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Wolf body mass, skull morphology, and mitochondrial DNA haplotypes in the Riding Mountain National Park region of Manitoba, Canada

Astrid V. Stronen, Graham J. Forbes, Tim Sallows, Gloria Goulet, Marco Musiani, and Paul C. Paquet

Abstract: Two types of wolves, gray (*Canis lupus* L., 1758) and eastern (*Canis lupus lycaon* Schreber, 1775 or *Canis lycaon*) or Great Lakes wolves, representing Old World (OW) and New World (NW) mitochondrial DNA (mtDNA) haplotypes, have been reported in eastern Canada and the Great Lakes region. Both haplotypes were found in Duck Mountain Provincial Park and Forest, Manitoba. Only OW haplotypes have been reported from the isolated Riding Mountain National Park (RMNP), 30 km to the south. Wolves with NW haplotypes hybridize with *C. lupus* and coyotes (*Canis latrans* Say, 1823) and could mediate gene flow between canids. We examined available data on wolf body mass, skull morphology, and mtDNA from the RMNP region, as well as mtDNA from Manitoba and Saskatchewan, to assess the occurrence of NW haplotypes in wolves and possible canid hybridization. Mean body mass of female ($n = 54$) and male ($n = 42$) RMNP wolves during 1985–1987 was higher than that of females ($n = 12$) and males ($n = 8$) during 1999–2004. Thirteen skull measures from 29 wolf skulls did not suggest significant differences between RMNP and Duck Mountain wolves. Nineteen of 20 RMNP samples had OW haplotypes, whereas one clustered together with NW haplotypes.

Résumé : On a signalé deux types de loups, le loup gris (*Canis lupus* L., 1758) et le loup de l'est ou loup des Grands Lacs (*Canis lupus lycaon* Schreber, 1775 ou *Canis lycaon*), représentant des haplotypes d'ADN mitochondrial (ADNmt) de l'Ancien Monde (OW) et du Nouveau Monde (NW), dans l'est du Canada et la région des Grands Lacs. Les deux haplotypes se retrouvent dans la Forêt et le Parc provincial de Duck Mountain, Manitoba. Seuls les haplotypes OW ont été signalés au Parc national du Mont-Riding (RMNP), une région isolée 30 km plus au sud. Les loups possédant des haplotypes NW s'hybrident avec *C. lupus* et avec les coyotes (*Canis latrans* Say, 1823) et pourraient favoriser le flux génique entre les canidés. Nous examinons les données disponibles sur la masse corporelle, la morphologie des crânes et l'ADNmt des loups de la région du RMNP ainsi que sur l'ADNmt du Manitoba et de la Saskatchewan afin d'évaluer l'occurrence des haplotypes NW chez les loups et la possibilité d'hybridation entre les canidés. La masse corporelle moyenne des loups femelles ($n = 54$) et mâles ($n = 42$) du RMNP en 1985–1987 était plus importante que celle des loups femelles ($n = 12$) et mâles ($n = 8$) en 1999–2004. Treize mesures faites sur 29 crânes de loups n'indiquent aucune différence significative entre les loups du RMNP et de Duck Mountain. Dix-neuf des 20 échantillons provenant du RMNP possédaient des haplotypes OW et un échantillon se regroupait avec les haplotypes NW.

[Traduit par la Rédaction]

Introduction

The gray wolf (*Canis lupus* L., 1758) was historically widely distributed across Eurasia and North America with high rates of gene flow (Kurtén and Anderson 1980; Vilà et al. 1999) but has experienced extensive range contraction, primarily owing to hunting, poisoning, and reduced prey abundance (Paquet and Carbyn 2003; Leonard et al. 2005).

Recent genetic research indicates that two types of wolves, gray and eastern (*Canis lupus lycaon* Schreber, 1775; use of *Canis lycaon* proposed by Wilson et al. 2000) or Great Lakes (Leonard and Wayne 2008) wolves, occur in east-central North America. Gray wolves are considered to have evolved in Eurasia (Kurtén and Anderson 1980) and represent Old World (OW) mitochondrial DNA (mtDNA) haplotypes. Eastern or Great Lakes wolves are characterized by

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mtDNA haplotypes closely related to the North American native coyote (*Canis latrans* Say, 1823), and have been found in the Canadian provinces of Quebec, Ontario, and Manitoba (Wilson et al. 2000; Grewal et al. 2004) and in Great Lakes states that include Minnesota, Wisconsin, and Michigan (Kobl Müller et al. 2009a; Fain et al. 2010). They hybridize with *C. lupus* and *C. latrans* in Ontario (Grewal 2001; Wilson et al. 2009) and the Great Lakes states (Leonard and Wayne 2008; Kobl Müller et al. 2009a; but see Fain et al. 2010).

The evolutionary history and conservation status of wolves with haplotypes closely related to coyotes is unresolved (Wilson et al. 2000, 2009; Leonard and Wayne 2008, 2009; Cronin and Mech 2009; Kays et al. 2009; Kobl Müller et al. 2009a, 2009b; Mech 2009, 2010; Schwartz and Vucetich 2009; Wheeldon and White 2009; Fain et al. 2010; Rutledge et al. 2010). However, we hereafter refer to these wolves, and to coyotes, as representing New World (NW) haplotypes. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has listed the eastern wolf (referred to as gray wolf subspecies *C. l. lycaon*) as Special Concern for which *C. latrans* introgression is considered a threat (COSEWIC 2001). The introgression of *C. latrans* does not appear to constitute a risk to *C. lupus* where the two species overlap in western North America (Pilgrim et al. 1998; COSEWIC 2001).

Wilson et al. (2000) reported a mixture of OW and NW mtDNA haplotypes in Duck Mountain Provincial Park and Forest (hereafter the Duck Mountains) in southwestern Manitoba, whereas nuclear microsatellite analyses placed wolves from Riding Mountain National Park (RMNP), approximately 30 km to the south, close to populations from the Northwest Territories and Pukaskwa National Park in northern Ontario. Pukaskwa wolves have been identified as having primarily OW mtDNA with only few occurrences of NW haplotypes, although the latter appear common outside the Park (Grewal 2001; Wilson et al. 2009). Other mtDNA studies have found RMNP wolves to be distinct from other North American populations (Geffen et al. 2004). A mtDNA haplotype found in RMNP grouped with northern and western North American and Eurasian wolves and was not reported elsewhere (Lehman et al. 1991).

