

ONTARIO FEDERATION OF ANGLERS & HUNTERS



Ontario Conservation Centre

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November 24, 2014

Ms. Deb Stetson, Manager
Wildlife Policy Section
Ministry of Natural Resources and Forestry
300 Water Street
Peterborough, Ontario
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Dear Ms. Stetson

Subject: Management of Wolves and Coyotes across Northeast and Northwest Ontario

Grey wolves, eastern wolves, and coyotes are currently managed as a single species/population in primary wolf range in Ontario. It is our understanding these regulatory measures were put in place where wolf and coyote ranges overlap to address (at the time) the perceived difficulty in distinguishing wolves and coyotes in the field.

Recent findings by Wheeldon and Patterson (attached) suggest there is little evidence of eastern wolves north of the French River and, in areas without eastern wolves, the physical differences between wolves and coyotes are large enough to make them distinguishable to hunters. In light of new information and monitoring activities, the OFAH believes a review of the current wolf and coyote regulations is warranted.

Applying an adaptive management approach, we request the Ministry of Natural Resources and Forestry (MNR) to consider revising the following conservation approaches:

Harvest Controls for Coyotes in WMUs 1A, 1C, 1D, and 2-42

The OFAH believes, from a biological point of view, separate management of wolves and coyotes (across northeastern and northwestern Ontario) are practical and desirable from a wildlife management perspective. The current harvest restrictions on coyote harvest in Wolf Ecological Zones 4 and 5 are of particular concern, due to ongoing issues with livestock predation in these areas. Compensation records continue to demonstrate that coyotes are the primary cause of losses of livestock and poultry in Ontario.

Based on new empirical evidence, the OFAH requests the MNR to remove harvest controls for coyotes in WMUs 1A, 1C, 1D, and 2-42. For greater clarity, we are requesting the seal requirement for coyotes be removed (only a small game licence would be required) with a year-round season in the above-mentioned WMUs.

Small Game Licence Restriction

The small game licence restriction in most of northern/central Ontario from June 16 to August 31 does not apply in southern Ontario in order to provide landowners with additional tools to address human-wildlife conflict (e.g. livestock depredation) through regulated hunting. Falconers are also exempt from the small game licence restriction due to the need for them to exercise their birds year-round. A similar extension would be required to allow for a year-round open season for coyotes in WMUs 1A, 1C, 1D, and 2-42.

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What is the justification for maintaining the small game restriction in northern and central Ontario? If there is no legitimate rationale for the restriction, the OFAH recommends removing it altogether. This would simplify the current hunting regulations without having any negative impacts on wildlife management.

Wolf Hunting Season Closed From April 1 to September 14

In many years, closing the hunting season on April 1 is too early in Ontario. It can impact wolf hunting at a time of year when wolves can be a significant mortality factor on sheep and other livestock operations. We recommend extending the season to April 30.

Maximum of Two Wolf Seals Per Year

While we support the seal requirement for wolves in northern Ontario, we do not believe there should be a limit on the number of seals made available to resident wolf hunters. The vast majority of wolf hunters will not purchase more than one seal, but there will be some who could use more than two; so why continue to restrict the number available? Ontario's wolf populations are healthy; therefore, the negligible increase in harvest resulting from this change would not be a sustainability concern.

Party Hunting

We also recommend removing current restrictions on "party hunting" for wolves in WMUs 1A, 1C, 1D, and 2-42; and where coyote seal requirements remain in core wolf range (i.e. WMUs 46-50 and 53-58). This would allow a hunter to continue to hunt in a party, even if the person has previously attached their game seal.

In closing, we believe revising these conservation approaches will create substantial benefits (e.g. social, economic, etc.) across northeastern and northwestern Ontario, which are consistent with the Strategy for Wolf Conservation in Ontario (i.e. managing for sustainable harvest levels and hunting opportunities).

At your earliest convenience, we welcome the opportunity to discuss our recommendations with you further.

Yours in Conservation,



Matt DeMille, M.Sc.
Manager, Fish and Wildlife Services

MD/ds/gh
Attach.

cc: OFAH Board of Directors
OFAH Sporting Dogs/Small Game Advisory Committee
Angelo Lombardo, OFAH Executive Director
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Genetic and morphological differentiation of wolves (*Canis lupus*) and coyotes (*Canis latrans*) in northeastern Ontario

Tyler J. Wheeldon and Brent R. Patterson

Abstract: Gray wolves (*Canis lupus* L., 1758), eastern wolves (*Canis lycaon* Schreber, 1775), and coyotes (*Canis latrans* Say, 1823) are presently managed as a single biological population in primary wolf range in Ontario with the intent of minimizing incidental harvest of wolves. This management strategy is based on the assumption that wolves and coyotes cannot be reliably distinguished because of hybridization, and the resulting restrictions on coyote harvest are unpopular with hunters and farmers. We genetically and morphologically characterized a sample of sympatric wolves and coyotes harvested in the Lesser Clay Belt area of northeastern Ontario in 2006–2009 to test the hypothesis that these species cannot be reliably distinguished. We found that wolves and coyotes were genetically and morphologically distinct, with minimal hybridization between them. Our findings suggest that wolves and coyotes in the sampled area can be reliably distinguished, but further sampling is required to determine the full extent of areas in Ontario where wolves and coyotes are reliably distinguishable. We discuss unresolved issues regarding the feasibility of separate management for these species. We also discuss implications of our findings regarding wolf recovery in the northeastern United States.

Key words: *Canis latrans*, coyote, *Canis lupus*, gray wolf, *Canis lycaon*, eastern wolf, microsatellite genotype, mitochondrial DNA, Y chromosome, haplotype, morphology.

Résumé : Le loup gris (*Canis lupus* L., 1758), le loup de l'est (*Canis lycaon* Schreber, 1775) et le coyote (*Canis latrans* Say, 1823) sont actuellement gérés comme s'ils constituaient une seule population biologique dans l'aire de répartition principale des loups en Ontario, et ce, afin de minimiser la récolte fortuite de loups. Cette stratégie de gestion repose sur l'hypothèse voulant que les loups et les coyotes ne puissent être distingués avec certitude en raison de leur hybridation, et les restrictions visant la récolte de coyotes en découlant ne sont pas appréciées des chasseurs et des agriculteurs. Nous avons effectué la caractérisation génétique et morphologique d'un échantillon de loups et de coyotes sympatriques prélevé dans la région de Lesser Clay Belt du nord-est de l'Ontario, de 2006 à 2009, afin de vérifier cette hypothèse. Nos résultats ont démontré que les loups et les coyotes étaient distincts du point de vue tant génétique que morphologique, et que l'hybridation entre les deux espèces était minimale. Si ces résultats suggèrent que les loups et les coyotes dans la région échantillonnée peuvent être distingués avec certitude, un échantillonnage plus grand est nécessaire pour déterminer l'étendue des régions ontariennes dans lesquelles les loups et les coyotes peuvent être distingués avec certitude. Nous abordons des questions touchant à la faisabilité d'une gestion distincte de ces espèces ainsi que les conséquences de nos résultats en ce qui concerne le rétablissement du loup dans le nord-est des États-Unis.

Mots-clés : *Canis latrans*, coyote, *Canis lupus*, loup gris, *Canis lycaon*, loup de l'est, génotype de microsatellites, ADN mitochondrial, chromosome Y, haplotype, morphologie.

