves reported south of the St. Lawrence River in Canada and through the northeastern United States have generally been considered of wild origin (Elder 2000; Villemure and Jolicoeur 2004). However, Kays and Feranec (2011) recently determined that among eight animals collected in New England, most were of captive origin, although three were likely of wild origin. In addition, the assignment of free-ranging animals to specific types within the genus *Canis*, especially in regions of hybridization among Coyotes, domestic dogs, and wolves, is rarely obvious phenotypically (Chambers *et al.* 2012). The origin and identity of wolf-like canids in the northeastern United States and Canada south of the St. Lawrence River can, therefore, not be assumed, although such occurrences could be significant to potential wolf re-introduction or re-establishment in these regions.

Here we identify the genetic status and origin (wild versus captive) of a purported wolf shot in New Brunswick in 2012. We also report on the origin of two wolves from Quebec south of the St. Lawrence River killed in 2002 and 2006 and discuss the significance of these results. Acknowledging that wolf taxonomy in North America remains controversial, we follow a three-species model (Chambers *et al.* 2012; Rutledge *et al.* 2012) that includes the Gray Wolf (*Canis lupus*), Eastern Wolf (*C. lycaon*), and Coyote (*C. latrans*), and their hybrids, rather than the more traditional two-species approach, *C. lupus* and *C. latrans* (Koblmüller *et al.* 2009; vonHoldt *et al.* 2011).

Methods

Collection and Necropsy

On 6 April 2012, a large canid was shot at a bait station in a regenerating clear-cut forest about 3 km southwest of Caraquet (47.7280°N, 64.9474°W; Figure 1), in northwest New Brunswick, Canada. New Brunswick Department of Natural Resources (NB DNR) personnel collected tissue for genetic analysis and the carcass of the animal was subsequently turned over to NB DNR. A full necropsy of the animal by a qualified veterinary pathologist (JPG) was performed; standard external measurements and body mass were recorded and alimentary tract contents were collected for later examination. The skeleton and taxidermied hide were deposited in the New Brunswick Museum (NBM M11985). We used the tooth wear criteria of Gipson *et al.* (2000) developed for Gray Wolves, in conjunction with a series of wolf skulls from northern Quebec and Labrador deposited in the NBM mammal collection that had been aged by tooth-sectioning (Parker and Luttich 1986), to estimate the age of the animal. We refer to this specimen hereafter as the "Caraquet wolf."

Genetic Analysis

DNA was extracted from tongue tissue of the Caraquet wolf with a Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, Ontario, Canada). Maternal, paternal, and bi-parental genetic markers were investigated for species identification. The mitochondrial DNA (mtDNA) control region was amplified as in Wheeldon *et al.* (2010), and the polymerase chain reaction product was sequenced in both forward and reverse directions on an ABI3730 DNA analyzer (Life Technologies, Burlington, Ontario, Canada). Sequences were edited in MEGA (version 5; Tamura *et al.* 2011), and the consensus sequence was assigned a specific haplotype based on a search of the National Centre for Biotechnology Information sequence database using the Basic Local Alignment Search Tool (BLAST) and comparison with previously described sequences (Wilson *et al.* 2000).

Four Y-chromosome microsatellite loci and 12 autosomal microsatellite loci were genotyped as in Wheeldon *et al.* (2010). The Y-chromosome microsatellite genotype was combined into a haplotype. The autosomal microsatellite genotype of the Caraquet wolf was analyzed in the Bayesian-clustering program STRUCTURE (version 2.3; Pritchard *et al.* 2000; Hubisz *et al.* 2009) using default settings (i.e., F-model, infer alpha), including genotypes from five reference populations (data from Wheeldon 2009; Wheeldon *et al.* 2013): Coyotes from southeastern Ontario (n = 100); Eastern Wolves from Algonquin Provincial Park, Ontario (n = 62); Gray–Eastern Wolf hybrids from northeastern Ontario (n = 62; which are known to cluster together with wolves from parts of Quebec; Wheeldon 2009); Gray Wolves from Northwest Territories (n = 55); and Domestic Dogs (*C. lupus familiaris*; n = 75). The admixture model of STRUCTURE was run five times assuming $K = 5$ for 10^6 iterations following an initial burn-in of 10^5 iterations and Q values for the Caraquet canid were averaged. We used an exclusion test with 10 000 simulated genotypes and the frequencies-based method (Paetkau *et al.* 1995, 2004) in GENECLASS (version 2; Piry *et al.* 2004) to determine the probability of the Caraquet wolf originating from each of the five reference populations.

