

Genetic nature of eastern wolves: Past, present and future

C.J. Kyle^{1,2,*}, A.R. Johnson^{2,3}, B.R. Patterson¹, P.J. Wilson², K. Shami², S.K. Grewal² & B.N. White²

¹Wildlife Research and Development Section, Ontario Ministry of Natural Resources, Trent University, Peterborough, ON, K9J 7B8, Canada; ²Natural Resources DNA Profiling and Forensic Centre, Department of Biology, Trent University, Peterborough, ON, K9J 7B8, Canada; ³Department of Biology, McMaster University, Hamilton, ON, K9H 3Y7, Canada (*Corresponding author: Phone: + (705)-755-2293; Fax: + (705)-755-2276; E-mail: chris.kyle@nrdfc.ca)

Received 20 June 2005; accepted 3 October 2005

Key words: *Canis latrans*, *Canis lupus*, *Canis lycaon*, eastern wolf, hybridization

Abstract

Eastern North American wolves have long been recognized as morphologically distinct from both coyotes and gray wolves. This has led to questions regarding their origins and taxonomic status. Eastern wolves are mainly viewed as: (1) a smaller subspecies of gray wolf (*Canis lupus lycaon*), potentially the result of historical hybridization between gray wolves (*C. lupus*) and red wolves (*C. rufus*), (2) a hybrid, the result of gray wolf (*C. lupus*) and coyote (*C. latrans*) interbreeding, or (3) a distinct species, *C. lycaon*, closely related to the red wolf (*C. rufus*). Although debate persists, recent molecular studies suggest that the eastern wolf is not a gray wolf subspecies, nor the result of gray wolf/coyote hybridization. Eastern wolves were more likely a distinct species, *C. lycaon*, prior to the eastward spread of coyotes in the late 1800s. However, contemporary interbreeding exists between *C. lycaon* to both *C. lupus* and *C. latrans* over much of its present range complicating its present taxonomic characterization. While hybridization may be reducing the taxonomic distinctiveness of *C. lycaon*, it should not necessarily be viewed as negative influence. Hybridization may be enhancing the adaptive potential of eastern wolves, allowing them to more effectively exploit available resources in rapidly changing environments.

Introduction

Numerous authors have long recognized that wolves found in eastern North America are morphologically distinct from western and northern populations of gray wolves (*C. lupus*) and coyotes (*C. latrans*) (e.g., Miller 1912; Pockock 1935; Young and Goldman 1944; Hall and Kelson 1959; Peterson 1966; Kolenosky and Standfield 1975; Theberge 1991; Brewster and Fritts 1995; Nowak 1995, 2002; see Figures 1 and 2). The distinction is most often based on the morphologically intermediate features of eastern wolves to these other *Canis* species (Nowak 2002). In fact, the first tax-

onomic descriptions of the eastern wolf by Schreber in 1775 (in Nowak 1995) referred to a distinct species, *C. lycaon*, found in southern regions of Ontario and Quebec extending southwards to a poorly defined boundary. Miller (1912) and Pockock (1935) also recognized *C. lycaon* as a distinct species of wolf in eastern North America. However, most morphological studies maintain that the eastern wolf is a subspecies of gray wolf, *C. lupus lycaon* (e.g., Young and Goldman 1944; Peterson 1966; Kolenosky and Standfield 1975; Nowak 1979, 1995, 2002). In either event, the eastern wolf was viewed as morphologically distinct from both gray wolves and western coyotes. The

taxonomic debate surrounding the eastern wolf was rekindled when molecular markers were used to investigate their genetic make-up (e.g., Lehman et al. 1991, 1992; Roy et al. 1994; Wilson et al. 2000). Currently, there are three main hypotheses regarding the taxonomic status of this animal:

- (1) It is a smaller subspecies of the gray wolf (*Canis lupus lycaon*), potentially resulting from post-Pleistocene/pre-European settlement hybridization between gray wolves (*C. lupus*) and red wolves (*C. rufus*) (see Nowak 2002).
- (2) It is a hybrid, and not a distinct species, resulting from gray wolf (*C. lupus*) and coyote (*C. latrans*) hybridization (see Lehman et al. 1991; Roy et al. 1994; Wayne and Vila 2003).
- (3) It is a distinct species (*C. lycaon*) closely related to red wolves (*C. rufus*) from the southeastern United States (see Wilson et al. 2000).

Further understanding of the genetic nature and evolutionary potential of the wolf-like canid that exists in eastern North America is needed to put current *Canis* conservation and management strategies into perspective. Implications could include: (a) recognizing the presence of another potentially threatened *Canis* species, *C. lycaon*, distinct to the Great Lakes region; (b) the misclassification of, and overestimation of, *C. lupus* numbers in the Great Lakes region, a population that has recently been moved from “endangered” to “threatened” status by the US Fish and Wildlife Service; and (c) if eastern wolves are hybrids between *Canis* species, even if one of the congeners is an endangered species, they would not be afforded a level of legal protection under the US Endangered Species Act (<http://www.fws.gov/le/pdf/ESA.doc>). Here we attempt to address the historical and contemporary taxonomic status of the eastern wolf by summarizing molecular studies and analyses that support or refute the aforementioned biological hypotheses and speculate as to the nature of the wolf that may inhabit this region in the future.

Canis species distribution pre-European settlement

It is important to put current taxonomic debates into context by briefly reviewing the distribution of

wolves in North America prior to European settlement. At this time, patterns of *Canis* species and subspecies distributions were likely influenced by habitat and prey selection (Mech 1970; Kolenosky and Standfield 1975; Moore and Parker 1992; Nowak 1995; Geffen et al. 2004). It is thought that gray wolves inhabited most of North America, with the exception of the deciduous forests of eastern North America (Nowak 1995), primarily preying on larger ungulates (Kolenosky and Standfield 1975; Nowak 2002). Historically, coyotes were restricted to the plains and deserts of central North America (Bekoff and Wells 1986; Moore and Parker 1992), however, they expanded their range into eastern North America in the early 1900s (Moore and Parker 1992; Nowak 2002). In addition, in eastern North America, a morphologically distinct wolf has long been recognized (Miller 1912; Pockock 1935; Young and Goldman 1944; Hall and Kelson 1959; Peterson 1966; Kolenosky and Standfield 1975; Theberge 1991; Brewster and Fritts 1995; Nowak 1983; 1995, 2002; see Figures 1 and 2). The distribution of the eastern wolf coincided approximately with the deciduous forests east of the Mississippi, from the Gulf Coast to southern Ontario (Nowak 2002; Figure 1), and the distribution of white-tailed deer (*Odocoileus virginianus*) (Mech 1970), its preferred prey species (Mech and Karns 1977; Forbes and Theberge 1995).

