Processes Underlying Rabies Virus Incursions across US–Canada Border as Revealed by Whole-Genome Phylogeography

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Disease control programs aim to constrain and reduce the spread of infection. Human disease interventions such as wildlife vaccination play a major role in determining the limits of a pathogen's spatial distribution. Over the past few decades, a raccoon-specific variant of rabies virus (RRV) has invaded large areas of eastern North America. Although expansion into Canada has been largely prevented through vaccination along the US border, several outbreaks have occurred in Canada. Applying phylogeographic approaches to 289 RRV whole-genome sequences derived from isolates collected in Canada and adjacent US states, we examined the processes underlying these outbreaks. RRV incursions were attributable predominantly to systematic virus leakage of local strains across areas along the border where vaccination has been conducted but also to single stochastic events such as long-distance translocations. These results demonstrate the utility of phylogeographic analysis of pathogen genomes for understanding transboundary outbreaks.

Control measures are often used at the edges of a pathogen's range to limit geographic spread and prevent incursions of infection into areas free from disease. Although geopolitical boundaries generally do not directly affect spread of infectious diseases, human-imposed control measures are often structured around national or international borders. Where such control measures fail, the resulting outbreaks can prove extremely costly in terms of public health and economic and political consequences. It is therefore vital to understand the events involved in such transboundary outbreaks of infection and particularly how these events relate to the control measures applied at the boundary.

Rabies virus is a major zoonotic pathogen worldwide. In the United States, the geographic range of the raccoon variant of rabies virus (RRV) has expanded in recent decades and is now endemic throughout the eastern seaboard area (*1*). Further spread of RRV has been largely contained through oral vaccination of raccoons along the edge of its range (*2*). However, multiple incursions across the vaccination corridor have occurred at the northern edge of the RRV range, corresponding to the US–Canada border; the resulting outbreaks in the Canadian provinces of Ontario, Quebec, and New Brunswick have necessitated large-scale control operations to prevent the establishment of RRV in Canada. By focusing specifically on these outbreaks of RRV in Canada, we are by default highlighting points at which transboundary controls have failed. However, our doing so does not imply that the control measures at the Canada border have been unsuccessful. Despite the epidemic expansion of RRV covering 40–60 km/year in the absence of controls (*3*–*6*), spatial spread of RRV has been static in most areas of the Canada border for >15 years (*7*).

RRV was first reported in the US state of New York in 1990. By 1994, it had spread to reach the Canada border at Niagara in western New York, and by 1996, it had reached the New York–Ontario border at the St. Lawrence River. Implementation of rabies vaccination started along the Niagara River in 1994 and at potential crossing points along the St. Lawrence River from 1995 on, later replaced by larger scale oral vaccination (*8*). The first RRV outbreak in Ontario occurred in the southeastern part of the province during 1999–2005; 126 cases were confined to an area of the mainland adjacent to the St. Lawrence River, and 6 cases occurred on Wolfe Island between Ontario and New York at the mouth of Lake Ontario (*8*,*9*). More recently, in 2015, an outbreak was identified west of the Niagara area and is ongoing (*7*).

At the US–Canada border between Quebec and Vermont, oral rabies vaccination was implemented in the late 1990s in response to the northward spread of RRV through Vermont. The first outbreak of RRV in Quebec occurred during 2006–2009, near the Vermont border (*10*). Another isolated case of raccoon rabies was reported in 2015 at the border with New York in southwestern Quebec (*7*).

In New Brunswick, RRV outbreaks occurred during 2000–2002 and 2014–2016, both in the southwestern part

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of the province near the border with Maine (*7*). RRV vaccination was conducted at the New Brunswick–Maine border from 2001 through 2008 and is currently in place after the 2015 outbreak.

In this study, we used RRV whole-genome sequences to investigate the processes giving rise to these outbreaks in Canada, particularly with respect to the effectiveness of the vaccine area at the US–Canada border, and to determine whether these processes were comparable across different outbreaks. We generated 289 RRV whole-genome sequences: 140 sequences from RRV cases covering each of the Canada outbreaks and 149 sequences from cases in the neighboring US states of New York, Vermont, and Maine. Using Bayesian phylogeographic approaches, we addressed the following 3 questions: 1) Did the Canada outbreaks result from multiple simultaneous incursions of RRV or from single introductions? 2) Is there evidence of backflow of RRV from Canada into the United States? 3) Did the Canada outbreaks originate from RRV lineages circulating locally, or are they attributable to long-distance movement?

Materials and Methods

Samples and Sequencing

In eastern Canada, brain samples from animals suspected to be infected with rabies virus are submitted to the Centre of Expertise for Rabies of the Canadian Food Inspection Agency in Ottawa for diagnosis. In addition, any rabies-positive wildlife cases diagnosed by provincial authorities during enhanced surveillance activities are confirmed by the laboratory in Ottawa. We selected a temporally and spatially representative subset of RRV samples for sequencing from the Canada outbreaks in Ontario (n = 57), Quebec (n = 51), and New Brunswick (n = 32). For comparison, we obtained another set of confirmed rabies virus samples, in most cases collected over the same period as the Canada outbreaks, for sequencing from the relevant state rabies laboratories of New York, Vermont, and Maine. The high-density sampling in New York and Vermont enabled us to focus sampling on cases within 75 km of the Canada border in these 2 states (54 sequenced samples in New York and 62 in Vermont), with the aim of capturing representatives of most RRV lineages circulating near the border. A lower density of samples was available from Maine, collected in 2013 and 2014 only, and therefore we sequenced RRV from throughout this state (33 sequences). Details of sequenced samples are shown in online Technical Appendix Table 1 (https://wwwnc.cdc.gov/EID/article/23/9/17-0325-Techapp1.pdf).

We performed RRV genome extraction and sequencing as described in detail by Nadin-Davis et al. (*11*). Viral RNA was extracted from brain tissue of animals with confirmed infection by using Trizol (Life Technologies Inc., Carlsbad, CA, USA) and further purified by using a MagMax instrument (Applied Biosystems, Foster City, CA, USA). RNA was amplified as 3 overlapping amplicons covering the whole RRV genome. Purified amplicons from a single sample were pooled and used to generate indexed libraries with Nextera XT kits (Illumina, Inc., San Diego, CA, USA); libraries were sequenced as 200- or 250-bp paired end reads on an Illumina MiSeq machine. Genomes were sequenced with high depth of coverage (average >1,000×), and reference-based assembly was conducted by use of the NGen program in the DNASTAR Lasergene software package, version 11 (*12*), with either the RRV reference genome (GenBank accession no. EU311738) or more genetically related sequences generated during this study that better facilitated complete assembly, to generate consensus sequences.

Phylogeographic Analyses

To estimate RRV transitions between geographic regions, we conducted discrete trait phylogeographic analysis (*13*). Sequences were grouped into 8 groups according to location: western Ontario, eastern Ontario, western New York, northwestern New York, New Brunswick, Maine, Quebec, and Vermont (this latter group includes 2 New York sequences, New York.1995.3745 and New York.2011.5590 [online Technical Appendix Table 1], which clustered geographically and genetically with Vermont sequences). Independent incursions into Canada were identified as lineages of the maximum clade credibility phylogeny stemming from a most recent common ancestor with a >90% posterior probability of occurring in Ontario, Quebec, or New Brunswick, according to discrete trait ancestral state reconstruction.

We conducted analyses in BEAST version 1.8.2 (*14*) with the BEAGLE library (*15*) by using the generalized time-reversible model with gamma distributed rate variation among sites and separate partitions for coding and noncoding regions and a relaxed molecular clock (*12*) with branch rates drawn from an exponential distribution (identified by model selection as the best fitting models for these data; online Technical Appendix text and Table 2). Asymmetric transition rates were allowed between regions. Significant transitions were identified by using Bayesian stochastic search variable selection to calculate Bayes factors in the program SPREAD version 1.0.6 (*16*), and the number of transitions between regions was estimated by using Markov Jump counts (*17*).

Identifying Long-Distance Movement

If an outbreak in Canada were the result of local spread of infection, we would expect the responsible viruses to be genetically similar to US RRV lineages circulating near the

Canada border. However, even where dense sampling and sequencing was conducted near the Canada border (i.e., in New York and Vermont), sampling was not exhaustive. Therefore, the absence of a virus closely related to that causing the Canada outbreak in neighboring US regions may indicate an outbreak initiated by long-distance virus movement but could also be the result of incomplete sampling of RRV transmission chains circulating in the local area. We extracted the coalescent time between the most recent common ancestor of a Canada outbreak and the most genetically similar US sequence as a measure of the length of time the outbreak lineage had been circulating unsampled. To generate an expectation for the length of time lineages would circulate undetected under our sampling regimen, we took the distribution of coalescent times for US sequences within each of the 3 major clades. Coalescent times falling outside the 95th percentile interval of US times indicate an outbreak that was probably initiated by long-distance movement. Depending on the (unknown) level of long-distance movement underlying the US samples, this 95th percentile might be overly conservative in identifying long-distance movements.

Results

The sequenced samples fell into 3 well-supported major clades (Figure 1) that are largely structured by region. Clade I consists predominantly of sequences from Quebec and Vermont, although it also includes 5 sequences from northwestern New York and from the 1 case in western Ontario (Ontario-15); clade II is restricted to samples from New Brunswick and Maine; and clade III contains samples from Ontario and New York, with the addition of the 1 isolate from western Quebec in 2015 (Quebec-15). Clades I and III correspond to lineages identified in a previous study of RRV in New York (*18*); clade I corresponds to lineage 3A (found in southeastern New York in the previous study) and clade III to 3B (found in western and northern New York). Because our sampling scheme focuses on RRV infections near the Canada border in western and northern New York, only 5 of the samples from New York sequenced here fall into clade I.