Stable Isotope Analyses

Stable isotopes are increasingly used in wildlife ecology and forensics to determine the geographic origin or diet of animals (Bowen *et al.* 2005; Moore and Semmens 2008; Hobson *et al.* 2012). Here, we used a multi-tissue stable isotope approach to determine the wild versus captive origin of wolf samples based on short- and long-term trophic history, following the method of Kays and Feranec (2011). The method assumes that captive-origin animals have been fed a diet derived from C_4 plant material (i.e., corn), whereas wild-origin wolves have fed on tissue derived from C_3 plants, which are dominant in northeastern North America. Free-ranging urban or suburban canids may rely on food from domestic sources (i.e., C_4) to some degree. This should be reflected in a carbon stable isotope signature that is intermediate between those of captive and wild animals. As isotope turnover rates vary among tissues, the stable isotope composition of different tissues can incorporate trophic information from specific time periods (Tieszen *et al.* 1983). For mammals, hair samples provide isotopic data since the last molt, while bone should provide information over the entire life of a canid (Kays and Feranec 2011). Kays and Feranec (2011) acknowledge that interpreting such signatures with the limited data currently available can sometimes present challenges, and they review the limitations and caveats of the approach. They also investigated the use of nitrogen stable isotopes to discriminate between wild- and captive-origin wolves, but did not find this useful.

Collagen was extracted from bone (caudal vertebrae, scapula, and metatarsals) and hair of the Caraquet wolf in the manner of Kays and Feranec (2011). To place the Caraquet wolf in a broader context, we also obtained hair and bone for stable carbon isotope analysis ($\delta^{13}C$) from two wolves killed in 2002 and 2006 in Quebec south of the St. Lawrence (Figure 1).

The 29.1-kg male "Lingwick wolf" was snared in January 2002 near the village of Sainte-Marguerite-de-Lingwick (45.6042°N, 71.2875°W). Villemure and Jolicoeur (2004) reported that the mtDNA profile for this animal was consistent with an Eastern Wolf–Coyote hybrid, the microsatellite genotype suggesting 95.0% shared ancestry with Eastern Wolf from Algonquin Provincial Park, Ontario. On this basis, Villemure and Jolicoeur (2004) identified the animal as an Eastern