Post-settlement Canis species distribution in eastern North America

The advent of European settlement, and concomitant agricultural and deforestation practices, fur harvests, and predator control programs led to severe declines in the number of wolves and large ungulates in eastern North America (Young and Goldman 1944; Nowak 2002). Wolves were extirpated from much of the eastern United States and Atlantic Canada by 1900 (Young and Goldman 1944; Parker 1995). Specifically, *C. lupus* was extirpated from southeastern Ontario and Quebec between the 1850s and 1900 (Peterson 1955; Kolenosky and Standfield 1975; Franzmann and Schwartz 1997). In the absence of *C. lupus*, it is hypothesized that

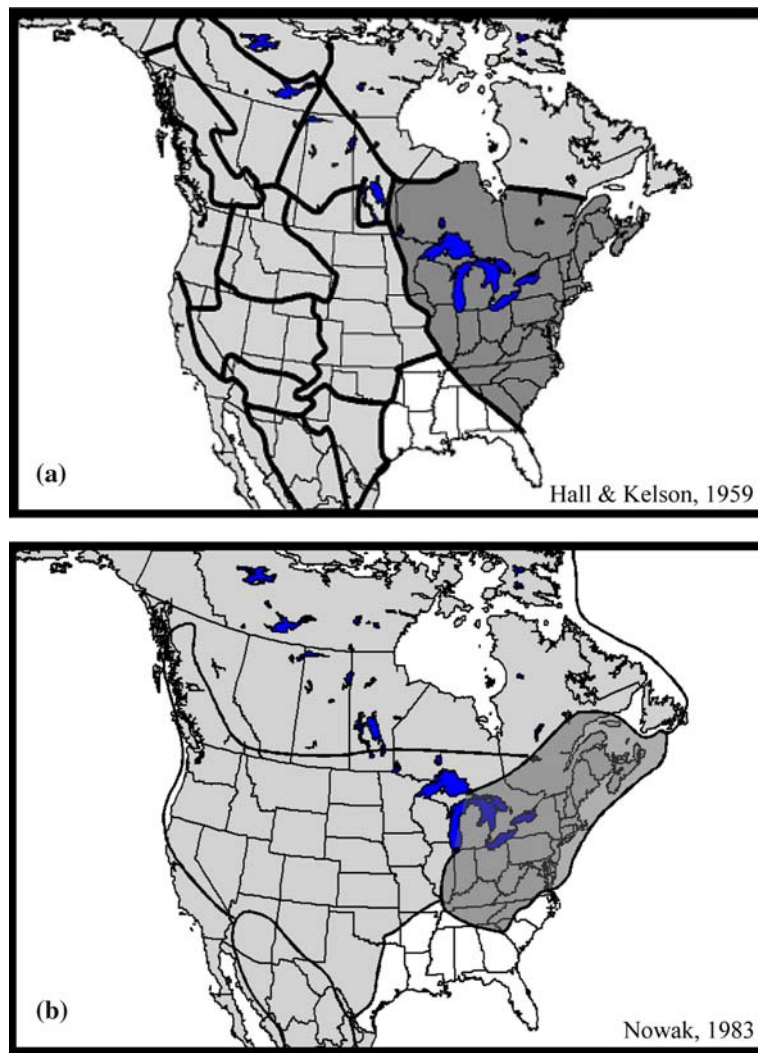


Figure 1. Interpretations by several authors of post-Pleistocene distributions of wolf species and subspecies in North America: (a) Distribution of gray wolves in North America showing the eastern wolf as a distinct entity (adapted from Hall and Kelson 1959). Light gray areas indicate gray wolf occurrence; dark gray area indicates gray wolf subspecies, *C. l. lycaon*, occurrence (similar *C. l. lycaon* distribution reported in Young and Goldman 1944); (b) Geographic distribution of two species of wolf in North America (adapted from Nowak 1983). Light gray areas indicate gray wolf (*C. lupus*) occurrence, white area indicates red wolf (*C. rufus*) occurrence, dark gray area indicates gray wolf subspecies, *C. l. lycaon*, occurrence; (c) Geographic distribution of two species of wolf in North America (adapted from Nowak 1995). Light gray areas indicate gray wolf (*C. lupus*) occurrence, white area indicates red wolf (*C. rufus*) occurrence, dark gray area indicates gray wolf subspecies, *C. l. lycaon*, occurrence. Nowak (2002) interpreted *C. l. lycaon* to be a subspecies formed by post-Pleistocene hybridization between gray wolves and red wolves; (d) Geographic distribution of wolves and coyotes in North America following a three species evolutionary model (as per Wilson et al. 2000; Grewal 2001). Light gray areas indicate gray wolf occurrence, dark gray area indicates eastern wolf occurrence, dotted area indicates coyote occurrence sympatric with gray wolves. It should also be noted that Grewal (2001) suggests the distribution of *C. lycaon* extends into Manitoba as well; (e) Hypothetical pre-European settlement geographic distribution of wolves and coyotes in North America showing the two species evolutionary model suggested by Moore and Parker (1992) and maintained by subsequent authors (e.g., Lehman et al. 1992, Wayne and Lehman 1992). Light gray areas indicate gray wolf distribution, dark gray area indicates coyote distribution. Lehman et al. (1991) interpreted *C. rufus* to be a hybrid as a result of recent (post-European colonization) hybridization between the gray wolf and coyote.

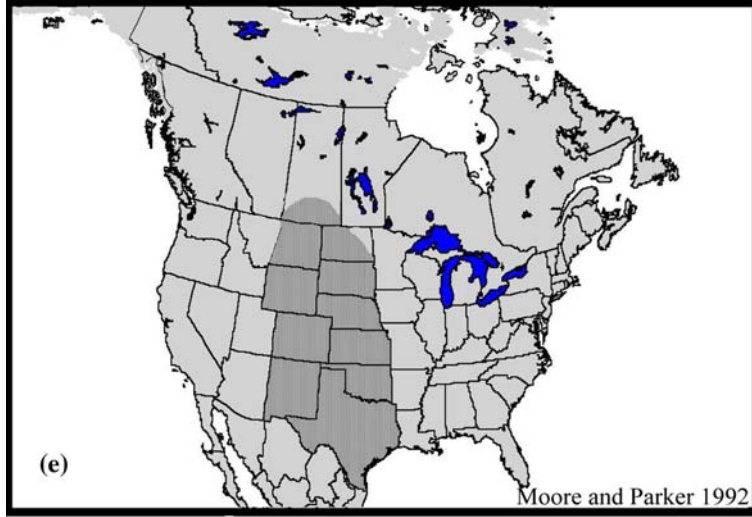
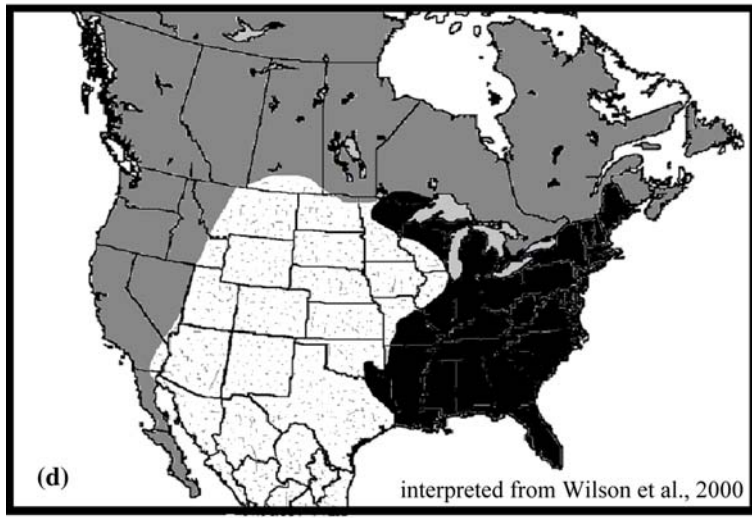
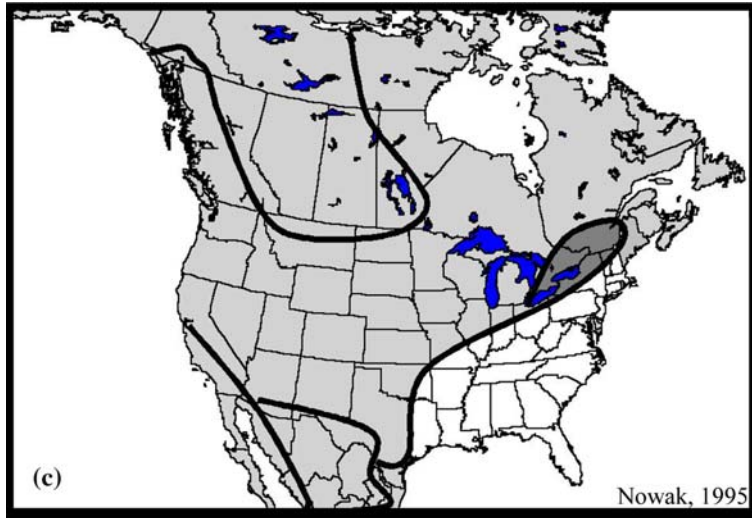