[Traduit par la Rédaction]

Introduction

Ontario is inhabited by three putative species of *Canis*: the coyote (*Canis latrans* Say, 1823), the eastern wolf (*Canis lycaon* Schreber, 1775), and the gray wolf (*Canis lupus* L., 1758). Genetic and morphological studies have demonstrated that eastern wolves have hybridized with both coyotes and gray wolves in Ontario (e.g., Schmitz and Kolenosky 1985;

Sears et al. 2003; Wheeldon 2009; Rutledge et al. 2010); the intermediate size of eastern wolves may contribute to their ability to hybridize with both species. Where these canid types occur sympatrically, hybridization produces individuals with varying phenotypes and genetic compositions (e.g., Sears et al. 2003; Rutledge et al. 2010; Benson et al. 2012), thereby blurring distinctiveness and complicating management of these canids. Consequently, within primary wolf

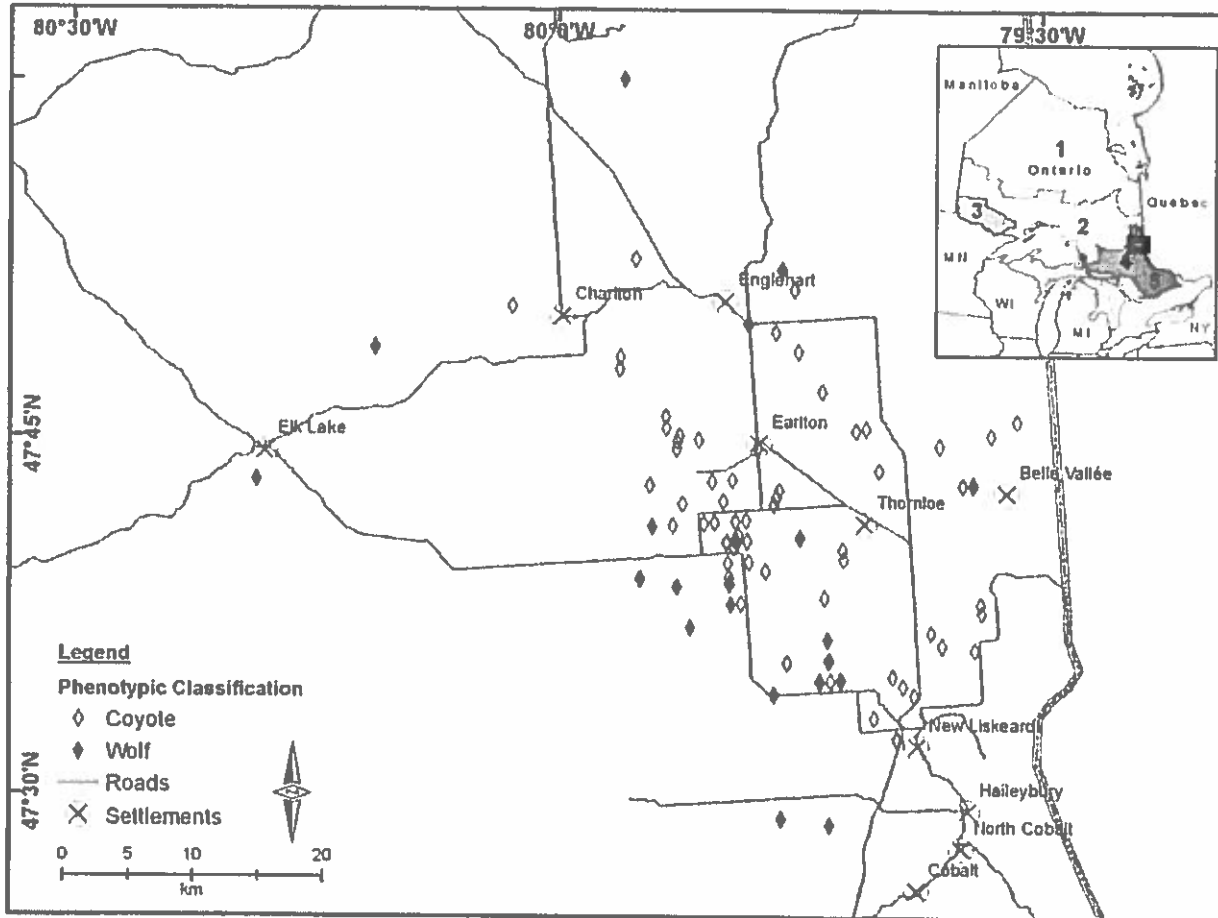
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Fig. 1. Harvest locations of phenotypically classified wolves (*Canis lupus*) and coyotes (*Canis latrans*) collected from the Lesser Clay Belt area of northeastern Ontario, Canada, in 2006–2009. For simplicity, the individual that was phenotypically classified as unknown was grouped with wolves based on its morphological measurements. Sample locations are approximate to lot and concession or township; some points are overlapping. The inset map depicts wolf ecological zones in Ontario; the study area is indicated by the square. Primary wolf range is represented by zones 1–5 where wolves and coyotes are managed as a single biological population. Note that eastern wolves (*Canis lycaon*) primarily occur in zone 5 and are rare in the other zones where gray wolves predominate. Coyotes range across the majority of Ontario except the far north region.



range (PWR) in Ontario (Fig. 1), wolves and coyotes are managed as a single biological population with the intent of minimizing incidental harvest of wolves (OMNR 2005).

Conflicting interpretations of genetic and morphological data have led to ongoing debate on the evolutionary history and taxonomic status of *Canis* species in eastern North America; specifically regarding the eastern wolf (e.g., Kobl-müller et al. 2009; Nowak 2009; Fain et al. 2010; Rutledge et al. 2010; vonHoldt et al. 2011). Taxonomic debate notwithstanding, empirical data demonstrate that the wolf population occurring in and around Algonquin Provincial Park (APP) in central Ontario, which contains genes suggested to be of eastern wolf origin, is genetically (Rutledge et al. 2010) and morphologically (Schmitz and Kolenosky 1985; Benson et al. 2012) distinct from surrounding populations of gray wolves and coyotes, despite hybridization. Thus, disagreement over historic lineages and hybridization patterns should not preclude recognizing this contemporary wolf population

as distinct at a regional scale. Accordingly, we hereafter refer to the unique wolf that occurs in and around APP as the eastern wolf. We use the terms “eastern wolf” and “APP wolf” synonymously, with the understanding that the former describes the species and the latter describes a contemporary population putatively representative of the eastern wolf.

Managing wolves and coyotes as a single biological population in PWR in Ontario is premised on the assumption that phenotypic¹ ambiguity associated with wolf–coyote hybridization precludes wolves and coyotes being reliably distinguishable by hunters “in the field” (OMNR 2005). However, this assumption is primarily based on the presumed phenotypic ambiguity of hybrids between eastern wolves and coyotes in central Ontario. In northern Ontario, where larger gray wolves occur, phenotypic ambiguity of wolves and coyotes is less likely to be an issue and thus may be weak justification for managing wolves and coyotes as a single biological population.

¹Use of the term “phenotypic” throughout is intended to define only an animal’s physical characteristics.

This management prescription has serious implications for coyote harvest in PWR in Ontario. Outside of PWR in Ontario (i.e., in southeastern Ontario) coyotes may be hunted year-round with no bag limits, but following the introduction of the strategy for wolf conservation (OMNR 2005), wolves and coyotes in PWR in Ontario may only be hunted 15 September – 31 March with each hunter being limited to a maximum of two wolves or coyotes per year. The trapping season for wolves and coyotes in PWR in Ontario is 15 September – 31 March and there are no bag limits for trappers on their own registered traplines. Outside of PWR in Ontario foothold trapping is legal, but the use of suspended neck snares is generally not. Regardless, the change in hunting allowance for coyotes in northern Ontario is an issue for hunters and farmers in the region, with many believing the relatively strict harvest limits on canids have contributed to recent increases in wolf and coyote abundance in the area (Fig. 2a). At the same time, claims for wolf-coyote damage paid by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) under the Ontario Wildlife Damage Compensation Program have increased dramatically across much of Ontario, with most losses being attributable to coyotes (Fig. 2b; OMAFRA, unpublished data).