Wolves and coyotes are sympatric throughout RMNP and occupy different ecological niches with no evidence of hybridization (Carbyn 1982a; Paquet 1992). The possibility of hybridization has nonetheless been noted as a concern for this isolated population (Carbyn 1980). Wolves are the primary predators of elk (*Cervus elaphus* L., 1758) in RMNP and conserving predation processes is vital for the ecological integrity of the Park (Parks Canada 2004), which is located at the western edge of the currently proposed range of wolves with NW haplotypes (Kyle et al. 2006). As wolves with NW haplotypes seem capable of mediating gene flow between canid types (Wilson et al. 2009), their potential presence may have significant consequences for genetic and ecological exchangeability (Templeton 1989) in RMNP and the farmlands that now surround the Park and encompass much of the transition zone between the Prairie and the Boreal Plain ecozones (Environment Canada 1993).

Conservation management of North American canids requires further information on the range of wolves with NW

haplotypes and whether wolves with divergent, but presumably neutral (nonselective), mtDNA haplotypes are ecologically different. Molecular genetics, behaviour, morphology, range, and distribution should therefore be evaluated together when examining taxonomy and differentiation between populations (Ryder 1986; O'Brien and Mayr 1991; Frankham et al. 2002). Contemporary evolution can be both diversifying and homogenizing (see review in Carroll 2008) and human-dominated environments appear, at times, to promote homogenizing selection in canids (Kolenosky and Standfield 1975; Schmitz and Kolenosky 1985; Schmitz and Lavigne 1987; Sears et al. 2003). Multiple factors could also influence the extent and direction of canid hybridization, including the presence of an intermediate-size canid (Hailer and Leonard 2008). Recent findings from Minnesota, where wolves with OW and NW haplotypes appear to meet (Lehman et al. 1991; Wilson et al. 2000; Leonard and Wayne 2008; Fain et al. 2010), indicate a cline in body mass from the northwest to the northeast (Mech and Paul 2008). If coyotes and wolves with NW haplotypes are sympatric and interbreed in the Duck Mountains, we expect Duck Mountain wolves to be smaller than RMNP wolves, with less massive skulls and coyote-like features such as a narrower muzzle (Kolenosky and Stanfield 1975). However, we also expect that wolves with NW haplotypes are now dispersing into Riding Mountain National Park. We examine available data on wolf body mass and skull morphology from the RMNP region, report mtDNA findings from the region and beyond, and discuss these results with respect to their ecological significance for RMNP and other small wolf populations in human-dominated landscapes.

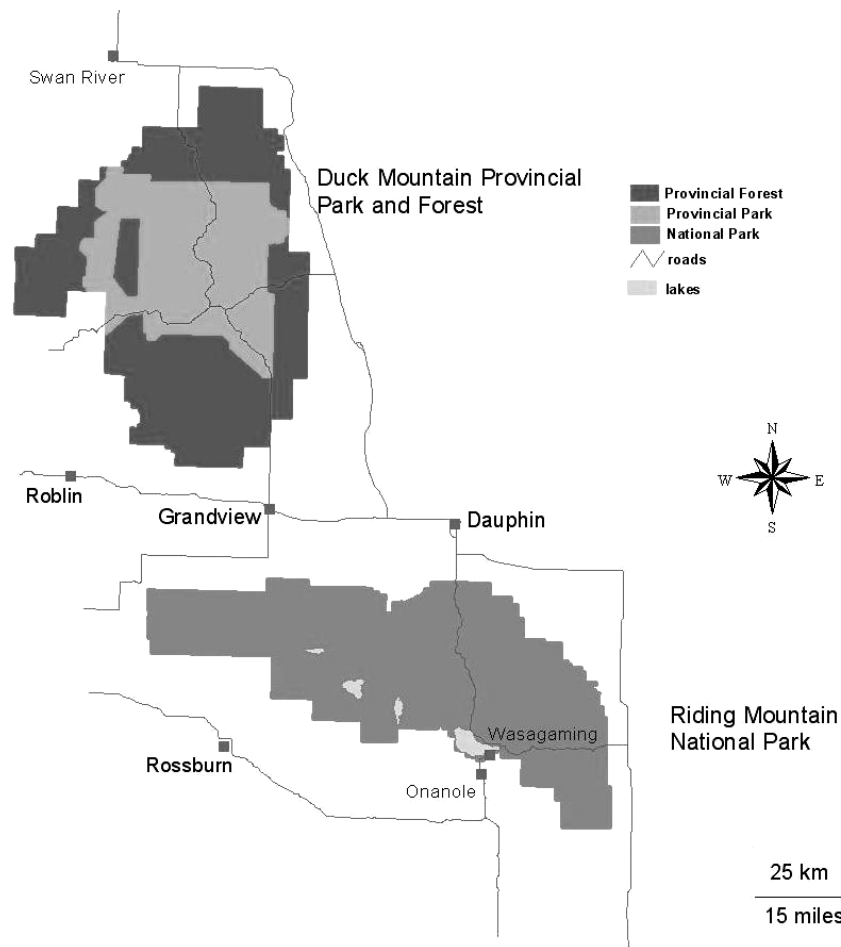
Materials and methods

Study area

Our core study area encompasses the Duck Mountains (5184 km²) and Riding Mountain Biosphere Reserve (15000 km²), where the latter comprises the core protected area RMNP (2974 km²) and 15 surrounding rural municipalities. RMNP is located approximately 30 km south of the Duck Mountains (Fig. 1). The area is part of the Manitoba Escarpment, a series of highlands in western Manitoba separated by broad valleys that include numerous lakes and ponds; deciduous, boreal and mixed forest; rough fescue grasslands; and extensive marshes and wetlands (Manitoba Conservation 2004; Parks Canada 2006). The climate is continental interior, with cold winters and moderate snow depths (Carbyn 1982b). The growing season is variable but averages 72 days (Parks Canada 2004). Considerable human-caused landscape fragmentation has occurred in the region surrounding RMNP (McNamee 1993). Forest cover was almost continuous between RMNP and the Duck Mountains until the 1950s, but only 14% remained by 1991 and intense agricultural development between RMNP and the Duck Mountains effectively severed RMNP from other forested areas (Walker 2001). Agriculture is now the dominant land use and occupies approximately 58% of the area around RMNP (35% cropland and 23% rangeland), whereas managed public land (including parks) makes up 16% (Parks Canada 2004).