Figure 1. Continued

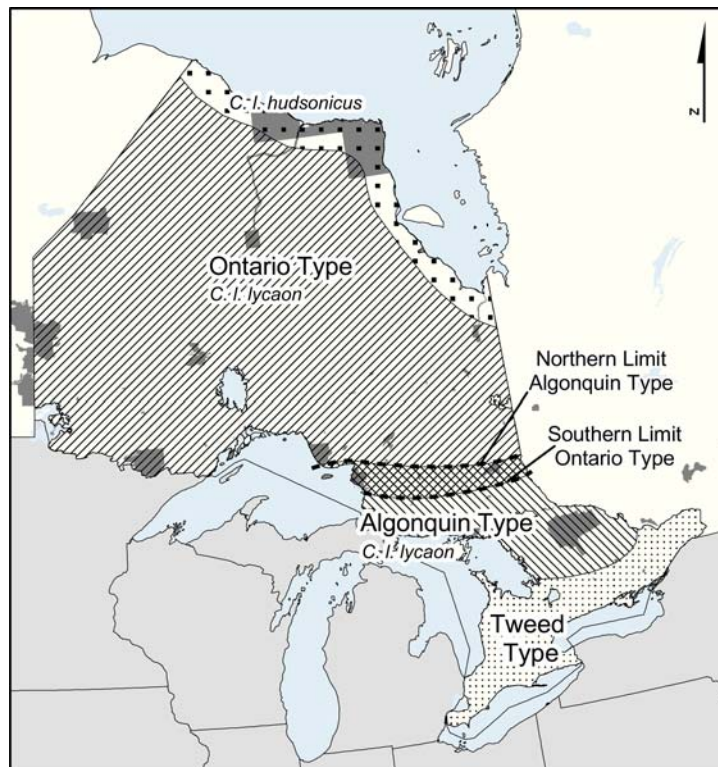


Figure 2. Clinal distribution of four wolf “types” found in Ontario in 1970’s (adapted from Kolenosky and Standfield 1975) including: gray wolf along Hudson Bay; “Ontario type” of wolf across most of Ontario; “Algonquin type” of wolf in central Ontario; and a “Tweed type” of wolf in southern Ontario. One interpretation is that the Tweed wolf is a coyote–eastern wolf hybrid, the Algonquin type is an eastern wolf less impacted by hybridization with coyotes, and the Ontario type is an eastern wolf/gray wolf hybrid. National and Provincial Parks in Canada are identified by gray shading.

eastern wolves extended their range northward, as anthropogenic landscape changes promoted a northward extension of the range of white-tailed deer (Kolenosky and Standfield 1975; Nowak 2002, 2003). Similarly, wolf control programs and anthropogenic modification of the landscape led to the expansion of coyote populations to include the whole of the conterminous USA and most of south and northwest Canada (Moore and Parker 1992). Coyotes were rare in the Great Lakes region until about 1890 (Nowak 1979; Moore and Parker 1992), reaching south-eastern Ontario around 1920 (although possibly as early as 1905), Quebec in 1945, New Brunswick in 1958 and Maine in 1970 (Kolenosky and Standfield 1975; Moore and Parker 1992).

It is important to note that there is little evidence of coyote hybridization with eastern wolf populations between 10,000 and 100 ybp (Graham

and Lundelius 1994; Nowak 2002). However, the early to mid 1900s arrival of coyotes to eastern North America did lead to eastern wolf/coyote hybridization (Silver and Silver 1969; Kolenosky and Standfield 1975). For example, in Ontario, subsequent to the expansion of *C. latrans*, Kolenosky and Standfield (1975) recognized the “Tweed wolf” (also termed the eastern coyote; Parker 1995), an animal they suggest resulted from the hybridization of the “Algonquin type” of gray wolf (*C. l. lycaon*) and *C. latrans*. In contrast to the observations of hybridization between coyotes and eastern wolves in eastern North America, no hybridization has been observed between western populations of gray wolves and coyotes (Pilgrim et al. 1998), nor is there evidence of Mexican gray wolves (*C. l. baileyi*) interbreeding with coyotes where they occur in sympatry (Garcia-Moreno et al. 1996; Hedrick et al. 1997).

Competing hypotheses on the origin and taxonomic status of eastern wolves

C. lupus subspecies hypothesis

The eastern wolf phenotype is a continuum of sizes ranging between that of *C. lupus* and *C. latrans*. This morphological variation has been attributed to either interspecific hybridization between *C. lupus* and *C. latrans* (Nowak 1979, 1995) or a phenotypic plastic response to changes in prey size (Young and Goldman 1944; Kolenosky and Standfield 1975; Schmitz and Kolenosky 1985; Thurber and Peterson 1991; Brewster and Fritts 1995; Nowak 1995). In either case, the eastern wolf has been viewed as a *C. lupus* subspecies, *C. l. lycaon*, by the aforementioned authors.

Nowak (2002) suggests that the modern range of the smaller eastern wolf, *C. l. lycaon*, was historically occupied by a larger *C. lupus* that had moved from the west following the retreat of the glaciers at the end of the Pleistocene. Nowak (2002, 2003) then hypothesized that *C. rufus*, considered by this author to be a distinct southeastern United States *Canis* species, would have moved northward post-glaciation and these two species would have hybridized at their distributional interface, resulting in the eastern wolf (Figure 1). Cranial measurements of eastern wolf fossils from southeastern Ontario and southern Quebec were found intermediate in size to *C. rufus* and western *C. lupus* and these findings are taken as evidence of a natural hybridization of those two species. As such, Nowak (1979, 1992, 1995, 2002, 2003) considered eastern wolves to be a *C. lupus* subspecies.

Genetic data pertaining to the C. lupus subspecies hypothesis

The *C. lupus* subspecies hypothesis is rejected by most recent molecular data (e.g., Lehman et al. 1991; Roy et al. 1994, 1996; Wilson et al. 2000, 2003; Grewal 2001; Grewal et al. 2004). These studies have all found extensive evidence of *C. latrans* or *C. rufus* alleles and haplotypes within eastern wolf populations, but a general lack of *C. lupus* alleles and haplotypes that would be expected for the *C. lupus* subspecific designation to be valid. Importantly, Wilson et al. (2003) found that two historic eastern wolf samples (pre-coyote presence) had mitochondrial DNA haplotypes not found in other extant gray wolf populations.

In contrast to other molecular studies, however, Y chromosome intron work by Shami (2002) provides a less clear distinction between eastern wolves and gray wolves. Shami (2002) elucidated four Y-intron haplotypes, one of which was interpreted as *C. lupus* specific, and found in high frequency in some extant eastern wolf populations. It is unclear if the presence of a putatively *C. lupus* specific haplotype in extant populations of eastern wolves is indicative of a historic hybridization event between *C. rufus*/*C. lupus* (*sensu* Nowak 2002) or if this haplotype is simply ancestral to both species.

