

Rapid adaptive evolution of northeastern coyotes via hybridization with wolves

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The dramatic expansion of the geographical range of coyotes over the last 90 years is partly explained by changes to the landscape and local extinctions of wolves, but hybridization may also have facilitated their movement. We present mtDNA sequence data from 686 eastern coyotes and measurements of 196 skulls related to their two-front colonization pattern. We find evidence for hybridization with Great Lakes wolves only along the northern front, which is correlated with larger skull size, increased sexual dimorphism and a five times faster colonization rate than the southern front. Northeastern haplotype diversity is low, suggesting that this population was founded by very few females moving across the Saint Lawrence River. This northern front then spread south and west, eventually coming in contact with an expanding front of non-hybrid coyotes in western New York and Pennsylvania. We suggest that hybridization with wolves in Canada introduced adaptive variation that contributed to larger size, which in turn allowed eastern coyotes to better hunt deer, allowing a more rapid colonization of new areas than coyotes without introgressed wolf genes. Thus, hybridization is a conduit by which genetic variation from an extirpated species has been reintroduced into northeastern USA, enabling northeastern coyotes to occupy a portion of the niche left vacant by wolves.

Keywords: hybridization; adaptive introgression; range expansion; genetic variation; morphological variation

1. INTRODUCTION

Dramatic expansions in the distribution of a species without being introduced by humans are rare, and are typically explained by habitat change or release from competitors (Sakai *et al.* 2001). The coyote (*Canis latrans*) evolved as hunter of small prey in the Great Plains, but has rapidly colonized all of eastern North America in the last 90 years. The spread of agriculture and the extinction of wolves (*C. lupus sensu lato*) in parts of the region are thought to have facilitated coyote expansion, but genetic interchange with remnant wolf populations may have played a role. Coyote colonization was fivefold faster via the northern

route through Ontario, which exposed them to wolf populations, compared with the southern route through Ohio, where wolves were extirpated prior to coyote expansion (figure 1).

The hybridization of colonizing coyotes with wolves (*C. lupus lycaon*) in Ontario has been demonstrated by recent studies (Leonard & Wayne 2008; Koblmüller *et al.* 2009; Schwartz & Vucetich 2009; Wheeldon & White 2009; Wilson *et al.* 2009). Mitochondrial phylogenies reveal three main lineages within Ontario wolves (grey wolf, Great Lakes wolf (GLW) and coyote), suggesting high rates of hybridization in the region. Nuclear loci reveal similar patterns, but suggest that the GLW remains a discrete ecotype despite hybridization.

Less attention has been paid to the effect of this hybridization on eastern coyotes, which are now the largest predator in the region, are abundant in many areas, and are thus thought to play important ecological roles. Although northeastern coyotes are clearly smaller than wolves, they are larger than western coyotes, and have a unique ecology (Lawrence & Bossert 1969; Parker 1995; Kays *et al.* 2008). Here we examine both genetics and morphology from a large sample of coyotes to evaluate the potential introgression of adaptive variation through hybridization with wolves.

2. MATERIAL AND METHODS

Coyote specimens were obtained by New York State Museum specimen salvage efforts, donations by fur trappers, hunters, state and provincial government agencies, scat collection (Kays *et al.* 2008), and by loans from other museums (see the electronic supplementary material, table S1). Our sample includes three large wolf-like canids from Vermont and New York that were not judged to be escaped pets based on the condition of their claws and teeth, and therefore are presumed to be recent immigrants (USFWS 2002, 2004, 2007). DNA was extracted using FastDNA (MP Biomedicals) or QIAmp Stool (Qiagen) kits. We amplified and sequenced a 369 bp part of the 5' end of the mitochondrial control region for 687 individuals using primers L15926 5'-TCAAAGCTTACACCAGTCTTG TAAAC-3' and H16498 5'-CCTGAAGTAGGAACCAGATG-3' (modified from Vilà *et al.* 1999). Double-stranded sequences were aligned and edited in SEQUENCHER v. 3.1.1. DNASP v. 5 (Librado & Rosas 2009) was used to calculate nucleotide diversity (π), haplotypic diversity and the population genetic parameter $\theta = 2N_e\mu$, all which we use to estimate relative levels of polymorphism. The HKY + Γ model of DNA evolution was selected by the Akaike information criterion implemented in MODELTEST 3.6.6 (Posada & Crandall 1998) and used in a heuristic, maximum likelihood tree search conducted in PAUP* v. 4.0b10. Support for nodes was assessed by bootstrapping (100 replicates).

