Top predators are disappearing worldwide, significantly changing ecosystems that depend on top-down regulation. Conflict with humans remains the primary roadblock for large carnivore conservation, but for the eastern wolf (Canis lycaon), disagreement over its evolutionary origins presents a significant barrier to conservation in Canada and has impeded protection for grey wolves (Canis lupus) in the USA. Here, we use 127,235 single-nucleotide polymorphisms (SNPs) identified from restriction-site associated DNA sequencing (RAD-seq) of wolves and coyotes, in combination with genomic simulations, to test hypotheses of hybrid origins of Canis types in eastern North America. A principal components analysis revealed no evidence to support eastern wolves, or any other Canis type, as the product of grey wolf/C2 western coyote hybridization. In contrast, simulations that included eastern wolves as a distinct taxon clarified the hybrid origins of Great Lakes-boreal wolves and eastern coyotes. Our results support the eastern wolf as a distinct genomic cluster in North America and help resolve hybrid origins of Great Lakes wolves and eastern coyotes. The data provide timely information that will shed new light on the debate over wolf conservation in eastern North America.

1. Introduction
Carnivores are disappearing at an alarming rate, threatening top-down regulation, ecosystem resiliency and biodiversity worldwide [1]. With their need for expansive spaces and sufficient prey, large predators have an ecological life history that directly conflicts with human interests. This incompatibility has caused a long-standing history of widespread persecution that brought top predators like wolves to the brink of extinction in Europe [2] and North America [3]. The twenty-first century, however, has witnessed some recovery of large carnivores in Europe [4] and the USA [1], suggesting restoration is possible.

Few species present more of a challenge to conservation than those within the Canis genus. Eastern wolves (Canis lycaon) from Algonquin Provincial Park, Ontario [5] faced targeted extermination during the first half of the twentieth century [6], and high mortality owing to hunting and trapping outside park boundaries during the second half [7]. Since the turn of the millennium, however, the biggest threat to their long-term persistence has been disagreement over their evolutionary history. Although genomics holds promise for improving conservation efforts [8], varying interpretation of genome-wide single-nucleotide polymorphism (SNP) data from wolves and coyotes [9,10] has, so far, only added to the confusion among non-geneticists and policy...
2. Results and discussion

We obtained high-quality RADSeq data with a median depth of coverage between 27 and 93× (electronic supplementary material, table S2). There were 197,263 putative RAD loci in the final filtered set, which represents 17.8 Mbp of putative low copy grey wolf genomic reference sequence. After filtering for bi-allelic SNPs (see electronic supplementary material), genotypes at 127,235 SNP loci for each of 17 individuals of five different Canis types (electronic supplementary material, table S2) were generated for further analysis.

Following the genetic clusters species concept [23], a principal components analysis of SNP genotypes was consistent with the existence of a distinct eastern wolf species (figure 1). Simulated grey wolf × western coyote hybrid genotypes failed to overlap with any other Canis types when projected on the factorial map, but simulated grey wolf × eastern wolf genotypes overlapped with observed data for Great Lakes-boreal wolves and simulated eastern wolf × western coyote genotypes overlapped with observed data for eastern coyotes (figure 1). These patterns are consistent with previous suggestions that the eastern wolf is a conduit of gene flow between grey wolves and coyotes [10,21,24], but are in contrast to previous work that analysed 48K SNPs (based on the dog genome) and concluded (under the assumption of the two-species model) that the eastern wolf from Algonquin Park was a grey wolf × western coyote hybrid [9]. Our work, however, differs from that presented in reference [9] in that our dataset is (i) unfettered by ascertainment bias or assumptions of a two-species model (see electronic supplementary material), (ii) uses 80K more loci and (iii) implements a sampling and analytical design intended to specifically test hypotheses of hybrid origins for the different Canis types.