C. lupus/C. latrans hybridization hypothesis

Several authors have interpreted their genetic data as evidence of hybridization between gray wolves and coyotes in the Great Lakes region (e.g., Lehman et al. 1991; Wayne et al. 1992; Roy et al. 1994, 1996; Reich et al. 1999). These authors suggest the lack of unique alleles and haplotypes within eastern wolves (and southern red wolves) indicates that they are of hybrid origin from the interbreeding of gray wolves and coyotes, and therefore they should not be considered distinct species. If these animals are of hybrid origin, there are important conservation and management implications such as hybrids not warranting any level of protection in the United States (US *Endangered Species Act*, 2004: <http://www.fws.gov/le/pdf/ESA.doc>). In contrast, hybrids would be protected based on the status of the parent species in Ontario (as per the *Fish and Wildlife Conservation Act*, 1997: <http://www.e-laws.gov.on.ca:81/ISYSquery/IRL18AE.tmp/9/doc> and the *Heritage Hunting and Fishing Act*, 2002: <http://www.e-laws.gov.on.ca:81/ISYSquery/IRL18B5.tmp/2/doc>). This raises the question of allocating resources to conserving an animal that is the result of recent hybridization likely due to anthropogenic influences on the landscape allowing for the eastern range expansion of coyotes. The main proponents of the hybrid origins hypothesis do concede that hybridization between coyotes and gray wolves may have occurred pre-European settlement, and as such the contemporary eastern wolf would warrant a level of conservation. These authors also suggest that their results reflect a unidirectional introgression from male gray wolves to female coyotes; viable hybrids would be able to

back-cross with gray wolves, but not coyotes (Lehman et al. 1991; Wayne and Jenks 1991; Roy et al. 1994, 1996; Wayne 1995; but see Kolenosky 1971; Schmitz and Kolenosky 1985). They attribute hybridization between gray wolves and coyotes in eastern populations, something that is not observed between western populations of these species (Pilgrim et al. 1998), to high coyote densities and low gray wolf densities in the east. They contend that this would have led to an Allee effect for gray wolves that would have then mated with coyotes.

Genetic data pertaining to the C. lupus/C. latrans hybridization hypothesis

Lehman et al. (1991) suggested that a gray wolf/coyote hybrid zone exists from western Ontario and northern Minnesota through to north shore of the St. Lawrence. Based on mtDNA restriction data, a significant proportion of wolves from these regions were found to have haplotypes identical to those found in western coyote populations, and as such these wolves were viewed as hybrids. West of the Great Lakes region, no introgression was observed between gray wolves and coyotes where wolf aggression towards coyotes has been observed (e.g., Carbyn 1982), similar to observations by Mech (1966) on Isle Royale, Michigan. This hybrid zone matches well with the morphotypes of *C. l. lycaon* described by Kolenosky and Standfield (1975; Figure 2). Lehman et al. (1991) suggested that northern Ontario wolves, "boreal type," would have no coyote genetic material; central Ontario wolves, described as an "Algonquin type," represent a slow yet steady influx of coyote genes into wolf populations, and the third type of *C. l. lycaon*, "Tweed" wolf, also known as the eastern coyote, would be composed primarily of western coyote genetic material. Lehman et al. (1991), conclude their study by stating that, "wolves may be increasingly threatened by interbreeding with coyotes."

To further explore the level of coyote introgression into gray wolf populations, Roy et al. (1994) used 10 autosomal microsatellite markers to analyze populations of both non-hybridizing and hybridizing populations of *C. lupus* and *C. latrans* from North America (hybridizing populations from Minnesota, Maine, and southern Quebec). Forty wolves from a *C. rufus* captive population were also analyzed in this study. No alleles were

found to be unique to *C. rufus* or the Minnesota, Maine, and southern Quebec populations, and as such, these data were considered not to refute the hybrid origin hypothesis. A tree of the genetic distances between populations suggested that the red wolf (captive population) and hybridizing wolves from Minnesota and southern Quebec were closely related and intermediate to "pure" *C. lupus* and *C. latrans* populations. Again, this was viewed as not refuting the hybrid origin hypothesis. Roy et al. (1994) suggested that, although coyote introgression into wolf populations in the Great Lakes region is less extensive than that observed in the captive red wolf population, continued hybridization is expected to undermine genetic integrity of wolves in this region given continued habitat changes that favor an increase in coyote populations.

To investigate if the hybridization of coyotes and gray wolves occurred historically, Roy et al. (1996) studied the mtDNA cytochrome b gene from 17 pre-1940 red wolves (although there is some question as to the origin of the pelts, see Nowak 2002). Most red wolf haplotypes seemed to be closely related to coyote haplotypes, but some unique red wolf haplotypes were observed. Roy et al. (1996), however, found that red wolf and coyote clades were interspersed throughout the most parsimonious phylogenetic tree and not monophyletic as might be expected if the red wolf was a distinct species. The lack of unique groupings of red wolf haplotypes would therefore not refute the hybrid origin hypothesis. However, the authors do point out that the most parsimonious tree could have coyote haplotypes in a monophyletic clade with one more mutational step, and red wolf haplotypes in a monophyletic clade with 9 more mutational steps. A potential problem with this study is that samples from the Great Lakes regions were interpreted as being either of coyote or gray wolf origin only, hence animals with intermediate characteristics were automatically considered to be of hybrid origin. However, the similarity of captive and pre-1940 red wolves to Minnesota and southern Quebec wolf populations, both with characteristics intermediate to both coyotes and gray wolves, could as easily be interpreted as belonging to a third category of *Canis* instead of being indicative of introgression between coyotes and gray wolves.

In order to determine when coyotes and gray wolves might have hybridized, Reich et al. (1999) analyzed the microsatellite data from Roy et al. (1994) using a step-wise mutation model. They found that red wolves were the result of a relatively recent (with very broad confidence limits) hybridization. This work was later supported by Wang (2003), who using Roy et al.'s (1994) data set, found similar levels of hybridization with a maximum-likelihood method to estimate admixture proportions.

In a recent review of canid genetics, Wayne and Vila (2003) seem to soften their stance on the hybrid origins of the eastern wolf based on recent molecular data. They state, "*C. lycaon* might have been a distinct red wolf-like species that is now interbreeding with *C. lupus* and migrated into Canada after the last glaciation." However, the authors maintain that the main argument against a distinct species hypothesis is that haplotypes that seem to be exclusive to eastern wolves are not reciprocally monophyletic (*sensu* Moritz 1994) to define an evolutionary significant unit (ESU). It should be noted, however, that reciprocal monophyly is not observed for many species where there is no debate concerning their validity as a species (e.g., Crandall et al. 2000). Furthermore, most of the eastern wolf molecular analyses to date have only studied small fragments of the mtDNA genome (350 bp or less). By studying larger mtDNA fragments, the lack of reciprocal monophyly may be partially resolved. It should also be noted that given the significant decline in wolf numbers in the mid 1800s through 1900s, several intermediate haplotypes may have been lost (see Leonard et al. 2005). Hence, the observed phylogenetic tree may not be completely representative of this species' evolutionary history.

Finally, one would expect that if hybridization were possible between western gray wolves and coyotes when gray wolf populations are at very low densities that western regions with low density, recolonizing populations of wolves (e.g., Montana and Wyoming) would exhibit a similar mechanism. Hybrids have yet to be observed in these regions (Pilgrim et al. 1998). Interestingly, there is also no evidence of hybridization between Mexican gray wolves (*C. l. baileyi*) and coyotes where they are sympatric (Garcia-Moreno et al.

1996; Hedrick et al. 1997), further undermining the hypothesis of gray wolf and coyote hybridization when wolves are found at low density.