BEAST analysis revealed a molecular clock rate of 3.28×10^{-4} nucleotide substitutions/site across the genome for these sequences (95% highest posterior density [HPD] 2.83×10^{-4} to 3.76×10^{-4}). The time to most recent common ancestor was estimated as 1990 for clade I (95% HPD 1983–1994), 1994 for clade II (95% HPD 1988–1998), and 1987 for clade III (95% HPD 1979–1991). These estimates suggest that the 3 clades were probably already diverged before RRV entered the region, as indicated by Szanto et al. (*18*).

We identified 10 independent incursions of RRV, giving rise to the 6 Canada outbreaks, from the maximum clade credibility tree (Figure 1). We analyzed each of these outbreaks to determine the number of recorded cases and whether evidence exists for multiple virus introductions, backflow to the United States, or long-distance movement (Table).

Ontario

Ontario was subject to a persistent outbreak of RRV from 1999 through 2005, resulting in reported cases on Wolfe Island and the Leeds/Grenville area in mainland Ontario. Discrete traits reconstruction (Figure 1, eastern Ontario outbreak shown in dark blue) suggested that the Wolfe Island viruses (Ontario-99b) are part of a separate incursion to the mainland (Ontario-99a), confirming previous suggestions from partial genome data (*19*). Our results give no indication that the mainland Ontario incursion was caused by multiple invading lineages, and we found no evidence for backflow of infection from Ontario to the United States (online Technical Appendix Figure 1). Sequences from the eastern Ontario outbreak were genetically closely related to sequences circulating locally on the US side of the border with eastern Ontario (Figure 2), suggesting that local spread of infection is responsible for initiating this outbreak. The spatial-genetic spread of this Ontario outbreak is described in more detail by Nadin-Davis et al. (*11*).

By contrast, the outbreak in western Ontario (represented here by 1 sequence, Ontario-15) falls into a completely separate clade (clade I) than other viruses circulating in the neighboring area of New York (clade III; light orange branches in Figure 1). Even in comparison with other sequences in clade I, the Ontario-15 sequence is considerably divergent; coalescent time is >20 years, and it falls distinctly outside the 95th percentile of coalescent times for US sequences in this clade (Figure 2). These findings provide evidence that the variant represented by isolate Ontario-15 was the result of long-distance movement from outside the study area, rather than local spread across the border. Although our results provide some statistical support for the Ontario-15 incursion originating in Vermont (online Technical Appendix Figure 1), a more full exploration of the origins of the Ontario-15 incursion would require a more comprehensive study in which sampling is not restricted to RRV samples within 75 km of the Canada border. An additional isolate from this ongoing outbreak, sequenced subsequent to preparation of this article, differs from the Ontario-15 isolate at 13 of 11,924 sites. This finding represents a genetic similarity of 99.9%, which would place these 2 samples in the same phylogenetic group (data not shown).

Quebec

With the exception of the 1 case in eastern Quebec in 2015 (Quebec-15), all cases of RRV infection from Quebec were

reported from 2006 through 2009 and were again focused on a relatively small area (Figure 3). The combination of the high level of discrimination provided by whole-genome sequencing plus high-density sequencing of samples from close to the Canada border in this study have made it possible to attribute this single temporal outbreak to several separate incursions of RRV into Quebec (lineages Quebec-06, Quebec-07a, Quebec-07b, and Quebec-07c; Figure 1). The last 2 reported cases of RRV in this outbreak, although located near the border, are shown here to be part of an ongoing circulation of lineage Quebec-07b within Quebec, rather than a separate introduction, as might have been assumed in the absence of sequencing.

Incursions of lineages Quebec-07a, Quebec-07b, and Quebec-07c involved viruses closely genetically related to others circulating locally near the Quebec border (Figure 2), indicating that these were probably the result of local spread from RRV circulating near the border. Although the Quebec-06 lineage seems more genetically distinct from other sequences circulating near the border (and also from the other Quebec lineages; Figure 1), it does fall within the 95th percentile interval for coalescent times in this clade (Figure 2, panel A); therefore, there is no evidence to rule out the possibility that local spread from Vermont initiated this incursion. Phylogenetic analysis using discrete traits provides strong statistical support (online Technical Appendix Figure 1) for backflow of RRV infection from the Quebec outbreak back into the United States. These instances of backflow (Vermont-07 and Vermont-08; Figure 1) were each identified within 30 km of the Quebec border, again consistent with local transmission of disease.

The 1 case reported in 2015, Quebec-15, was located at the western edge of the Quebec border with the United States. Contrary to the previous Quebec outbreak, the sequence for this case falls into clade III (Figure 1), indicating that it is linked to the lineage that has spread north through New York, as opposed to the predominantly Vermont-associated clade I implicated in the 2006–2009 outbreak. This finding is not surprising because Quebec-15 was found in an area directly adjacent to New York (Figure

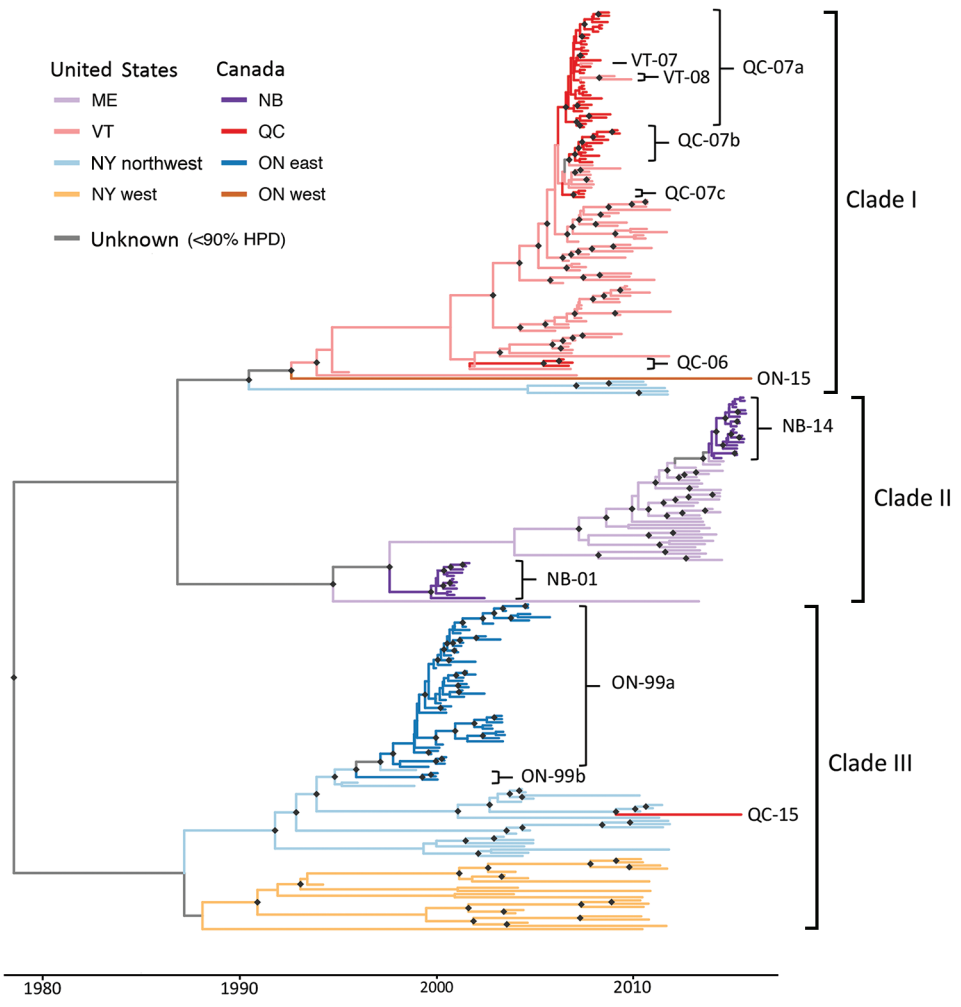

Figure 1. Time-scaled maximum clade credibility phylogeny of sequenced genomes of raccoon-specific variant of rabies virus, US–Canada border. Branches are colored by inferred geographic region. Samples belonging to Canada lineages are labeled by province and year of first sample, as is backflow of infection from Canada into Vermont. Black diamonds indicate nodes with >90% posterior support. HPD, highest posterior density; NB, New Brunswick; ON, Ontario; QC, Quebec.

Table. Summary of Canada outbreaks and evidence of multiple introductions of raccoon-specific variant of rabies virus, backflow from Canada to the United States, and long-distance movement initiating an outbreak

Location	Time	No. recorded cases	Evidence of multiple introductions	Evidence of backflow to the United States	Evidence of long-distance movement
Ontario (east)	1999–2005	132	Yes	No	No
Ontario (west)	2015–ongoing	307*	No	No	Yes
Quebec	2006–2009	104	Yes	Yes (strong)	No
Quebec	2015–2015	1	No	No	No
New Brunswick	2000–2002	64	No	No	No
New Brunswick	2014–ongoing	30*	No	Yes (weak)	No

*Number correct as of April 30, 2017.

3) and was reported soon after cases of RRV reached Franklin County, New York, adjacent to the Quebec border. The Quebec-15 case was located in an area where vaccination had previously not been conducted and has not been followed by any further reports of RRV in Quebec. Although on first examination the Quebec-15 sequence does exhibit some divergence from other sequences in the clade, it is again within the 95th percentile of coalescent times for this clade (Figure 2, panel B), further confirming that this case is probably the result of local spread of viruses from the New York side of the Quebec border.