Elk, moose (*Alces alces* (L., 1758)), beaver (*Castor cana-*

Fig. 1. Study area in southwestern Manitoba, Canada. Riding Mountain National Park is located 265 km northwest of the city of Winnipeg.



densis Kuhl, 1820), and white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)) are abundant in the region, although moose is rarely reported from the agricultural landscape between RMNP and the Duck Mountains (Brook 2007). Endemic mule deer (*Odocoileus hemionus* (Rafinesque, 1817)) are now rare and have been replaced by white-tailed deer, which have extended their range over the past century in response to human-induced landscape change. Other large mammals include wolves, black bears (*Ursus americanus* Pallas, 1780), coyotes, lynx (*Lynx canadensis* Kerr, 1792), red fox (*Vulpes vulpes* L., 1758), snowshoe hare (*Lepus americanus* Erxleben, 1777), and several mustelid species.

Wolves have been present in southern Manitoba for at least 5000 years (Goulet 1993). The species occupied the RMNP region until a combination of hunting, trapping, land clearing, and poisoning probably caused a local extirpation around 1900 (Carbyn 1980). By the 1930s, reports from Park wardens and residents confirmed that wolves had returned, possibly via dispersal from forested regions north of RMNP (Fritts and Carbyn 1995). RMNP wolves have been tracked for several multiyear studies since 1974 with no evidence of successful dispersal from the Park to surrounding areas (Carbyn 1980; Paquet 1992; Stronen 2009).

Body mass

We measured the body mass of 96 adult wolves >3 years

old ($n = 54$ females and $n = 42$ males) in good condition (based on body fat) killed inside or within a 10 km radius of RMNP between 1985 and 1987. Wolves <3 years old were segregated from older animals based on types (deciduous or permanent), number, and stage of eruption of teeth, as well as ossification of skull sutures and fusion of epiphyses on long bones. Subsequently, we measured the body mass of 20 adult wolves from the same area ($n = 12$ females and $n = 8$ males) between 1999 and 2004, of which 6 females and 3 males were live-captured and radio-collared as part of a study of dispersal in RMNP (Stronen 2009). No data are available from the Duck Mountains, and little more than a decade separates the two intervals of measurements from RMNP. However, we compare these available data to assess whether the body mass of adult RMNP wolves appears to be unchanged, or whether it may be declining and therefore might indicate wolf-coyote hybridization.

Skull morphology

We made 13 morphological measurements (Goulet 1993; Nowak 1995) on 29 Manitoba wolf skulls (17 males and 12 females) from RMNP and the Duck Mountains available at the Manitoba Museum in Winnipeg (Table 1) to assess whether Duck Mountain wolves are smaller than RMNP wolves and have narrower muzzles. Nowak (1995) suggests that these characters express major adaptive features of the wolf skull regarding overall size, cranial protection, grasping

Table 1. Morphological measures on Riding Mountain National Park and Duck Mountain Provincial Park and Forest wolf skulls at the Manitoba Museum, Winnipeg, Canada, 2005.

Number	Measure	Description
1*	length	Greatest length of skull
2	zyg. width	Greatest distance across zygomata
3	P1–M2	Alveolar length of maxillary tooth row (P ¹ –M ²)
4	cheek	Maximum width across upper cheek teeth (carnassials, P ⁴)
5	p. width	Palatal width at first premolars (P ¹)
6	fr. shield	Width of frontal shield
7	M1–orbit	Height from tooth row to orbit
8	jugal	Depth of jugal
9	carnas.	Crown length of upper carnassial (P ⁴)
10	M2	Crown width of second upper molar (M ²)
11	a–a	Braincase width at widest point
12	b–b	Length from second upper molar (M ²) to depression in front of auditory bullae
13	g–g	Least width across frontals at constriction behind postorbital processes

Note: All skulls were considered fully grown based on (i) full eruption of canine teeth and (ii) closing of skull sutures. Measures 1–10 described with images in Nowak (1995), whereas measures 11–13 are described in Goulet (1993). Measures were recorded to the nearest 0.05 mm.

*Larger caliper required; measured to nearest millimetre.

Table 2. Adult wolf body mass (kg; mean and SE) from Riding Mountain National Park, Manitoba, Canada, 1985–2004.

Period	Females				Males			
	<i>n</i>	Mean (SE)	Minimum	Maximum	<i>n</i>	Mean (SE)	Minimum	Maximum
1985–1987	54	38.76 (0.64)	31.0	48.0	42	45.95 (0.80)	36.0	53.0
1999–2004	12	35.62 (1.00)	31.7	43.1	8	39.00 (1.18)	34.0	43.0

Note: Wolves were collected inside or within 10 km of RMNP. All individuals, with the exception of one female in 2004, were noted as being in fair to good condition.

Table 3. Wolf skull measures (mean and SE) from Riding Mountain National Park and Duck Mountain Provincial Park and Forest, Manitoba, Canada.