Distinct species, C. lycaon, hypothesis (sensu Wilson et al. 2000)

Gray wolves were thought to have occupied much of Ontario prior to European colonization (Bates 1958). Intense predator control programs along with fur and forest harvesting in the 1800s led to declines in the number of wolves and large ungulates in southern and eastern Ontario (Peterson 1955; Kolenosky and Standfield 1975; Franzmann and Schwartz 1997; Boitani 2003). This led to the influx of white-tailed deer into the region better suited to this modified habitat. It is then hypothesized (e.g., Wilson et al. 2000; Grewal 2001; Grewal et al. 2004) that this in turn led to smaller eastern wolves (thought to be the same species as the southern red wolf, *C. rufus*, by these authors) expanding their range northwards during the 1800s from their original distribution associated with deciduous forests east of the Mississippi (Nowak 1995). This hypothesis suggests that the eastern wolf is a distinct species of *Canis*, sharing a recent common ancestor with coyotes that originated in North America. The close relationship of eastern wolves and coyotes explains the ability of these two species to readily hybridize, whereas more distantly related gray wolves do not seem to hybridize with coyotes (Garcia-Moreno et al. 1996; Hedrick et al. 1997; Pilgrim et al. 1998).

Genetic data pertaining to the distinct species, C. lycaon, hypothesis (sensu Wilson et al. 2000)

All molecular papers prior to 1998 suggest hybridization explains the origin of the red wolf and eastern wolf until Bertorelle and Excoffier (1998) developed a test for inferring admixture proportions from molecular data based on a coalescent approach. They applied this statistical estimator to admixed *Canis* populations based on microsatellite information from Roy et al. (1994). Simulations of three potential models of admixture between these groups suggested that the evolutionary process that led to the red wolf species was likely something other than a simple hybridization event. Their model suggests that red wolves and coyotes likely shared a recent common ancestor. This has definite implications for the eastern wolf's

origin given the similarities observed between eastern wolves and red wolves in earlier genetic studies (Roy et al. 1994, 1996).

Further evidence pertaining to this hypothesis came from a study of eastern wolves by Wilson et al. (2000). Using both autosomal microsatellites and the mtDNA control region, a genetic similarity was observed between red wolves and eastern wolves in the form of similar microsatellite allele frequencies and closely related mtDNA haplotypes. Furthermore, several haplotypes were independent of those found in both non-hybridizing coyote and gray wolf populations. Wilson et al. (2000) suggested that red wolves and eastern wolves may be the same species sharing a recent common ancestor with coyotes (150,000–300,000 ybp), independent of the gray wolf lineage. Given their results, Wilson et al. (2000) proposed that the eastern wolf retain its original designation, *C. lycaon* (Schreber 1775 (in Nowak 2002); Miller 1912; Pockock 1935).

The idea that the red wolf, *C. rufus*, and eastern wolf, *C. lycaon*, were indeed a distinct third species of *Canis* in North America resulted in much controversy. To further test their hypothesis, Wilson et al. (2003) examined two wolf samples thought to predate the eastward spread of coyotes. A northern New York state wolf sample from around 1890 and another from Maine around 1880 were analyzed. Both samples were found to have haplotypes that were more coyote-like, and clearly not of *C. lupus* origin. The Maine sample clustered with previously identified eastern wolf and *C. rufus* mtDNA sequences. The New York sample clustered with modern western-coyote populations. They suggest that the clustering of the New York samples with coyote populations could be evidence for either (1) an earlier than presumed (by 40 years) coyote presence and introgression into eastern wolves or (2) that *C. latrans* and *C. lycaon* share a recent common ancestor. They argue that, *C. lupus* and *C. latrans* do not seem to hybridize in western populations based on field observations (Thurber and Peterson 1991) and genetic studies (Lehman et al. 1991; Wayne and Lehman 1992; Roy et al. 1994; Pilgrim et al. 1998; Arjo and Pletcher 1999), but there is evidence that eastern wolves and gray wolves could (Mech and Federoff 2002; Grewal et al. 2004). Hence, the more probable scenario is that *C. latrans* and *C. lycaon* share a common ancestor. A

potential weakness of the studies by Wilson et al. (2000, 2003), however, is that only western *C. lupus* and *C. latrans* were used as baselines for “pure” populations of coyotes and gray wolves (although “pure” *C. latrans* may not exist in eastern North America, Schmidz and Kolenosky 1985). Using only western populations could pose a problem as *C. lupus* has been found to have a significant degree of genetic structuring (Roy et al. 1994; Forbes and Boyd 1997; Sharma et al. 2004; Leonard et al. 2005), such that alleles and haplotypes found in western populations may be different from those in northeastern populations of *C. lupus*.

Hedrick et al. (2002) examined the major histocompatibility complex (MHC) class II gene *DRB1* from a captive red wolf population and coyotes from Texas and North Carolina. They found only four alleles among the 48 captive red wolves, two of which were found in coyote populations, one from a previous gray wolf MHC study, and another allele unique to red wolves, that was found in the highest frequency. The allele unique to red wolves was very closely related to that found in the coyote populations. From this the authors suggest that red wolves are more closely related to coyotes than to gray wolves supporting the work of Bertorelle and Excoffier (1998) and Wilson et al. (2000). However, MHC genes are most often not appropriate for phylogenetic studies as there can be more intraspecific variation than interspecific variation within this region of the genome. The authors acknowledge the possibility that further examination of gray wolves may reveal other alleles identical or more similar to the red wolf alleles.

Grewal (2001) investigated the extent of the eastern wolf’s distribution in Ontario by analyzing 269 animals using mtDNA control region sequences and eight autosomal microsatellite loci. Grewal (2001) found northwestern Ontario through to northeast Ontario populations of wolves mostly had haplotypes unique to eastern wolves, but also a number of haplotypes also found in western *C. lupus* populations. This was taken as evidence of contemporary *C. lupus*/*C. lycaon* hybridization in these regions. In the southeastern portion of the province, wolf populations again were found to have mostly eastern wolf specific haplotypes, but also a significant number of haplotypes also found in western coyote populations. This was taken to



Figure 3. Approximate present day geographic distribution of eastern North American wolves with a genetic composition that is predominately *C. lycaon* with more *C. latrans* material to the south and east of the distribution and more *C. lupus* material to the northwest (adapted from Grewal 2001). Stippled area indicates zone of wolves identified as coyote and gray wolf hybrids by Lehman et al. (1991). Putative present day distribution of the eastern wolf shown by stippled area (Ontario and Québec). Dotted outline show regions where eastern wolves and gray wolves may be sympatric (Manitoba, Minnesota, Wisconsin and Michigan). Stripped locations in North Carolina indicate present day red wolf distributions (Captive program and Alligator River Refuge). Note that Pukaskwa National Park (PNP) wolves were identified as *C. lupus* with very little *C. lycaon* genetic introgression (Grewal 2001).

suggest that eastern wolves had hybridized with *C. latrans* in the southern portions of the province (see Figures 2 and 3). A remnant pocket of a *C. lupus* population, free of eastern wolf specific or western coyote genetic material was also found adjacent to the southeastern part of Lake Superior (Pukaskwa National Park, Figure 3). Interestingly, the Pukaskwa National Park region is dominated by boreal forests, with the predominant ungulate being moose. The distribution of eastern wolf populations, with varying degrees of *C. lupus* introgression in north and *C. latrans* introgression in the south, fits very closely with the Ontario “races” of wolves defined by Kolenosky and Standfield (1975) in Figure 2. One interpretation is the “Ontario type” found in boreal forests would

be represented by the remnant *C. lupus* population in the Pukaskwa region; the “Algonquin type” in the deciduous forests would be representative of the majority of the present populations in the northeastern, northwestern, and central regions (eastern wolf with some coyote and gray wolf introgression), and the “Tweed wolf”/eastern coyote in the south and southeast would be representative of the eastern wolf/western coyote hybrids found in the southeastern portions of the province (see Figure 2). An alternate interpretation is the “Ontario type” represents *C. lupus* impacted by *C. lycaon* stretching across northern Ontario from east to west, while the “Algonquin type” represents non-hybridized eastern wolf in central regions, and the “Tweed wolf” or eastern

coyote represents a *C. latrans* hybrid (impacted with *C. lycaon*). The original gray wolf thought to have inhabited most of Ontario may be represented by the remnant *C. lupus* population in the Pukaskwa region. In either event, the eastern wolf cannot be regarded as a *C. lupus* subspecies based on these data.

