

## NOTE

# $\alpha_1$ -Antitrypsin polymorphism and systematics of eastern North American wolves

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**Abstract:** We used data on the polymorphic status of  $\alpha_1$ -antitrypsin ( $\alpha_1$ AT) to study the relationship of Minnesota wolves to the gray wolf (*Canis lupus*), which was thought to have evolved in Eurasia, and to red wolves (*Canis rufus*) and coyotes (*Canis latrans*), which putatively evolved in North America. Recent evidence had indicated that Minnesota wolves might be more closely related to red wolves and coyotes. Samples from wild-caught Minnesota wolves and from captive wolves, at least some of which originated in Alaska and western Canada, were similarly polymorphic for  $\alpha_1$ AT, whereas coyote and red wolf samples were all monomorphic. Our findings, in conjunction with earlier results, are consistent with the Minnesota wolf being a gray wolf of Eurasian origin or possibly a hybrid between the gray wolf of Eurasian origin and the proposed North American wolf.

**Résumé :** Nous avons utilisé des données sur le statut polymorphe de l' $\alpha_1$ -antitrypsine ( $\alpha_1$ AT) pour définir les relations entre les loups du Minnesota et le loup gris (*Canis lupus*), que l'on croit avoir évolué en Eurasie, le loup roux (*Canis rufus*) et le coyote (*Canis latrans*), qui ont probablement évolué en Amérique du Nord. Des données récentes indiquent que les loups du Minnesota semblent plus apparentés aux loups roux et aux coyotes. Des échantillons provenant de loups capturés en nature et de loups en captivité, dont au moins quelques-uns sont originaires d'Alaska et de l'ouest du Canada, se sont avérés également polymorphes quant à l' $\alpha_1$ TA, alors que les échantillons prélevés sur des loups roux et des coyotes étaient tous monomorphes. Nos résultats, combinés à ceux d'études antérieures, entérinent l'idée que le loup du Minnesota est un loup gris d'origine eurasiennne, ou encore possiblement un hybride entre le loup gris d'origine eurasiennne et le loup nord-américain proposé.

[Traduit par la Rédaction]

## Introduction

A recent genetic analysis of red wolves (*Canis rufus*) and gray wolves (*Canis lupus*) using microsatellite loci suggested that the wolf in Minnesota was more closely related to the red wolf than to the gray wolf (Fig. 1 in Wilson et al. 2000). Federoff and Kueppers (2000) suggested that the red wolf and the coyote may have evolved in North America, sharing a common ancestor, which Wilson et al. (2000) subsequently confirmed. In addition, Wilson et al. (2000) suggested that the eastern Canadian wolf, which they named *Canis lycaon*, evolved in North America along with the red wolf and coyote, whereas the gray wolf evolved in Eurasia. Further, the study

concluded that the range of the eastern Canadian wolf extends westward through Minnesota into Manitoba.

However, the distribution of the gray wolf also extends into Minnesota from Alaska and the Yukon eastward (Nowak 1995). Thus, the identity of the Minnesota wolf is in question, and the possibility exists that the Minnesota wolf is a hybrid between the gray wolf and the eastern Canadian wolf. Indeed, some mitochondrial DNA (mtDNA) haplotypes found in Minnesota wolves are the same as those in gray wolves of Alaska and northwestern Canada, whereas others are similar to those in the *Canis* spp. that putatively evolved in North America, i.e., coyotes or eastern Canadian wolves (Lehman et al. 1991).

Thus, with the genetic identity of the wolf in Minnesota unclear (Fig. 1), any new information bearing on the subject is valuable. We present here the results of our comparison of the polymorphic status of  $\alpha_1$ -antitrypsin ( $\alpha_1$ AT) (Federoff and Kueppers 2000) among red wolves, Minnesota wolves, gray wolves from areas other than Minnesota, and with coyotes (*Canis latrans*), which putatively are also related to North American wolves (Wilson et al. 2000).