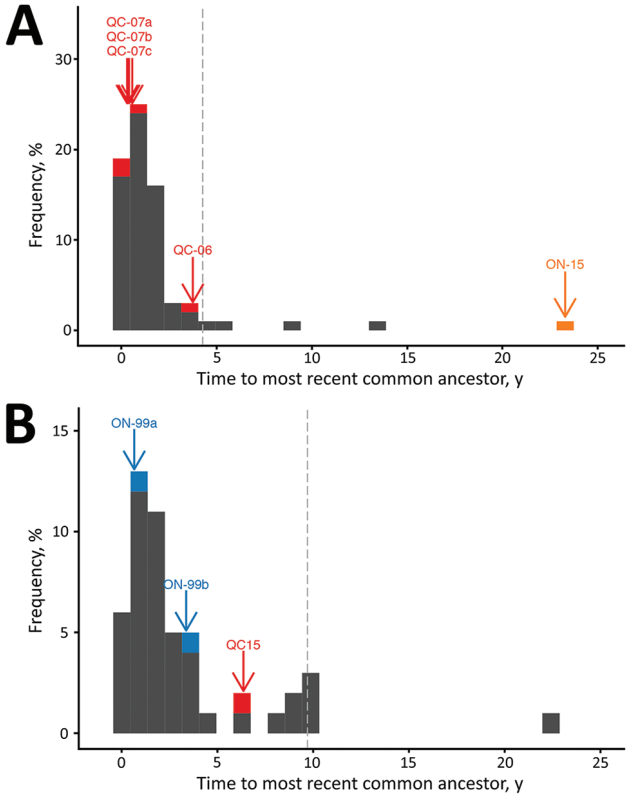

Figure 2. Distribution of coalescent times for raccoon-specific variant of rabies virus near the US–Canada border, clade I (A) and clade III (B). Gray histograms give the distribution of coalescent times for each US sample in the clade, and colored bars and labels indicate the coalescent times for the most recent common ancestor of each Canada lineage in the specified clade. Gray dashed lines indicate the 95th percentiles of the coalescent times for virus from the United States. ON, Ontario; QC, Quebec.

New Brunswick

Our results provide no evidence that either of the outbreaks in New Brunswick (2000–2002 and 2014–2015) was initiated by multiple incursions into New Brunswick; however, discrete trait phylogeographic analysis gives some limited statistical support for backflow of RRV from New Brunswick into Maine (online Technical Appendix Figure 1; note that this backflow is not represented in the maximum clade credibility tree in Figure 1). Sequences from the second New Brunswick outbreak seem closely genetically related to sequences circulating in Maine; however, to examine further the suggestion of backflow from New Brunswick into Maine, and to assess whether the 2000–2002 outbreak is the result of local spread or of long-distance translocation of infection as was suggested at the time (*21*), more extensive sampling and sequencing of RRV from cases near the New Brunswick border would be necessary.

Discussion

The use of high-throughput RRV sequencing enabled us to investigate events giving rise to a series of transboundary RRV outbreaks in eastern provinces of Canada that border the United States. By generating high-resolution whole-genome sequences and comparing results across multiple Canada outbreaks, we were able to discriminate between different epidemiologic scenarios and gain generalizable insights that would not be possible to gain from single-outbreak data.

In most instances, results were consistent with the outbreaks being initiated by 1 lineage, possibly representing 1 infected individual. A major exception was the outbreak in Quebec in 2006–2009, which involved at least 4 cross-border incursions. The Quebec 2006–2009 outbreak also showed strong evidence of backflow of infection from Quebec across the border into the United States. The combination of multiple introductions and backflow of infection indicates that the Quebec–Vermont border and adjacent areas of vaccination were relatively permeable to the spread of RRV during 2006–2008. These results suggest that this particular outbreak was related to systematic challenges in maintaining an effective vaccine corridor at this location and time, as opposed to rare stochastic events. A likely contributing factor for the

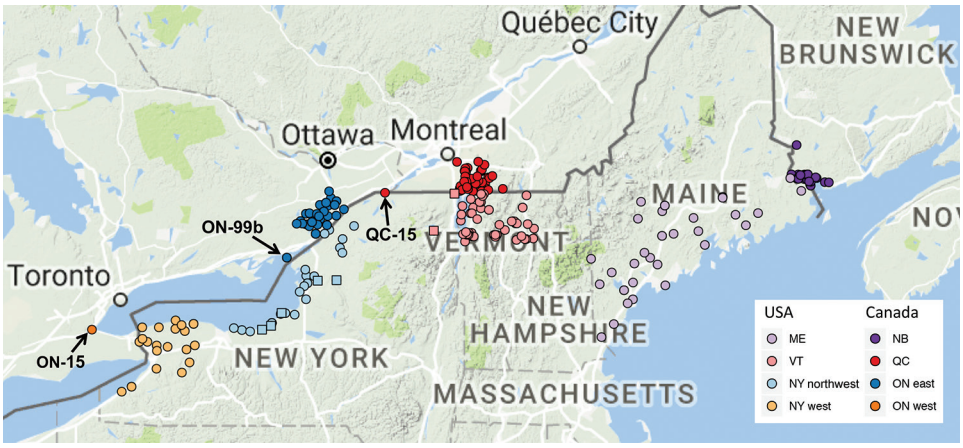

Figure 3. Locations of sequenced samples from Canada outbreaks of raccoon-specific variant of rabies virus infection in western Ontario (n = 1), eastern Ontario (n = 56), Quebec (n = 51), and New Brunswick (n = 32); and from the United States within 75 km of the border in western New York (n = 23), northwestern New York (n = 29, including 5 samples into clade I, indicated by squares), and Vermont (n = 64, including 2 samples from New York that grouped within this clade, indicated by squares); and from throughout Maine (n = 33). Map generated by using ggmap package (*20*). NB, New Brunswick; ON, Ontario; QC, Quebec.

higher transboundary transmission in Quebec is the lack of natural barriers along this border, compared with the major rivers or lakes that reinforce the border between Ontario and the United States. A similar argument can be made for the 1999–2005 Ontario outbreak, given the evidence for 2 separate introductions into Wolfe Island and the mainland, although no indications of backflow or further introductions were found. Weak evidence for backflow of RRV into the United States was also found for New Brunswick, although more in-depth sampling from Maine would be necessary to confirm this; other outbreaks showed no indication of backflow of infection into the United States. Transmission of infection in both directions across the US–Canada border highlights the need for coordination of surveillance programs. For future detection of such backflow events, however, surveillance strategies will probably need detailed genomic data and dense geographic sampling, as described here for New York and Vermont.

Introductions of RRV into Canada were predominantly attributable to viruses closely genetically related to lineages circulating near the US–Canada border. This finding indicates that for many outbreaks, whether multiple introductions or backflow of RRV were evident in the data, the largest risk for introduction stems from local pressure of infection resulting in RRV spreading through the areas of vaccination across the international boundary. This finding is consistent with previous findings of an observed breach of the vaccine corridor within the United States into the state of Ohio, which also implicated local spread of virus lineages through the vaccinated area (*22*). However, we demonstrate that at least 1 introduction into Canada (Ontario-15) was attributable to movement of infection across an exceptionally large distance. The index case was found 64 km from the Ontario–US border, and our results indicate that it is highly unlikely to have originated from US territory within the scale of our sampling (75 km from the border). Most raccoon movements are <5 km (*23*–*27*); however, long-distance dispersal of raccoons covering ≥100 km has been reported and is generally attributed to human-mediated translocation, whether deliberate or inadvertent (*4*,*28*,*29*). The Ontario-15 introduction was adjacent to the Niagara region, an area where vaccination has been conducted on both sides of the border for over a decade and where the border is further strengthened by the large Niagara River. It is also the area on the US–Canada border that was first reached by the northward expansion of RRV in 1994, resulting in the longest potential for transboundary incursions. The absence of any local spread of infection suggests that the local barrier to transboundary incursion of RRV here is particularly strong. However, our results highlight that such areas are still vulnerable to long-distance translocation events, effectively allowing RRV infection to bypass areas of vaccination completely.

Long-distance translocations are likely to be stochastic events and therefore difficult to predict and prevent; however, on the basis of our evidence, these events seem to be relatively rare compared with breaches of the vaccination corridor by locally circulating viruses. Although such breaches could occur anywhere along the US–Canada border, it is apparent that some areas experience multiple incursions, either in short succession (Ontario, Quebec) or separated by several years (New Brunswick), suggesting deterministic factors. Identification of these factors, which are probably related to temporal and spatial variation in raccoon demography or vaccination coverage affecting local pressure of infection, is an area for future work.

Irrespective of the underlying mechanisms, these results demonstrate the utility of whole-genome data and bioinformatics approaches for resolving transmission processes in sensitive areas such as international borders for infectious diseases of high public concern. Increased

efforts are needed to make these tools available to government agencies dealing with transboundary diseases and to facilitate international collaboration toward controlling and ultimately eliminating the spread of infection.