Shami (2002) used Y-chromosome studies as an independent test to previous mtDNA work to elucidate the phylogenetic relationships among eastern wolves, coyotes, and gray wolves. Shami (2002) genotyped 383 animals using Y-chromosome microsatellite regions and found 46 haplotypes. Shami (2002) found that although eastern wolves and eastern coyotes contain western coyote mtDNA, they lack western coyote Y-chromosomes. This suggests that there has been asymmetric mating between western coyotes and eastern wolves (Lehman et al. 1991). Eastern wolves possess two predominant haplotypes, neither of which is found in gray wolves. A phylogenetic tree of the Y-haplotypes group animals from central Ontario with other coyote and eastern *Canis* haplotypes suggests they are more closely related to coyotes than to gray wolves. Shami (2002) found no overlapping haplotypes between the Northwest Territories wolves and western coyote populations. With few exceptions, the number of alleles present in each region is quite high, despite some regions having low sample sizes. Hence, the sampling in this study was probably not sufficient to determine the genetic structure of many of the sampled populations and resulted in a high number of unclassified eastern *Canis* haplotypes. There is a need to analyze more coyote and gray wolf populations to get a clear indication of the inter-relationships of the Y-haplotypes, however, the findings from this study do not refute the hypothesis of a distinct species of eastern wolf, *C. lycaon*, closely related to coyotes.

Shami (2002) also investigated a Y-chromosome intron region (slower evolving region of Y-chromosome than the Y-microsatellites). In this study, four intron sequence haplotypes were found: one in Northwest Territories gray wolves, one in western coyotes, one thought to be exclusive to eastern wolves, and one intron was found to be ancestral to the other three. It should be noted that both putatively gray wolf Y-intron and eastern wolf introns were found in some canids from Ohio, Vermont, and New York. Shami (2002)

interpreted these data as suggesting a historic presence of gray wolves in these regions given no coyote Y-chromosome haplotypes were observed in western populations.

Conclusions

Of the three main taxonomic hypotheses regarding eastern wolves, molecular data formally reject the hypothesis that eastern wolves are a *C. lupus* subspecies due to lack of *C. lupus* genes in eastern wolves and predominant presence of coyote-like genes in these populations (e.g., Lehman et al. 1991; Roy et al. 1994, 1996; Wilson et al. 2000, 2003; Grewal 2001; Shami 2002; Grewal et al. 2004). The hypothesis that eastern wolves are the result of *C. lupus/C. latrans* hybridization cannot be rejected by all of the molecular data. These data include the fact that there are no microsatellite alleles unique to eastern wolves and the presence of presumably gray wolf Y-chromosome haplotypes in eastern wolf populations (Shami 2002). However, given the lack of *C. lupus* haplotypes in eastern wolves prior to the mid- to late-1800s expansion of coyotes into the region (Wilson et al. 2003), we are led to reject this hypothesis. In addition, many of the genes (mtDNA and Y-chromosome) found in eastern wolves were not found in either western coyote or northern gray wolf populations and seem to be exclusive to eastern wolves (Wilson et al. 2000; Grewal 2001; Shami 2002) further undermining the hybrid hypothesis. Although debate persists, the distinct species hypothesis is currently not rejected by any of the aforementioned molecular studies. Our review supports the proposal of Wilson et al. 2003 that suggests eastern wolves were likely a distinct species, *C. lycaon*, prior to the eastern expansion of coyotes. This follows from observations by authors who have long-recognized a morphologically distinct, deer-eating wolf in eastern North America (e.g., Miller 1912; Pockock 1935; Young and Goldman 1944; Hall and Kelson 1959; Peterson 1966; Kolenosky and Standfield 1975; Theberge 1991; Brewster and Fritts 1995; Nowak 1983, 1995, 2002, see Figure 1).

With the exception of the chosen nomenclature, the distribution maps of eastern wolves shown by Nowak (1983, 1995, 2002; see Figure 1) should not be discounted if eastern wolves and red

wolves are indeed a closely related species (*sensu* Wilson et al. 2000). The red wolf and eastern wolf have been shown to share many similarities (morphological, behavioral, and molecular characteristics; Wilson et al. 2000; Nowak 2002). Based on these similarities, and the lack of geographic barriers separating these putatively different species (*C. rufus* and *C. l. lycaon*, *sensu* Nowak 2002), it would seem that the red wolf and the eastern wolf could be considered remnants of an eastern North American evolved wolf with no recent connection to the gray wolf that evolved in Eurasia. Hence, eastern wolves could represent the remaining natural populations of this once widespread species complex (Wilson et al. 2000).

The pre-European settlement, morphological distinctiveness of eastern wolves, relative to gray wolves and western coyotes was likely the result of glacial barriers (Nowak 2002) that were subsequently maintained by reproductive barriers attributable to habitat and prey specificities of these *Canis* species (Kolenosky and Standfield 1975; Moore and Parker 1992; Nowak 1995; Geffen et al. 2004). We suggest that subsequent to European settlement, agricultural and forestry practices along with predator control programs led to the breakdown of reproductive barriers between *C. lupus* and *C. lycaon* in the northwestern portions of *C. lycaon*'s distribution and *C. latrans* in the southeastern portion of its range. Hence, the contemporary hybridization between these *Canis* species led to the various morphotypes described in Kolenosky and Standfield (1975). The anthropogenic changes in eastern North America that led to the demise of most gray wolf populations promoted a habitat more suited to deer and an influx of western coyotes in the late 1800s and early 1900s (Moore and Parker 1992; Quinn 2004). A northward extension of eastern wolves into habitats previously dominated by *C. lupus*, followed by the influx of coyotes from the west has resulted in varying degrees of contemporary interbreeding of eastern wolves to both *C. latrans* and *C. lupus* over much of its present range. Hence, the current taxonomic distinctiveness of eastern wolves has been somewhat clouded (Figures 2 and 3).

Recent molecular studies indicate that the eastern wolf's relatively narrow present distribution includes central and western Quebec, central and northeastern Ontario, possibly extending around the Great Lakes region, and through to

southern Manitoba (Lehman et al. 1991; Grewal 2001; see Figure 3). To the north and west of this distribution, increasing levels of *C. lupus* genetic material are observed, in contrast, to the south and east of the distribution, increasing levels of *C. latrans* genetic material are observed. However, a remnant pocket of a *C. lupus* population, with minimal evidence of eastern wolf genetic material, was observed at the core of this distribution (Figure 3). More extensive sampling at the periphery of the eastern wolf's distribution needs to take place to clearly outline where *C. lycaon* and *C. lupus* populations persist and where *C. latrans* continues to invade.