Measure	Males			Females		
	Duck Mountain (SE); <i>n</i> = 7	RMNP (SE); <i>n</i> = 10	Mean (SE); <i>n</i> = 17	Duck Mountain (SE); <i>n</i> = 8	RMNP (SE); <i>n</i> = 4	Mean (SE); <i>n</i> = 12
1. length	255.43 (3.64)	261.20 (4.24)	258.8 (2.92)	255.50 (4.47)	243.50 (2.75)	251.5 (3.48)
2. zyg. width	137.43 (2.17)	138.70 (3.05)	138.2 (1.96)	138.37 (1.68)	134.55 (1.40)	137.1 (1.29)
3. P1–M2	85.35 (0.90)	88.02 (1.11)	86.9 (0.80)	84.94 (1.53)	81.44 (1.54)	83.7 (1.21)
4. cheek	79.66 (1.11)	82.65 (1.31)	81.4 (0.94)	80.18 (1.41)	79.74 (0.88)	80.0 (0.96)
5. p. width	32.22 (0.88)	32.36 (0.79)	32.3 (0.57)	32.52 (0.74)	31.43 (0.68)	32.2 (0.55)
6. fr. shield	63.14 (2.93)	63.04 (2.02)	63.1 (1.64)	62.16 (1.43)	63.89 (1.79)	62.7 (1.11)
7. M1–orbit	39.02 (0.68)	40.56 (0.71)	39.9 (0.52)	39.61 (0.79)	37.09 (0.81)	38.8 (0.67)
8. jugal	19.36 (0.56)	19.16 (0.51)	19.2 (0.37)	19.33 (0.49)	18.06 (0.01)	18.9 (0.37)
9. carnas.	25.14 (0.37)	26.17 (0.38)	25.7 (0.29)	24.99 (0.53)	23.99 (0.30)	24.7 (0.39)
10. M2	13.64 (0.39)	14.01 (0.15)	13.9 (0.18)	13.76 (0.21)	13.21 (0.14)	13.6 (0.16)
11. a–a	66.45 (1.47)	65.39 (0.64)	65.8 (0.70)	66.84 (0.52)	63.24 (0.96)	65.6 (0.68)
12. b–b	68.36 (1.32)	68.09 (1.42)	68.2 (0.97)	67.27 (1.58)	63.50 (1.40)	66.0 (1.24)
13. g–g	41.63 (1.20)	39.40 (0.82)	40.3 (0.72)	40.65 (1.50)	39.40 (1.08)	40.2 (1.04)

Note: All measures are in millimetres. Measurement details are provided in the Materials and methods.

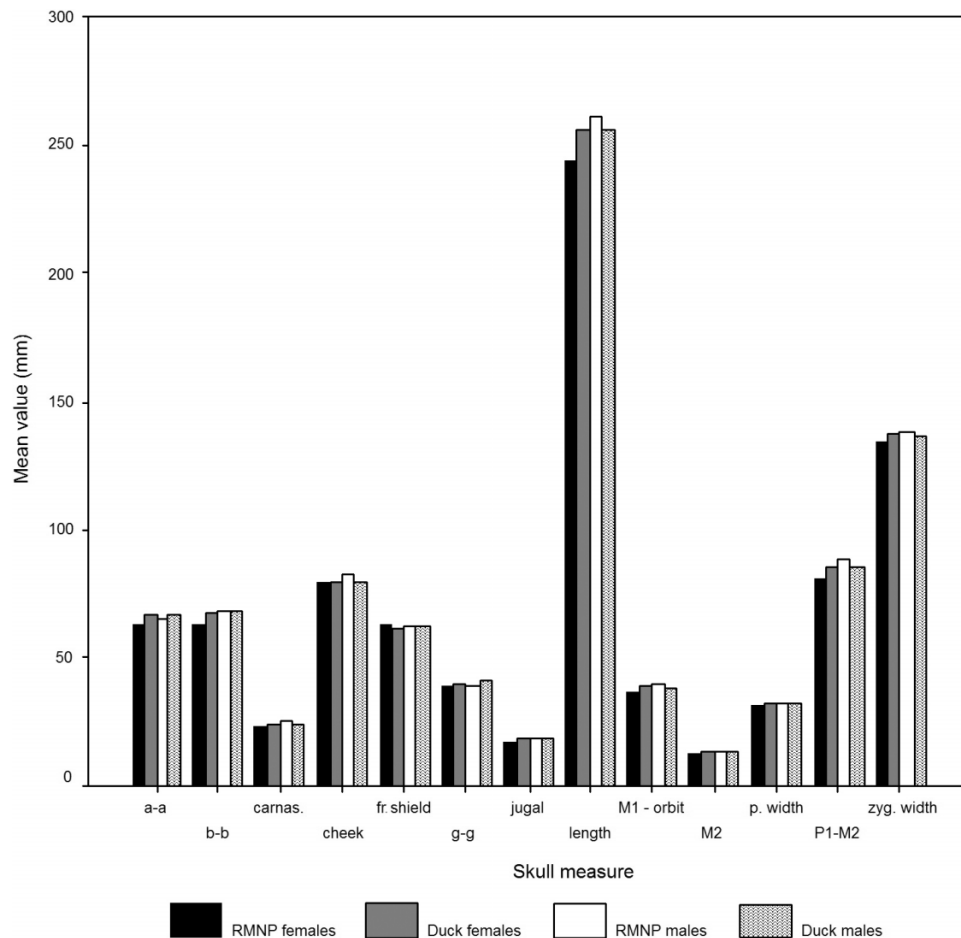
power, and capacity for cutting and crushing. The measure of skull length required a larger calliper and we measured this distance to the nearest millimetre; all other measures were done to the nearest 0.05 mm.

mtDNA analyses

We opportunistically collected tissue samples available from human-caused and accidental mortality from 2003 to

2005. mtDNA from the control region (D-loop) was amplified by polymerase chain reaction (PCR) and purified for 10 wolves and 2 coyotes collected inside or near RMNP. We used primers THR-L 5'-GAATTCCCCGGTCTTG-TAAACC-3' and H16498 5'-CCTGAAGTAGGAACCA-GATG-3'. Aliquots of 2–6 µL were taken from each sample and mixed with primer and a premix containing fluorescently labelled nucleotides and *Taq* DNA polymerase

Fig. 2. Mean values of wolf skull measurements from Riding Mountain National Park and Duck Mountain Provincial Park and Forest, Manitoba, Canada.