We took 10 cranial and four mandibular measurements on 196 coyote skulls, including 126 animals with corresponding mtDNA data. All measurements were made by one person (A.C.), with an estimated error of 0.15 per cent. All specimens were of known sex and judged to be adults by the fusion of the basioccipital-basisphenoid suture. We used STATISTICA6 to conduct analysis of variance (ANOVA), grouping specimens into three regions based on colonization history and our genetic results; northeast ($n = 154$), west ($n = 21$) and Ohio ($n = 21$). Western specimens were represented by specimens from Montana ($n = 2$), Nebraska ($n = 8$) and Arizona ($n = 11$).

3. RESULTS

The phylogeny placing our new haplotypes within the context of published haplotypes is consistent with previous studies (Koblmüller *et al.* 2009) in showing that the eastern coyote population has both western coyote and GLW mtDNA, indicative of a history of hybridization among mitochondrial lineages (inset figure 2; complete tree in the electronic supplementary material, figure S1). From the geographical distribution of haplotypes and historical records of first occurrences in

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2009.0575> or via <http://rsbl.royalsocietypublishing.org>.

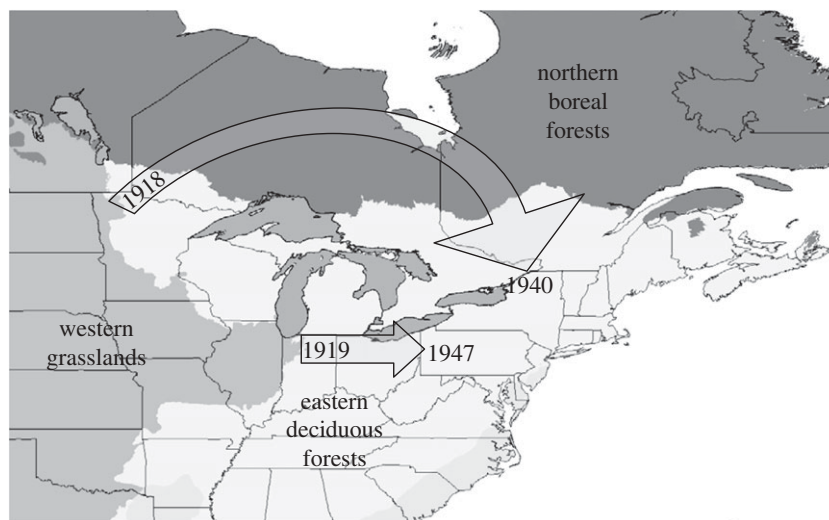


Figure 1. Colonization routes of coyotes moving from their historic range in the grasslands of western states into eastern deciduous forests (shading shows biomes). Dates are for the first coyote records from Ontario (Young & Jackson 1951), New York (Fener *et al.* 2005), Ohio (Weeks *et al.* 1990) and western Pennsylvania (Williams *et al.* 1985).

