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Arctic and Arctic-like rabies viruses: distribution, phylogeny and evolutionary history

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SUMMARY

Forty-one newly sequenced isolates of Arctic and Arctic-like rabies viruses, were genetically compared to each other and to those available from GenBank. Four phylogenetic lineages of Arctic viruses were identified. Arctic-1 viruses circulate in Ontario, Arctic-2 viruses circulate in Siberia and Alaska, Arctic-3 viruses circulate circumpolarly, and a newly described lineage Arctic-4 circulates locally in Alaska. The oldest available isolates from Siberia (between 1950 and 1960) belong to the Arctic-2 and Arctic-3 lineages and share 98·6–99·2% N gene identity with contemporary viruses. Two lineages of Arctic-like viruses were identified in southern Asia and the Middle East (Arctic-like-1) and eastern Asia (Arctic-like-2). A time-scaled tree demonstrates that the time of the most recent common ancestor (TMRCA) of Arctic and Arctic-like viruses is dated between 1255 and 1786. Evolution of the Arctic viruses has occurred through a northerly spread. The Arctic-like-2 lineage diverged first, whereas Arctic viruses share a TMRCA with Arctic-like-1 viruses.

INTRODUCTION

Animal diseases compatible clinically with rabies have been described in the Arctic and sub-Arctic areas for over a century. At least 150 years ago extensive epizootics among Arctic foxes (*Alopex lagopus*) and sled dogs were documented [1, 2]. The disease was frequently referred to as 'Polar madness', 'Eskimo dog disease', 'Arctic dog disease' [3, 4], and *dikovanie* or *dikusha* in Russian [5]. As Negri bodies were not detected in Seller-stained brain impressions, and human rabies was rarely reported from the Arctic and sub-Arctic territories, the identification of the aetiological agent as rabies virus (RABV) was not confirmed until the 1940s, when serological relatedness was demonstrated [6, 7]. A variety of methods, such as the fluorescent antibody test, electron microscopy, and typing with monoclonal antibodies (mAbs), confirmed the agent as RABV [4].

The use of mAbs for antigenic typing has significantly improved the differentiation of RABV variants. For example, mAb P-41, obtained as the result of immunization of mice with a RABV isolate from an Arctic fox in Yakutia, reacts selectively with the nucleocapsid of Arctic RABV isolates [8]. The P-41 reactive viruses were found in Arctic and sub-Arctic

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areas circumpolarly, and antigenic patterns of isolates from Alaska and Canada are identical or similar to those of viruses circulating in Siberia. The P-41-reactive viruses have also been identified in raccoon dogs (*Nyctereutes procyonoides*) and red foxes (*Vulpes vulpes*) in Baltic regions. It has been proposed that Arctic viruses have been translocated to this territory, and thereafter established circulation in new host species [9].

Nucleotide sequencing has facilitated a more precise differentiation and subsequent phylogenetic placement of viruses isolated during the last several decades [10, 11]. Three phylogenetic groups of Arctic RABV have been described [12]: one of these groups was identified in North America, and includes viruses circulating in Ontario, Maine and Greenland (at least formerly); a second group includes viruses circulating in Siberia and Alaska; the third group includes Arctic viruses with apparent circumpolar circulation patterns. The first of these three groups was described in earlier studies as having been introduced into Ontario with a wave of Arctic rabies during the 1950s, and further established independent circulation among red foxes and striped skunks (*Mephitis mephitis*) [13, 14].

Recent studies have shown that several RABV lineages related phylogenetically to the Arctic viruses are present in the Middle East, and southern and eastern Asia. These viruses were referred to as either the Arctic or Arctic-like RABVs [11, 15–19]. The most western isolation point of these viruses was described in northern Iran [15].

In contrast, Baltic isolates do not belong phylogenetically to the Arctic or Arctic-like lineages, but form a clade within the European fox lineage, which is part of the 'cosmopolitan' canine RABV lineage [20]. Reactivity of MAb P-41 with these viruses remains incompatible with their phylogeny. One common feature of both Arctic and Baltic viruses is the amino-acid substitution (Asp to Gly) at position 115 of the nucleoprotein (N) gene. It has been suggested that this substitution could have facilitated switching of RABV from Arctic foxes to raccoon dogs, and further to red foxes [20]. However, further comparisons have demonstrated that raccoon dogs in eastern Asia maintain circulation of Arctic-like viruses with the presence of Asp115, whereas Gly115 was detected in some isolates from corsac foxes (*Vulpes corsac*) in eastern Siberia. The substitution with Gly115 may facilitate a positive reaction with mAb P-41, but the evolutionary or functional significance of this substitution, if any, is unclear [21].