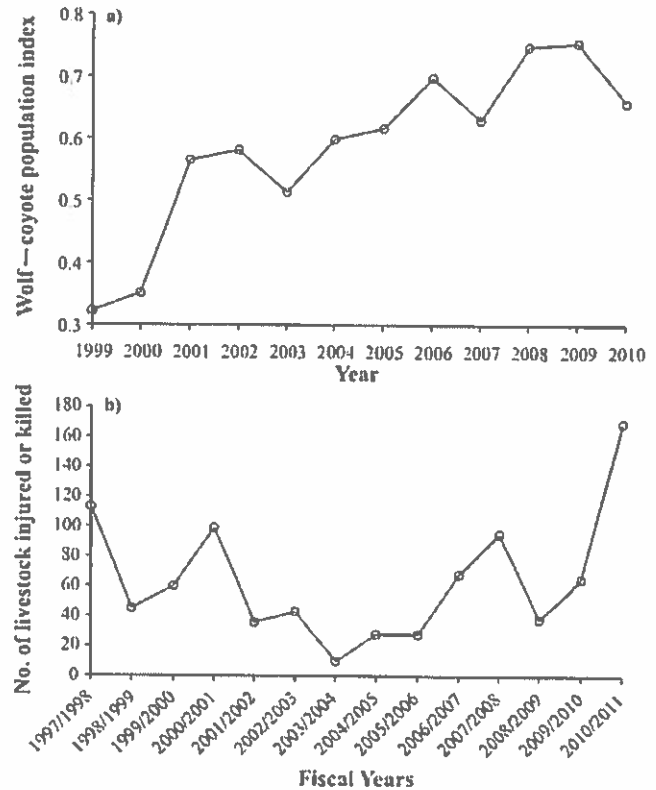
The relatively restrictive harvest allowance for canids in northern Ontario is perceived by many hunters and farmers to hinder their ability to adequately control coyote numbers and to preemptively reduce livestock depredation. Regardless of whether preemptive harvest of coyotes has any impact on coyote numbers or rates of livestock depredation, determining the feasibility of separate management of wolves and coyotes in northern Ontario is important to agricultural stakeholders. As such, a primary objective of this study was to determine the ability of hunters in northern Ontario to distinguish wolves from coyotes in the field based on premortem observations and tracks. We genetically and morphologically characterized a sample of sympatric wolves and coyotes harvested in northeastern Ontario (NEON) to test the hypothesis that these two species cannot be reliably distinguished. We acknowledge the mixed ancestry of wolves and coyotes in Ontario (e.g., Wheeldon 2009; Rutledge et al. 2010; Benson et al. 2012), but herein the wolves and coyotes sampled in NEON are considered representative of wolf-like and coyote-like canids, respectively. When referring to wolves sampled in NEON, it is implied that we are referring to putative gray wolves, to distinguish them from putative eastern wolves in APP. We also discuss implications of our findings regarding wolf recovery planning for the northeastern United States (NEUS).

Materials and methods

Study area

We conducted our study in the Lesser Clay Belt area of NEON (47°42'N, 79°51'W); a vast tract of fertile soil running from Englehart down to the Wabi River to the northern tip of Lake Timiskaming (Fig. 1). The Clay Belt is surrounded by Canadian Shield, forming an island of "southern flatlands" in the midst of hilly and rocky surroundings. The Clay Belt has mean monthly temperatures ranging from -15 °C in January to 18 °C in July, and mean annual precipitation is 95 cm with most falling as rain during spring-

Fig. 2. (a) Wolf (*Canis lupus*) – coyote (*Canis latrans*) population trends and (b) reported livestock losses to coyotes and wolves in the Lesser Clay Belt area of northeastern Ontario, Canada. The wolf-coyote population index was calculated from combined observations of wolves and coyotes reported by deer and moose hunters on post-hunt report cards.



autumn. Low-elevation areas are dominated by black spruce (*Picea mariana* (Mill.) B.S.P.) and as elevation rises the forest composition changes to a mixture of balsam poplar (*Populus balsamifera* L.), paper birch (*Betula papyrifera* Marshall), black spruce, white spruce (*Picea glauca* (Moench) Voss), and balsam fir (*Abies balsamea* (L.) Mill.). There are three important exceptions to this mixed forest: pure jack pine (*Pinus banksiana* Lamb.) on very dry sandy or rocky sites; pure poplar on tills; and a tamarack (*Larix laricina* (Du Roi) K. Koch) – black spruce association in the muskegs.

Sample collection and carcass necropsy

We obtained skinned and unskinned carcasses of wolves and coyotes harvested in the study area (Fig. 1) between October 2006 and December 2009 (91% harvested during December–March). Hunters and trappers classified the animals as wolf or coyote based on physical characteristics (i.e., phenotypic classification) observed in the field; one carcass was classified as unknown. Hunters were instructed to classify animals based on premortem observations and tracks, and although premortem classification of animals by trappers was not possible for those captured in suspended neck snares, trappers were instructed to classify animals observed in foothold traps premortem when possible. Thus the phenotypic

classifications of the carcasses obtained include those made pre-mortem and post-mortem; however, hunter-submitted carcasses were much more prevalent in our sample than trapper-submitted carcasses. The specimens examined were considered to be representative of the wolf and coyote populations in the study area because participants in the study submitted the carcasses of all the wild canids that they harvested during the study period. We collected a tissue sample from each carcass for genetic profiling and recorded the gender of the animal. Because hunters sometimes incorporated the size of tracks in snow into their assessment of the species they were pursuing, we were interested in comparing foot sizes of wolves and coyotes. For carcasses with front foot pads intact, we measured front left foot length (i.e., length from back edge of heel pad to tip of toe pad) and width (i.e., distance between outer edges of outer toe pads); the right foot was measured in cases where the left foot could not be measured. Carcasses obtained with pelt intact were weighed and then skinned. We then weighed each skinned carcass and measured body length (i.e., length from tip of nose to base of tail measured along dorsal midline contour) and chest girth (i.e., circumference around torso behind shoulders). Note that carcass masses predominantly reflect winter masses based on the harvest dates (see above). We omitted anatomically incomplete carcasses for which morphology could not be assessed, but we included carcasses for which incomplete anatomy did not preclude morphological assessment and was predicted to constitute less than a 0.5 kg reduction in body mass (i.e., tail missing, paw(s) missing, evidence of minor scavenging). Age was estimated after necropsy based on tooth wear (e.g., Gipson et al. 2000) and (or) fusion of the basioccipital–basisphenoid suture as observed on cleaned skulls (e.g., Landon et al. 1998). We identified 117 yearlings–adults in our sample, comprising 85 coyotes (39 females and 46 males), 31 wolves (11 females and 20 males), and 1 unknown individual; we omitted juveniles from further analysis. Most of the 117 yearling–adult carcasses were obtained skinned, but 38 were obtained with the pelt intact, thus we performed a linear regression of unskinned mass (y axis) versus skinned mass (x axis) and found a strong relationship ($y = 1.13x + 0.21$; $R^2 = 0.99$), which was used to convert the skinned masses of carcasses obtained without pelts to predicted unskinned masses to facilitate comparison with other studies.

Genetic profiling and analyses

We extracted DNA from the tissue samples collected from the 117 yearling–adult carcasses using a Qiagen DNeasy Blood and Tissue Kit. For each sample, we amplified a 343–347 base pair (bp) fragment of the mitochondrial DNA (mtDNA) control region using published primers (AB13279: Pilgrim et al. 1998; AB13280: Wilson et al. 2000) and generated autosomal microsatellite genotypes based on 12 loci (Ostrander et al. 1993, 1995: *cxr225*, *cxr2*,² *cxr123*, *cxr377*, *cxr250*, *cxr204*, *cxr172*, *cxr109*, *cxr253*, *cxr442*, *cxr410*, *cxr147*) following the methods of Wheelton et al. (2010). For male samples, we generated Y-chromosome

microsatellite genotypes based on four loci (Sundqvist et al. 2001: *MS34A*, *MS34B*, *MS41A*, *MS41B*) following the methods of Wheelton et al. (2010). We performed all sequencing and genotyping on a MegaBACE 1000 (GE Healthcare) or an ABI3730 (Applied Biosystems) and scored microsatellite alleles in GeneMarker version 1.7 (SoftGenetics LLC), accounting for allele size shifts between instruments with multiple control samples.