Tests of admixture were contradictory: ALDER did not identify eastern wolves as admixed between grey wolves × western coyotes, but was limited by sample size (see electronic supplementary material). The β results suggested various wolf × coyote admixture in both eastern wolves and Great Lakes-boreal wolves, with increasing standard error and decreasing significance as the number of SNP re-sampling blocks increased (see electronic supplementary material). The β analysis failed, however, to detect admixture in eastern coyotes—a cluster for which wolf × coyote ancestry is well documented [24,25]. Further, the β results contradict known behavioural [26] and probable biological [27] reproductive barriers between grey wolves and western coyotes. We suspect that the broad estimates of divergence between grey wolves and western coyotes (approx. 1 Ma) and between eastern wolves and western coyotes (approx. 300,000 Ma), in combination with the contemporary admixture observed in the eastern North American populations, may be impacting the ability of these tests to accurately estimate ancestry. We therefore recommend cautious interpretation of both ALDER and β results presented here, and suggest more extensive sampling may provide more robust results with sophisticated analytical software (see the electronic supplementary material).

Based on 63 diagnostic SNPs, previous research identified eastern coyotes as a mix of western coyotes, western wolves, eastern wolves and domestic dogs [21]. However, the sample of ‘eastern wolves’ used in reference [21] originated primarily from the Great Lakes states (n = 14) and only included three samples from Ontario. That sample of eastern wolves,
therefore, represents Great Lakes-boreal wolves as a parental reference population rather than what are currently considered representative eastern wolves [22]. If one considers (i) the differences in terminology, and (ii) the lack of representative eastern wolf samples from Algonquin Park in previous genomic studies, our results are consistent with SNP results from other researchers who identified the eastern coyote as having wolf/C2 coyote hybrid ancestry [21], and who suggest Great Lakes wolves are admixed [9]. The differences arise based on the interpretation and representation of the eastern wolf, which in our data is represented by animals from Algonquin Park. These differences in terminology and geographical sampling have hindered conservation of eastern wolves in Canada and grey wolves in the USA. We suggest that the most parsimonious explanation of all genetic data to date, including that of mitochondrial DNA [28], Y-chromosome [24] and genome-wide SNP data (this manuscript and [10]), support eastern wolves from Algonquin Park as a distinctive remnant entity of a historical wolf that most likely occurred across the eastern United States.

3. Conclusion

Our findings represent important information for implementing effective endangered species policy in North America. We demonstrate support for the eastern wolf centralized in Algonquin Provincial Park as a distinct genomic cluster, thus providing support for the three-species model of Canis evolution. Additionally, our data support previous work indicating wolves in the Great Lakes states as originating from grey
wolf × eastern wolf origins [29]. Although we were unable to test alternative evolutionary scenarios (e.g. hybridization followed by drift) with this dataset, and specific admixture tests were inconclusive and contradictory, future work with species-specific SNPs and broader sampling may allow a more comprehensive comparison of the alternatives. The recognition of the eastern wolf as a separate species does not exclude the possibility that a grey wolf × eastern wolf hybrid animal (previously identified as Canis lupus lycaon, boreal/Ontario-type [30]), similar to a Great Lakes-boreal wolf currently located in the Great Lakes states and across Manitoba, northern Ontario, and northern Quebec, historically inhabited the northeastern United States alongside eastern wolves, and there is some evidence to support the historical presence of both Canis types [10]. The recognition of C. lycaon should not, therefore, influence grey wolf delisting decisions in the USA. In the light of the current funding gap for biodiversity conservation [17], and the increased biodiversity, reduced disease and control of invasive species that occurs with top-down regulation [31], wolf conservation could provide a fundamental, cost-effective approach to reduce herbivory, conserve ecosystems and improve biodiversity in the troubled landscapes of eastern North America.

Data accessibility. Wolf/Coyote DNA Bank at Trent University.

Authors’ contributions. L.Y.R. conceived/designed the study; laboratory work/data analyses; drafted manuscript. S.D. analyses/methods; J.Q.B. RAD-sequencing/methods; P.A.H. data analyses/manipulation/methods; B.N.W. sample/genetic database. All authors provided intellectual input and edited/approved the manuscript.

Competing interests. We declare we have no competing interests.

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