The objective of this study is presentation of new data on the distribution and phylogenetics of Arctic and Arctic-like viruses. These include the oldest viruses available, isolated in north-eastern Siberia (Yakutia) during 1950–1960. In addition, the implementation of a time-based evolutionary analysis, using a relaxed molecular clock, has enabled a deeper insight into the time-scale of RABV spread within Arctic regions.

METHODS

Viral isolates

Viruses isolated from Arctic foxes in Yakutia during 1950–1960 were obtained from the Russian State Collection of Viruses (Ivanovsky Institute of Virology, Moscow, Russia) as frozen mouse brain suspensions. Other viruses described here for the first time were isolated during surveillance in Siberia, Alaska and southern Asia during the period 1980–2006. A positive sample from an Iraqi dog was kindly provided by Dr L. Fuhrmann (Veterinary Laboratory Europe, Kirchberg, Germany). Initial diagnosis was performed using the standard direct fluorescent antibody test (protocol available at http://www.cdc.gov/ncidod/dvrd/rabies/professional/publications/DFA_diagnosis/DFA_protocol-b.htm). Virus isolation by intracerebral inoculation of inbred laboratory mice was performed in some instances.

RT–PCR, gene sequencing and phylogenetic analysis

Total RNA was extracted from infected material (either original host brain or mouse brain following limited passages), using TRIzol™ (Gibco-BRL Inc., Gaithersburg, MD, USA) according to the manufacturer's recommendations. The RT–PCR was performed as described previously [22] with primers for amplification of the entire N gene (1350 bp). The PCR products were purified and subjected to direct sequencing using the ABI Prism™ 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Primary assembly, alignment, consensus generation and DNA translation were performed using BioEdit [23]. Neighbour joining (NJ) tree analysis was performed using either the Kimura-2-parameter or Jukes–Cantor model of nucleotide substitution and performed using MEGA version 2.1 [24]. Bootstrap support was estimated for 1000 replicates.

Previously published gene sequences were retrieved from GenBank for comparison (Table 1).

Estimation of the evolutionary rate and application of a relaxed molecular clock

Estimates of the rate of molecular evolution (μ; substitutions per site per year) for complete N gene alignments were obtained using a relaxed molecular clock [25] implemented using a Bayesian Markov Chain Monte Carlo (MCMC) method within the BEAST program (available from http://www.beast.bio.ed.ac.uk). Sequences were dated with the year of isolation and identical sequences with the same year of isolation excluded. For sequences with an imprecise isolation date, a random subset was selected and sequences dated with the midpoint of the isolation range. For each dataset, the maximum-likelihood model of nucleotide substitution was selected using the Modeltest software [26] and the selected model used as the basis for BEAST analysis. A lognormal distribution of rates was used with Jeffrey's priors on μ and population size [27]. Two models of population dynamics were used and their likelihood compared [28]. MCMCs were run for a minimum length of 5×10^6 with a 1% burn-in ensuring all effective sample sizes were >100. A minimum of 10 000 trees was used to produce a time-scaled tree with TreeAnnotator which was displayed and edited using FigTree (both available from http://www.beast.bio.ed.ac.uk). Details of all models used are available from the authors on request.

Three datasets were used for substitution rate analysis: (1) a combined analysis of Arctic/Arctic-like RABV isolates using SG1, SG15 and SG91 dated as 1955 ($n=32$); (2) 17 European red fox isolates (GenBank accession nos.: AF033905, U22474-6, U22480, U42605-7, U42700-2, U42704, U42706, U42707, U43432-4); and (3) the Arctic/Arctic-like and European red fox datasets combined with the addition of 11 further isolates from the same lineage [29] (GenBank accession nos.: DQ837383, DQ837385, DQ837446, DQ837448, DQ837463, U22481, U22482, U22484, U22627, U22629, U22852).

RESULTS

Phylogenetic relationships

The NJ trees show 42 viruses as members of the Arctic and Arctic-like phylogenetic clades. The majority of the Arctic RABV isolates belonged to the same phylogenetic groups to which they have been previously assigned [12]. Group Arctic-1 (Fig. 1) was comprised of viruses circulating in Ontario among red foxes and striped skunks [13, 14]. Group Arctic-2 consisted of viruses circulating in north-eastern Siberia and Alaska, predominantly among Arctic foxes. Group Arctic-3 included viruses that circulate circumpolarly in Siberia, Alaska and Canada. Despite the circumpolar distribution, the percentage identity of these viruses is remarkable. For example, isolate RVHK obtained in Norilsk (north-central Siberia), shared 98·5–99·3% nucleotide identity with isolates from Alaska and Canada. The oldest available viruses, isolated between 1950 and 1960 in north-eastern Siberia (Yakutia), all belonged to the Arctic-2 and Arctic-3 groups, and shared 98·6–99·2% identity with the more recent isolates, including those obtained during 2007 in Alaska. Mansfield *et al.* [12] considered groups Arctic-2 and Arctic-3 as (*a*) subgroups and (*b*) of one group, Arctic-2. However, bootstrap support for this node is low in this and the previous study. Since these viruses also exhibit different circulation patterns, we designate them as distinct groups.