(BigDye Terminator V3.1 Kit) for a total PCR volume of 10 μ L. For each sample, two reactions were prepared, one for the forward and one for the reverse primer. Following an initial denaturing step at 96 °C for 5 min, 25 cycles of amplification were performed for each reaction. Each cycle consisted of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s, and an extension at 60 °C for 4 min. This was followed by a hold at 4 °C, with varying lengths of time. Ethanol precipitation was then performed twice, to remove any remains of the fluorescent nucleotides, and the samples were spun upside down for 1 min to dry the pellets. Ten microlitres of the DNA denaturing agent formamide was then added to dissolve the DNA. Sequencing was performed using a 3730XL DNA Analyser supplied by Applied Biosystems. The mtDNA sequences have been submitted to GenBank (accession nos. HM014451–HM014467).

Statistical analyses

We calculated values (mean \pm SE) for skull measurements and body-mass data. We examined sequence divergence for 239 base pairs of the mtDNA control region, comparing recent samples with previously identified haplotypes from within our primary study area and other regions of Manitoba and Prince Albert National Park (PANP) in Saskatchewan (P. Paquet, unpublished data). Furthermore, we compared our results with haplotypes from eastern North America (ob-

tained from NCBI GenBank) examined by Wilson et al. (2000), who first reported wolves with NW haplotypes from our study area. This data set included haplotypes from the red wolf (*Canis rufus* Audubon and Bachman, 1851) captive breeding program, Ontario, Texas, and Ohio that grouped close to the NW haplotype found in a Manitoba wolf. We also included haplotypes from Europe (Ellegren et al. 1996) examined by Wilson et al. (2000). We aligned haplotypes using CLC Free Workbench version 3.2 (Knudsen et al. 2005), made manual adjustments, and created a neighbour-joining tree of haplotypes using 1000 bootstrap replicates. Subsequently, we used the program TCS (Clement et al. 2000) to estimate gene genealogies with a method that incorporates the possibility of recombination (Avice 1991; Bromham et al. 2003). The method is well suited to analyses at the population level (Clement et al. 2000). We excluded gaps in the haplotypes for this analysis. This can reduce the number of haplotypes (where some haplotypes become identical when gaps are removed), which can simplify the output and provide better overview of the relationship between a large number of haplotypes.

Results

Body mass and skull morphology

Mean body mass was lower for male and female wolves

Table 4. North American canid mtDNA haplotypes per location, with sample sizes.

Sample code	Sample description	Haplotype with number of samples	Number of samples
NWMan	Northwestern Manitoba	C22 (5), C23 (2), 13 (1), 14 (1), 15 (1)	10
NEMan	Northeastern Manitoba	C22 (1)	1
EMan	Eastern Manitoba	C23 (2)	2
Duck	Duck Mountains, Manitoba	C3 (3), C22 (1), C23 (3), 12 (1)	8
PANP	Prince Albert National Park, Saskatchewan	C22 (4), 16 (18), 17 (2)	24
PA latrans	Prince Albert <i>Canis latrans</i>	4 (1)	1
RMNP	Riding Mountain National Park, Manitoba	C23 (13), 5 (1), 6 (1), 7 (1), 8 (1) 9 (1), 10 (1), 11 (1)	20
RM latrans	Riding Mountain <i>Canis latrans</i>	1 (1), 2 (1), 3 (1)	3
Montana	Montana, USA	C22 (1)	1
Rufus	<i>Canis rufus</i>	C2 (9), C19 (3), 18 (1)	13
Algonquin	Algonquin Provincial Park, Ontario	C1 (7), C9 (1), C14 (3), C17 (1), C19 (1)	13
NAlgonquin	North of Algonquin Provincial Park, Ontario	C1 (1), C16 (1), C23 (1)	3
OH latrans	Ohio, USA, <i>Canis latrans</i>	C5 (1)	1
TX latrans	Texas, USA, <i>Canis latrans</i>	C4 (1), C6 (2), C7 (1), C8 (1), C10 (1), C11 (1), C12 (1), C15 (1), C18 (2), C19 (12), C20 (2), C21 (2)	27
NWT	Northwest Territories	C23 (1)	1
N QC	Northern Quebec	C23 (1)	1
NW ON	Northwest Ontario	C13 (2), C24 (1)	3
Nbor ON	Northern boreal Ontario	C23 (1)	1
FF ON	Fort Francis, Ontario	C23 (1)	1
S ON	Southern Ontario	C1 (1), C9 (1), C14 (2), C19 (4)	8

Note: Haplotypes with C-prefix are from Wilson et al. 2000. C22 also was identified in a Montana *C. lupus* reference sample provided to P. Paquet and 18 from a *C. rufus* sample contributed by J. Leonard.

sampled after 1990 (Table 2). The change in mean mass, as well as minimum and maximum mass, was larger for male wolves, and all three measures decreased with time. Minimum female mass was the only measure that showed an increase after 1990. Mean skull measures were generally larger for RMNP males than for Duck Mountain males, whereas the opposite relationship was seen in females (Table 3, Fig. 2). The results did not suggest that Duck Mountain wolves had narrower muzzles than that of RMNP wolves. Cheek width was slightly smaller in Duck Mountain males than Riding Mountain males, but the opposite was seen in females. Palatal width was similar in males from both areas, whereas Duck Mountain females had slightly wider palates.

mtDNA haplotype frequencies and sequence divergence

We found 13 haplotypes within the primary study area and an additional 7 from other regions of Manitoba and Saskatchewan (Tables 4, 5). Of these, four Manitoba and one Saskatchewan variants were from *C. latrans*. Haplotypes C22 and C23 are found in both provinces. Northern Manitoba had three haplotypes that were only found in this area (13, 14, 15), whereas PANP had two (16, 17). Seven haplotypes (5–12) were found only in RMNP, and C3 and 12 only in the Duck Mountains. Haplotypes from northern and eastern Manitoba, RMNP, and PANP generally clustered together and close to OW haplotypes from North America and Europe in a neighbour-joining tree (Fig. 3). In the Duck Mountains, C3 and 12 grouped together and near C1 from Ontario's Algonquin Provincial Park region and southern Ontario.