We obtained 117 autosomal microsatellite genotypes based on 10 ($n = 1$), 11 ($n = 3$), and 12 ($n = 113$) loci. To test for admixture between wolves and coyotes in the study area, we analyzed the autosomal microsatellite genotype data in STRUCTURE version 2.3 (Pritchard et al. 2000; Hubisz et al. 2009) with settings FREQSCORR = 1, INFERRALPHA = 1, and ANCESTDIST = 1 (90% probability intervals of q values). We ran the admixture model at $K = 2$ for 10 repetitions of 10^6 iterations following a burn-in period of 250 000 iterations and obtained individual assignments from the run having the highest posterior probability and lowest variance. We chose to analyze the genotype data at $K = 2$ because prior investigations suggested that migrant APP wolves were uncommon in NEON despite evidence of APP admixture in NEON canids (Wheelton 2009; Holloway 2009; Rutledge et al. 2010). We analyzed the data at $K = 3$ (settings same as above) including 45 highly assigned (i.e., $q_i > 0.95$) APP wolves (data from Rutledge et al. 2010) and assessed that $K = 2$ was appropriate for our data set (Supplementary Fig. S1).³

We edited mtDNA sequences to 223–228 bp in length using BIOEDIT version 7.0.9 (Hall 1999) and assigned haplotypes, denoted as C(#), corresponding to previously described sequences (Wilson et al. 2000). We used a previously identified diagnostic indel to distinguish between mtDNA sequences of gray wolf origin and those of coyote or eastern wolf origin (Pilgrim et al. 1998; Wilson et al. 2000).

We combined the genotypes of the four Y-chromosome microsatellite loci into haplotypes and classified them taxonomically based on the allele present at locus *MS41A*, for which allele 208 is putatively specific to gray wolves (and domestic dogs), and alleles 212 and 214 are putatively specific to coyotes and eastern wolves (Sundqvist et al. 2001, 2006; Hailer and Leonard 2008; Fain et al. 2010; Rutledge et al. 2010). We inferred the Y-chromosome haplotypes for two samples with missing allele data for *MS41A* based on their Y-intron sequence (data not shown; see Wilson et al. 2012).

Due to the extensive introgressive hybridization that has occurred between coyotes and eastern wolves in central Ontario, here we do not distinguish between the observed coyote and eastern wolf mtDNA or Y-chromosome haplotypes because nearly all of them occur in both species and their hybrids (Grewal et al. 2004; Rutledge et al. 2010). We simply refer to them as coyote–eastern wolf (C–EW) haplotypes. However, we recognize that prior to this introgressive hybridization, these haplotypes putatively had distinct taxonomic origins, as suggested by phylogenetic analysis (Wilson et al. 2003, 2012).

²This locus was erroneously labeled *cxr200* in previous studies (e.g., Rutledge et al. 2010; Wheelton et al. 2010).

³Supplementary Fig. S1 and Table S1 are available with the article through the journal Web site (<http://nrcresearchpress.com/doi/suppl/10.1139/z2012-090>).

Morphological comparisons

The STRUCTURE assignments were used to reclassify individuals as wolves ($q_{\text{wolf}} \geq 0.80$), coyotes ($q_{\text{coyote}} \geq 0.80$), or admixed ($0.20 < q_i < 0.80$) (e.g., Vähä and Primmer 2006). We calculated gender-specific mean unskinned masses for wolves and coyotes; these mean values included the observed unskinned masses of carcasses obtained with pelts and the predicted unskinned masses (i.e., determined with the regression equation) of carcasses obtained without pelts. We then calculated the means and 95% confidence intervals, and determined the ranges, of the four morphological measurements (note: skinned mass) and compared them between wolves and coyotes, separated by gender, except for foot size for which genders were pooled; we multiplied foot length by width to obtain a composite measure of foot size. We used a two-factor multiple analysis of variance (MANOVA) to test for effects of species and gender, as well as a gender \times species interaction, on mass, body length, and chest girth. After having determined overall significance, we used two-factor ANOVA to test for morphological differences (i) between species while controlling for gender and (ii) between genders while controlling for species. Because many of the carcasses were missing feet, smaller sample sizes precluded our including measures of foot size in the MANOVA modeling. Accordingly, to test for differences in foot size between species, we performed a two-tailed two-sample *t* test assuming unequal variances (e.g., Ruxton 2006) in Excel 2007 (Microsoft Corporation). We verified the suitability of the data for parametric statistical modeling by examining histograms of residuals, normal quantile-quantile plots of residuals, and plots of residuals versus fit (Venables and Ripley 2002). All statistical analyses were conducted in S-Plus version 6.2 (Insightful Corporation), unless stated otherwise.

Results

Genetic differentiation

The $K = 2$ STRUCTURE analysis of the individual autosomal microsatellite genotypes assigned 83 as coyotes, 30 as wolves, and 4 as admixed (Fig. 3). None of the individuals assigned as coyotes or wolves had 90% probability intervals that overlapped zero for the population to which they were assigned, but three of the individuals assigned as admixed had 90% probability intervals that ranged either precisely or approximately from zero to one for both population assignments (Supplementary Table S1).³ The genetic assignment of individuals as wolves or coyotes was concordant with their phenotypic classification, except for one individual that was genetically assigned as a coyote but phenotypically classified as a wolf (Fig. 3; see Discussion). The individual that was phenotypically classified as unknown was genetically assigned as a wolf. The four individuals assigned as admixed comprised three coyotes and one wolf based on phenotypic classification (Fig. 3); further consideration of these individuals is presented in the Discussion. References hereafter to wolves or coyotes in the data set are based on the STRUCTURE assignments, unless stated otherwise.

We observed seven mtDNA haplotypes in our sample: two were of gray wolf origin and five were of C-EW origin (Table 1). The two gray wolf haplotypes were observed in wolves but not in coyotes, and four of the five C-EW haplo-

types were observed in coyotes, but only two of those four were observed in wolves (Table 1). The remaining C-EW haplotype (i.e., C17) was only observed in wolves (Table 1).

We obtained Y-chromosome microsatellite genotypes for all but one of the males and observed 10 haplotypes in our sample: 6 were of gray wolf origin and 4 were of C-EW origin (Table 2). Five of the gray wolf haplotypes were observed in wolves, but only one of those was observed in a coyote; the remaining gray wolf haplotype (i.e., FF) was only observed in coyotes (Table 2) and is putatively of domestic dog origin (see Discussion). All four C-EW haplotypes were observed in coyotes, but only one of those was observed in wolves (Table 2).

The combined maternal and paternal haplotype composition of males revealed evidence of mixed ancestry, specifically C-EW mtDNA was observed in males with gray wolf or putative dog Y chromosomes, but notably gray wolf mtDNA was observed only in males with gray wolf Y chromosomes (Table 3). The mtDNA haplotypes that were only observed in wolves (i.e., C17, C22, and C23) were only observed in males with gray wolf Y chromosomes, whereas the mtDNA haplotypes that were only observed in coyotes (i.e., C9 and C19) were observed in males with C-EW or putative dog Y chromosomes but only in one male with a gray wolf Y chromosome (Table 3).

Morphological differentiation

The unskinned masses (mean \pm SE) of female and male coyotes were 14.7 ± 0.3 kg ($n = 38$, range 10.9–18.0 kg) and 17.3 ± 0.4 kg ($n = 44$, range 11.2–22.8 kg), respectively, and those of female and male wolves were 28.0 ± 1.3 kg ($n = 10$, range 23.4–36.7 kg) and 32.7 ± 1.7 kg ($n = 20$, range 17.2–46.5 kg), respectively. MANOVA revealed that species ($F_{[3,104]} = 175.6$, $P < 0.001$) and gender ($F_{[3,104]} = 10.5$, $P < 0.001$) both significantly influenced the three morphological measures. More specifically, when controlling for gender, wolves were heavier ($F_{[1,108]} = 308.4$, $P < 0.001$), longer ($F_{[1,109]} = 409.6$, $P < 0.001$), and possessed larger chest girths ($F_{[1,107]} = 278.4$, $P < 0.001$) than coyotes (Fig. 4). Similarly, when controlling for species, males were heavier ($F_{[1,108]} = 38.5$, $P < 0.001$), longer ($F_{[1,109]} = 39.4$, $P < 0.001$), and possessed larger chest girths ($F_{[1,107]} = 53.0$, $P < 0.001$) than females (Fig. 4). Of particular interest was that female wolves were heavier, longer, and possessed larger chest girths than male coyotes (mass: $t_{[10]} = -7.95$, $P < 0.001$; body length: $t_{[16]} = -11.62$, $P < 0.001$; chest girth: $t_{[14]} = -8.31$, $P < 0.001$) (Fig. 4). Finally, wolves also had larger foot sizes than coyotes regardless of gender ($t_{[10]} = -6.61$, $P < 0.001$) (Fig. 4).