Several viruses from Alaska, isolated from foxes and dogs during 2006–2007, were not included in the Arctic-2 and Arctic-3 groups, but were placed ancestrally, within a well-formed new group that we designated Arctic-4. In fact, viruses isolated in Alaska during the last 2 years represent all three major phylogenetic lineages except Arctic-1. However, distribution patterns are different: Arctic-3 viruses were isolated only along the northern coast, supporting their circumpolar circulation with animals migrating along the pack ice. Arctic-2 viruses were isolated in the west, and are probably maintained by Arctic foxes migrating between Siberia and Alaska across the Bering Strait which is frozen in winter. Arctic-4 viruses were found in the south-western area, and probably circulate within the local population of Arctic foxes (Fig. 2).

Arctic-like viruses formed two clades. The Arctic-like-1 clade contained viruses circulating in the Middle East and southern Asia (Iraq, Iran, Pakistan, India), and the Arctic-like-2 clade contained viruses circulating in eastern Asia (south-eastern Siberia, Russian Far East, Japan (formerly) and Korea. The same topology was obtained for the limited gene sequences available in GenBank from a previous study [19]. Interestingly, the RABV isolate '994 dog', from Chita Province (south-eastern Siberia) isolated during

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Table 1. *Rabies viruses used in the present study*

Isolate name	Animal species	Year	Territory	Reference	GenBank accession no.
sg91	Arctic fox	1950–1960	Yakutia	This study	EF611834
sg12	Arctic fox	1950–1960	Yakutia	This study	EF611837
sg15	Arctic fox	1950–1960	Yakutia	This study	EF611840
sg16	Arctic fox	1950–1960	Yakutia	This study	EF611836
sg3	Arctic fox	1950–1960	Yakutia	This study	EF611832
sg92	Arctic fox	1950–1960	Yakutia	This study	EF611835
sg1	Arctic fox	1950–1960	Yakutia	This study	EF611839
sg10	Arctic fox	1950–1960	Yakutia	This study	EF611838
sg22	Arctic fox	1987	Yakutia	This study	EF611831
sg20	Arctic fox	1987	Yakutia	This study	EF611830
sg23	Arctic fox	1988	Yakutia	This study	EF611833
sg19	Arctic fox	1987	Yakutia	This study	EF611829
sg21	Arctic fox	1987	Yakutia	This study	EF611828
743a	Arctic fox	1988	Yakutia	[16]	AY352488
483a	Arctic fox	1986	Yakutia	[16]	AY352487
3510w	Wolf	1995	Yakutia	[16]	AY352486
RVHK	Human (ex wolf)	1998	North-central Siberia	[16]	AY352462
1090DG	Dog	1993	Canada/Arctic	[13]	U03769
8480FX	Red fox	1993	Canada/Arctic	[13]	U03768
4055DG	Dog	1992	Canada/Arctic	[13]	U03770
A1421	Red fox	1988	Alaska	[16]	AY352500
A4795	Dog	1988	Alaska	[16]	AY352498
A6054	Red fox	2006	Alaska	This study	EF611846
A6013	Red fox	2006	Alaska	This study	EF611848
A0904	Red fox	2006	Alaska	This study	EF611854
A0906	Arctic fox	2006	Alaska	This study	EF611856
A7026	Arctic fox	2006	Alaska	This study	EF611852
A7031	Arctic fox	2006	Alaska	This study	EF611851
A7033	Red fox	2007	Alaska	This study	EF611845
A6091	Red fox	2006	Alaska	This study	EF611849
A7032	Red fox	2007	Alaska	This study	EF611850
A0905	Dog	2006	Alaska	This study	EF611853
A0903	Dog	2006	Alaska	This study	EF611855
A7027	Red fox	2007	Alaska	This study	EF611843
A7007	Red fox	2007	Alaska	This study	EF611841
A7006	Dog	2007	Alaska	This study	EF611844
A6086	Red fox	2006	Alaska	This study	EF611842
A6053	Red fox	2006	Alaska	This study	EF611847
ON.T1	Red fox	1991	Canada/Ontario	[13]	L20673
ON.T2	Red fox	1993	Canada/Ontario	[13]	U11735
ON.T3	Arctic fox	1991	Canada/Ontario	[13]	L20675
ON.T4	Red fox	1990	Canada/Ontario	[13]	L20676
V704IRN	Sheep	2000	Iran	[15]	AY224183
196p	Cow	Unknown	Pakistan	[16]	AY352495
277p	Goat	Unknown	Pakistan	[16]	AY352496
Iraq_dog	Dog	2005	Iraq	This study	EF611869
RV61	Human (ex dog)	1988	UK (ex India)	[16]	AY352493
AY956319	Human (ex dog)	2004	Germany (ex India)	Pfefferle *et al.* 2005 (unpublished obs.)	AY956319
I_141	Unknown	Unknown	India	This study	EF611858
I_145	Unknown	Unknown	India	This study	EF611857
I_123	Unknown	Unknown	India	This study	EF611860
I_129	Unknown	Unknown	India	This study	EF611859
I_116	Unknown	Unknown	India	This study	EF611862
I_114	Unknown	Unknown	India	This study	EF611863