Nineteen of 20 RMNP samples had OW haplotypes. The

most frequent RMNP haplotype (C23) is common and shared with the Duck Mountains, but the remaining RMNP haplotypes were not observed outside the Park. RMNP haplotype 5, found in a fecal sample from within the Park, is located closer to NW haplotypes from the Duck Mountains, Algonquin, *C. rufus*, and *C. latrans* than to other RMNP variants. OW and NW haplotypes form two separate groups in the TCS gene genealogy with gaps in the haplotypes excluded (Fig. 4). Here, haplotypes C3 and 12 from the Duck Mountains are grouped with C1 and closer to OW haplotypes than haplotype 5.

Discussion

Body mass and skull morphology

We found a decline in mean wolf body mass in RMNP. If the presence of intermediate-sized canids with NW haplotypes facilitates hybridization (Hailer and Leonard 2008), smaller RMNP wolves might be more likely to hybridize with other canids in and around the Park. Our results from available skull samples do not support obvious divergence in skull shape or size between RMNP and the Duck Mountains. Samples of Duck Mountain males had a slightly narrower muzzle (palatal and cheek teeth width) and narrower second upper molars compared with samples from RMNP. However, the difference is small and the opposite relationship was seen in females. Nowak (1995) found male Algonquin wolves (referred to as *C. l. lycaon*) to have narrower palates than male wolves grouped with western Ontario *C. l. lycaon* and *Canis lupus hudsonicus* Goldman, 1941 (including northern Manitoba), but Algonquin wolves also had wider upper second molars.

Table 5. North American canid mtDNA haplotypes per location, with sample sizes and data listed by haplotype.

Haplotype	Location	Number of samples
1	Riding Mountain National Park (RMNP), Manitoba (1)	1
2	RMNP, Manitoba (1)	1
3	RMNP, Manitoba (1)	1
4	Prince Albert National Park (PANP), Saskatchewan (1)	1
5	RMNP, Manitoba (1)	1
6	RMNP, Manitoba (1)	1
7	RMNP, Manitoba (1)	1
8	RMNP, Manitoba (1)	1
9	RMNP, Manitoba (1)	1
10	RMNP, Manitoba (1)	1
11	RMNP, Manitoba (1)	1
12	Duck Mountains, Manitoba (1)	1
13	Northwestern Manitoba (1)	1
14	Northwestern Manitoba (1)	1
15	Northwestern Manitoba (1)	1
16	PANP, Saskatchewan (18)	18
17	PANP, Saskatchewan (2)	2
18	<i>Canis rufus</i> (1)	1
C1	Algonquin Provincial Park, Ontario (7); north of Algonquin (1); southern Ontario (1)	9
C2	<i>Canis rufus</i> (9)	9
C3	Duck Mountains, Manitoba (3)	3
C4	Texas (1)	1
C5	Ohio (1)	1
C6	Texas (2)	2
C7	Texas (1)	1
C8	Texas (1)	1
C9	Algonquin Provincial Park, Ontario (1); southern Ontario (1)	2
C10	Texas (1)	1
C11	Texas (1)	1
C12	Texas (1)	1
C13	Northwest Ontario (2),	2
C14	Algonquin Provincial Park, Ontario (3); southern Ontario (2),	5
C15	Texas (1)	1
C16	North of Algonquin, Ontario (1)	1
C17	Algonquin Provincial Park, Ontario (1)	1
C18	Texas (2)	2
C19	Texas (12); <i>Canis rufus</i> (3); Algonquin Provincial Park, Ontario (1); southern Ontario (4)	20
C20	Texas (2)	2
C21	Texas (2)	2
C22	Northwestern Manitoba (5); northeastern Manitoba (1); Duck Mountains, Manitoba (1); PANP (4); Montana (1)	12
C23	Northwestern Manitoba (2); eastern Manitoba (2); Duck Mountains, Manitoba (3); RMNP (13); north of Algonquin, Ontario (1); Northwest Territories (1); northern Quebec (1); northern boreal Ontario (1); Fort Francis Ontario (1)	25
C24	Northwest Ontario (1)	1

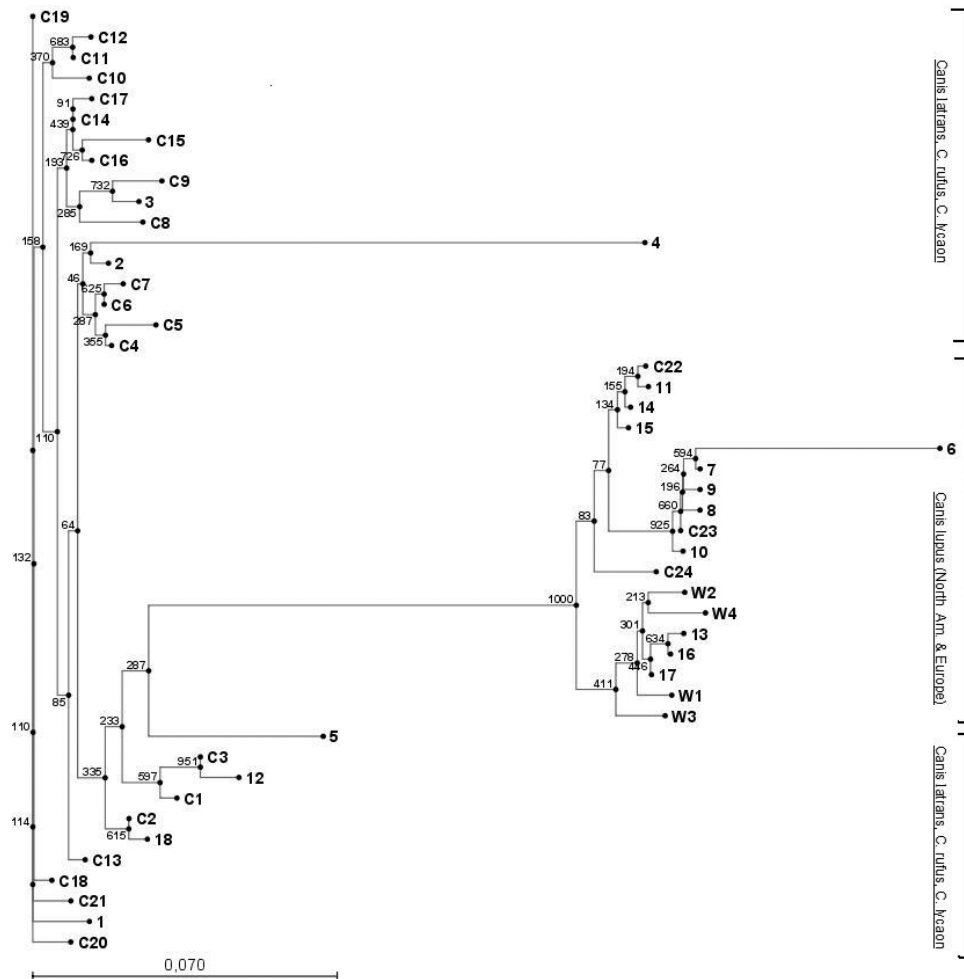
Note: Haplotypes with C-prefix are from Wilson et al. (2000). C22 was also identified in a Montana *C. lupus* reference sample provided to P. Paquet and 18 from a *C. rufus* sample contributed by J. Leonard. For abbreviations of sample locations see Table 4.