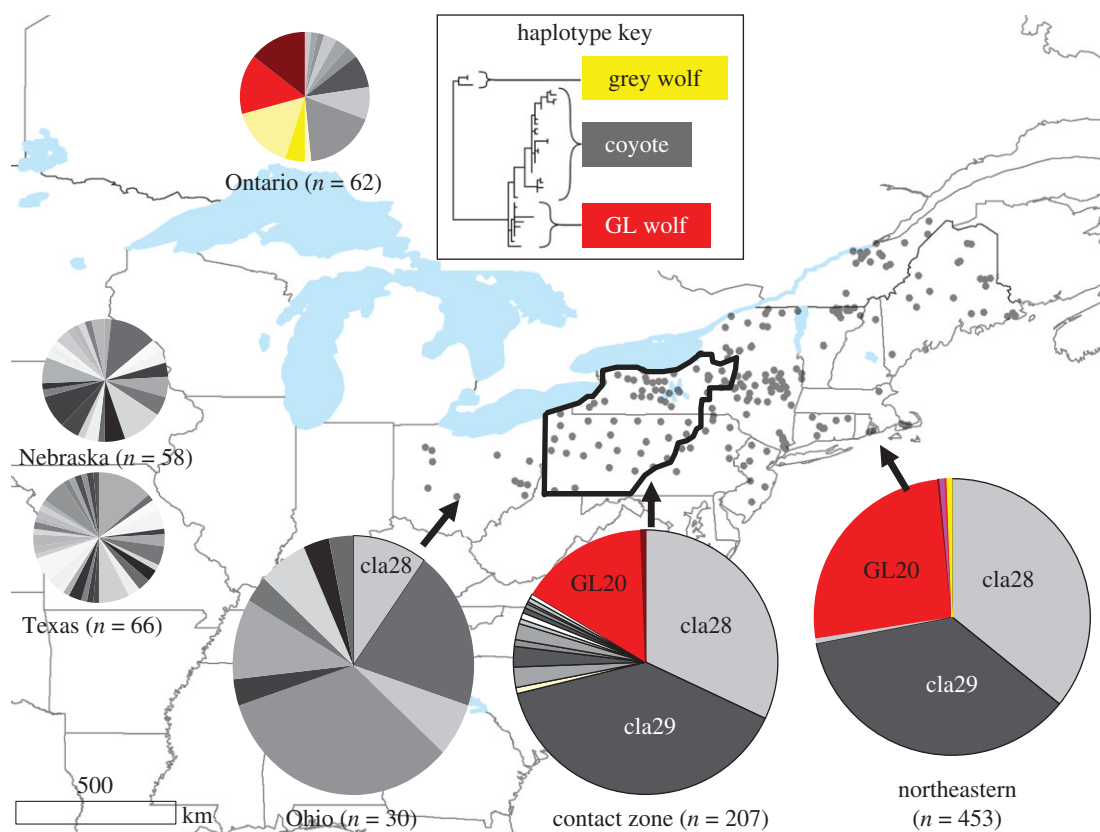


Figure 2. Mitochondrial haplotype frequencies of eastern coyotes and source populations. Sampling localities from this paper are shown as grey dots and summarized as large pie charts showing the proportion of animals with haplotypes categorized according to their position on the phylogeny. Smaller pie charts show haplotype frequencies representative of the two source populations: Ontario wolves and coyotes (Wilson *et al.* 2000; Leonard & Wayne 2008) and coyotes from western states (Hailer & Leonard 2008).

Ontario and eastern states (figure 1), we infer a region in western New York and Pennsylvania representing the contact zone between two advancing fronts of colonizing coyotes (figure 2). Haplotype diversity was highest in the west (Ohio) and lowest in the northeast (table 1). Our subsample east of the contact zone had the lowest value of θ , consistent with a very small

founding population. Comparison with the previously published surveys indicates that our sample from Ohio comprises a subset of the diversity found in western coyote populations, whereas nearly all northeastern coyotes (447 of 453 individuals) carry one of three common haplotypes (cla28, cla29, GL20; figure 2). The six remaining individuals in the

Table 1. Summary of genetic diversity observed in the total sample and three geographical subsamples of coyotes (figure 1).

	total	Ohio	contact	northeast
sample size	687	30	207	450
haplotypes	22	11	16	6
haplotype diversity	0.708	0.844	0.721	0.664
nucleotide diversity, π (per site)	0.0158	0.0133	0.0152	0.0158
θ (per site)	0.0128	0.0179	0.0140	0.0077
average pairwise number of nucleotide differences, k	5.57	4.69	5.36	5.56

northeastern sample include three coyotes carrying rare haplotypes (GL21, GL22 and cla37), and three large wolves (two lu32, one GL23, not included in our coyote population genetics calculations). In addition, one partial sequence of a dog-like haplotype was obtained from a coyote in Vermont. The inferred contact zone has an intermediate value of haplotype diversity (table 1), but the highest number of haplotypes, indicating admixture between the diverse western coyote source and the coyote-wolf hybridized source.

Northeast coyote skulls from both sexes were larger than those from Ohio and western states (table 2), especially in skull width (zygomatic width (ZW), 4–9% larger) and the area of muscle attachment on the mandible (width anterior portion of the ramus (WAR), 8–15% larger). In addition, unlike western coyotes, northeastern coyotes were sexually dimorphic in all measurements (table 2). Within the northeast, we found correlations between genotype and phenotype, but these varied by sex. In males, two measurements were related to mtDNA haplotype (ANOVA, d.f. = 62, $p < 0.005$ and < 0.05 , respectively) in that animals with the GL20 (Tukey HSD post hoc tests $p < 0.0$) and cla29 ($p < 0.05$) had wider skulls (ZW) than individuals with cla28, and that WAR was larger in males with cla29 than those with cla28 ($p < 0.05$). In northeastern female coyotes the height from the base of the first upper molar to the orbit was correlated with haplotype (ANOVA, d.f. = 51, $p < 0.05$), which was primarily driven by larger skulls in animals with GL20 compared with cla28 (Tukey HSD post hoc tests $p < 0.05$).