Table 1 (*cont.*)

Isolate name	Animal species	Year	Territory	Reference	GenBank accession no.
I_117	Unknown	Unknown	India	This study	EF611861
I_81	Unknown	Unknown	India	This study	EF611866
I_107	Unknown	Unknown	India	This study	EF611864
I_96	Unknown	Unknown	India	This study	EF611865
I_76	Unknown	Unknown	India	This study	EF611867
304c	Corsac fox	1977	Southern Siberia	[16]	AY352459
248c	Corsac fox	1977	Southern Siberia	[16]	AY352460
994_dog	Dog	1980	Southern Siberia	This study	EF611868
857r	Raccoon dog	1980	Russian Far East	[16]	AY352458
Komatsugawa	Dog	1940s	Japan	[16]	AY352494
KRH2-04	Raccoon dog	2004	Korea	[17]	AY730595
KRH3-04	Dog	2004	Korea	[17]	AY730596
KRC5-04	Dog	2004	Korea	[17]	AY730597
RV1590	Human (ex fox)	Unknown	Western Siberia	[16]	AY352472
8692EGY	Human	1979	Egypt	[11]	U22627
8631MOZ	Dog	1986	Mozambique	[11]	U22484
8698GAB	Dog	1986	Gabon	[11]	U22630
9107MAR	Human	1990	Marocco	[11]	U22852
8681IRA	Dog	1986	Iran	[11]	U22482
8706ARS	Red fox	1987	Saudi Arabia	[11]	U22481
Mz5644	Dog	1998	Israel	[29]	DQ837448
329	Human	1996	Israel	[29]	DQ837383
Mu3996	Red fox	2000	Israel	[29]	DQ837446
Ab2437	Red fox	2004	Israel	[29]	DQ837385
9215HON	Human	1991	Hungary	[20]	U43025
9339EST	Raccoon dog	1991	Estonia	[20]	U42707
9342EST	Raccoon dog	1991	Estonia	[20]	U43432
9142EST	Raccoon dog	1985	Estonia	[11]	U22476
8653YOU	Wolf	1986	Yugoslavia	[20]	U42704
86106YOU	Red fox	1972	Yugoslavia	[11]	U22839
9212ALL	Red fox	1991	Germany	[11]	U22475
9202ALL	Red fox	1991	Germany	[20]	U42701
8618POL	Raccoon dog	1985	Poland	[11]	U22840
9213ALL	Red fox	1991	Germany	[20]	U42702
86111YOU	Red fox	1986	Yugoslavia	[20]	U42706
9223FRA	Red fox	1974	France	[20]	U43433
8903FRA	Red fox	1989	France	[20]	U42606
8661FRA	Hedgehog	1984	France	[20]	U43434
9616FRA	Sheep	1996	France	[20]	AF033905
8663FRA	Red fox	1984	France	[20]	U42605
9244FRA	Red fox	1992	France	[20]	U42607
9147FRA	Red fox	1991	France	[11]	U22474
9445FRA	Red fox	1994	France	[20]	U42700

an extensive dog outbreak in the late 1970s to early 1980s, was phylogenetically more closely related to viruses circulating in the Far East than to viruses circulating in south-eastern Siberia in wild canids.

Substitution rates and time-scaled trees

For all datasets the exponential model of population size was significantly favoured above the constant one (results not shown). Although the mean substitution rate for the European red fox dataset is higher than that of the other two, substitution rates for all three datasets were shown to have widely overlapping confidence intervals (Table 2) and are comparable to those previously estimated for the RABV N gene using the same method [29, 30]. The low value of the coefficient of variation of rates (σ_r) for the Arctic/Arctic-like dataset suggests a more clock-like evolution