mtDNA haplotype frequencies and sequence divergence

The majority of Manitoba and all Saskatchewan haplotypes collected from wolves grouped with OW haplotypes from North America and Europe. The high bootstrap value suggests that the division between OW and NW haplotypes is distinct. A wide zone of geographic overlap is observed, with haplotype C22 found throughout Manitoba and PANP (this study); in Ontario and Quebec (Wilson et al. 2000; Grewal et al. 2004; Rutledge et al. 2010); Michigan, Wis-

consin, and Minnesota (Wheeldon 2009; Fain et al. 2010), and haplotype C3 reported from Manitoba (Wilson et al. 2000; this study), Ontario (Rutledge et al. 2010), western Quebec (Grewal et al. 2004), and Michigan, Wisconsin, and Minnesota (Wheeldon 2009; Fain et al. 2010). Our haplotype findings from the Duck Mountains concur with that of Wilson et al. (2000). We found haplotype C3 and the closely related haplotype 12. The results from RMNP suggest that the Park is occupied primarily by *C. lupus*. The

Fig. 3. Neighbour-joining tree of sequence divergence for 239 base pairs of the mtDNA control region for canid haplotypes. Bootstrap values show support for nodes based on 1000 bootstrap trees (replicates). European haplotypes W1–W4 from Ellegren et al. (1996) are included for reference, whereas haplotypes with C-prefix are from Wilson et al. (2000). Sample locations for each haplotype are as follows: 1–3 (RMNP, Manitoba); 4 (PANP, Saskatchewan); 5–11 (RMNP, Manitoba); 12 (Duck Mountains, Manitoba); 13–15 (northwestern Manitoba); 16–17 (PANP, Saskatchewan); 18 (*Canis rufus*); C1 (Algonquin Provincial Park, north of Algonquin Provincial Park, and southern Ontario); C2 (*C. rufus*); C3 (Duck Mountains, Manitoba); C4 (Texas); C5 (Ohio); C6–C8 (Texas); C9 (Algonquin Provincial Park and southern Ontario); C10–C12 (Texas); C13 (northwestern Ontario); C14 (Algonquin Provincial Park and southern Ontario); C15 (Texas); C16 (north of Algonquin Provincial Park, Ontario); C17 (Algonquin Provincial Park, Ontario); C18 (Texas); C19 (Texas, *C. rufus*, Algonquin Provincial Park, and southern Ontario), C20–C21 (Texas); C22 (Duck Mountains, northwestern and northeastern Manitoba; PANP, Saskatchewan; Montana); C23 (Duck Mountains, RMNP, northwestern and eastern Manitoba; Fort Francis, north of Algonquin Provincial Park, and northern boreal Ontario; northern Quebec; Northwest Territories); and C24 (northwest Ontario).

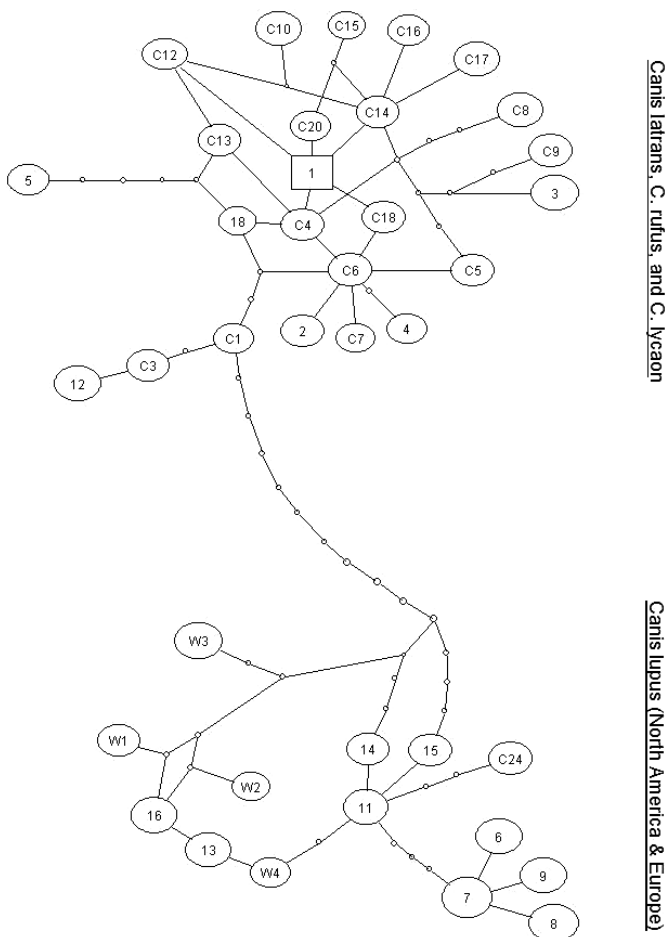


most frequent RMNP haplotype (C23) is common and shared with the Duck Mountains, but the remaining RMNP haplotypes were not observed outside the Park. More extensive analyses are required to assess whether these RMNP haplotypes are rare or unique.