4. DISCUSSION

The ecological differences between western and northeastern coyotes, on average, are that northeastern animals eat more deer (*Odocoileus* sp.) but fewer small mammals (Parker 1995), and show no avoidance of forested habitats (Kays *et al.* 2008). The larger body size of northeastern coyotes is widely accepted as advantageous for hunting large prey, but there has been debate about the origin of this variation through hybridization versus phenotypic plasticity

(Lariviere & Crete 1993; Peterson & Thurber 1993). Our results show that northeastern coyote populations are a hybrid swarm resulting from the widespread introgression of GLW genes. This suggests that hybridization introduced genetic variation for the rapid adaptation of more efficient predation on deer, including larger predator body size and skull dimensions. This is further supported by our finding that northeastern coyotes were larger than those from Ohio, which are living in similar eastern forests, but have not hybridized with wolves. Mitochondrial genes are surely not responsible for the large body size, so the observed associations of particular haplotypes with skull morphology suggest that this hybrid swarm is young. That is, the linkage of mtDNA and morphology has not had sufficient time to break down through recombination.

Northeastern coyote skulls are not simply larger versions of their western relatives, but show additional craniodental characteristics similar to wolves, supporting the hypothesis of the introgression of genetic variation; northeastern skulls are proportionally broader, with greater areas of attachment for masticatory musculature. In large-prey hunters, such as wolves, these traits are associated with strong bite forces and resistance to the mechanical stresses imposed by large, struggling prey (Slater *et al.* 2009). Furthermore, the sexual dimorphism we found in northeastern coyotes is absent in western coyotes, but similar to that reported for wolves (Gittleman & Van Valkenburgh 1997). We suggest that these traits confer similar adaptive advantages in northeastern coyotes and allow them to be more proficient in the capture of deer than western and Ohio coyotes. These adaptations presumably allowed the rapid movement of coyote-wolves through Ontario, in comparison with the slower colonization rate of the smaller non-hybridized coyotes across Ohio.

The geographical pattern of genetic diversity we found in eastern coyotes reflects the reported colonization route (Parker 1995), with a pure coyote front advancing from the west and meeting a coyote-wolf hybrid swarm that colonized earlier from the north. The low number of haplotypes in the northeast is consistent with the idea that the population was founded by a few animals crossing the Saint Lawrence River, with little or no genetic contribution from the scattered introductions of coyotes by hunt clubs (Fener *et al.* 2005). The presence of *C. lupus* haplotypes in two large wolf-like animals suggest that there is limited migration of large wolves into the region, but that they are not contributing to the coyote gene pool via hybridization as Canadian wolves are. Likewise, our finding of only one dog-like haplotype suggests that hybridization with dogs has not been significant, at least not between male northeastern coyotes and female dogs.

Our course-grained genetic approach allowed us to show the widespread distribution of introgressed wolf mitochondria across many hundreds of northeastern coyotes but does not provide the detailed ancestry of individuals. Additional analyses with nuclear markers will be needed to provide more resolution on the relative contributions of coyote, GLW, grey wolf and dog to this hybrid swarm, and evaluate the related

Table 2. Sexual and regional differences in skull measurements for male = M and female = F coyotes. (Mean sexual differences (MSD) are calculated as male/female and the number of specimens measured from each group is in parenthesis. Mean values (mm): length upper carnassial (LP4), width upper second molar (WM2), alveolar length of maxillary toothrow (ALM), maximum (MXP) and minimum (MNP) palate width, greatest length skull (GLS), zygomatic width (ZW), height jugal (HJ), height from the base of the first upper molar to the orbit (M1O), width postorbital processes (WPOP), height coronoid process (HCP), width anterior portion of the ramus (WAR), width mandible at carnassial (WMm1), height mandible at carnassial (HMm1). Significance of an ANOVA using Tukey's HSD post hoc test: * $p \leq 0.05$, ** $p \leq 0.01$, $p > 0.05 = \text{n.s.}$)