The finding that there is another North American *Canis* species has numerous conservation and management implications. If *C. lycaon* is recognized as another *Canis* species in the Great Lakes region, current numbers of *C. lupus* would be overestimated, potentially influencing its conservation status. Further, in the southern USA, attempts have been made to identify and remove both hybrid *C. rufus* individuals with *C. latrans* DNA and *C. latrans* individuals within certain animal reserves to promote a pure genetic stock of *C. rufus* (see Adams et al. 2003; Miller et al. 2003). Such a program is likely not practical, nor desirable for eastern wolves that now comprise a continuum of morphotypes in the Great Lakes region, from larger *C. lycaon* animals with *C. lupus* genetic material in the northwest to smaller *C. lycaon* with *C. latrans* genetic material in the southeast of its current range (Grewal 2001; Figure 3). In our opinion, less emphasis should be given to preserving the eastern wolf's phenotype; the concern should be conserving the evolutionary process (*sensu* Moritz 1999, 2002). This can be accomplished by protecting the genetic diversity found in *Canis* species, that if lost is not recoverable, whereas adaptive phenotypes can be recovered through recurrent selection (Moritz 2002). *Canis* species are a highly vagile and fast adapting species. This is reflected by the rapid invasion of *C. latrans* to eastern North America, and the presence of *C. latrans/lycaon* hybrids that are well adapted to the anthropogenically modified landscapes of southern Ontario and Quebec where other species would likely not thrive. As such, irrespective of the eastern wolf's taxonomic origins, current hybridization in eastern *Canis* populations should not always be viewed as

negative. For instance, if hybridization has occurred naturally between migrating gray wolves and eastern wolves after the last glaciation, it should be allowed to continue. In fact, similar processes are observed in many plant species where hybridization may be the mechanism protecting their genetic diversity (Soltis and Gitzendanner 1999). Management policies should allow eastern wolves to continue to adapt to their changing environment as an efficient means towards establishing a *Canis* population that is able to effectively exploit the available habitat and prey-base. There is some evidence (see Grewal 2001; Grewal et al. 2004) of reproductive barriers between eastern coyotes and eastern wolves in central Ontario that has been associated with the changing prey-base in this region (Quinn 2004). In some regions of central Ontario the predominant ungulate is moose, a species that is not easily preyed upon by a smaller eastern wolf/coyote hybrids (Forbes and Theberge 1992, 1995; Quinn 2004). Natural selection should favor the presence of larger *C. lycaon* or *C. lycaon/C. lupus* animals where moose predominate.

Overall, it should be noted that molecular investigations of eastern North American wolf populations have provided much insight into the ecology, behavior, and conservation issues surrounding these species. These studies need to continue given that a significant amount of hybridization has occurred between the three species that currently inhabit this region, and that hybrid zones are dynamic, quickly changing in size with varying levels of habitat conservation and management (Wayne and Vila 2003).

Acknowledgements

We would like to thank Dr. J.C. Davies and D. Strickland (Ontario Ministry of Natural Resources), Dr. D. Murray (Trent University), and the anonymous reviewers for their comments on this manuscript.

References

Adams JR, Kelly BT, Waits LP (2003) Using fecal DNA sampling and GIS to monitor hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*). *Mol. Ecol.*, **12**, 2175–2186.

Arjo WM, Pletcher DH (1999) Behavioral responses of coyotes to wolf recolonization in northwestern Montana. *Can. J. Zool.*, **77**, 1919–1927.

Bates DN (1958) *History of the Timber Wolf and Coyote in Ontario*, Ontario Department of Lands and Parks, Ontario.

Bekoff M, Wells MC (1986) Social ecology and behavior of coyotes. *Adv. Stud. Behav.*, **16**, 251–338.

Bertorelle G, Excoffier L (1998) Inferring admixture proportions from molecular data. *Mol. Biol. Evol.*, **15**, 1298–1311.

Boitani L (2003) Wolves: behavior, ecology, and conservation. In: Chapter 13, *Wolf Conservation and Recovery* (eds. Mech LD, Boitani L), pp. 317–340. University of Chicago Press, Chicago.

Brewster WG, Fritts SH (1995) Taxonomy and genetics of the gray wolf in western North America: a review. In: *Ecology and Conservation of Wolves in a Changing World* (eds. Carbyn LN et al.), pp. 353–374. Canadian Circumpolar Institute, Edmonton, Alberta, Canada.

Carbyn LN (1982) Coyote population fluctuations and spatial distribution in relation to wolf territories in Riding Mountain National Park, Manitoba. *Can. Field Nat.*, **96**, 176–183.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.*, **15**, 290–295.

Forbes GJ, Theberge JB (1992) Implications of scavenging on moose by wolves in Algonquin Park, Ontario. *Alces*, **28**, 235–241.

Forbes GJ, Theberge JB (1995) Influences of migratory deer herd on wolf movements and mortality in and near Algonquin Provincial Park, Ontario. In: *Ecology and Conservation of Wolves in a Changing World* (eds. Carbyn LN, Fritts SH, Seip DR), pp. 303–313. Canadian Wildlife Service, Edmonton, Alberta, Canada.

Forbes SH, Boyd DK (1997) Genetic structure and migration in native and reintroduced Rocky Mountain wolf populations. *Conserv. Biol.*, **11**, 1226–1234.

Franzmann AW, Schwartz C (1997) *Ecology and Management of the North American Moose*, Smithsonian Institution Press, Washington, DC.

Garcia-Moreno J, Matocq MD, Roy MS, Geffen E, Wayne RK (1996) Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. *Conserv. Biol.*, **10**, 376–389.

Geffen E, Anderson MJ, Wayne RK (2004) Climate and habitat barriers to dispersal in the highly mobile gray wolf. *Mol. Ecol.*, **13**, 2481–2490.

Graham RW, Lundelius EL Jr (1994) *FAUNMAP: A Database Documenting Late Quaternary Distributions of Mammal Species in the United States*, Illinois State Museum, Springfield, IL pp 690.

Grewal SR (2001) A Genetic Analysis of the Eastern Timber Wolf. M.Sc. Thesis, McMaster University, 173pp.

Grewal SR, Wilson PJ, Kung TK, Shami K, Theberge MT, Theberge JB, White BN (2004) A genetic assessment of the eastern wolf, *Canis lycaon* in Algonquin Park. *J. Mammal.*, **85**, 625–632.

Hall RE, Kelson KR (1959) *The Mammals of North America*, Vol. 2, The Ronald Press Company, NY.

Hedrick PW, Miller PS, Geffen E, Wayne RK (1997) Genetic evaluation of three captive Mexican wolf lineages. *Zool. Biol.*, **16**, 47–69.