Discussion

We genetically and morphologically characterized a sample of sympatric wolves and coyotes from NEON and found differences in both respects. Specifically, body lengths of wolves and coyotes were essentially nonoverlapping, although masses, chest girths, and foot sizes exhibited some overlap (Fig. 4). Notably, two of the three wolves with masses within the maximum range for coyotes were noted as being "emaciated" or "very skinny". Although body condition of animals could potentially affect the ability of hunters

Fig. 3. Individual admixture proportions (Q) of wolves (*Canis lupus*) and coyotes (*Canis latrans*) genotyped at 12 autosomal microsatellite loci. Each partitioned vertical bar represents an individual's proportional membership to the $K = 2$ populations analyzed in STRUCTURE. For simplicity, the individual that was phenotypically classified as unknown was grouped with wolves based on its morphological measurements.

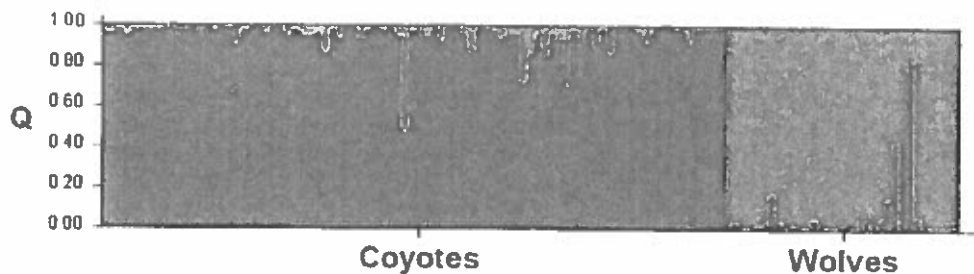


Table 1. Mitochondrial DNA haplotype frequencies in wolves (*Canis lupus*) and coyotes (*Canis latrans*).

Haplotype	Frequency	STRUCTURE assignments	GenBank accession
C1*	17 (10 F + 7 M)	14C; 2W; 1A	AY267718
C9†	28 (10 F + 18 M)	25C; 3A	AY267726
C14*	14 (5 F + 9 M)	1C; 13W	AY267731
C17*	4 (2 F + 2 M)	4W	AY267734
C19	43 (20 F + 23 M)	43C	AY267736
C22*	9 (3 F + 6 M)	9W	FJ687608
C23*	2 (2 M)	2W	FJ687609
Total	117 (50 F + 67 M)		

Note: Gender-specific haplotype frequencies (F, female; M, male) are indicated in parentheses. STRUCTURE assignments are from the $K = 2$ analysis of the autosomal microsatellite genotypes: C = $q_{Coyote} \geq 0.80$; W = $q_{Wolves} \geq 0.80$; A = $0.20 < q_{Coyote} < 0.80$.

*Haplotype of coyote or eastern wolf (*Canis lycaon*) origin.

†Haplotype of gray wolf origin.

to visually distinguish wolves and coyotes, the typically bushy coats make visual assessment of condition difficult, and thus skeletal dimensions probably have a greater influence on hunters' perceptions of the size of wolves and coyotes. Regardless, there was nearly complete concordance between the phenotypic classification and the genetic assignment of individuals, except for one individual that was phenotypically classified as a wolf but genetically assigned as a coyote. The morphological measurements of the misclassified individual were within the maximum range for coyotes and below the minimum range for wolves (note: except for mass of an emaciated 15 kg (skinned) wolf that lowered the minimum mass range for wolves below that of the misclassified individual), thus the genetic assignment seems valid; the animal was misclassified based on phenotype. In general, our data suggest that wolves and coyotes in the sampled area can be reliably distinguished based on physical characteristics; accounting for the individual classified as unknown, phenotypic classification accuracy was 98%. The assessment of pelage is recommended for future studies comparing wolves and coyotes because some hunters may use this character, which can be variable within and between species (e.g., Kolenosky and Standfield 1975), for distinguishing these species. Furthermore, shoulder height would be a more appropriate morphological variable to assess than chest girth because the former is a more perceivable physical characteristic. We measured shoulder height on carcasses but reduced sample size because

of many carcasses missing feet precluded inclusion of this morphological variable in our analyses.

The four individuals assigned as admixed had haplotype compositions and morphologies generally consistent with their phenotypic classifications (Supplementary Table S1).³ Considering the uncertainty in the STRUCTURE assignments of these individuals, as indicated by wide probability intervals, we simply acknowledge that some admixture is apparent between wolves and coyotes in NEON, even when accounting for admixture from APP wolves (Supplementary Fig. S1).³

We observed a low proportion of individuals exhibiting admixture between wolves and coyotes in NEON (Fig. 3), but the haplotypes they shared (i.e., C1, C14, AA, CE) are also observed in APP wolves, which have hybridized with coyotes and NEON wolves (Wheeldon 2009; Rutledge et al. 2010). Thus, it is difficult to assess patterns of introgression. For instance, the presence of C-EW haplotypes (i.e., C1, C14, AA) in wolves in NEON may be the result of introgression from coyotes or APP wolves, and the presence of Y-chromosome haplotype CE in a coyote in NEON may be the result of introgression from a NEON wolf or an APP wolf. Notably, C-EW haplotype C17 was observed in wolves and not coyotes in NEON, but it occurs in APP wolves (Rutledge et al. 2010), indicating that its presence in NEON wolves is probably the result of introgression from APP wolves. Similarly, C-EW haplotype C14 was rare in coyotes ($n = 1$) but common in wolves ($n = 13$) in NEON, and it is common in APP wolves (Rutledge et al. 2010), suggesting that it was likely introgressed into NEON wolves from APP wolves, but also plausibly from coyotes. The low amount of admixture that we observed between wolves and coyotes in NEON (Fig. 3) further supports the introgression of some C-EW haplotypes into NEON wolves from APP wolves, as previously reported (Rutledge et al. 2010). Furthermore, analysis of our data in STRUCTURE at $K = 3$ including APP wolves, revealed admixture from APP wolves in our sample of NEON wolves (Supplementary Fig. S1).³ However, that admixture was detected between wolves and coyotes in NEON suggests that some shared haplotypes are plausibly the result of wolf-coyote hybridization that occurs at low levels in NEON. In summary, wolves and coyotes in NEON are genetically differentiated but with some shared ancestry that derives from a combination of low-level wolf-coyote hybridization in NEON and introgression from APP wolves. Notably, the admixed nature of wolves in NEON seems to be reflected in

Table 2. Y-chromosome haplotype frequencies in male wolves (*Canis lupus*) and coyotes (*Canis latrans*).

Haplotype	MS34A	MS34B	MS41A	MS41B	Frequency	STRUCTURE assignments
AA ^a	172	180	212	212	25	22C; 2W; 1A
AF ^b	172	180	208	222	4	4W
BB ^a	170	182	212	226	3	3C
CC ^b	172	178	208	214	2	2W
CD ^a	172	178	214	210	4	3C; 1A
CE ^b	172	178	208	216	4	1C; 3W
CM ^a	172	178	214	218	10	9C; 1A
CS ^b	172	178	208	226	5	5W
CT ^b	172	178	208	220	3	3W
FF ^c	174	178	208	222	6	6C
Total					66	

Note: First letter of haplotype indicates allele combination for loci MS34A/B and second letter of haplotype indicates allele combination for loci MS41A/B. STRUCTURE assignments are from the $K = 2$ analysis of the autosomal microsatellite genotypes: C = $q_{\text{coyote}} \geq 0.80$; W = $q_{\text{wolf}} \geq 0.80$; A = $0.20 < q_i < 0.80$.