measurement	sexual dimorphism									regional comparisons			
	northeastern			Ohio			western			northeastern versus Ohio		northeastern versus western	
	M (88)	F (66)	MSD	M (13)	F (8)	MSD	M (10)	F (11)	MSD	M	F	M	F
LP4	20.9	19.9	1.05**	20.5	19.8	1.04	20.1	19.6	1.03	n.s.	n.s.	n.s.	n.s.
WM2	12.3	11.7	1.05**	11.6	12.1	0.96	11.9	11.3	1.05	*	n.s.	n.s.	n.s.
ALM	72.5	70.0	1.04**	73.5	70.7	1.04*	71.5	70.2	1.02	n.s.	n.s.	n.s.	n.s.
MXP	61.3	58.1	1.06**	59.8	57.6	1.04	54.5	53.6	1.02	n.s.	n.s.	**	**
MNP	22.7	21.4	1.06**	22.1	21.7	1.02	19.7	19.5	1.01	n.s.	n.s.	**	**
GSL	201.6	192.8	1.05**	201.5	195.4	1.03*	194.4	189.6	1.03	n.s.	n.s.	*	n.s.
ZW	106.8	101.8	1.05**	101.7	97.9	1.04*	98.2	95.4	1.03	**	*	**	**
HJ	14.1	13.2	1.07**	13.6	12.9	1.05	12.1	12.4	0.98	n.s.	n.s.	**	n.s.
M1O	28.2	26.8	1.05**	27.4	25.8	1.06	25.8	25.2	1.02	n.s.	n.s.	**	*
WPOP	50.1	47.8	1.05**	47.1	45.6	1.03	45.1	44.5	1.01	*	n.s.	**	**
HCP	55.3	52.2	1.06**	53.5	52.6	1.02	50.8	49.9	1.02	n.s.	n.s.	**	*
WAR	9.1	8.7	1.05**	8.4	8.0	1.05	7.9	8.0	0.99	*	*	**	*
WMm1	10.2	9.6	1.06**	10.1	9.2	1.10*	9.2	9.1	1.01	n.s.	n.s.	**	n.s.
HMm1	23.1	21.8	1.06**	21.9	21.5	1.02	20.7	20.4	1.01	*	n.s.	**	**

taxonomic and conservation implications of the dynamic population genetics of the region's *Canis*.

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Comment

Colonization history and ancestry of northeastern coyotes

Recently Kays *et al.* (2010) reported on mitochondrial DNA (mtDNA) sequence data and skull measurements to demonstrate a hybrid origin for northeastern coyotes. They suggested that, as western coyotes (*Canis latrans*) expanded their range in the last century, they colonized Ontario from Minnesota and hybridized with wolves, and subsequently colonized the northeastern United States via movement across the Saint-Lawrence River. We support the

conclusion that northeastern coyotes are derived from wolf–coyote hybridization, and we agree that introduced adaptive variation resulting in larger body size and more wolf-like cranial features probably allowed them to better hunt deer and facilitated their colonization of the northeast. However, data do not support the proposed route of western coyote colonization into Ontario from Minnesota and we criticize their use of the term ‘Great Lakes wolf’ (GLW) in describing *Canis* species taxonomy. We provide mtDNA data from southern Ontario (SON) coyotes and refer to the literature to demonstrate that the most probable route of western coyote colonization was from the lower Michigan peninsula near Detroit into SON, where hybridization with eastern wolves (*Canis lycaon*) occurred (figure 1).

We observed 10 mtDNA haplotypes in our SON sample ($n = 310$); two were of eastern wolf origin and the other eight were of coyote origin (see the electronic supplementary material, figure S1). The predominant haplotypes were C1, C9 and C19, with other haplotypes observed in low frequency, similar to the haplotype composition reported by Kays *et al.* in the northeast (figure 1; see the electronic