## Methods

$\alpha_1$ AT is a major mammalian protease inhibitor and is a highly polymorphic glycoprotein.  $\alpha_1$ AT has activity against trypsin, chymotrypsin, cathepsin G, elastase, and probably

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**Table 1.**  $\alpha_1$ -Antitrypsin (Pi) phenotypes (M, MS, and S) in coyotes and various types of wolves.

Sample	<i>n</i>	M	MS	S
Minnesota wolves <sup>a,b</sup>	6	2 (33)	3 (50)	1 (17)
Captive wolves <sup>a,c</sup>	23	9 (39)	14 (61)	0
Red wolves <sup>b</sup>	27	0	0	27 (100) <sup>d</sup>
Coyotes	24			24 (100) <sup>d</sup>

**Note:** Values in parentheses are percentages.

<sup>a</sup>ns,  $P = 0.14$ ,  $\chi^2_2 = 4.0$ .

<sup>b</sup> $P < 0.0001$ ,  $\chi^2_2 = 26.5$ .

<sup>c</sup>Natural origins are unknown for most, but some are from Alaska and Canada.

<sup>d</sup>Federoff and Kueppers (2000).

other serin proteinases.  $\alpha_1$ AT occupies a single locus with two alleles. Three phenotype band patterns are designated M, S, and MS. M and S are homozygotes, and MS is a heterozygote.

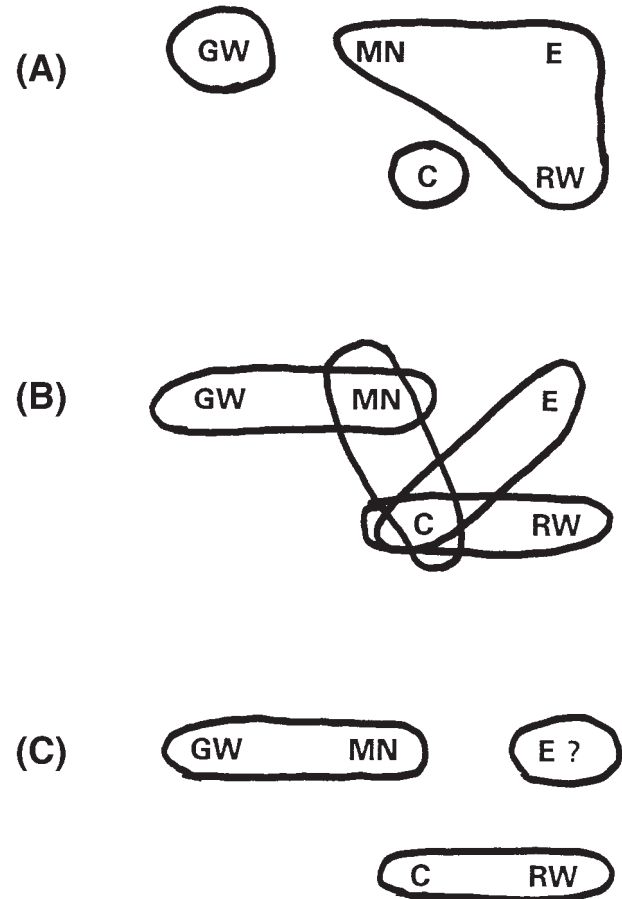
Using  $\chi^2$  tests, we reanalyzed data on  $\alpha_1$ AT polymorphic status from a study by Federoff and Kueppers (2000). That study examined  $\alpha_1$ AT in *Canis* spp. samples from various sources. We used the data from 6 wild-caught Minnesota wolves, 23 captive wolves (including 4 known to have origins in Alaska and (or) western Canada and others of unknown origins), 27 captive red wolves, and 24 captive coyotes originating in Utah. Our objective was to compare the  $\alpha_1$ AT locus in canids that had evolved in Eurasia (gray wolves) with those that had evolved putatively in North America (coyotes and red wolves). In particular, we sought to test wolves from Minnesota, where the two wolf types supposedly meet (Wilson et al. 2000).