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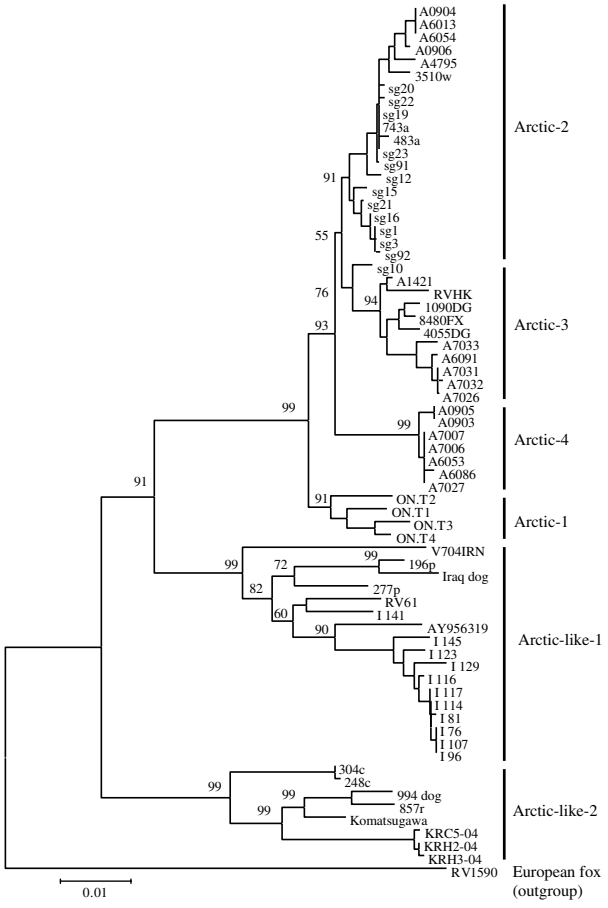

Fig. 1. Neighbour-joining phylogenetic tree of Arctic and Arctic-like rabies viruses (entire N gene) used in the present study (with a European fox virus RV1590 as outgroup). Bootstrap support for 1000 replicates is provided for nodes. Branch lengths are drawn to scale.

than for the other datasets [25]. The use of the larger, lineage-based dataset, selected on the basis of the global time-scaled phylogeny of RABV [29], has enabled an estimation of the divergence times of the branches which led to current diversity of European/Middle-Eastern RABV and Arctic/Arctic-like RABV (Fig. 3). The time of the most recent common ancestor (TMRCA) of all these variants is estimated as 652 years [1353, 95% highest probability distribution (HPD) 1000–1663]. The TMRCA of Arctic lineages is similar when the variants are analysed alone (1524, HPD 1255–1763) or as part of the larger lineage dataset (1610, HPD 1394–1786). Moreover, both analyses suggest that Arctic-like RABV variants diverged slightly earlier than Arctic RABV variants (tree for Arctic/Arctic-like dataset not shown, available from the authors upon request). Of the Arctic variants, the divergence of the Arctic-1 lineage (RABV circulating in Ontario) appears to have occurred first (TMRCA

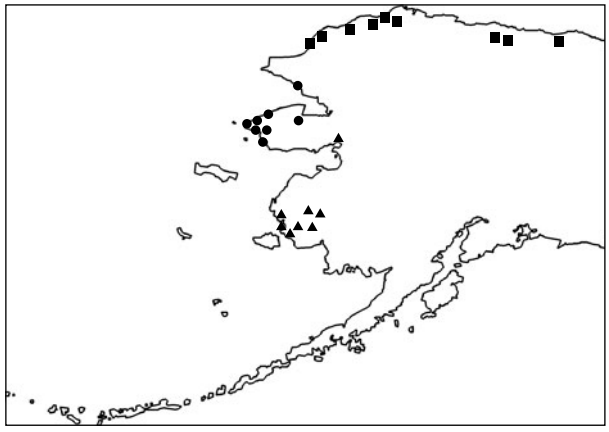

Fig. 2. Isolation points of Arctic-2 (●), Arctic-3 (■) and Arctic-4 (▲) rabies viruses in Alaska during 2005–2007.

of current Arctic-1 diversity: 1921, HPD 1874–1959) and represents a distinct lineage from other Arctic variants.

In agreement with a previous study [29], we show that the emergence of European red fox rabies (currently distributed in moderate latitudes of Eurasia) is a recent event. The lower mean substitution rate for the lineage dataset leads to a larger estimate of the root height of the European red fox variant (1834, HPD 1748–1910) than when the variant is analysed alone (1921, HPD 1839–1974).

DISCUSSION

This phylogenetic study is the first to include the oldest known Arctic RABV isolates. Analysis of these isolates demonstrates that Arctic-2 and Arctic-3 groups were already well established over 50 years ago, and that these viruses show a high degree of similarity to those currently circulating in Arctic regions. The fact that the Arctic-2 viruses appear to be restricted to Siberia and Alaska, whereas lineage Arctic-3 has a circumpolar distribution, may be connected to migratory activity of the main wildlife virus reservoir, the Arctic fox, or intercontinental movements of humans and dogs, as suggested in Figure 2. Indeed, phylogenetically these groups are closely related, and their separation could have occurred about 100 years ago. Given the previous estimation that Asian (dog) RABV variants appear at the root of a time-scaled tree [29], Arctic RABV variants probably evolved via a northerly spread, as recently proposed [19]. Furthermore, Arctic-like viruses appear to have compartmentalized earlier than Arctic viruses.