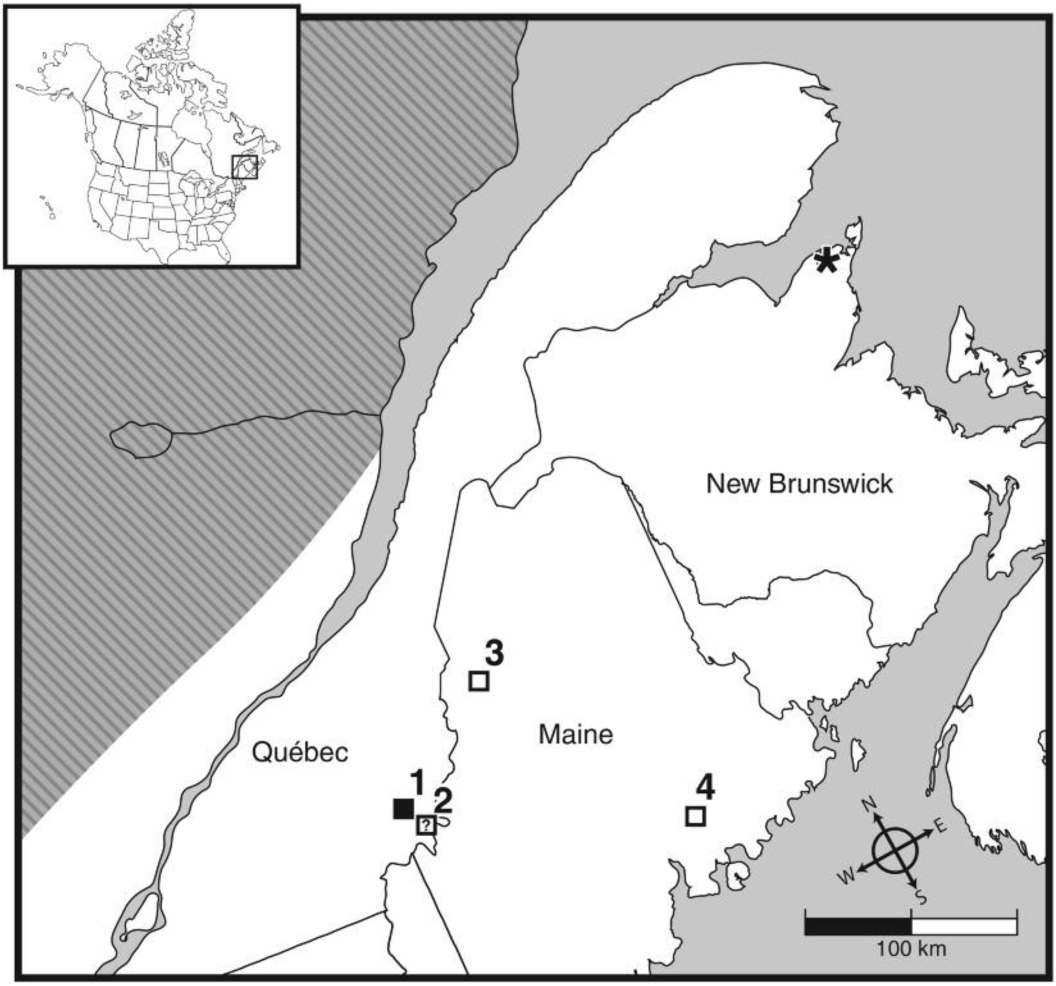

FIGURE 1. Collection location of the Caraquet wolf (*), a Gray–Eastern Wolf hybrid (*Canis lupus* × *C. lycaon*), and extralimital records of wolves in adjacent Maine and Quebec determined by $\delta^{13}C$ to be wild (■), domestic (□), or of unknown origin (?). Wolves 3 and 4 are the most northerly records analyzed by Kays and Feranec (2011). The stippled area marks the southeastern margin of the current distribution of Gray and Eastern Wolves and their hybrids (*C. lupus, C. lycaon, C. lupus* × *C. lycaon*) in Quebec. 1 = Lingwick wolf, 2 = Sainte-Marguerite wolf, 3 = Museum of Comparative Zoology, Harvard (MCZ) 62506, 4 = MCZ 62507.

Wolf. In a more recent study using 12 nuclear markers, this animal clustered with Quebec Coyotes (Stronen *et al.* 2012). However, the sample size of Eastern Wolves from Algonquin Park in Stronen *et al*. (2012) was insufficient to identify a distinct cluster of Eastern Wolves in the program STRUCTURE. The Lingwick sample may, therefore, have been inaccurately assigned and A. V. Stronen (Aalborg University, Denmark, personal communication) has acknowledged that this animal should be considered a wolf–coyote hybrid. Although the skull of the Lingwick canid is decidedly smaller and less robust than that of the Caraquet wolf (or the Sainte-Marguerite wolf, see below; Figure 2), the body weight of this animal is above the mean for male Coyote, Coyote–Eastern Wolf hybrids, or Eastern Wolves provided by Benson *et al.* (2012). The skull and mounted skin of this animal are now in the Musée de la nature et des sciences in Sherbrooke, Quebec (accession no. 2003.2).

The 48.6-kg "Sainte-Marguerite wolf" was trapped in November 2006 near Sainte-Marguerite-de-Beauce (45.514°N, 70.9415°W) and has been identified genetically as an Eastern–Gray Wolf hybrid (J.-F. Dumont, Québec Ministère des Forêts, de la Faune et des Parcs, personal communication); the mounted skin is in a private collection and the skull is held by the Ministère des Ressources naturelles et de la Faune, Quebec.