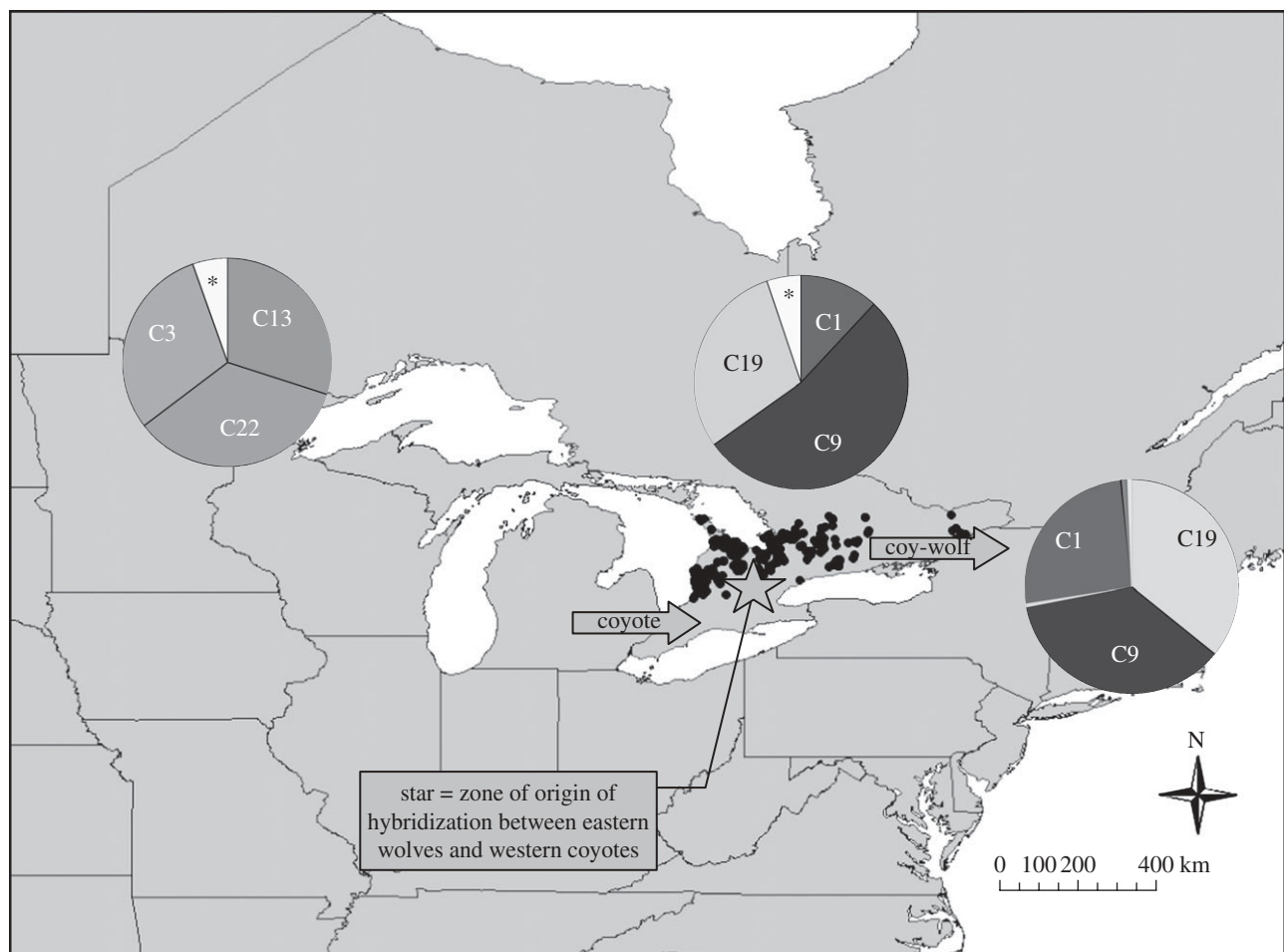


Figure 1. Sampling distribution of southern Ontario coyotes, and mtDNA haplotype frequencies of southern Ontario coyotes ($n = 310$), northeastern coyotes ($n = 453$; modified from Kays *et al.* 2010, fig. 2), and Minnesota/northwestern Ontario wolves ($n = 130$; data from Wheeldon 2009). Asterisk in pie charts indicates combined low frequency haplotypes. Arrows indicate colonization paths.

The accompanying reply can be viewed at <http://dx.doi.org/doi:10.1098/rsbl.2009.1022>.

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supplementary material, table S1). The haplotype composition of Minnesota and northwestern Ontario wolves is different from that of eastern coyotes (figure 1), and there is no morphological or biological evidence to support wolf–coyote hybridization occurring in the western Great Lakes region (Nowak 2009). Eastern wolf–western coyote hybridization has been extensive in SON, supported by genetic (Wilson *et al.* 2009) and morphological data (Sears *et al.* 2003), suggesting it is the probable origin of the wolf–coyote hybrids that colonized the northeast.

Way *et al.* (in press) also presented mtDNA sequences and microsatellite genotype data to demonstrate the hybrid nature of northeastern coyotes (*C. latrans* × *C. lycaon*). They used genetic distance measures to demonstrate the colonization path of ‘coywolves’ from southeastern Ontario into New York, followed by expansion into the northeast. This independent study confirms the hybrid nature of the animals reported by Kays *et al.* but supports western coyote colonization from the lower Michigan peninsula and hybridization in SON.

The use of the term GLW as a species designation by Kays *et al.* ignores literature on the eastern wolf, and perpetuates confusion over *Canis* taxonomy. Although Leonard & Wayne (2008) presented mtDNA data to demonstrate the GLW was a distinct species, Wheeldon & White (2009) demonstrated that the GLW sequences were similar or identical to those of the eastern wolf. Thus, it is disturbing that Kays *et al.* cite Wheeldon & White (2009) in the sentence preceding their use of the term GLW. In reporting on the same samples as Leonard & Wayne (2008), Koblmüller *et al.* (2009) did not recognize the GLW as a distinct species in their mtDNA phylogeny. This further demonstrates that the term GLW used to describe a phylogenetically distinct mtDNA lineage lacks support and consistency. Additionally, the failure on the part of Koblmüller *et al.* (2009) to distinguish between coyote and eastern wolf lineages obscures the view that western Great Lakes region wolves contain grey and eastern wolf genetic material, and thus are grey–eastern wolf hybrids (*C. lupus* × *lycaon*) and not wolf–coyote hybrids (Wheeldon 2009; Wheeldon & White 2009; Wilson *et al.* 2009). In addition to a more careful analysis of the probable colonization routes of western coyotes into northeastern North America, Kays *et al.* should have considered this alternative hypothesis,

which has considerable support in the literature (see Kyle *et al.* 2006).