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References

1. Wandeler AI, Rosatte RC, Williams D, Lee TK, Gensheimer KF, Montero JT, et al.; Centers for Disease Control and Prevention. Update: raccoon rabies epizootic—United States and Canada, 1999. MMWR Morb Mortal Wkly Rep. 2000;49:31–5.
2. Sterner RT, Meltzer MI, Shwiff SA, Slate D. Tactics and economics of wildlife oral rabies vaccination, Canada and the United States. Emerg Infect Dis. 2009;15:1176–84. http://dx.doi.org/10.3201/eid1508.081061
3. Moore DA. Spatial diffusion of raccoon rabies in Pennsylvania, USA. Prev Vet Med. 1999;40:19–32. http://dx.doi.org/10.1016/S0167-5877(99)00005-7
4. Wilson ML, Bretsky PM, Cooper GH Jr, Egbertson SH, Van Kruiningen HJ, Cartter ML. Emergence of raccoon rabies in Connecticut, 1991–1994: spatial and temporal characteristics of animal infection and human contact. Am J Trop Med Hyg. 1997;57:457–63. http://dx.doi.org/10.4269/ajtmh.1997.57.457
5. Lucey BT, Russell CA, Smith D, Wilson ML, Long A, Waller LA, et al. Spatiotemporal analysis of epizootic raccoon rabies propagation in Connecticut, 1991–1995. Vector Borne Zoonotic Dis. 2002;2:77–86. http://dx.doi.org/10.1089/153036602321131878
6. Biek R, Henderson JC, Waller LA, Rupprecht CE, Real LA. A high-resolution genetic signature of demographic and spatial expansion in epizootic rabies virus. Proc Natl Acad Sci U S A. 2007;104:7993–8. http://dx.doi.org/10.1073/pnas.0700741104
7. Stevenson B, Goltz J, Massé A. Preparing for and responding to recent incursions of raccoon rabies variant into Canada. Can Commun Dis Rep. 2016;42:125–9 [cited 2017 Jun 22]. http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/16vol42/dr-rm42-6/assets/pdf/16vol42_6-ar-03-eng.pdf
8. Rosatte RC, Donovan D, Allan M, Bruce L, Buchanan T, Sobey K, et al. The control of raccoon rabies in Ontario Canada: proactive and reactive tactics, 1994–2007. J Wildl Dis. 2009;45:772–84. http://dx.doi.org/10.7589/0090-3558-45.3.772
9. Rosatte R, MacDonald E, Sobey K, Donovan D, Bruce L, Allan M, et al. The elimination of raccoon rabies from Wolfe Island, Ontario: animal density and movements. J Wildl Dis. 2007;43:242–50. http://dx.doi.org/10.7589/0090-3558-43.2.242
10. Shwiff S, Aenishaenslin C, Ludwig A, Berthiaume P, Bigras-Poulin M, Kirkpatrick K, et al. Bioeconomic modelling of raccoon rabies spread management impacts in Quebec, Canada. Transbound Emerg Dis. 2013;60:330–7. http://dx.doi.org/10.1111/j.1865-1682.2012.01351.x
11. Nadin-Davis SA, Colville A, Trewby H, Biek R, Real L. Application of high-throughput sequencing to whole rabies viral genome characterisation and its use for phylogenetic re-evaluation of a raccoon strain incursion into the province of Ontario. Virus Res. 2017;232:123–33. http://dx.doi.org/10.1016/j.virusres.2017.02.007
12. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. PLoS Biol. 2006;4:e88. http://dx.doi.org/10.1371/journal.pbio.0040088
13. Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. PLOS Comput Biol. 2009;5:e1000520. http://dx.doi.org/10.1371/journal.pcbi.1000520
14. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 2012; 29:1969–73. http://dx.doi.org/10.1093/molbev/mss075
15. Ayres DL, Darling A, Zwickl DJ, Beerli P, Holder MT, Lewis PO, et al. BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. Syst Biol. 2012;61:170–3. http://dx.doi.org/10.1093/sysbio/syr100
16. Bielejec F, Rambaut A, Suchard MA, Lemey P. SPREAD: spatial phylogenetic reconstruction of evolutionary dynamics. Bioinformatics. 2011;27:2910–2. http://dx.doi.org/10.1093/bioinformatics/btr481
17. Minin VN, Suchard MA. Fast, accurate and simulation-free stochastic mapping. Philos Trans R Soc B 2008;363:3985–95. http://dx.doi.org/: 10.1098/rstb.2008.0176
18. Szanto AG, Nadin-Davis SA, Rosatte RC, White BN. Genetic tracking of the raccoon variant of rabies virus in eastern North America. Epidemics. 2011;3:76–87. http://dx.doi.org/ 10.1016/j.epidem.2011.02.002
19. Nadin-Davis SA, Muldoon F, Wandeler AI. A molecular epidemiological analysis of the incursion of the raccoon strain of rabies virus into Canada. Epidemiol Infect. 2006;134:534–47. http://dx.doi.org/10.1017/S0950268805005108
20. Kahle D, Wickham H. ggmap: spatial visualization with ggplot2. The R Journal. 2013;5:144–61 [cited 2017 June 22]. http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf
21. MacInnes C. Raccoon rabies in New Brunswick. Rabies report. Peterborough (Ontario, Canada); Ontario Ministry of Natural Resources; 2000; vol. 11. p. 10.
22. Henderson JC, Biek R, Hanlon CA, O'Dee S, Real LA. Rabies virus in raccoons, Ohio, 2004. Emerg Infect Dis. 2008;14:650–2. http://dx.doi.org/10.3201/eid1404.070972
23. Cullingham CI, Pond BA, Kyle CJ, Rees EE, Rosatte RC, White BN. Combining direct and indirect genetic methods to estimate dispersal for informing wildlife disease management decisions. Mol Ecol. 2008;17:4874–86. http://dx.doi.org/10.1111/j.1365-294X.2008.03956.x
24. Dharmarajan G, Beasley JC, Fike JA, Rhodes OE. Population genetic structure of raccoons (*Procyon lotor*) inhabiting a highly fragmented landscape. Can J Zool. 2009;87:814–24. http://dx.doi.org/10.1139/Z09-072
25. Rosatte R, Ryckman M, Ing K, Proceviat S, Allan M, Bruce L, et al. Density, movements, and survival of raccoons in Ontario, Canada: implications for disease spread and management. J Mammal. 2010;91:122–35. http://dx.doi.org/10.1644/08-MAMM-A-201R2.1
26. Rosatte R, Sobey K, Donovan D, Bruce L, Allan M, Silver A, et al. Behavior, movements, and demographics of rabid raccoons

in Ontario, Canada: management implications. J Wildl Dis. 2006;42:589–605. http://dx.doi.org/10.7589/0090-3558-42.3.589
27. Rosatte R, Sobey K, Donovan D, Allan M, Bruce L, Buchanan T, et al. Raccoon density and movements after population reduction to control rabies. J Wildl Manage. 2007; 71:2373–8. http://dx.doi.org/10.2193/2006-549
28. Rosatte R, Donovan D, Allan M, Howes LA, Silver A, Bennett K, et al. Emergency response to raccoon rabies introduction into Ontario. J Wildl Dis. 2001;37:265–79. http://dx.doi.org/10.7589/0090-3558-37.2.265
29. Nettles VF, Shaddock JH, Sikes RK, Reyes CR. Rabies in translocated raccoons. Am J Public Health. 1979;69:601–2. http://dx.doi.org/10.2105/AJPH.69.6.601

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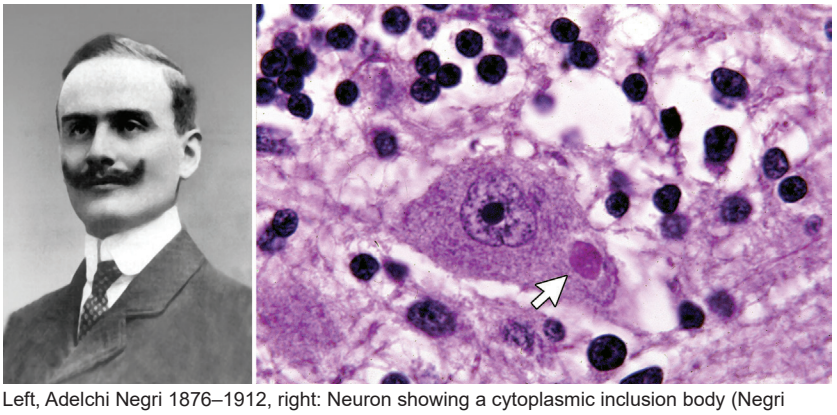
etymologia

Negri [neg′rē] Bodies

Ronnie Henry, Frederick A. Murphy

Negri bodies are cytoplasmic inclusions in neurons that are composed of rabies virus proteins and RNA. Adelchi Negri, an assistant pathologist working in the laboratory of Camillo Golgi, observed these inclusions in rabbits and dogs with rabies. These findings were presented in 1903 at a meeting of the Società Medico-Chirurgica of Pavia. Negri was convinced the inclusions were a parasitic protozoon and the etiologic agent of rabies. Later that same year, however, Paul Remlinger and Rifat-Bey Frasheri in Constantinople and, separately, Alfonso di Vestea in Naples showed that the etiologic agent of rabies is a filterable virus. Negri continued until 1909 to try to prove that the intraneuronal neurons named after him corresponded to steps in the developmental cycle of a protozoan. In spite of his incorrect etiologic hypothesis, Negri's discovery represented a breakthrough in the rapid diagnosis of rabies, and the detection of Negri bodies was used for many years until the development of modern diagnostic methods.



Left, Adelchi Negri 1876–1912, right: Neuron showing a cytoplasmic inclusion body (Negri body, arrow). Courtesy Frederick A. Murphy.

Sources

1. Centers for Disease Control and Prevention. Histologic examination. Apr 22, 2011 [cited 2017 Jun 21]. https://www.cdc.gov/rabies/diagnosis/histologic.html
2. Di Vestea A. On the finding of the filtration of the virus of anger [in Italian]. Annali di Igiene Sperimentale. 1905;15:147.
3. Jackson AC. Rabies and other rhabdovirus infections. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's principles of internal medicine. 18th ed. New York: McGraw Hill; 2012. p. 1611–7.
4. Remlinger PA, Frasheri RB. Rabies virus crosses the Berkefeld filter [in French]. Comptes Rendus de la Société de Biologie. 1903;55:730–1.

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Processes Underlying Rabies Virus Incursions across US–Canada Border as Revealed by Whole-Genome Phylogeography

Technical Appendix

Supporting Information

Phylogenetic model selection

The appropriate nucleotide substitution model for these data was identified using jModelTest v2.1.7 (*1*). The GTR model with gamma-distributed rate variation among sites (GTR+G) was identified as the best fitting nucleotide substitution model by both BIC and DIC methods, and as the second best fitting model by AIC and AICc methods (Δ AIC <2.5). A maximum likelihood (ML) phylogeny was then generated in PhyML v3.0 (*2*) using the GTR+G model of nucleotide substitution. The R^2 correlation between root-to-tip distance and date of sampling in the resulting phylogeny was 18.7%, indicating a measurably evolving signal within these data.