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Table 2. *Relaxed molecular clock testing*

Dataset	*n*	μ* (95% HPD)	Root height (95% HPD)	σ_r†
Arctic/Arctic-like	32	0·000123 (0·000068–0·000183)	481 (242–750)	0·18
European red fox	17	0·000389 (0·000051–0·000670)	75 (22–157)	0·43
Lineage	60	0·000148 (0·000081–0·000223)	651 (342–1005)	0·34

HPD, Highest probability distribution.
* Substitution rate per site per year.
† Coefficient of rate variation.

Phylogenetic methods which apply a molecular clock must include the uncertainty inherent in estimation of substitution rates, and in turn, reflected in the confidence limits of dating divergence times. The recent development of methods allowing for a relaxed molecular clock to be used has obvious advantages to those which use a strict molecular clock which assumes a single substitution rate across a phylogeny [25]. The TMRCA estimates here (and the dating of the divergence of the Arctic/Arctic-like lineage) are greater than those previously estimated using a strict molecular clock [29], although the topology of both time-scaled trees is congruent. In this analysis, the high posterior probabilities associated with major nodes (1 for all major branching events) allows a high degree of confidence to be placed on the direction and relative timing of divergence events in a tree, even when the confidence limits of a timed event (i.e. a TMRCA) are quite large.

According to the available epidemiological data, Arctic-1 RABV entered Ontario from Arctic regions during the 1950s [31]. Unfortunately, the progenitor viruses for this event are not available for comparison, but observations within the Arctic-2 and Arctic-3 groups does suggest that substitutions which differentiate Arctic-1 RABV from these groups are unlikely to have accumulated within this time-frame; a fact very much supported by our time-scaled phylogeny. Our age estimate suggests that the Arctic-1 group is the oldest within the Arctic RABV lineages. In this context it is interesting to note that the 8486GRO, isolated from Greenland in 1981 [11], belongs to the Arctic-1 group, whereas six Greenland RABV isolated more recently (1990–2002) all belong to the Arctic-3 group (designated Arctic-2b; see ref. [12]). Mansfield *et al.* [12] proposed that this could be a result of the short-term incursion of Arctic-1 viruses from Canada into Greenland, without sustained circulation. Alternatively, these viruses may have co-circulated undetected within that (and potentially other) region for many years. As the density of the human population in northern Canada and Greenland is quite low, many epizootic events are undoubtedly not detected, such that only a limited number of isolates are available from those territories for comparison.

Whether genetic markers exist which provide evidence for RABV adaptation to arctic host species is currently unclear. The RABV genes analyzed to date appear to be under a high degree of purifying selection [28, 30]. Several substitutions have been suggested to be specific for Arctic-1 viruses, such as the T/A^{379} in the nucleoprotein; L/V^{183}, Q/P^{244} and A/S^{483} in the glycoprotein [13]. However, they are not conserved within the large dataset of Arctic viruses from this study.

Similarly, the isolates from Alaska, that form the Arctic-4 group in our study, could represent a lineage of RABV that has circulated in Alaska for a long time (the divergence date according to our estimations goes to the beginning of 20th century) but were not present in previous studies, due to the lack of adequate surveillance. Viruses of this group were isolated during our study only from red foxes and dogs in the south-western Alaska. Further observation is needed to establish their distribution and circulation patterns.

The geographical area of Arctic RABV variants circulation is separated from that of Arctic-like RABV variants by a wide band of conifer taiga forests, lying to the south of the tundra-forest zone in Siberia. These forests are considered largely free of rabies, perhaps because the population density of wild canids appears too low to maintain active virus circulation [16]. Among the Arctic-like viruses, those genetically most closely related to the Arctic viruses currently circulate in the Middle East and southern Asia (Arctic-like-1). However, where the progenitor