Bone samples were decalcified using 0.5 N HCl at room temperature for 24–48 h. Samples were rinsed with distilled water and then decanted once the mineral portion of the bone was fully dissolved. Lipids were extracted in a 2:1 (v/v) chloroform:methanol solution and the resulting bone collagen was oven dried. Hair samples were washed in the same solution to remove surface oils and air dried. Samples (about 1 mg) were analyzed for $\delta^{13}C$ using a continuous flow isotope-ratio mass spectrometer (Thermo-Finnigan, Bremen, Germany) at the Stable Isotopes in Nature Laboratory, Fredericton, New Brunswick. Carbon isotope measurements were expressed as isotope delta (δ) in parts per thousand (‰) relative to the international standard, Vienna Pee Dee Belemnite. Isotope values were normalized using in-house standards calibrated against International Atomic Energy Agency reference materials. Analytical precision, estimated by repeated analyses of laboratory standards, was better than ± 0.2‰.

Canid $\delta^{13}C$ values were adjusted using the same diet-tissue discrimination values that Kays and Feranec (2011) applied to wolves and Coyotes from the northeastern United States (+5.0‰ for bone collagen and +2.6‰ for hair; Roth and Hobson 2000). Here, we assume that the isotopic composition of the wild diet of canids in eastern Canada is similar to that of canids in the northeastern United States. Although potential

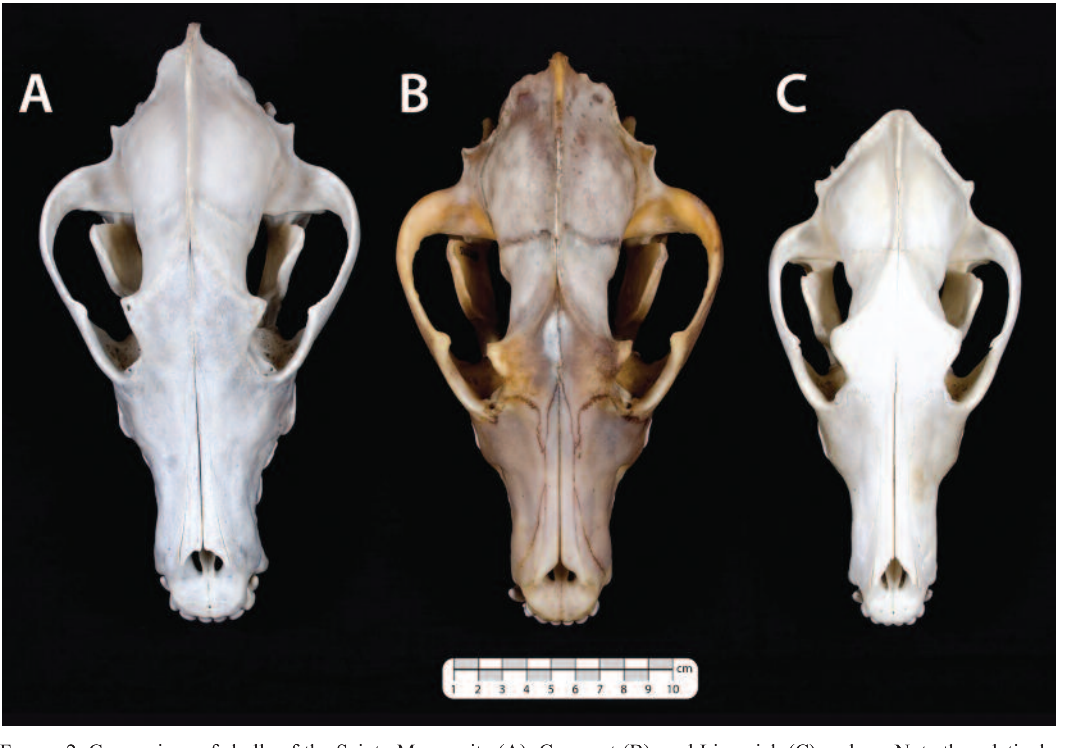

FIGURE 2. Comparison of skulls of the Sainte-Marguerite (A), Caraquet (B), and Lingwick (C) wolves. Note the relatively smaller size and less robust character of C, an Eastern Wolf–Coyote hybrid (*Canis lycaon* × *C. latrans*) compared with A and B, both Gray–Eastern Wolf hybrids (*C. lupus* × *C. lycaon*). Photo: New Brunswick Museum.

regional isotopic variation could introduce uncertainty, we believe it should be negligible given that eastern Canada and New England are geographically proximate and that Kays and Feranec (2011) found relatively good separation in isotopic values between wild and captive canid diets.