Further model selection was carried out in BEAST v1.8.2 (*3*) to identify the most appropriate gene and codon partitions, and the best fitting molecular clock model for the data. The following gene and codon partitions were tested, with variation in substitution rate, rate heterogeneity, and base frequencies allowed between partitions:

1) Partitions for concatenated non-coding regions and for each for the five genes separately, with:

   a) Three separate partitions for codon positions (CPs) 1, 2 and 3 separately

   b) No codon partitions

2) Separate partitions for concatenated non-coding regions and concatenated gene regions with:

   a) Three separate partitions for codon positions (CPs) 1, 2 and 3 separately

b) No codon partitions

3) No gene or codon partitions.

Each of these sets of gene/codon partitions was tested with a relaxed molecular clock with branch rates drawn from an exponential distribution (UCED clock); and a relaxed molecular clock with branch rates drawn from a log normal distribution (UCLD clock) (*4*). Tip dates were calibrated using the date associated with each sample in the laboratory records.

Each model was run in duplicate using the GTR+G nucleotide substitution model and the ML phylogeny (above) as starting tree, and the Bayesian skyline model as a flexible demographic prior (*5*). The clock rate prior was set to a normal distribution with mean of $1.44x10^{-4}$ nucleotide substitutions per site (based on results from [*6*]), truncated to 0 and 0.15 nucleotide substitutions per site, and with a wide standard deviation of 0.0144 to allow for variation from the prior mean.

BEAST analyses were run until MCMC chains had converged, as determined by visual checking in Tracer v1.6.0 and by effective sample size values greater than 200. Model selection was carried out using marginal likelihood estimates as generated by Path Sampling and Stepping Stone Sampling (*7*,*8*).

The model selection analysis for the UCLD clock with five gene partitions and three separate partitions for each codon position (1b) failed to converge. Results for the other models tested (Table S2) indicate that the UCED molecular clock, with separate partitions for coding and non-coding regions, but no partitioning by codon position (2b) was best fitting model for these data.

References

1. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. [Nature Publishing Group.]. Nat Methods. 2012;9:772. PubMedhttp://dx.doi.org/10.1038/nmeth.2109

2. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 2003;52:696–704. PubMedhttp://dx.doi.org/10.1080/10635150390235520

3. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012;29:1969–73. PubMedhttp://dx.doi.org/10.1093/molbev/mss075

4. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. PLoS Biol. 2006;4:e88. PubMedhttp://dx.doi.org/10.1371/journal.pbio.0040088

5. Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol Biol Evol. 2005;22:1185–92. PubMedhttp://dx.doi.org/10.1093/molbev/msi103

6. Brunker K, Hampson K, Horton DL, Biek R. Integrating the landscape epidemiology and genetics of RNA viruses: rabies in domestic dogs as a model. Parasitology. 2012;139:1899–913. PubMedhttp://dx.doi.org/10.1017/S003118201200090X

7. Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA, Alekseyenko AV. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol Biol Evol. 2012;29:2157–67. PubMedhttp://dx.doi.org/10.1093/molbev/mss084

8. Baele G, Li WLS, Drummond AJ, Suchard MA, Lemey P. Accurate model selection of relaxed molecular clocks in bayesian phylogenetics. Mol Biol Evol. 2013;30:239–43. PubMedhttp://dx.doi.org/10.1093/molbev/mss243

Technical Appendix Table 1. Detailed information for sequenced samples

Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
ME.2013.0002		2013-01-03	Skunk	USA	Maine	Kennebec	China	ME
ME.2013.0025		2013-02-04	Raccoon	USA	Maine	York	Wells	ME
ME.2013.0034		2013-02-13	Raccoon	USA	Maine	Penobscot	Dixmont	ME
ME.2013.0052		2013-02-28	Skunk	USA	Maine	Penobscot	Milford	ME
ME.2013.0064		2013-03-21	Raccoon	USA	Maine	Kennebec	Litchfield	ME
ME.2013.0067		2013-03-22	Raccoon	USA	Maine	Somerset	Skowhegan	ME
ME.2013.0098		2013-04-12	Skunk	USA	Maine	York	Eliot	ME
ME.2013.0101		2013-04-17	Skunk	USA	Maine	Cumberland	Windham	ME
ME.2013.0102		2013-04-17	Raccoon	USA	Maine	Hancock	Mariaville	ME
ME.2013.0109		2013-04-25	Raccoon	USA	Maine	Cumberland	Windham	ME
ME.2013.0110		2013-04-26	Raccoon	USA	Maine	Androscoggin	Lewistion	ME
ME.2013.0113		2013-04-30	Skunk	USA	Maine	Franklin	Farmington	ME
ME.2013.0131	KY026414	2013-05-21	Raccoon	USA	Maine	Waldo	Northport	ME
ME.2013.0157		2013-06-06	Red Fox	USA	Maine	Oxford	Hiram	ME
ME.2013.0158		2013-06-10	Skunk	USA	Maine	Waldo	Waldo	ME
ME.2013.0187		2013-06-25	Raccoon	USA	Maine	Lincoln	Southport Island	ME
ME.2013.0233		2013-07-19	Raccoon	USA	Maine	Oxford	Stow	ME
ME.2013.0247		2013-07-31	Gray Fox	USA	Maine	Cumberland	South Portland	ME
ME.2013.0332		2013-08-19	Raccoon	USA	Maine	Kennebec	Monmouth	ME
ME.2013.0425		2013-10-11	Skunk	USA	Maine	Hancock	Franklin	ME
ME.2013.0444		2013-10-28	Skunk	USA	Maine	Franklin	New Portland	ME
ME.2013.0491		2013-12-20	Skunk	USA	Maine	Kennebec	Sidney	ME
ME.2013.0498		2013-12-31	Skunk	USA	Maine	Cumberland	Scarborough	ME
ME.2014.0038		2014-02-27	Raccoon	USA	Maine	Cumberland	North Yarmouth	ME
ME.2014.0079		2014-04-22	Skunk	USA	Maine	Penobscot	Old Town	ME

Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
ME.2014.0084		2014-05-02	Raccoon	USA	Maine	Somerset	Cornville	ME
ME.2014.0090		2014-05-07	Skunk	USA	Maine	Somerset	Cornville	ME
ME.2014.0091		2014-05-08	Raccoon	USA	Maine	Washington	Charlotte	ME
ME.2014.0095		2014-05-13	Raccoon	USA	Maine	Kennebec	Sidney	ME
ME.2014.0103		2014-05-22	Raccoon	USA	Maine	Sagadahoc	Richmond	ME
ME.2014.0137		2014-06-13	Gray Fox	USA	Maine	Cumberland	Naples	ME
ME.2014.0169		2014-06-26	Raccoon	USA	Maine	Washington	Deblois	ME
ME.2014.0197		2014-07-11	Raccoon	USA	Maine	Washington	Baileyville	ME
NB.2000.4394		2000-09-12	Skunk	Canada	New Brunswick	Charlotte	Heathland	NB
NB.2000.5443		2000-10-31	Skunk	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2000.5579		2000-11-07	Raccoon	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2000.5733		2000-11-16	Skunk	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2000.5735		2000-11-16	Raccoon	Canada	New Brunswick	Charlotte	St. George	NB
NB.2001.0005		2001-01-02	Raccoon	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2001.0006		2001-01-02	Skunk	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2001.1588		2001-04-11	Raccoon	Canada	New Brunswick	Charlotte	Little Ridge	NB
NB.2001.3484		2001-04-26	Raccoon	Canada	New Brunswick	Charlotte	Dufferin	NB
NB.2001.6249		2001-06-21	Raccoon	Canada	New Brunswick	Charlotte	Cockerhill	NB
NB.2001.9134		2001-08-22	Raccoon	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2002.3420		2002-05-30	Raccoon	Canada	New Brunswick	Charlotte	Valley Rd.	NB
NB.2014.0486		2014-06-02	Raccoon	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2015.0046		2015-01-22	Raccoon	Canada	New Brunswick	Charlotte	Oak Bay	NB
NB.2015.0149		2015-03-03	Raccoon	Canada	New Brunswick	Charlotte	Waweig	NB
NB.2015.0155		2015-03-04	Raccoon	Canada	New Brunswick	Charlotte	Bayside	NB
NB.2015.0156		2015-03-04	Raccoon	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2015.0183		2015-03-10	Raccoon	Canada	New Brunswick	Charlotte	Bocabec	NB
NB.2015.0185		2015-03-10	Raccoon	Canada	New Brunswick	Charlotte	Waweig	NB
NB.2015.0201		2015-03-18	Raccoon	Canada	New Brunswick	Charlotte	Burnt Hill	NB
NB.2015.0202		2015-03-18	Raccoon	Canada	New Brunswick	Charlotte	St. Stephen	NB
NB.2015.0207		2015-03-19	Raccoon	Canada	New Brunswick	Charlotte	Cooks Lane	NB
NB.2015.0270		2015-04-14	Raccoon	Canada	New Brunswick	Charlotte	Waweig	NB
NB.2015.0343		2015-05-05	Raccoon	Canada	New Brunswick	Charlotte	Waweig	NB
NB.2015.0359		2015-05-07	Raccoon	Canada	New Brunswick	Charlotte	Valley Rd.	NB
NB.2015.0583		2015-06-23	Raccoon	Canada	New Brunswick	Charlotte	St. Andrews	NB
NB.2015.0671		2015-07-14	Raccoon	Canada	New Brunswick	Charlotte	St. Andrews	NB
NB.2015.0672		2015-07-14	Raccoon	Canada	New Brunswick	Charlotte	Chamcook	NB
NB.2015.0774		2015-07-29	Skunk	Canada	New Brunswick	Charlotte	Bocabec	NB
NB.2015.0835		2015-08-06	Raccoon	Canada	New Brunswick	Charlotte	Bayside	NB
NB.2015.0921		2015-08-18	Raccoon	Canada	New Brunswick	Charlotte	Pennfield	NB
NB.2015.1049		2015-09-01	Raccoon	Canada	New Brunswick	York	McAdam	NB
NY.1994.1330		1994-03-24	Raccoon	USA	New York	Orleans	Kendall	NY west

Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
NY.1995.3745		1995-07-12	Raccoon	USA	New York	Clinton	Black Brook	VT
NY.1995.7951	KY026416	1995-12-22	Raccoon	USA	New York	Jefferson	Hounsfield	NY north-west
NY.1998.8982	KY026417	1998-11-09	Raccoon	USA	New York	St Lawrence	Canton	NY north-west
NY.1998.9581	KY026418	1998-12-08	Skunk	USA	New York	St Lawrence	Morristown	NY north-west
NY.2003.1694		2003-05-21	Raccoon	USA	New York	Cayuga	Sterling	NY north-west
NY.2003.1995		2003-06-06	Raccoon	USA	New York	Orleans	Kendall	NY west
NY.2003.7809		2003-11-24	Skunk	USA	New York	Orleans	Ridgeway	NY west
NY.2003.8109		2003-12-22	Raccoon	USA	New York	Niagara	Wheatfield	NY west
NY.2003.8176		2003-12-25	Raccoon	USA	New York	Orleans	Albion	NY west
NY.2004.0023		2004-01-05	Skunk	USA	New York	Oswego	Scriba	NY north-west
NY.2004.0353		2004-02-06	Raccoon	USA	New York	Wyoming	Middlebury	NY west
NY.2004.0953	KY026419	2004-04-06	Skunk	USA	New York	Jefferson	Brownville	NY north-west
NY.2004.1133		2004-04-21	Raccoon	USA	New York	Jefferson	Adams	NY north-west
NY.2004.1285		2004-05-04	Raccoon	USA	New York	Wayne	Williamson	NY north-west
NY.2004.1600		2004-05-20	Raccoon	USA	New York	Niagara	Niagara Falls	NY west
NY.2004.1974		2004-06-03	Raccoon	USA	New York	Cayuga	Sterling	NY north-west
NY.2004.2753		2004-06-30	Raccoon	USA	New York	Jefferson	Adams	NY north-west
NY.2004.4289		2004-08-04	Raccoon	USA	New York	Erie	Newstead	NY west
NY.2004.4875		2004-08-13	Raccoon	USA	New York	Orleans	Kendall	NY west
NY.2004.5301		2004-08-18	Raccoon	USA	New York	Wayne	Sodus	NY north-west
NY.2004.6938		2004-09-17	Skunk	USA	New York	Oswego	Oswego City	NY north-west
NY.2004.7916		2004-11-16	Skunk	USA	New York	Cayuga	Sterling	NY north-west
NY.2004.7972		2004-11-18	Skunk	USA	New York	Cayuga	Sterling	NY north-west
NY.2004.8025		2004-11-23	Raccoon	USA	New York	Jefferson	Watertown	NY north-west
NY.2010.1066		2010-04-08	Skunk	USA	New York	St Lawrence	Hammond	NY north-west
NY.2010.1178		2010-04-20	Raccoon	USA	New York	St Lawrence	Gouveneur	NY north-west
NY.2010.1233		2010-12-10	Raccoon	USA	New York	St Lawrence	Gouveneur	NY north-west
NY.2010.1234		2010-04-23	Skunk	USA	New York	Niagara	Cambria	NY west
NY.2010.1349		2010-05-04	Raccoon	USA	New York	Orleans	Yates	NY west
NY.2010.1444		2010-05-11	Raccoon	USA	New York	Chautauqua	Sherman	NY west
NY.2010.1962		2010-06-02	Raccoon	USA	New York	Chemung	Horseheads	NY west
NY.2010.1986		2010-06-02	Raccoon	USA	New York	Erie	Orchard Parl	NY west
NY.2010.2167		2010-06-09	Raccoon	USA	New York	Genesee	Byron	NY west
NY.2010.2369		2010-06-18	Skunk	USA	New York	Cayuga	Sterling	NY north-west
NY.2010.2456		2014-06-23	Raccoon	USA	New York	Niagara	Niagra	NY west
NY.2010.3956		2010-08-05	Skunk	USA	New York	Oswego	Oswego City	NY north-west
NY.2010.5558		2010-09-21	Raccoon	USA	New York	Niagara	Porter	NY west
NY.2010.5667		2010-09-29	Skunk	USA	New York	Chautauqua	Hanover	NY west
NY.2010.5742		2010-10-06	Raccoon	USA	New York	Orleans	Carlton	NY west
NY.2010.5965		2010-10-26	Skunk	USA	New York	Wyoming	Wethersfield	NY west
NY.2011.0862		2011-04-05	Raccoon	USA	New York	Jefferson	Adams	NY north-west
NY.2011.1089		2011-04-26	Raccoon	USA	New York	Erie	Amherst	NY west
NY.2011.1548	KY026421	2011-05-31	Raccoon	USA	New York	St Lawrence	De Kalb	NY north-west
NY.2011.2028		2011-06-16	Raccoon	USA	New York	Jefferson	Ellisburg	NY north-west
NY.2011.2880		2011-07-22	Cat	USA	New York	Wayne	Wolcott	NY north-west
NY.2011.4498		2011-08-19	Raccoon	USA	New York	Genesee	Batavia City	NY west
NY.2011.5028		2011-09-02	Raccoon	USA	New York	Erie	Amherst	NY west
NY.2011.5139		2011-09-08	Skunk	USA	New York	Jefferson	Water	NY north-west
NY.2011.5196		2011-09-13	Skunk	USA	New York	Lewis	Croghan	NY north-west
NY.2011.5335		2011-09-21	Skunk	USA	New York	Oswego	Mexico	NY north-west
NY.2011.5545		2011-10-06	Cat	USA	New York	Wayne	Ontario	NY north-west
NY.2011.5590		2011-10-07	Skunk	USA	New York	Clinton	Champlain	VT
NY.2011.5763		2011-10-21	Gray Fox	USA	New York	Oswego	Sandy Creek	NY north-west
ON.1999.3545	EU311738	1999-07-26	Raccoon	Canada	Ontario	Leeds	North Augusta	ON east
ON.1999.5025	KY026423	1999-09-17	Raccoon	Canada	Ontario	Grenville	Oxford Station	ON east
ON.1999.6417	KY026424	1999-12-10	Raccoon	Canada	Ontario	Frontenac	Wolfe Island	ON east
ON.1999.6479	KY026425	1999-12-16	Raccoon	Canada	Ontario	Leeds	North Augusta	ON east
ON.2000.0112	KY026426	2000-01-12	Raccoon	Canada	Ontario	Frontenac	Wolfe Island	ON east
ON.2000.0155	KY026427	2000-01-13	Raccoon	Canada	Ontario	Frontenac	Wolfe Island	ON east
ON.2000.0222	KY026428	2000-01-19	Raccoon	Canada	Ontario	Grenville	North Augusta	ON east
ON.2000.0484	KY026429	2000-02-14	Raccoon	Canada	Ontario	Grenville	Garretton	ON east
ON.2000.0771	KY026430	2000-03-07	Raccoon	Canada	Ontario	Leeds	North Augusta	ON east
ON.2000.1357	KY026432	2000-04-18	Raccoon	Canada	Ontario	Grenville	Bishops Mills	ON east
ON.2000.1730	KY026433	2000-05-17	Raccoon	Canada	Ontario	Grenville	Merrickville	ON east
ON.2000.1818	KY026434	2000-05-23	Raccoon	Canada	Ontario	Grenville	North Augusta	ON east
ON.2000.2067	KY026435	2000-06-05	Raccoon	Canada	Ontario	Leeds	New Dublin	ON east
ON.2000.2201	KY026436	2000-06-12	Raccoon	Canada	Ontario	Leeds	Addison	ON east

Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
ON.2000.2342	KY026437	2000-06-19	Raccoon	Canada	Ontario	Grenville	Bishops Mills	ON east
ON.2000.2398	KY026438	2000-06-20	Raccoon	Canada	Ontario	Grenville	Algonquin	ON east
ON.2000.4360	KY026439	2000-09-08	Raccoon	Canada	Ontario	Leeds	North Augusta	ON east
ON.2000.4461	KY026440	2000-09-15	Raccoon	Canada	Ontario	Leeds	Addison	ON east
ON.2000.4474	KY026441	2000-09-18	Raccoon	Canada	Ontario	Leeds	RR#5 Brockville	ON east
ON.2000.5320	KY026442	2000-10-25	Raccoon	Canada	Ontario	Leeds	Frankville	ON east
ON.2000.6152	KY026443	2000-12-14	Raccoon	Canada	Ontario	Leeds	Athens	ON east
ON.2001.0312	KY026444	2001-01-26	Raccoon	Canada	Ontario	Leeds	Black Church Rd.	ON east
ON.2001.0889	KY026445	2001-03-12	Raccoon	Canada	Ontario	Leeds	Graham Lake Rd.	ON east
ON.2001.1190	KY026446	2001-03-26	Raccoon	Canada	Ontario	Leeds	Upper Oak Leaf Rd.	ON east
ON.2001.12052	KY026447	2001-12-13	Raccoon	Canada	Ontario	Leeds	Bastard Twp	ON east
ON.2001.12053	KY026448	2001-12-13	Raccoon	Canada	Ontario	Leeds	Toledo	ON east
ON.2001.1891	KY026449	2001-04-18	Raccoon	Canada	Ontario	Leeds	Athens	ON east
ON.2001.1895	KY026450	2001-04-18	Skunk	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2001.3744	KY026451	2001-04-30	Raccoon	Canada	Ontario	Leeds	Athens	ON east
ON.2001.3913	KY026452	2001-05-04	Raccoon	Canada	Ontario	Leeds	Charleston Village	ON east
ON.2001.4148	KY026453	2001-05-16	Raccoon	Canada	Ontario	Leeds	Rocksprings	ON east
ON.2001.6195	KY026454	2001-06-19	Raccoon	Canada	Ontario	Leeds	Athens	ON east
ON.2001.7006	KY026455	2001-07-03	Raccoon	Canada	Ontario	Leeds	Philipsville	ON east
ON.2001.7160	KY026456	2001-07-06	Raccoon	Canada	Ontario	Leeds	Athens	ON east
ON.2001.8399	KY026457	2001-08-08	Raccoon	Canada	Ontario	Leeds	Delta	ON east
ON.2001.9303	KY026458	2001-08-24	Raccoon	Canada	Ontario	Leeds	Brockville	ON east
ON.2002.3398	KY026459	2002-05-29	Raccoon	Canada	Ontario	Leeds	Kitley Twp	ON east
ON.2002.3853	KY026460	2002-06-21	Raccoon	Canada	Ontario	Leeds	Elgin	ON east
ON.2002.7095	KY026461	2002-10-02	Raccoon	Canada	Ontario	Grenville	Ventnor Rd.	ON east
ON.2002.7849	KY026462	2002-10-24	Raccoon	Canada	Ontario	Grenville	Ventnor Rd.	ON east
ON.2002.8206	KY026463	2002-11-05	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2003.0941	KY026464	2003-02-21	Raccoon	Canada	Ontario	Grenville	Spencerville	ON east
ON.2003.1519	KY026465	2003-03-19	Raccoon	Canada	Ontario	Grenville	Prescott	ON east
ON.2003.2673	KY026466	2003-04-14	Raccoon	Canada	Ontario	Grenville	Spencerville	ON east
ON.2003.2760	KY026467	2003-04-17	Raccoon	Canada	Ontario	Leeds	Athens	ON east
ON.2003.2794	KY026468	2003-04-22	Raccoon	Canada	Ontario	Leeds	Spencerville	ON east
ON.2003.3406	KY026469	2003-05-05	Raccoon	Canada	Ontario	Leeds	Cardinal	ON east
ON.2003.3574	KY026470	2003-05-12	Raccoon	Canada	Ontario	Leeds	Kemptville	ON east
ON.2003.4680	KY026471	2003-06-10	Raccoon	Canada	Ontario	Grenville	Prescott	ON east
ON.2003.5257	KY026472	2003-06-30	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2004.6275	KY026473	2004-08-13	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2004.6277	KY026474	2004-08-13	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2004.6953	KY026475	2004-08-31	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2004.7702	KY026476	2004-09-22	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2005.4941	KY026477	2005-09-22	Raccoon	Canada	Ontario	Leeds	Mallorytown	ON east
ON.2015.1361		2015-12-04	Raccoon	Canada	Ontario	Hamilton	Hamilton	ON west
QC.2006.2049		2006-06-02	Raccoon	Canada	Quebec	Brome-Missisquoi	Dunham	QC
QC.2006.4930		2006-09-06	Raccoon	Canada	Quebec	Brome-Missisquoi	Cowansville	QC
QC.2006.5982		2006-11-15	Raccoon	Canada	Quebec	Brome-Missisquoi	Dunham	QC
QC.2007.0909		2007-08-01	Raccoon	Canada	Quebec	Brome-Missisquoi	Mont-Saint-Gregoire	QC
QC.2007.0910		2007-08-02	Raccoon	Canada	Quebec	Brome-Missisquoi	Mont-Saint-Gregoire	QC
QC.2007.1065		2007-08-29	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Jean-sur-Richelieu	QC
QC.2007.1122		2007-08-30	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Jean-sur-Richelieu	QC
QC.2007.2234		2007-05-11	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Armand	QC
QC.2007.2783		2007-06-11	Raccoon	Canada	Quebec	Brome-Missisquoi	Frelighsburg	QC
QC.2007.2892		2007-06-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Armand	QC
QC.2007.2893		2007-06-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Frelighsburg	QC
QC.2007.2937		2007-06-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Armand	QC

Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
QC.2007.2939		2007-06-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Pierre-de-Veronne-a-Pike-River	QC
QC.2007.2941		2007-06-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Stanbridge East	QC
QC.2007.2942		2007-06-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Armand	QC
QC.2007.2975		2007-06-14	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Armand	QC
QC.2007.2986		2007-06-16	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Pierre-de-Veronne-a-Pike-River	QC
QC.2007.3122		2007-06-21	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Pierre-de-Veronne-a-Pike-River	QC
QC.2007.3211		2007-06-29	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Sebastien	QC
QC.2007.3246		2007-07-03	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Sebastien	QC
QC.2007.3657		2007-07-05	Raccoon	Canada	Quebec	Brome-Missisquoi	Frelighsburg	QC
QC.2007.3658		2007-07-14	Raccoon	Canada	Quebec	Brome-Missisquoi	Noyan	QC
QC.2007.3659		2007-07-15	Raccoon	Canada	Quebec	Brome-Missisquoi	Noyan	QC
QC.2007.3870		2007-07-19	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Pierre-de-Veronne-a-Pike-River	QC
QC.2007.4485		2007-08-03	Raccoon	Canada	Quebec	Brome-Missisquoi	Saint-Armand	QC
QC.2007.5318		2007-08-23	Raccoon	Canada	Quebec	Brome-Missisquoi	Bedford	QC
QC.2007.5827		2007-09-21	Raccoon	Canada	Quebec	Le Haut-Richelieu	Sainte-Anne-de-Sabrevois	QC
QC.2007.6184		2007-10-11	Raccoon	Canada	Quebec	Brome-Missisquoi	Stanbridge Station	QC
QC.2007.6234		2007-10-12	Skunk	Canada	Quebec	Brome-Missisquoi	Cowansville	QC
QC.2007.6292		2007-11-01	Raccoon	Canada	Quebec	Le Haut-Richelieu	Saint-Jean-sur-Richelieu	QC
QC.2007.6389		2007-11-01	Skunk	Canada	Quebec	Le Haut-Richelieu	Saint-Alexandre	QC
QC.2007.6390		2007-10-20	Raccoon	Canada	Quebec	Le Haut-Richelieu	Noyan	QC
QC.2007.6442		2007-11-15	Red Fox	Canada	Quebec	Le Haut-Richelieu	Saint-Jean-sur-Richelieu	QC
QC.2007.6659		2007-11-19	Raccoon	Canada	Quebec	Le Haut-Richelieu	Noyan	QC
QC.2008.0750		2008-03-04	Raccoon	Canada	Quebec	Brome-Missisquoi	Farnham	QC
QC.2008.1096		2008-03-17	Raccoon	Canada	Quebec	Brome-Missisquoi	Farnham	QC
QC.2008.1237		2008-03-21	Raccoon	Canada	Quebec	Le Haut-Richelieu	Noyan	QC
QC.2008.1411		2008-04-15	Raccoon	Canada	Quebec	Brome-Missisquoi	Sutton	QC
QC.2008.1846		2008-05-07	Raccoon	Canada	Quebec	Le Haut-Richelieu	Noyan	QC
QC.2008.1848		2008-05-08	Raccoon	Canada	Quebec	Le Haut-Richelieu	Farnham	QC
QC.2008.1894		2008-05-13	Raccoon	Canada	Quebec	Brome-Missisquoi	Farnham	QC
QC.2008.1970		2008-05-13	Raccoon	Canada	Quebec	Le Haut-Richelieu	Noyan	QC
QC.2008.2444		2008-06-16	Raccoon	Canada	Quebec	Le Haut-Richelieu	Saint-Georges-de-Clarenceville	QC
QC.2008.4180		2008-07-31	Skunk	Canada	Quebec	Le Haut-Richelieu	Sainte-Anne-de-Sabrevois	QC
QC.2008.4262		2008-08-25	Raccoon	Canada	Quebec	Brome-Missisquoi	Sainte-Sabine	QC
QC.2008.4808		2008-09-03	Raccoon	Canada	Quebec	Le Haut-Richelieu	Henryville	QC