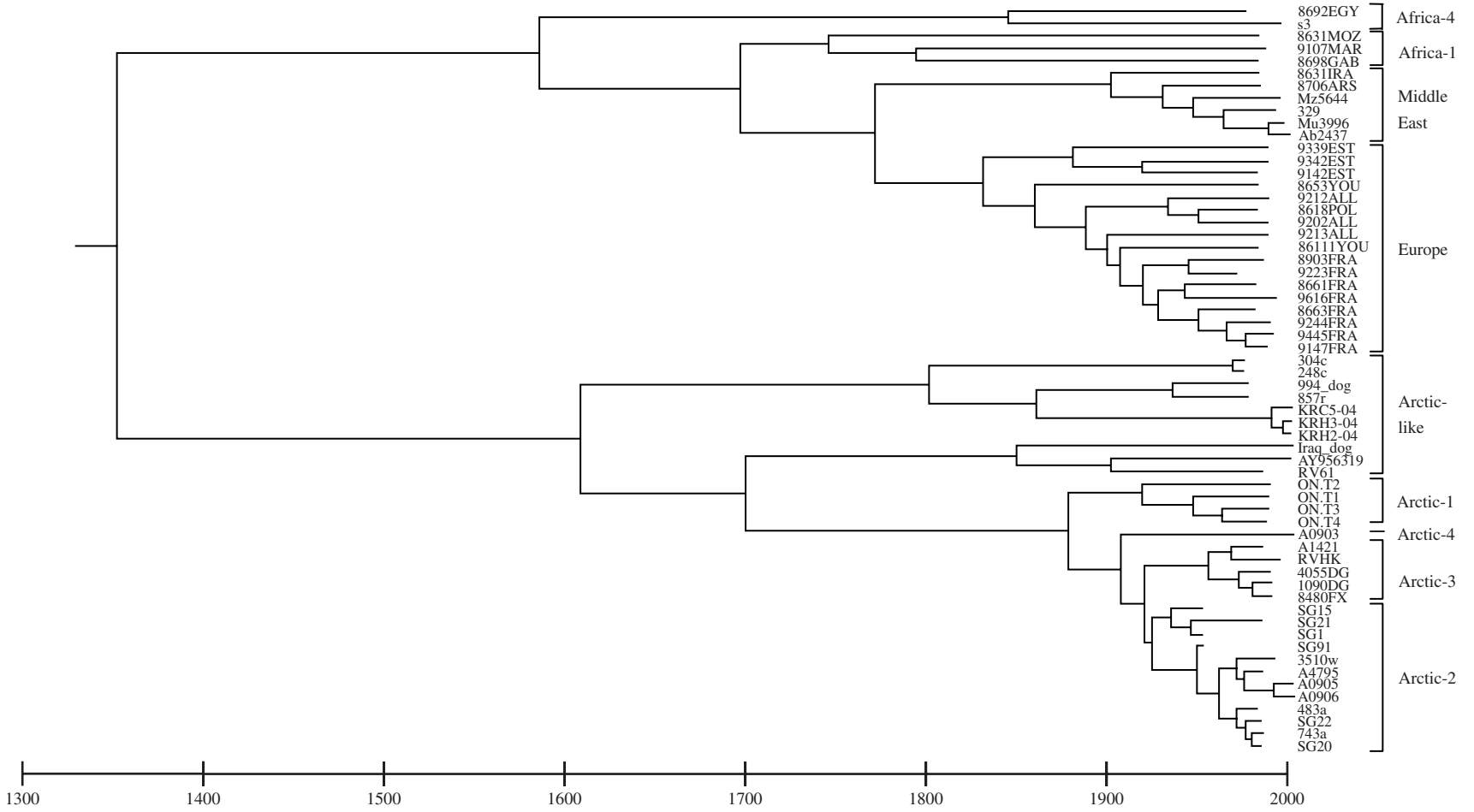

Fig. 3. Time-scaled phylogenetic tree of the rabies virus N gene dataset estimated using a relaxed molecular clock.

virus circulated before the incursion to the Arctic is unknown. The incursion probably happened several hundred years ago, and no isolates are available from that time. Our finding of the related virus in Iraq provides the most westerly isolation point to date. However, it is uncertain whether this represents a recent emergence, a sporadic incursion, or part of sustained circulation. Extensive rabies surveillance in Israel did not show the presence of Arctic-like viruses [29].

Circulation areas of Arctic-like-1 and Arctic-like-2 viruses are separated from each other, as well as being separated phylogenetically and historically. Mountainous areas of the Himalayas and Tibet may serve as natural barriers for viral populations circulating in wild animals. Political and cultural distinctions of human society might prevent translocations of these viruses with companion dogs. Unfortunately, no RABV sequences are available from northern China. We can expect that Arctic-like-2 viruses might be found there, as they circulate in the bordering parts of Russia and Korea. In central and southern China, no Arctic-like viruses have been recovered to date [32].

Numerous human migrations into northern territories have occurred over the last centuries, bringing with them the possible spread of rabies. Arctic (and perhaps the initial pre-Arctic) RABV appear best adapted to the principal Arctic host, the Arctic fox, whereas other variants may not be able to establish long-term circulation, due perhaps to a relative reduction in fitness. The mechanisms of such adaptation, which may occur on the organism or population level, are unclear. For example, we do not know why the 'cosmopolitan' canine RABV, broadly disseminated in moderate latitudes of Eurasia, the Americas and Africa, does not circulate in the Arctic. Further, we do not understand why the 'cosmopolitan' lineage could not establish circulation in southern and eastern Asia (except for several isolates described in China, which were similar to the vaccine RABV strains, suggesting a probability that they could derive from poorly attenuated veterinary vaccines [32]). Similarly, why Arctic viruses do not circulate in areas besides the Arctic is unresolved. Multiple situations may have arisen for Arctic RABV to be translocated with rabid dogs.