We categorized New Brunswick and Quebec wolves as "wild" or "captive" using an assignment test based on likelihood analysis (Rogers *et al.* 2012). From each measured wolf δ^{13}C value, we depicted the likely origin of individuals and summarized the likelihood of origin for each wolf based on the average probability across our replicate samples. The likelihood that a wolf sample originated from a captive versus wild animal was determined by $f(y^* | \mu_f, \sigma_f)$, assessing a normal probability density function as follows:

$$f(y^* | \mu_f, \sigma_f) = \frac{1}{\sqrt{2\pi\sigma_f^2}} \exp\left[-\frac{1}{2\sigma_f^2}(y^* - \mu_f)^2\right]$$

for each wolf subsample (y^*), given the expected mean (μ_f) and standard deviation (σ_f) of δ^{13}C for individuals growing their tissues on domestic food (mean = −18.2, standard deviation [SD] = 3.4 for commercial meat and dog foods typically given to captive canids) or wild food sources (mean = −27.8, SD = 1.8 for medium and large mammals and wild fruits that generally dominate their natural diet) analyzed by Kays and Feranec (2011). Thus, values from replicate samples for each wolf showed a probability of origin for both domestic and wild food sources, with the highest values of $f(y^* | \mu_f, \sigma_f)$ indicating the greatest likelihood of the animal being associated with that food source.

Results

Collection and Necropsy

The Caraquet wolf (Figure 3) was a male; testes were scrotal and measured 40.6 mm by 29.1 mm. Shortly after death, the animal weighed 39.9 kg. Tooth wear suggests that it was 3–4 years old at time of death. Measurements for the animal were as follows: total length 1573 mm, tail vertebrae 397 mm, hind foot 278 mm, ear 107.4 mm. Physically, the animal was in good body condition; it was negative for mange and carried 12.2–22.6 mm of extra-visceral body fat across the lateral pelvic region, about 2 mm over the ribs, and 819.2 g of visceral fat. Stomach contents (266.4 g wet

FIGURE 3. Taxidermied skin of the Caraquet wolf, a Gray–Eastern Wolf hybrid (*Canis lupus* × *C. lycaon*) (NBM M11985). Photo: New Brunswick Museum.

weight) consisted mostly of pig meat from the bait. However, some residual hairs in the stomach and a 20.4-g (wet weight) fecal pellet of hair proved to be Moose (*Alces americanus*). Mature taenid cestodes were present in the intestine.

Genetic Analysis

Genetic analysis identified the Caraquet wolf as a Gray–Eastern Wolf hybrid. The mtDNA sequence (C22; Wilson *et al.* 2000) was of Gray Wolf origin and the Y-chromosome haplotype (AA; Wilson *et al.* 2012) was of Eastern Wolf origin. STRUCTURE assigned the Caraquet wolf 96% to the northeastern Ontario Gray–Eastern Wolf hybrid reference population and GENECLASS analysis excluded (i.e., $P < 0.01$) all populations except northeastern Ontario Gray–Eastern Wolf hybrids ($P = 0.305$) as a probable population of origin.

Stable Isotope Analyses

The $\delta^{13}C$ value for bone collagen of −26.85 ± 0.38 for the Caraquet wolf clearly places it within the "wild" category (range −30.2 to −24.6) of Kays and Feranec (2011), although hair samples hint at some reliance on domestic food sources since the last molt (Table 1). The Lingwick wolf, with a $\delta^{13}C$ value for bone collagen of −28.34 ± 0.16 is also classified as of wild origin. Hair samples from the Lingwick wolf likewise suggest an animal that fed on wild food sources since its last molt. Analysis of bone collagen from the Saint-Marguerite wolf suggests that this animal spent most of its life subsisting on prey that included substantial domestic food sources, and hair samples strongly suggest that this animal had been feeding on or was fed largely domestic food sources since its last molt. Although the overall $\delta^{13}C$ value for the Saint-Marguerite wolf, −23.34 ± 1.02, is ambiguous in terms of categorizing this animal as a once-captive or a free-ranging urban animal, it strongly suggests that this wolf did not spend most of its life as a free-ranging wild animal. Likelihood-based assignment methods support these characterizations (Table 1).