RMNP haplotype 5 originated from a fecal sample collected on a hiking trail used by wolves, coyotes, and dogs (*Canis lupus familiaris* L., 1758). Population genetic analyses of 13 nuclear microsatellite markers placed the individual in a cluster with other RMNP wolves (Stronen 2009), whereas a dog or coyote likely would have been identified as an immigrant to RMNP according to our suite of microsatellite alleles (Thiessen 2007). However, it is also possible that an offspring from a breeding between a coyote female and a wolf male, the most likely route of introgression

(Roy et al. 1994), could have crossed back into the wolf population. Wheeldon and White (2009) found mtDNA haplotypes C1 and C13 in three samples from the Great Lakes region collected >100 years ago. They further noted that all three samples clustered with eastern-gray wolves from Manitoba, Minnesota, and northwestern-northeastern Ontario according to frequencies of microsatellite alleles and had low assignment probability to both eastern and western coyotes. C1 and C13 have also been reported in recent samples from Michigan, Wisconsin, Minnesota, and Ontario (Wheeldon 2009; Fain et al. 2010; Rutledge et al. 2010). Both haplotypes C1 and C13 are placed relatively close to haplotype 5 from RMNP. Their occurrence in canids identified as wolves according to frequencies of microsatellite alleles appears consistent with our findings. If

Fig. 4. Gene genealogy for 239 base pairs of the mtDNA control region for canid haplotypes. We excluded gaps in the haplotypes for this analysis. Some haplotypes therefore became identical: 7 = 10 = C23; 11 = C22; 16 = 17; 1 = C19 = C21; 18 = C2; and C12 = C11. European haplotypes W1–W4 from Ellegren et al. (1996) are included for reference, whereas haplotypes with C-prefix are from Wilson et al. (2000). Sample locations for each haplotype are as follows: 1–3 (RMNP, Manitoba); 4 (PANP, Saskatchewan); 5–11 (RMNP, Manitoba); 12 (Duck Mountains, Manitoba); 13–15 (northwestern Manitoba); 16–17 (PANP, Saskatchewan); 18 (*C. rufus*); C1 (Algonquin Provincial Park, north of Algonquin Provincial Park, and southern Ontario); C2 (*C. rufus*); C3 (Duck Mountains, Manitoba); C4 (Texas); C5 (Ohio); C6–C8 (Texas); C9 (Algonquin Provincial Park and southern Ontario); C10–C12 (Texas); C13 (northwestern Ontario); C14 (Algonquin Provincial Park and southern Ontario); C15 (Texas); C16 (north of Algonquin Provincial Park, Ontario); C17 (Algonquin Provincial Park, Ontario); C18 (Texas); C19 (Texas; *C. rufus*; Algonquin Provincial Park and southern Ontario); C20–C21 (Texas); C22 (Duck Mountains, northwestern and northeastern Manitoba; PANP, Saskatchewan; Montana); C23 (Duck Mountains, RMNP, northwestern and eastern Manitoba; Fort Francis, north of Algonquin Provincial Park, and northern boreal Ontario; northern Quebec; Northwest Territories); and C24 (northwest Ontario).



haplotype 5 represents wolves with NW haplotypes, these canids nonetheless seem to be rare in RMNP.

The largest genetic distances found within species tend to be between regions believed to have acted as glacial refugia

(Hewitt 2000; Nichols 2001). If wolves with NW haplotypes represent a canid that evolved in North America independent of *C. lupus* (Wilson et al. 2000), these canids could be extending their range westward. An increasing zone of overlap between NW and OW haplotypes might be a consequence of natural dispersal and mixture following retreat of the most recent ice sheets (Nowak 1983; Kyle et al. 2006). Our results do not support the occurrence of NW haplotypes in northern Manitoba or Saskatchewan, but further research is required to determine the evolutionary history of wolves with NW haplotypes, whether their range is expanding, and the relative role of biophysical versus human factors in defining their range.

Overall assessment

Despite finding primarily OW haplotypes in RMNP, we noted a decline in wolf body mass within the Park. Few data were available from after 1990 and we cannot exclude the possibility that our results occurred by chance or that the population might be declining in body mass owing to unknown factors. Preliminary data suggest that inbreeding might lead to reduced size in wolves (Fredrickson and Hedrick 2002), and further isolation of RMNP and subsequent inbreeding could therefore possibly result in smaller wolves. The extent to which wolves with NW haplotypes have smaller skull features and body mass, as well as a different ecological niche, from wolves with OW mtDNA has not been resolved. The wolf found in Algonquin Park weighs approximately 29 kg (adult male) and Forbes and Theberge (1996) found that these wolves rarely killed moose. However, recent work by Loveless (2010) suggests that moose predation is now common. Wolves in northern Minnesota also prey on moose, and mean mass of males was reported here to range from 30.6 to 35.9 kg (Mech and Paul 2008). The wolves of Mauricie National Park, Quebec, are considered eastern wolves and prey primarily on moose, and mean mass of three collared males was 44.5 kg (Villemure 2003). Similar canids might occur within the western range of wolves with NW haplotypes.

Although the Great Plains is now an intensively human-managed landscape (Guertin et al. 1997), the RMNP region has no physical barriers to wolf movement and constitutes a historically well-connected landscape (Walker 2001) with high likelihood of gene flow. If wolves with NW haplotypes now extend around the Great Lakes and have resided in the region >100 years (Leonard and Wayne 2008; Koblmüller et al. 2009a; Wheelon and White 2009), their apparent rarity in Pukaskwa and RMNP is noteworthy. Further research should examine if there are adaptive genetic differences between wolves with OW and NW mtDNA haplotypes. We also need more information on why interbreeding between wolves with NW haplotypes and *C. latrans* appears to be more common in some areas. In particular, it is important to understand whether human-induced landscape change, and the loss or decline in large prey species that often follows, is an important predictor of where wolf–coyote interbreeding occurs or is likely to take place in the future.

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