^aHaplotype of coyote or eastern wolf (*Canis lycium*) origin.

^bHaplotype of gray wolf origin.

^cHaplotype of putative domestic dog origin.

Table 3. Mitochondrial DNA (mtDNA) and Y-chromosome haplotype composition of male wolves (*Canis lupus*) and coyotes (*Canis latrans*).

mtDNA haplotype	Y-chromosome haplotypes
C1 ^a	AA ^a (1C), AF ^b (1W), CM ^a (1C), CS ^b (1W), FF ^c (3C)
C9 ^a	AA ^a (11C; 1A), BB ^a (3C), CD ^a (1C; 1A), CM ^a (1A)
C14 ^a	AA ^a (2W; 1C), AF ^b (1W), CS ^b (2W), CT ^b (2W)
C17 ^a	CE ^b (1W), CS ^b (1W)
C19 ^a	AA ^a (9C), CD ^a (2C), CE ^b (1C), CM ^a (8C), FF ^c (3C)
C22 ^b	AF ^b (1W), CC ^b (2W), CE ^b (2W), CS ^b (1W)
C23 ^b	AF ^b (1W), CT ^b (1W)

Note: The frequency of each haplotype combination and the corresponding individual STRUCTURE assignment(s) from the $K = 2$ analysis of the autosomal microsatellite genotypes are indicated in parentheses: C = $q_{\text{coyote}} \geq 0.80$; W = $q_{\text{wolf}} \geq 0.80$; A = $0.20 < q_i < 0.80$.

^aHaplotype of coyote or eastern wolf (*Canis lycium*) origin.

^bHaplotype of gray wolf origin.

^cHaplotype of putative domestic dog origin.

their morphology, which ranges from that of gray wolves to eastern wolves (Holloway 2009; Rutledge et al. 2010; Benson et al. 2012).

A Y-chromosome haplotype of presumed gray wolf origin was observed in coyotes but not wolves in our sample (Table 2), and this haplotype is common in domestic dogs (Sundqvist et al. 2006: FF = H12, allele data obtained from authors for comparison), suggesting that it was plausibly introgressed into coyotes from dogs. The observation of a putative dog Y chromosome in coyotes in NEON is congruent with evidence of introgressed dog genes in coyotes based on single nucleotide polymorphisms (vonHoldt et al. 2011).

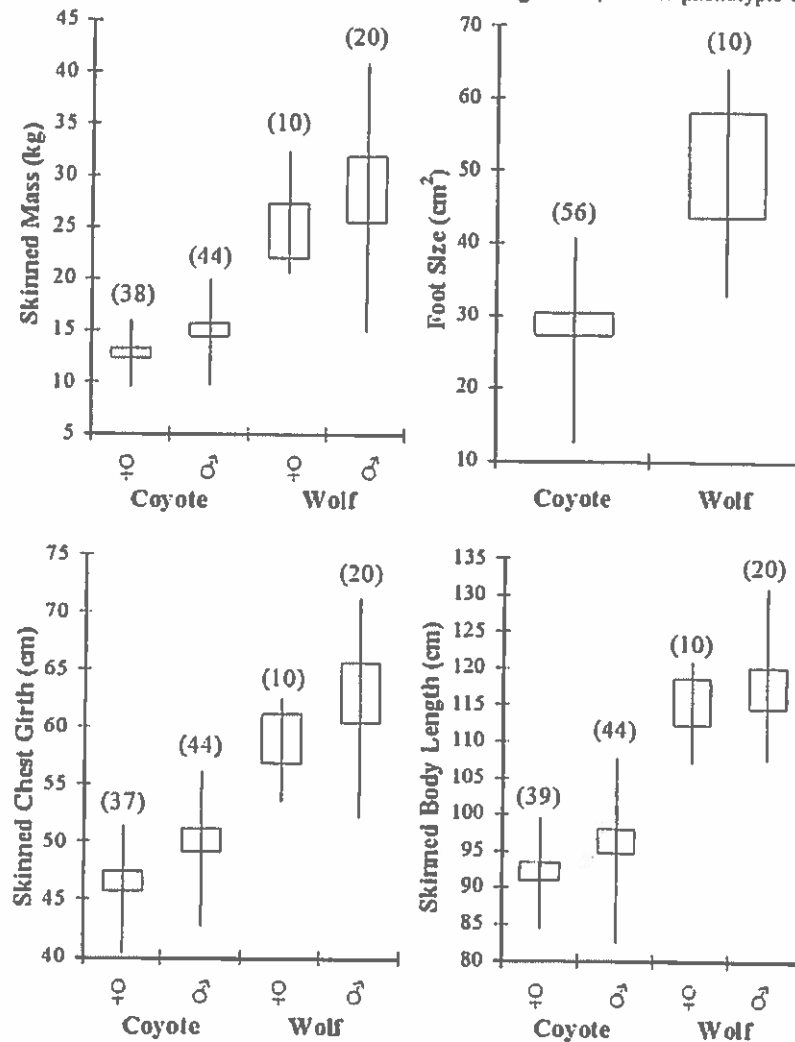
One of the original objectives of this study was to determine the ability of hunters in northeastern Ontario to distinguish wolves from coyotes in the field based on tracks and premortem observations. Indeed, we found a high degree of correspondence between the phenotypic classifications and the genetic assignments of the animals sampled. To the best

of our knowledge, the phenotypic classifications reported by the hunters were based on impressions prior to harvesting the animals, but because we cannot assess whether any hunters changed their classifications once animals were observed more closely after harvest, we must be cautious in drawing inference from these findings; misidentification rates may have been underestimated. The above issue also applies to trappers that observed animals premortem; however, trapped animals comprised a small portion of our sample.

Although we omitted carcasses of juveniles from our analysis, during late summer and early autumn a juvenile wolf will overlap in mass and measurements with yearling-adult coyotes, and thus might not be visually distinguishable from coyotes at a distance, especially during inclement weather and low light levels, which can reduce visibility. Considering that hunters have misidentified wolves as large "trophy" coyotes (e.g., CBC News 2012) or mistaken domestic dogs for coyotes (e.g., NFR 2010), some unintended wolf kill due to hunter misidentification should be anticipated if wolves and coyotes are managed separately where they occur sympatrically in Ontario. It is difficult to anticipate how common such unintended harvest might be, but we note that unintended harvest of elk (*Cervus elaphus* L., 1758) occurs at low levels during both white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)) and moose (*Alces alces* (L., 1758)) seasons in Ontario. Similarly, every year in Ontario a substantial number of cow moose are mistakenly shot by hunters possessing only calf tags. As presently occurs for the aforementioned ungulate species, an addition to existing hunter education programs aimed at providing guidance in distinguishing species may help minimize misidentification of canid species.