Sporadic rabies outbreaks, which could be caused by viruses other than those of the Arctic lineage, have been described in the Far North. For example, an epizootic among dogs in the delta of the Anadyr River (Chukotka Peninsula) occurred between 1953 and 1956, causing four cases of human rabies. Thereafter, no such epizootics or human mortality was reported from Chukotka, whereas rabies among the Arctic foxes, with limited spillover into dogs, was repeatedly reported [33].

Arctic RABV variants may have a lower pathogenicity than other canine RABV variants [34], although no truly robust study has been performed to date. Among 99 trapped Arctic foxes in Alaska, only one had RABV in neuronal tissue, but five had virus-neutralizing antibody in serum, suggesting a previous exposure or abortive infection [35]. In an experimental study, the mortality of Arctic foxes inoculated peripherally with the homologous virus strain was less than 100% [36].

The reduced pathogenicity of Arctic RABV to humans has also been repeatedly proposed, but never proven. For example, residents of the Ustinsky settlement in delta of Lena River lost all of their dogs in August, 1855 as the result of a disease clinically compatible to rabies. However, no cases of human rabies were recorded, even after bites from rabid dogs [2]. The limited evidence for a reduced virulence of Arctic RABV includes the presence of RABV neutralizing antibody in the serum of an Alaskan trapper who, although having never been vaccinated against RABV, had trapped animals for 47 years [37]. In general, very few cases of human rabies have been reported from the north. Most reported cases have occurred after severe, multiple animal bites (reviewed in ref. [12]) but one case was reported as the result of the skinning of a dead Arctic fox [38]. Unfortunately, most of the viruses that have caused human rabies in the Arctic are unavailable for typing. However, one isolate (RVHK; Fig. 1) was proven to be a typical Arctic RABV belonging to the Arctic-3 group [16]. In that case, an adult man who was severely bitten on the head, shin and hand by a wolf developed rabies after an incubation period of 24 days, despite initiation of post-exposure prophylaxis using a commercial rabies vaccine (immunoglobulin was unavailable). Therefore, at least severe exposure to the Arctic virus does result in rabies in humans. Besides reduced pathogenicity, other potential explanations of the infrequent reports of human rabies from the north include sparse human populations and protective outer garments used in the cold climate that protect against bites. Moreover, in many parts of the Far North surveillance is very limited, and human cases may go underreported.

In contrast, there is no evidence to suggest that Arctic-like viruses circulating in southern and eastern Asia show an altered pathogenicity for humans. In India, more than 20 000 human rabies cases occur every year. Arctic-like viruses are broadly distributed in this country [18, 19] and two human isolates of Indian origin are presented in our study, RV61 and AY956319. The last virus was responsible for three human rabies cases following organ transplantation in Germany.

Another interesting example is the extensive dog rabies outbreak that occurred in the Chita Province of eastern Siberia during the late 1970s–early 1980s, that caused multiple human deaths. The responsible RABV variant had a different reactivity pattern to mAbs than those viruses circulating locally among the red fox and corsac fox populations. It was proposed that the new variant may have been introduced to Chita from some other enzootic territory [9]. After the outbreak was eliminated, no other dog or human cases were reported from that area for decades. From phylogenetic analysis, both virus lineages, the locally circulating (304c and 248c) and the newly introduced one (994 dog), belong to the Arctic-like-2 group (Fig. 1). Further, the introduced strain is mostly related to isolates from the Far East (857r and Komatsugawa) rather than to the viruses from eastern Siberia. In contrast to eastern Siberia, human rabies occurs in the Far East quite frequently. In this example, we again encounter a potential indirect suggestion for distinct pathogenicity of these RABV variants.

Unfortunately, specific substitutions which may contribute to altered infection dynamics and consequent epidemiology of Arctic RABV variants are unknown. Even single amino-acid substitutions may be critical for pathogenicity of RABV (such as amino acid at position 333 in the glycoprotein ectodomain [39]). Climate change due to global warming raises further questions on future prospectives regarding Arctic rabies. For example, one possible repercussion is the potential extension of the geographic range of the red fox. The red fox is a well-established reservoir for RABV, and the possible northerly extension of its range may influence the epidemiology of Arctic rabies. Will Arctic RABV variants circulate in this species as readily as they circulate among Arctic foxes? If so, should we expect adaptive changes that will affect either the circulation properties of the virus and its pathogenicity for animals and humans? Additional surveillance, greater number of specimens, and extensive evolutionary analyses are necessary to address these questions.

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DECLARATION OF INTEREST

None.

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