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Invited reply

Reply to Wheeldon *et al.* ‘Colonization history and ancestry of northeastern coyotes’

The history of hybridization and range change of *Canis* in eastern North America has created an interesting evolutionary story that researchers are still untangling. We welcome the comment by Wheeldon *et al.* (in press) on our study on the evolution of northeastern coyote and the new data they present in their comment and new paper (Way *et al.* in press). Their comment raises two issues, one taxonomic and one biogeographic. Here we briefly defend our taxonomic treatment of northeastern wolves, and present new data supporting our original biogeographic interpretations.

Wheeldon *et al.* (in press) criticized our decision to refrain from using any formal (binomial) species designation for eastern wolves, choosing instead the geographical descriptor ‘Great Lakes Wolf’ (GLW). We recognize the ongoing controversy over wolf taxonomy and wished to avoid taking sides on a debate which our data did not directly address. Our data show that northeastern coyotes are the descendants of coyotes that hybridized with wolves, but do not contribute anything new to the understanding of the taxonomy of that wolf. Wheeldon *et al.* erroneously assert that Leonard *et al.* (2008) concluded that the GLW is a distinct species when that study actually made no taxonomic recommendations, and a more detailed follow-up (Koblmüller *et al.* 2009) considered the GLW an ecotype of *Canis lupus*, one of several ecotypes in North America (Munoz-Fuentes *et al.* 2009). Additionally, this diagnosis was based primarily on an analysis of 26 nuclear microsatellite loci, not mtDNA, as suggested by Wheeldon *et al.* The matter is hardly settled regarding the species status of the eastern wolves, but all data suggest that species limits in the genus are fluid in this region and poorly captured by traditional binomial taxonomy.

Wheeldon *et al.* (in press) also state that ‘data do not support the proposed route of western coyote colonization into Ontario from Minnesota’, and they presented new data which they suggest indicate a southern Ontario origin of hybridization following a colonization through the lower peninsula (LP) of Michigan near Detroit. Their data show that southern Ontario coyotes have hybridized with wolves. However, the presence of hybrids in southern Ontario

today does not bear on the geographic origin of this hybridization event, nor does it favour an immigration route through Detroit compared with a more northern route.

Our initial interpretation that coyotes immigrated into Ontario from Minnesota (MN) was based on a general history of coyotes across the entire region (Parker 1995). To examine the colonization in greater detail we surveyed museum collections for coyote specimens early in their range expansion (before 1940) in the Great Lakes region (figure 1). These records do not support an LP colonization route, but do show that western Ontario and Michigan’s upper peninsula (UP) had recorded multiple coyotes by 1940. This suggests early migration through western Ontario and the UP, both of which support our earlier conclusion (Kays *et al.* 2010) that a northern front of hybrid coyotes colonized much more rapidly than non-hybrid animals moving through Ohio.

Wheeldon *et al.* (in press) suggest that coyote colonization could not have started in MN or Western Ontario (WON) on the basis of new data, suggesting that the two coyote-like haplotypes we found in the northeast were not present in MN or WON (Wheeldon *et al.* in press; figure 1). However, these data were from wolves, not coyotes. Since our paper appeared, we have sequenced the same mtDNA region for 19 coyotes from MN and 18 from Michigan (five UP, 13 LP). The two coyote-like haplotypes characteristic of northeast coyotes (GenBank accession nos.: GQ863718.1 and GQ863719.1) are present in all these populations (MN: 4 cl28 and 2 cl29; UP: 3 cl28; LP: 11 cl28 and 1 cl29), as also noted by Wheeldon (2009). Furthermore, the wolf-like haplotype present in eastern coyotes was found in a wolf collected in Wisconsin near the time of coyote expansion (1908; Wheeldon & White 2009). Thus, all the genetic haplotypes common in eastern coyotes could have originated from this region.