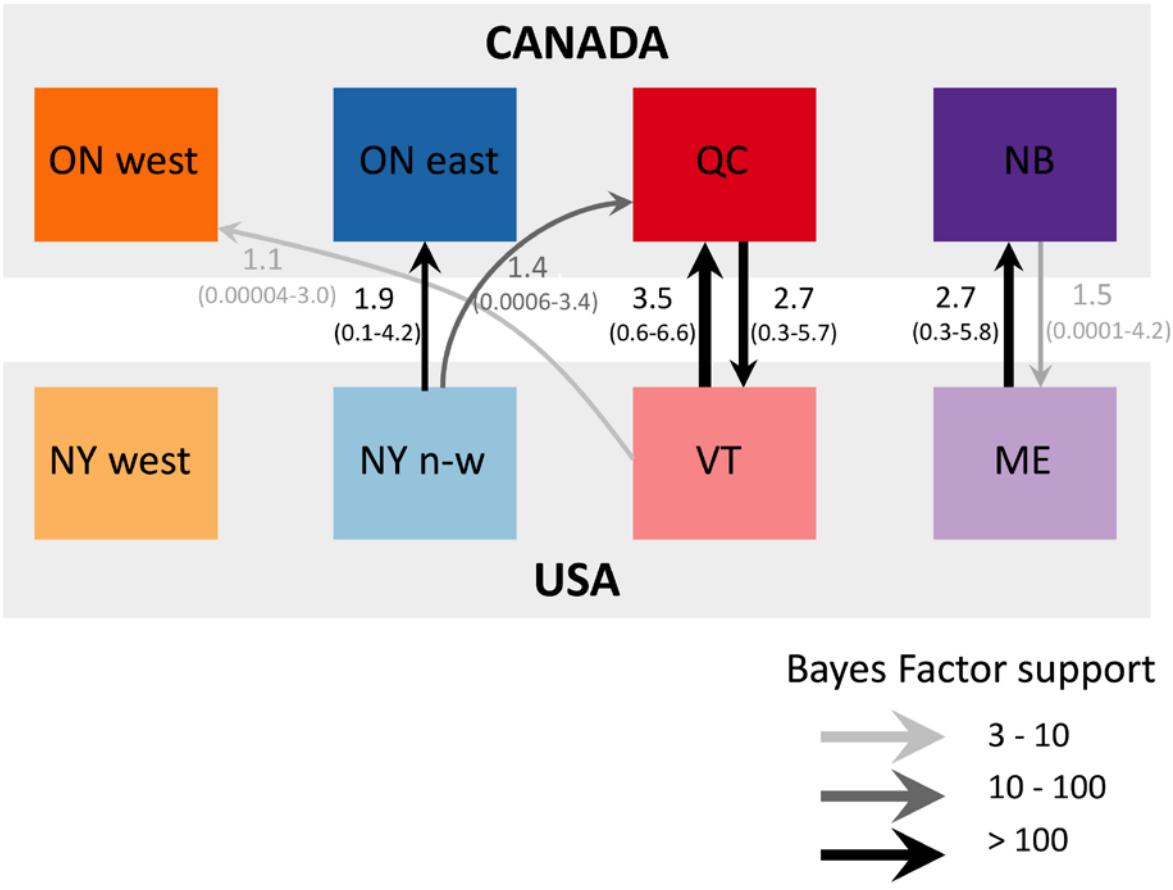
Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
QC.2008.5000		2008-09-17	Raccoon	Canada	Quebec	Rouville	Ange-Gardien	QC
QC.2008.5001		2008-09-20	Raccoon	Canada	Quebec	Rouville	Saint-Cesaire	QC
QC.2008.5337		2008-10-15	Skunk	Canada	Quebec	Le Haut-Richelieu	Henryville	QC
QC.2009.0709		2009-02-26	Skunk	Canada	Quebec	Le Haut-Richelieu	Clarenceville	QC
QC.2009.0819		2009-04-07	Skunk	Canada	Quebec	Le Haut-Richelieu	Clarenceville	QC
QC.2015.0488		2015-06-04	Raccoon	Canada	Quebec	Akwesasne Reservation	St Regis	ON east
VT.2005.0215		2005-12-29	Raccoon	USA	Vermont	Washington	Cabot	VT
VT.2006.0104		2006-08-29	Raccoon	USA	Vermont	Caledonia	St. Johnsbury	VT
VT.2006.0108		2006-08-30	Raccoon	USA	Vermont	Lamoille	Stowe	VT
VT.2006.0116		2006-09-05	Cow	USA	Vermont	Caledonia	Danville	VT
VT.2006.0117		2006-09-05	Skunk	USA	Vermont	Chittenden	South Burlington	VT
VT.2006.0153		2006-10-04	Skunk	USA	Vermont	Lamoille	Stowe	VT
VT.2006.0166		2006-10-11	Raccoon	USA	Vermont	Rutland	Clarendon	VT
VT.2006.0178		2006-10-30	Skunk	USA	Vermont	Addison	Panton	VT
VT.2006.0225	KY026478	2006-11-17	Raccoon	USA	Vermont	Lamoille	Stowe	VT
VT.2006.0332		2006-05-23	Skunk	USA	Vermont	Addison	Middlebury	VT
VT.2007.0084		2007-07-20	Skunk	USA	Vermont	Chittenden	Burlington	VT
VT.2007.0092		2007-07-25	Raccoon	USA	Vermont	Caledonia	Lyndon	VT
VT.2007.0297		2007-01-04	Raccoon	USA	Vermont	Lamoille	Stowe	VT
VT.2007.0308		2007-01-31	Raccoon	USA	Vermont	Bennington	Pownal	VT
VT.2007.0320		2007-09-11	Skunk	USA	Vermont	Chittenden	Williston	VT
VT.2007.0342		2007-03-02	Raccoon	USA	Vermont	Franklin	St. Albans City	VT
VT.2007.0380		2007-09-18	Raccoon	USA	Vermont	Franklin	Fairfield	VT
VT.2007.0392		2007-03-22	Raccoon	USA	Vermont	Chittenden	Burlington	VT
VT.2007.0398		2007-09-26	Skunk	USA	Vermont	Franklin	Georgia	VT
VT.2007.0412		2007-03-30	Raccoon	USA	Vermont	Chittenden	Essex	VT
VT.2007.0465		2007-10-15	Skunk	USA	Vermont	Franklin	Sheldon	VT
VT.2007.0479		2007-10-30	Skunk	USA	Vermont	Franklin	Swanton	VT
VT.2007.0489		2007-11-05	Raccoon	USA	Vermont	Grand Isle	North Hero	VT
VT.2007.0491		2007-11-05	Skunk	USA	Vermont	Franklin	Franklin	VT
VT.2007.0492		2007-04-25	Raccoon	USA	Vermont	Caledonia	Danville	VT
VT.2007.0496		2007-04-25	Raccoon	USA	Vermont	Caledonia	Danville	VT
VT.2007.0505		2007-04-30	Skunk	USA	Vermont	Caledonia	Danville	VT
VT.2007.0506		2007-11-14	Skunk	USA	Vermont	Franklin	Georgia	VT
VT.2007.0512		2007-11-20	Skunk	USA	Vermont	Franklin	Sheldon	VT
VT.2007.0523		2007-12-10	Skunk	USA	Vermont	Franklin	Franklin	VT
VT.2008.0237	KY026482	2008-09-29	Raccoon	USA	Vermont	Lamoille	Stowe	VT
VT.2008.0270		2008-11-06	Skunk	USA	Vermont	Lamoille	Elmore	VT
VT.2008.0310		2008-12-29	Cow	USA	Vermont	Franklin	Bakersfield	VT
VT.2009.0036		2009-07-20	Skunk	USA	Vermont	Franklin	Fairfax	VT
VT.2009.0092		2009-08-14	Raccoon	USA	Vermont	Grand Isle	South Hero	VT
VT.2009.0093		2009-08-20	Skunk	USA	Vermont	Orleans	Craftsbury	VT
VT.2009.0124		2009-10-04	Skunk	USA	Vermont	Chittenden	Williston	VT
VT.2009.0134		2009-10-12	Skunk	USA	Vermont	Caledonia	Sheffield	VT
VT.2009.0136		2009-10-19	Raccoon	USA	Vermont	Chittenden	Essex	VT
VT.2009.0146		2009-10-19	Raccoon	USA	Vermont	Orleans	Craftsbury	VT
VT.2009.0164		2009-11-05	Raccoon	USA	Vermont	Franklin	Franklin	VT
VT.2009.0169		2009-11-01	Skunk	USA	Vermont	Chittenden	Shelburne	VT
VT.2009.0194		2009-11-30	Raccoon	USA	Vermont	Chittenden	Williston	VT
VT.2009.0352		2009-02-26	Raccoon	USA	Vermont	Grand Isle	Grand Isle	VT
VT.2009.0370		2009-03-17	Raccoon	USA	Vermont	Lamoille	Stowe	VT
VT.2009.0371		2009-03-11	Cow	USA	Vermont	Orleans	Barton	VT
VT.2009.0397		2009-04-05	Cow	USA	Vermont	Orleans	Greensboro	VT
VT.2009.0423		2009-04-17	Raccoon	USA	Vermont	Orleans	Greensboro	VT
VT.2009.0428		2009-04-17	Raccoon	USA	Vermont	Franklin	Franklin	VT
VT.2009.0443		2009-05-12	Cow	USA	Vermont	Lamoille	Hyde Park	VT
VT.2010.0044		2010-07-29	Skunk	USA	Vermont	Chittenden	Burlington	VT
VT.2010.0054		2010-08-09	Raccoon	USA	Vermont	Chittenden	South Burlington	VT
VT.2010.0089		2010-08-20	Raccoon	USA	Vermont	Chittenden	Burlington	VT
VT.2010.0090		2010-08-23	Skunk	USA	Vermont	Chittenden	Winooski	VT
VT.2010.0093		2010-08-29	Skunk	USA	Vermont	Chittenden	Winooski	VT
VT.2010.0145		2010-10-14	Skunk	USA	Vermont	Orleans	Craftsbury	VT

Sequence ID	Accession no.	Date of testing	Host species	Country	Province/ State	County	Town	Geographic group
VT.2010.0172		2010-11-18	Skunk	USA	Vermont	Chittenden	South Burlington	VT
VT.2010.0370		2010-06-18	Skunk	USA	Vermont	Chittenden	Burlington	VT
VT.2011.0079		2011-08-27	Skunk	USA	Vermont	Chittenden	Burlington	VT
VT.2011.0122	KY026483	2011-10-23	Skunk	USA	Vermont	Caledonia	Walden	VT
VT.2011.0138		2011-11-04	Skunk	USA	Vermont	Orleans	Derby	VT
VT.2011.0198		2011-01-08	Raccoon	USA	Vermont	Chittenden	Burlington	VT

Technical Appendix Table 2. Summary of phylogenetic model selection path sampling (PS) and stepping stone sampling (SS) results.

Nt. subs. model	Gene partitions	Codon partitions	UCLD clock model		UCED clock model	
			PS	SS	PS	SS
GTR+G	5x genes + non-coding	1a) None	-41504	-40505	-39250	-38101
		1b) 3 partitions	*	*	-37408	-35381
	Coding regions + non-coding	2a) None	-41912	-40675	-18312	-18312
		2b) 3 partitions	-42270	-42274	-42278	-42283
	None	3) None	-43239	-43244	-43260	-43266

*UCLD model 1b) failed to converge.



Technical Appendix Figure. RRV transitions between different regions of Canada and USA, estimated using discrete trait phylogeography. Arrows are shaded according to statistical support for the transition. The estimated number of transitions between regions is given next to the relevant arrow, with the 95% Highest Posterior Density in brackets.