TABLE 1. Stable carbon isotope analysis of bone collagen and hair samples from New Brunswick and Quebec wolves south of the St. Lawrence River. Trophic discrimination values of $\delta^{13}C$ +5.0‰ for bone collagen; +2.6‰ for hair.

Sample source	Mean $\delta^{13}C$ ± SD	Likelihood of wild origin, %	Number of replicates
Caraquet wolf			
Collagen	−26.85 ± 0.38	98	6
Hair	−24.52 ± 0.18	67	4
All samples	−25.92 ± 1.19		10
Lingwick wolf			
Collagen	−28.34 ± 0.16	99	6
Hair	−26.79 ± 0.16	98	3
All samples	−27.82 ± 0.79		9
Sainte-Marguerite wolf			
Collagen	−24.26 ± 0.13	57	6
Hair	−22.42 ± 0.47	6	6
All samples	−23.34 ± 1.02		12

Discussion

Regardless of the historical genetic composition of Maritime wolves, the Caraquet wolf is the first free-ranging, wild-origin wolf recorded in New Brunswick since 1862, presumably dispersing south to the province from northeastern Ontario or western Quebec north of the St. Lawrence River. Wolves were extirpated from Maritime Canada by about 1900 (Harrison and Chapin 1998) and their possible re-establishment, whether by natural or assisted means, is controversial (Lohr *et al.* 1996; Nie 2001; Williams *et al.* 2002; Musiani and Paquet 2004). However, determining whether wolves outside their current distributional range represent the vanguard of re-establishment can be difficult. Such animals may represent nothing more than isolated occurrences of once-captive wolves or domesticated wolf–dog hybrids (Prendergast 1989; Kays and Feranec 2011), particularly as an estimated 300 000 wolf–dog hybrids are kept as companion animals in the United States (Fischer 2003). Complicating the sociopolitical issues surrounding wolf re-establishment has been an unresolved taxonomy (Chambers *et al.* 2012), lack of information on historical distribution of forms (Wilson *et al*. 2003; Rutledge 2010a), and possible contemporary hybridization among wolves, coyotes, and dogs (vonHoldt *et al.* 2011; Stronen *et al.* 2012; Monzón *et al.* 2013; Way 2013).

The literature suggests that wolves (of unknown genotype) were not common in Maritime Canada in the past, so it is not surprising that historical wolf specimens from New Brunswick do not appear to exist (Lohr and Ballard 1996). Wilson *et al.* (2000) include Maritime Canada within the historical range of the Gray Wolf. Wheeldon and White (2009) suggest that admixing of the Gray Wolf and the Eastern Wolf may pre-date European settlement in the western Great Lakes region, whereas Wilson *et al.* (2003) showed that historical samples from New York and Maine carried only Eastern Wolf mtDNA. Based on 16th century archaeological remains, Rutledge *et al.* (2010a) suggest that the Eastern Wolf, or perhaps an Eastern–Gray Wolf hybrid occupied the eastern temperate forest before European arrival. Kyle *et al.* (2006) state that after European settlement, it was the Gray Wolf that was extirpated from southeastern Ontario and Quebec; this was followed by land clearing, changes in forest cover, and the concomitant movement of the Eastern Wolf northward with White-tailed Deer (*Odocoileus virginianus*).

Unfortunately, none of this information resolves questions about the genetic makeup of the wolves that may have occupied New Brunswick historically. Harrison and Chapin (1998) and Wydeven *et al.* (1998) felt it was unclear whether wolves could recolonize available habitat in northeastern North America without human assistance and recommended a re-introduction program. Although Harrison and Chapin (1998) note that low human population and the extensive forests of northern New Brunswick provide suitable habitat for wolves, they also suggest that the St. Lawrence River and associated human development may present barriers to wolf dispersal south from Ontario and Quebec. Likewise, Larivière *et al.* (2000) were uncertain whether individual wolves from populations in Quebec north of the St. Lawrence River might disperse to available wolf habitat in the northeastern United States.