Considering that (i) wolf-coyote hybridization is spatially structured in Ontario (e.g., Wheeldon 2009; Rutledge et al. 2010) and (ii) eastern wolves are generally intermediate in size to gray wolves and coyotes (e.g., Rutledge et al. 2010; Benson et al. 2012) and have a limited distribution, levels of wolf-coyote hybridization and phenotypic ambiguity of can-

Fig. 4. Box and whisker plots of gender-specific morphological measurements of skinned carcasses of wolves (*Canis lupus*) and coyotes (*Canis latrans*). Boxes represent the mean and 95% confidence intervals, and whiskers indicate the range. Sample sizes are indicated in parentheses. The wolf and coyote classifications are based on the STRUCTURE assignments, not the phenotypic classifications.



ids will vary across PWR in Ontario. Specifically, in areas north and west of the study area (i.e., zones 1–3; Fig. 1), where wolf–coyote hybridization and eastern wolves are uncommon (Holloway 2009; Wheeldon 2009; Wheeldon et al. 2010), it is probable that wolves and coyotes can be reliably distinguished and thus managed separately with minimal misidentification by hunters. Conversely, in areas south of the study area (i.e., zones 4 and 5; Fig. 1), where wolf–coyote hybridization and eastern wolves are more prevalent (Wheeldon 2009; Rutledge et al. 2010; Benson et al. 2012), it is probable that wolves and coyotes cannot be reliably distinguished on a consistent basis and thus separate management may result in significant numbers of wolves and (or) hybrids being killed due to misidentification by hunters. Accordingly, the results of this study should not be generalized across all wolf range in Ontario. Further genetic and morphological assessment of harvested wolves and coyotes and the phenotypic classification accuracy by hunters and trappers is required in zones 4 and 5 to determine if these species are reliably distinguishable in areas where wolf–coyote hybridization and

eastern wolves are more prevalent. Determining more precisely the geographic distribution of eastern wolves in Ontario is relevant to predicting areas where phenotypic ambiguity of wolves and coyotes is likely. Furthermore, because visual observation is probably insufficient to distinguish hybrids from pure types, the issue remains of how to treat eastern wolf – coyote hybrids with respect to management; this issue is less relevant for gray wolf – eastern wolf hybrids because all wolves are presently governed by the same harvest regulations throughout PWR in Ontario (OMNR 2005).

The feasibility of separate management of wolves and coyotes is a separate issue to the ethical considerations around facilitating liberal harvest of coyotes just because it is unlikely to cause their extirpation. This is particularly true given the ambiguity around whether increased harvest of coyotes will help reduce livestock depredation (e.g., Knowlton et al. 1999; Wagner and Conover 1999; Berger 2006) or other conflicts with humans. Ethical issues and ecological value are traditionally not considered in management decisions for

most species, including coyotes (e.g., Minter and Collins 2005; Clapham et al. 2007), but this may be changing (e.g., Vucetich and Nelson 2010).

Debate regarding the potential for eastern coyotes to fill the ecological role of a top canid predator notwithstanding (e.g., Ballard et al. 1999; Crête et al. 2001), there is continued interest and pressure from some nongovernment organizations to restore wolves to their historic ranges in northeastern North America (e.g., Harrison and Chapin 1998; Elder 2000; Glowa et al. 2009; CREW 2012; but see also Lohr et al. 1996). The eastern wolf likely occupied much of northeastern North America historically (Wilson et al. 2003), but it readily hybridizes with coyotes, and as evidenced by the red wolf (*Canis rufus* Audubon and Bachman, 1851) recovery program in North Carolina, efforts to restore the eastern wolf to the NEUS would likely require intensive, expensive, and prolonged management intervention to minimize introgression from coyotes (Kelly et al. 1999; Adams et al. 2007). Furthermore, because of hybridization the eastern wolf may no longer exist in its original form; APP wolves may merely be the closest extant representation. Our findings provide further evidence that NEON wolves are less likely to hybridize with coyotes than are APP wolves (this study and Wheeldon 2009 versus Rutledge et al. 2010; Benson et al. 2012), possibly because the former tend to be larger than the latter and thus are more disparate in size to coyotes. Thus, wolves similar to those found in NEON may be desirable candidates for wolf reintroduction in the NEUS. The most likely candidates for natural wolf recolonization of the NEUS would come from Quebec and are probably similar to NEON wolves, as supported by the morphology and genetic composition of several wolves killed in the NEUS since the late 1990s (see Glowa et al. 2009 versus Grewal et al. 2004; Holloway 2009; Wheeldon 2009). In planning wolf recovery for the NEUS, the consideration of wolf type will likely be debated; hopefully such debate will contribute to greater understanding of the ecological and social consequences of wolf restoration in the region, but not distract from the overall goal of restoring a viable and ecologically functional population of wolves, regardless of taxonomy.

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References

- Adams, J.R., Lucash, C., Schutte, L., and Waits, L.P. 2007. Locating hybrid individuals in the red wolf (*Canis rufus*) experimental population area using a spatially targeted sampling strategy and faecal DNA genotyping. *Mol. Ecol.* 16(9): 1823–1834. doi:10.1111/j.1365-294X.2007.03270.x. PMID:17444895.
- Ballard, W.B., Whitlaw, H.A., Young, S.J., Jenkins, R.A., and Forbes, G.J. 1999. Predation and survival of white-tailed deer fawns in northcentral New Brunswick. *J. Wildl. Manage.* 63(2): 574–579. doi:10.2307/3802645.
- Benson, J.F., Patterson, B.R., and Wheeldon, T.J. 2012. Spatial genetic and morphologic structure of wolves and coyotes in relation to environmental heterogeneity in a *Canis* hybrid zone. *Mol. Ecol.* In press.
- Berger, K.M. 2006. Carnivore–livestock conflicts: effects of subsidized predator control and economic correlates on the sheep industry. *Conserv. Biol.* 20(3): 751–761. doi:10.1111/j.1523-1739.2006.00336.x. PMID:16909568.
- CBC News. 2012. Wolf killed in N.B. 1st in century. Available from <http://www.cbc.ca/news/canada/new-brunswick/story/2012/05/08/nb-wolf-dna-confirm.html> [accessed 9 May 2012].
- Clapham, P.J., Childerhouse, S., Gales, N.J., Rojas-Bracho, L., Tillman, M.F., and Brownell, R.L., Jr. 2007. The whaling issue: conservation, confusion, and casuistry. *Mar. Policy*, 31(3): 314–319. doi:10.1016/j.marpol.2006.09.004.
- Crête, M., Ouellet, J.-P., Tremblay, J.-P., and Arseneault, R. 2001. Suitability of the forest landscape for coyotes in northeastern North America and its implications for coexistence with other carnivores. *Ecoscience*, 8(3): 311–319. Available from http://www.cen.ulaval.ca/anticosti/pdf/CV_JPTremblay/Crete_et_al.2001.pdf [accessed 24 March 2012].
- CREW (Coalition to Restore the Eastern Wolf). 2012. The case for restoring the eastern wolf. Available from http://www.restore.org/Wildlife/wolf_brochure.pdf [accessed 11 July 2012].
- Elder, J. 2000. The return of the wolf: reflections on the future of wolves in the northeast. Middlebury College Press, Hanover, N.H.
- Fain, S.R., Straughan, D.J., and Taylor, B.F. 2010. Genetics outcomes of wolf recovery in the western Great Lakes states. *Conserv. Genet.* 11(5): 1747–1765. doi:10.1007/s10592-010-0068-x.
- Gipson, P.S., Ballard, W.B., Nowak, R.M., and Mech, L.D. 2000. Accuracy and precision of estimating age of gray wolves by tooth wear. *J. Wildl. Manage.* 64(3): 752–758. doi:10.2307/3802745.
- Glowa, J.M., Pepperman, W.L., Schadler, C.L., Butera, J., and Way, J.G. 2009. Petition submitted to protect wolves and allow wolf recovery in the northeast. Available from <http://easterncoyoteresearch.com/downloads/ESApetition2009final.pdf> [accessed 24 March 2012].
- Grewal, S.K., Wilson, P.J., Kung, T.K., Shami, K., Theberge, M.T., Theberge, J.B., and White, B.N. 2004. A genetic assessment of the eastern wolf (*Canis lycaon*) in Algonquin Provincial Park. *J. Mammal.* 85(4): 625–632. doi:10.1644/1545-1542(2004)085<0625:AGAOTE>2.0.CO;2.
- Hailer, F., and Leonard, J.A. 2008. Hybridization among three native North American *Canis* species in a region of natural sympatry. *PLoS ONE*, 3(10): e3333. [online.] doi:10.1371/journal.pone.0003333. PMID:18841199.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95–98 Available from <http://jwbrown.mbio.ncsu.edu/TWB/papers/1999Hall1.pdf> [accessed 24 March 2012].
- Harrison, D.J., and Chapin, T.G. 1998. Extent and connectivity of habitat for wolves in eastern North America. *Wildl. Soc. Bull.* 26(4): 767–775.
- Holloway, J. 2009. Size dependent resource use of a hybrid wolf (*C. lycaon* x *lupus*) population in northeast Ontario. M.Sc. thesis, Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ont.
- Hubisz, M.J., Falush, D., Stephens, M., and Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* 9(5): 1322–1332. doi:10.1111/j.1755-0998.2009.02591.x. PMID:21564903.
- Kelly, B.T., Miller, P.S., and Seal, U.S. 1999. Population and habitat viability assessment workshop for the Red Wolf (*Canis rufus*). Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, Minn. Available from <http://www.cbsg.org/cbsg/work>