## Results and discussion

The Minnesota and captive gray wolf samples were similarly polymorphic for  $\alpha_1$ AT, whereas coyote and red wolf samples were all monomorphic (Table 1). There is agreement in the findings of Federoff and Kueppers (2000) and, subsequently, Wilson et al. (2000), which suggests that the red wolf and coyote may have evolved from the same line, sharing a common ancestor (Fig. 1). Because we did not assay eastern Canadian wolves for  $\alpha_1$ AT, it is not known whether they are polymorphic or monomorphic at that locus. However, the conclusion from Wilson et al. (2000) would predict that they too are monomorphic for  $\alpha_1$ AT.

Regarding the contention that the eastern Canadian wolf is similar to the red wolf and that the eastern Canadian wolf range extends through Minnesota to Manitoba (Wilson et al. 2000), our findings might be explained in any of three ways: (1) the Minnesota wolf population might include both eastern Canadian wolves and gray wolves, and by chance our samples only represented gray wolves; (2) in Minnesota, eastern Canadian wolves and gray wolves have hybridized, and the locus we examined retained gray wolf behavior, i.e., being polymorphic rather than monomorphic for  $\alpha_1$ AT; or (3) the Minnesota wolves are gray wolves, some of whose ancestors have hybridized with coyotes (Lehman et al. 1992), and coyote introgression into red wolves and eastern Canadian wolves is the reason red wolves are 100% S for  $\alpha_1$ AT and why microsatellite analysis groups Minnesota wolves

**Fig. 1.** Conceptual diagram of three types of genetic analyses of the similarities among closely related *Canis* spp. considered in this paper: (A) microsatellite analysis (Wilson et al. 2000); (B) mtDNA analysis (Wayne and Jenks 1991); and (C)  $\alpha_1$ AT analysis (present study). GW, gray wolf; MN, Minnesota wolf; E, eastern Canadian wolf; RW, red wolf; and C, coyote. Encirclements denote entities that appear to be indistinguishable based on the given type of analysis; note that the power of each of these analyses is not the same.



with eastern Canadian and red wolves. A fourth explanation, that Minnesota wolves are eastern Canadian wolves and that perhaps eastern Canadian wolves are polymorphic for  $\alpha_1$ AT, would not accord with the findings of Wilson et al. (2000) that the coyote, red wolf, and eastern Canadian wolf had a common ancestor.

The weight of current evidence favors explanation 2. Explanation 1 is unlikely because the probability is low that all six of our samples would have been from gray wolves if our study population was composed of both eastern Canadian and gray wolf types. From the same population as our 6 Minnesota wolves, Lehman et al. found that 11 of 42 (0.2619) wolves possessed gray wolf haplotypes (Fig. 4 in Lehman et al. 1992). Thus, the probability that five of our six Minnesota samples were gray wolf types would be  $(0.2619)^5$  or 0.0001.

Explanations 2 and 3 are both supported by the findings that, in our Minnesota study population, some breeding packs include both individuals with mtDNA haplotypes found in gray wolves and individuals with mtDNA found in eastern

Canadian wolves and coyotes (Fig. 4 in Lehman et al. 1992). Because wolf packs are families, it seems far more likely that the occurrence of both such mtDNA haplotypes in the same packs resulted from hybridization rather than from association of two species (the eastern Canadian wolf and the gray wolf), especially decades or more after such sympatry would have first occurred.

However, evidence against explanation 3, which invokes hybridization with coyotes, would seem to come from microsatellite comparisons tending to refute coyote introgression into eastern Canadian wolves (Fig. 2 in Wilson et al. 2000). However, it is unclear whether comparisons were actually made between coyotes and Minnesota wolves (cf. p. 2158 and Fig. 2 in Wilson et al. 2000).

In any case, to further clarify the identity of the Minnesota wolves, we recommend that many other loci be examined. In addition, examination of the  $\alpha_1$ AT locus of a sample of eastern Canadian wolves and a larger sample of Alaskan or northwestern Canadian wolves would also better elucidate the relationships among the red wolf, the eastern Canadian wolf, the coyote, the gray wolf, and the Minnesota wolf.

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