Until 2002, wolves had not been reported in the wild in Canada south of the St. Lawrence River for more than a century (Wydeven *et al.* 1998; Villemure and Jolicoeur 2004). Nonetheless, $\delta^{13}C$ values suggest that the Caraquet and Lingwick wolves were of wild origin, indicating that wolves are capable of dispersing from north of the St. Lawrence River into southern Quebec and New Brunswick and probably the northeastern United States, in spite of natural and human barriers. Kays and Feranec (2011) also report that some free-ranging wolves in the northeastern United States appear to be of wild origin based on $\delta^{13}C$ values. Furthermore, in the course of our work, we were made aware of other purported (and assumed wild) wolves from Quebec south of the St. Lawrence River and New England (M. Hénault, Québec Ministère des Forêts, de la Faune et des Parcs, personal communication; M. McCollough, Endangered Species Specialist, United States Fish and Wildlife Service, personal communication; Monzón 2012), in addition to those examined by Kays and Feranec (2011).

The outcome of any future wolf dispersal into New Brunswick or New England is unclear. Although Coyotes in the east will readily interbreed with the Eastern Wolf (Kyle *et al*. 2006; Rutledge *et al.* 2010b), such hybridization occurs more rarely with Gray–Eastern Wolf hybrids (Wheeldon and Patterson 2012). Kyle *et al.* (2006) note that, although hybridization may be reducing the distinctiveness of the Eastern Wolf, it may also enhance the adaptive potential of wolves. Conversely, Coyote hybridization with wolves appears to have enhanced the adaptive ability of Coyotes in the northeast (Kays *et al.* 2010). Kyle *et al.* (2006) and Way (2013) both suggest that a Gray–Eastern Wolf hybrid is a more efficient predator of Moose than a Coyote–Eastern Wolf hybrid, which is adapted to prey on deer. Moose is the predominant ungulate in northern New Brunswick (Forbes *et al.* 2010).

Wydeven *et al.* (1998) observed that wolves in southern Ontario and Quebec appeared to be heavily exploited. Together with the fate of the Lingwick, Saint-Marguerite, and Caraquet wolves, this suggests that mortality among wolves dispersing into regions south of the St. Lawrence River may be high. In light of possible high mortality and the dense network of roads adjacent to the St. Lawrence River (well above the < 0.70 km/km^2 selected by Harrison and Chapin [1998] as a proxy for maximum levels of human presence compatible with wolf habitat), natural dispersal alone may be insufficient to re-establish the wolf in the northeast. Nonetheless, the importance of individual dispersers to the evolutionary potential of whatever wolf-like phenotype populates the region south of the St. Lawrence River in the future should not be underestimated (Vilà *et al.* 2003).

The $\delta^{13}C$ values for the Saint-Marguerite wolf are ambiguous, and we are unable to categorize this animal as either of captive or free-ranging urban origin. The $\delta^{13}C$ values of Kays and Feranec (2011) mark this animal as an "urban canid" and not wild, but attest to the statement of Darimont and Reimchen (2002) that the interpretation of isotopic signals can be challenging without relevant ecological information. Apparently, the animal had been resident for some time in an agricultural area and was feeding on domestic animals (J.-F. Dumont, Québec Ministère des Forêts, de la Faune et des Parcs, personal communication), but the origin of this wolf remains uncertain.

Although the approach presented by Kays and Feranec (2011) appears to have considerable utility in separating wild from formerly captive wolves, its value could be enhanced with a wider range of sample isotope values from animals of known diet and of wild, domestic, and urban origin. Wydeven *et al.* (1998) conclude that there is a need for better collection of data relating to dispersing wolves in the northeast, and Larivière *et al.* (2000) argue for the monitoring of wolf populations in Quebec outside wildlife reserves.

We concur, noting that we had difficulty locating the remains of the Lingwick and Saint-Marguerite wolves. Although, the collection of tissue for DNA analysis from purported wolves occurring outside their normal range now seems routine, ensuring that skeletal and hair samples from such animals are deposited in publicly maintained museum collections should likewise be a priority. The outcome of analyses of such samples may well influence future management decisions for the wolf in northeastern North America

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