- shopreports/23/red_wolf_phva_%281999%29.pdf [accessed 24 March 2012].
- Knowlton, F.F., Gese, E.M., and Jaeger, M.M. 1999. Coyote depredation control: an interface between biology and management. *J. Range Manage.* 52(5): 398–412. doi:10.2307/4003765.
- Kobl Müller, S., Nord, M., Wayne, R.K., and Leonard, J.A. 2009. Origin and status of the Great Lakes wolf. *Mol. Ecol.* 18(11): 2313–2326. doi:10.1111/j.1365-294X.2009.04176.x. PMID: 19366404.
- Kolenosky, G.B., and Standfield, R.O. 1975. Morphological and ecological variation among gray wolves (*Canis lupus*) of Ontario, Canada. In *The wild canids: their systematics, behavioral ecology and evolution*. Edited by M.W. Fox. Van Nostrand Reinhold, New York. pp. 62–72.
- Landon, D.B., Waite, C.A., Peterson, R.O., and Mech, L.D. 1998. Evaluation of age determination techniques for gray wolves. *J. Wildl. Manage.* 62(2): 674–682. doi:10.2307/3802343.
- Lohr, C., Ballard, W.B., and Bath, A. 1996. Attitudes toward gray wolf reintroduction to New Brunswick. *Wildl. Soc. Bull.* 24(3): 414–420.
- Minteer, B.A., and Collins, J.P. 2005. Why we need an “ecological ethics”. *Front. Ecol. Environ.* 3(6): 332–337. doi:10.1890/1540-9295(2005)003[0332:WWNAEE]2.0.CO;2.
- NFR (Niagara Falls Review). 2010. Coyote hunters shoot family dog. Available from <http://www.stcatharinesstandard.ca/2010/02/05/coyote-hunters-shoot-family-dog> [accessed 9 May 2012].
- Nowak, R.M. 2009. Taxonomy, morphology, and genetics of wolves in the Great Lakes region. In *Recovery of gray wolves in the Great Lakes region of the United States: an endangered species success story*. Edited by A.P. Wydeven, T.R. Van Deelen, and E.J. Heske. Springer-Verlag, New York. pp. 233–250.
- OMNR (Ontario Ministry of Natural Resources). 2005. Strategy for wolf conservation in Ontario. Available from <http://www.mnr.gov.on.ca/stdprodconsume/groups/tr/@mnr/@fw/documents/document/263811.pdf> [accessed 24 March 2012].
- Ostrander, E.A., Sprague, G.F., Jr., and Rine, J. 1993. Identification and characterization of dinucleotide repeat (CA)_n markers for genetic mapping in dog. *Genomics*, 16(1): 207–213. doi:10.1006/geno.1993.1160. PMID:8486359.
- Ostrander, E.A., Mapa, F.A., Yee, M., and Rine, J. 1995. One hundred and one new simple sequence repeat-based markers for the canine genome. *Mamm. Genome*, 6(3): 192–195. doi:10.1007/BF00293011. PMID:7749226.
- Pilgrim, K.L., Boyd, D.K., and Forbes, S.H. 1998. Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. *J. Wildl. Manage.* 62(2): 683–689. doi:10.2307/3802344.
- Pritchard, J.K., Stephens, M., and Donnelly, P.J. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155(2): 945–959. PMID:10835412.
- Rutledge, L.Y., Garroway, C.J., Loveless, K.M., and Patterson, B.R. 2010. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. *Heredity*, 105(6): 520–531. doi:10.1038/hdy.2010.6. PMID: 20160760.
- Ruxton, G.D. 2006. The unequal variance *t*-test is an underused alternative to Student's *t*-test and the Mann–Whitney *U* test. *Behav. Ecol.* 17(4): 688–690. doi:10.1093/beheco/ark016.
- Schmitz, O.J., and Kolenosky, G.B. 1985. Wolves and coyotes in Ontario: morphological relationships and origins. *Can. J. Zool.* 63(5): 1130–1137. doi:10.1139/z85-171.
- Sears, H.J., Theberge, J.B., Theberge, M.T., Thornton, I., and Campbell, G.D. 2003. Landscape influence on *Canis* morphological and ecological variation in a coyote-wolf *C. lupus x latrans* hybrid zone, southeastern Ontario. *Can. Field-Nat.* 117: 589–600. Available from <http://www.canadianfieldnaturalist.ca/index.php/cfn/article/view/828/828> [accessed 24 March 2012].
- Sundqvist, A.-K., Ellegren, H., Olivier, M., and Vilà, C. 2001. Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers. *Mol. Ecol.* 10(8): 1959–1966. doi:10.1046/j.1365-294X.2001.01326.x. PMID:11555240.
- Sundqvist, A.-K., Björnerfeldt, S., Leonard, J.A., Hailer, F., Hedhammar, A., Ellegren, H., and Vilà, C. 2006. Unequal contribution of sexes in the origin of dog breeds. *Genetics*, 172(2): 1121–1128. doi:10.1534/genetics.105.042358. PMID: 16219789.
- Vähä, J.-P., and Primmer, C.R. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Mol. Ecol.* 15(1): 63–72. doi:10.1111/j.1365-294X.2005.02773.x. PMID:16367830.
- Venables, W.N., and Ripley, B.D. 2002. *Modern applied statistics with S-PLUS*. 4th ed. Springer-Verlag, New York.
- vonHoldt, B.M., Pollinger, J.P., Earl, D.A., Knowles, J.C., Boyko, A.R., Parker, H., Geffen, E., Pilot, M., Jędrzejewski, W., Jędrzejewska, B., Sidorovich, V., Greco, C., Randi, E., Musiani, M., Kays, R., Bustamante, C.D., Ostrander, E.A., Novembre, J., and Wayne, R.K. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Res.* 21(8): 1294–1305. doi:10.1101/gr.116301.110. PMID:21566151.
- Vucetich, J.A., and Nelson, M.P. 2010. Sustainability: virtuous or vulgar? *Bioscience*, 60(7): 539–544. doi:10.1525/bio.2010.60.7.9.
- Wagner, K.K., and Conover, M.R. 1999. Effect of preventative coyote hunting on sheep losses to coyote predation. *J. Wildl. Manage.* 63(2): 606–612. doi:10.2307/3802649.
- Wheeldon, T. 2009. Genetic characterization of *Canis* populations in the western Great Lakes region. M.Sc. thesis, Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ont.
- Wheeldon, T.J., Patterson, B.R., and White, B.N. 2010. Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. *Mol. Ecol.* 19(20): 4428–4440. doi:10.1111/j.1365-294X.2010.04818.x. PMID:20854277.
- Wilson, P.J., Grewal, S., Lawford, I.D., Heal, J.N.M., Granacki, A.G., Pennock, D., Theberge, J.B., Theberge, M.T., Voigt, D.R., Waddell, W., Chambers, R.E., Paquet, P.C., Goulet, G., Cluff, D., and White, B.N. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Can. J. Zool.* 78(12): 2156–2166. doi:10.1139/z00-158.
- Wilson, P.J., Grewal, S., McFadden, T., Chambers, R.C., and White, B.N. 2003. Mitochondrial DNA extracted from eastern North American wolves killed in the 1800s is not of gray wolf origin. *Can. J. Zool.* 81(5): 936–940. doi:10.1139/z03-059.
- Wilson, P.J., Rutledge, L.Y., Wheeldon, T.J., Patterson, B.R., and White, B.N. 2012. Y-chromosome evidence supports widespread signatures of three-species *Canis* hybridization in eastern North America. *Ecol. Evol.* In press. doi:10.1002/ece3.301.