Finally, Wheeldon *et al.* argue against an MN or WON origin for the coyote–wolf hybridization on the basis of recent genetic (Wheeldon 2009) and morphological data (Nowak 2009); once again the papers they cite focus on wolves. In fact, a morphological study that included coyotes from the region did find evidence for hybridization, although not as extreme as we found in northeast coyotes. MN coyotes showed a shift toward a more wolf-like morphology, were intermediate between western coyotes and northeast coyotes in a linear discriminant function, and exhibited greater morphological variation than western coyotes, a characteristic of hybrid populations (Lawrence & Bossert 1969).

Taken together, these studies suggest that hybridization had different impacts on populations of coyotes and wolves. The lack of genetic and morphological signatures in wolf populations noted by Wheeldon *et al.* (in press) suggests that backcrosses of F1 coyote–wolf hybrids with wolves are not as successful as with coyotes. Conversely, the widespread hybrid signatures in the morphology and genetics of coyote populations throughout the northeast and southern Ontario suggests that hybrids backcrossing into the coyote population had offspring that were well adapted to

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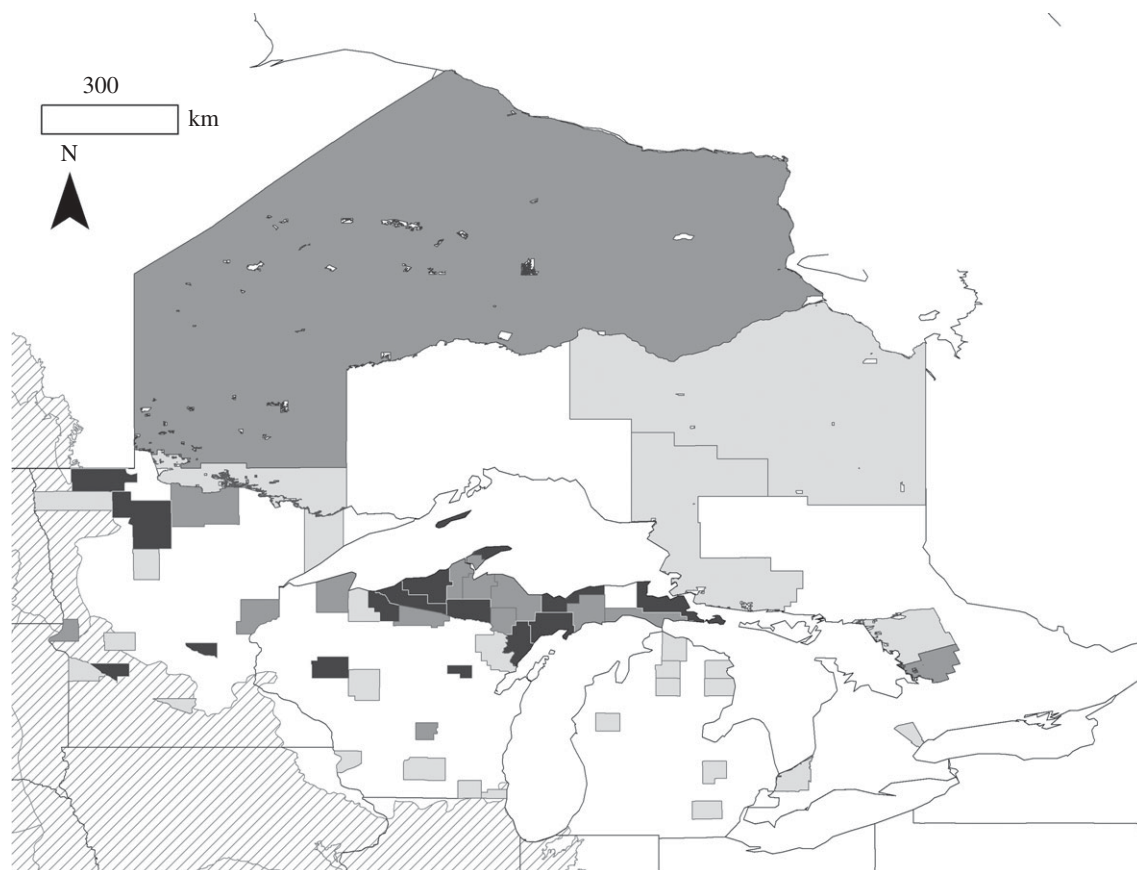


Figure 1. Coyote specimens recorded in the Great Lakes Region before 1940 suggests their eastward colonization moved along a route through Western Ontario and/or the Upper Peninsula of Michigan. The 311 specimen records come from 11 museums and are mapped per county. Hatching shows the original range of coyotes in western grasslands. Coyote specimens: light grey shading, 1–2; dark grey shading, 3–10; black shading, more than 11.

the human-dominated landscapes common in the region.

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