- Hedrick PW, Lee RN, Garrigan D (2002) Major histocompatibility complex variation in red wolves: evidence for common ancestry with coyotes and balancing selection. *Mol. Ecol.*, **11**, 1905–1913.
- Kolenosky GB (1971) Hybridization between wolf and coyote. *J. Mammal.*, **52**, 446–449.
- Kolenosky GB, Standfield R (1975) Morphological and ecological variation among gray wolves (*Canis lupus*) of Ontario, Canada. In: *The Wild Canids: Their Systematics, Behavioural Ecology and Evolution* (eds. Fox MW), pp. 62–72. Van Nostrand Reinhold, New York, NY.
- Lehman N, Eisenhawer A, Hansen K, Mech LD, Peterson RO, Gogan PJ, Wayne RK (1991) Introgression of coyote mitochondrial DNA genotype frequencies: estimation of the effective number of alleles. *Evolution*, **45**, 104–119.
- Lehman N, Clarkson P, Mech DL, Meier TJ, Wayne RK (1992) A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. *Behav. Ecol. Sociobiol.*, **30**, 83–94.
- Leonard JA, Vila C, Wayne RK (2005) Legacy lost: genetic variability and population size of extirpated US grey wolves (*Canis lupus*). *Mol. Ecol.*, **14**, 9–17.
- Mech LD (1966) *The Wolves of Isle Royale*. US National Park Service Fauna Series No. 7.
- Mech LD (1970) *The Wolf: The Ecology and Behaviour of an Endangered Species*, The Natural History Press, Garden City, New York.
- Mech LD, Karns PD (1977) *Role of the Wolf in a Deer Decline in the Superior National Forest*. USDA Forest Service Research Paper NC-148, North Central Forest Experiment Station, St. Paul, MN, 23pp.
- Mech LD, Federoff NE (2002) Alpha (1) – antitrypsin polymorphism and systematics of eastern North American wolves. *Can. J. Zool.*, **80**, 961–963.
- Miller GS (1912) *The Names of the Large Wolves of Northern and Western North America*. Smithsonian Miscellaneous Collection.
- Miller CR, Adams JR, Waits LP (2003) Pedigree-based assignment tests for reversing coyote (*Canis latrans*) introgression into the wild Red wolf (*Canis rufus*) population. *Mol. Ecol.*, **12**, 3287–3301.
- Moore GC, Parker GR (1992) Colonization by the eastern coyote (*Canis latrans*). In: *Ecology and Management of the Eastern Coyote* (eds. Boer AH), pp. 23–37. Wildlife Research Unit, University of New Brunswick, Fredericton, New Brunswick, Canada.
- Moritz C (1994) Defining “Evolutionarily Significant Units” for conservation. *Trends Ecol. Evol.*, **9**, 373–375.
- Moritz C (1999) Conservation units and translocations: strategies for conserving evolutionary processes. *Hereditas*, **130**, 217–228.
- Moritz C (2002) Strategies to protect biological diversity and the evolutionary process that sustains it. *Syst. Biol.*, **51**, 238–254.
- Nowak RM (1979) *North American Quaternary Canis*. University of Kansas Museum of Natural History, Monograph, no. 6.
- Nowak RM (1983) A perspective on the taxonomy of wolves in North America. In: *Wolves in Canada and Alaska* (ed. Carbyn LN), pp. 10–19. Can. Wildl. Serv. Rept. Ser., no. 45.
- Nowak RM (1992) The red wolf is not a hybrid. *Conserv. Biol.*, **6**, 593–595.
- Nowak RM (1995) Another look at wolf taxonomy In: *Ecology and Conservation of Wolves in a Changing World: Proceedings of the Second North American Symposium of Wolves* (eds. Carbyn LN, Fritts SH, Seip DR), pp. 375–398. Canadian Circumpolar Institute, University of Alberta, Edmonton, Canada.
- Nowak RM (2002) The original status of wolves in eastern North America. *Southeastern Nat.*, **1**, 95–130.
- Nowak RM (2003) Wolf evolution and taxonomy In: *Wolves: Behavior, Ecology, and Conservation* (eds. Mech LD, Boitani L), pp. 239–258. The University of Chicago Press, Chicago, IL.
- Parker G (1995) *Eastern Coyote: The Story of its Success*. Nimbus Publishing Limited, Halifax, Nova Scotia.
- Peterson RL (1955) *North American Moose*, University of Toronto Press, Toronto, Ontario, Canada.
- Peterson RL (1966) *The Mammals of Eastern Canada*, Oxford University Press, Toronto, Ontario, Canada.
- Pilgrim KL, Boyd DK, Forbes SH (1998) Testing for wolf–coyote hybridization in the Rocky Mountains using mitochondrial DNA. *J. Wildl. Manag.*, **62**, 683–689.
- Pockock RL (1935) The races of *Canis lupus*. *Proc. Zool. Soc. London*, **3**, 647–686.
- Quinn NWS (2004) The pre-settlement hardwood forests and wildlife of Algonquin Provincial park: a synthesis of historic evidence and recent research. *Forest. Chron.*, **80**, 705–717.
- Reich DE, Wayne RK, Goldstein DB (1999) Genetic evidence for a recent origin by hybridization of red wolves. *Mol. Ecol.*, **8**, 139–144.
- Roy MS, Geffen E, Smith D, Ostrander EA, Wayne RK (1994) Patterns of differentiation and hybridization in North American Wolflike Canids, revealed by analysis of microsatellite loci. *Mol. Biol. Evol.*, **11**, 553–570.
- Roy MS, Geffen E, Smith D, Wayne RK (1996) Molecular genetics of pre-1940 red wolves. *Conserv. Biol.*, **10**, 1413–1424.
- Schmitz OJ, Kolenosky GB (1985) Wolves and coyotes in Ontario: morphological relationships and origins. *Can. J. Zool.*, **63**, 1130–1137.
- Shami K (2002) Evaluating the Change in Distribution of the Eastern Timber Wolf (*Canis lycaon*) using the Y-Chromosome. M.Sc. Thesis, McMaster University, 73pp.
- Sharma DK, Maldonado JE, Jhala YV, Fleischer RC (2004) Ancient wolf lineages in India. *Proc. R. Soc. Lond. B (Suppl.)*, **271**, S1–S4.
- Silver H, Silver WT (1969) Growth and behaviour of the coyote-like canid of Northern New England with observations on canid hybrids. *Wildlife Monogr.*, **17**, 1–41.
- Soltis PS, Gitzendanner MA (1999) Molecular systematics and the conservation of rare species. *Conserv. Biol.*, **13**, 471–483.
- Theberge JB (1991) Ecological classifications, status, and management of the gray wolf, *Canis lupus*, in Canada. *Can. Field Nat.*, **105**, 459–463.
- Thurber JM, Peterson RO (1991) Changes in body size associated with range expansion in the coyote (*Canis latrans*). *J. Mammal.*, **72**, 750–755.
- Wayne RK (1995) The problematic red wolf. *Sci. Am.*, **273**, 36–42.

- Wang J (2003) Maximum-likelihood estimates of admixture proportions from genetic data. *Genetics*, **164**, 747–765.
- Wayne RK, Lehman N (1992) Mitochondrial DNA analysis of the eastern coyote: origins and hybridization. In: *Ecology and Management of the Eastern Coyote* (eds. Boer AH), pp. 9–22. Wildlife Research Unit, Fredericton, NB.
- Wayne RK, Lehman N, Allard MW, Honeycutt RL (1992) Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. *Conserv. Biol.*, **6**, 559–569.
- Wayne RK, Vila C (2003) Molecular genetics studies of wolves. In: *Wolves: Behavior, Ecology, and Conservation* (eds. Mech LD, Boitani L), pp. 218–238. The University of Chicago Press, Chicago, IL.
- Wayne RK, Jenks SM (1991) Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. *Nature*, **351**, 565–568.
- White BN, Wilson P, Johnson AR, Grewal S, Shami K (2001) Status of the eastern wolf (*Canis lycaon*). In: *O.M.N.R. The Eastern Wolf*, A Newsletter on Wolf Studies in and around Algonquin Provincial Park, Ontario. Vol. 1, Issue 1, 2003.
- Wilson PJ, Grewal S, Lawford ID, Heal JNM, Granacki AG, Pennock D, Theberge JB, Theberge MT, Voigt DR, Waddell W, Chambers RE, Paquet PC, Goulet G, Cluff D, White BW (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**, 2156–2166.
- Wilson PJ, Grewal S, McFadden T, Chambers RC, White BN (2003) Mitochondrial DNA extracted from eastern North American wolves killed in the 1800s is not of gray wolf origin. *Can. J. Zool.*, **81**, 936–940.
- Young SP, Goldman EA (1944) *The Wolves of North America, Part I*, American Wildlife Institute, Washington.