Landscape modelling spatial bottlenecks: implications for raccoon rabies disease spread

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A landscape genetic simulation modelling approach is used to understand factors affecting raccoon rabies disease spread in southern Ontario, Canada. Using the Ontario Rabies Model, we test the hypothesis that landscape configuration (shape of available habitat) affects dispersal, as indicated by genetic structuring. We simulated range expansions of raccoons from New York into vacant landscapes in Ontario, in two areas that differed by the presence or absence of a landscape constriction. Our results provide theoretical evidence that landscape constriction acts as a vicariant bottleneck. We discuss implications for raccoon rabies spread.

Keywords: raccoon rabies; landscape genetics; infectious disease; modelling; *Procyon lotor*

1. INTRODUCTION

Molecular ecology and landscape genetics offer innovative approaches to the field of disease ecology (Archie *et al.* 2009). Application of these methodologies promises to improve our understanding of disease spread by characterizing factors affecting movement and connectivity among individuals in the vector populations. We apply a model-based landscape genetic approach to explore factors affecting raccoon rabies spread in the Great Lakes region of North America (figure 1). Raccoons (*Procyon lotor*) are the primary vector of this rabies variant (Winkler & Jenkins 1991). Southern Ontario is at high risk for rabies because it is adjacent to endemic areas in New York (NY) and Quebec. For the past decade, the shortest geographical distance for raccoon movement with disease spread into Ontario has been from the Niagara (NIA) region in NY; however, rabies has never been detected in NIA, Ontario. The first Ontario cases were detected in 1999 in the St Lawrence region (STL; Wandeler & Salsberg 1999). It is possible that the Niagara River acts as a

greater barrier to raccoon movement in NIA than the St Lawrence River in STL (Rees *et al.* 2008a), thus, keeping the number of infected immigrants into NIA, Ontario below a threshold for which rabies is detected or becomes epizootic; however, the shape of available habitat (landscape configuration) can also affect the number of animals moving among regions (McRae & Beier 2007).

Genetic analysis of raccoons along the Ontario–NY border (Cullingham *et al.* 2009) revealed the presence of genetically distinct raccoon populations on either side of the Niagara River, but only one population spanning the St Lawrence River. Both regions have similar habitats and experience comparable rabies control efforts. In view of these factors, it appears that there is a greater resistance to cross-border movements in the NIA region, which we suspect is caused by the narrowing of land between NY and Ontario as bounded by lakes Ontario and Erie. Hence, we test the hypothesis that landscape configuration in NIA constricts movement of raccoons between NY and Ontario. We predict that the peninsular shape acts as a spatial bottleneck and creates two genetically distinct populations. We use the Ontario Rabies Model (ORM), an individual-based, spatially explicit stochastic genetic simulation model (see the electronic supplementary material) to simulate raccoon colonization from NY into Ontario separately in NIA and the STL, and analyse their genetic structures through time. The STL landscape acts as our control because it has no landscape constriction. Our approach (i) characterizes landscape constriction effects on gene flow and (ii) provides insight into raccoon rabies spread.

2. MATERIAL AND METHODS

The ORM simulates raccoon population dynamics (e.g. reproduction, mortality, dispersal) of individuals residing in a virtual landscape of contiguous hexagonal cells of 10.23 km², the approximate activity range of raccoons in Ontario (Rosatte 2000; see the electronic supplementary material). The model operates at weekly time steps (e.g. mating occurs at week 9 (end of February) and parturition occurs at week 18 (end of April)). ORM events are stochastically determined by randomly drawing a value from parameter distribution functions (Rees *et al.* 2008b). We used the ORM to simulate bi-parental genetic inheritance of microsatellite markers assuming no new alleles arise through mutation or recombination, since this is unlikely to be significant over the simulation period (Avice 2004). We used neutral genetic markers because they are not subject to selective pressures, thus distribute as a result of mating and dispersal processes, acting as a 'tag' to identify spatio-temporal patterns arising from these processes.

To test landscape configuration effects on gene flow, we modelled raccoon range expansions in NIA (43° N, 79° W) and STL (44° N, 75° W) (figure 1). The NIA study area is composed of 2255 cells representing approximately 23 070 km² on either side of the Niagara River. STL, the control landscape, is a rectangle of 2500 cells, approximately 25 575 km². We did not simulate the rivers in either landscape, to prevent confounding the effects of rivers and landscape constrictions on influencing dispersal movements.

In separate NIA and STL model simulations, raccoons expanded from NY to colonize 'uninhabited' Ontario. We expected genetic homogenization to occur more slowly in NIA because of the landscape constriction. Consequently, we ran the NIA colonization process for 2200 years and only 1000 years in STL. The NY 'origin' populations were tagged at time 0 with microsatellite markers at field-observed frequencies for which linkage and Hardy–Weinberg equilibrium had been confirmed (Cullingham *et al.* 2009). For NIA and STL, five simulations were run to capture variation from model stochasticity.

One raccoon was randomly sampled per cell from the entire landscape in NIA, subclassified as three regions (NIA_{origin}, NIA_{constriction} and NIA_{destination}). In the STL, we reduced analysis

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Figure 1. (a) Location of ORM landscapes in eastern North America. (b) NIA landscape and three sampling regions (A, NIA_{destination}; B, NIA_{constriction}; C, NIA_{origin}) for allelic diversity measures. Cell shade represents membership of one raccoon sampled for each cell to a unique cluster, as identified by STRUCTURE using an assignment probability of 0.8 or more, after 250 years of a colonization process. (c) STL landscape and two sampling regions (D, STL_{destination}; E, STL_{origin}) for allelic diversity measures.

time and still maintaining sufficient data for objective analysis by sampling two raccoons every two to three cells across the STL landscape. This landscape was subclassified as STL_{origin} and STL_{destination} (figure 1). This sampling scheme enabled us to assess population structure over the length of the landscapes, and compare that with the structure within the constriction. We used STRUCTURE v. 2.2 (Pritchard *et al.* 2000) to determine the number of genetic clusters ($K=1-5$) 250 years after colonization ($K>5$ is unlikely; Cullingham *et al.* 2009), the approximate time period during which raccoons have colonized NIA, Ontario (Trigger *et al.* 2000). We continued to test for $K=2$ in NIA at 50–200 year intervals up to 2200 years, and checked in the STL every 100 years up to 1000 years, after the start of colonization. STRUCTURE was run using a conservative admixture model that assumed correlated allele frequencies. Three independent tests were conducted for each K , with 500 000 Markov Chain Monte Carlo cycles each for burn-in and data collection. Individuals were assigned to a cluster if average probability of ownership was 0.8 or more. We determined the most likely number of genetic population clusters using the Evanno algorithm (Evanno *et al.* 2005) and calculated the number of individuals assigned to each cluster. From the same sample of raccoons, we calculated mean number of alleles (allelic richness) for the aforementioned regions in NIA and STL, approximately every 100 years, up to 1000 years since colonization, using CERVUS v. 3.0.3 (Kalinowski *et al.* 2007).

3. RESULTS

Despite not modelling the Niagara River barrier effect, the Evanno algorithm identifies two distinct genetic clusters on either side of the Niagara River as being the most likely population structure after 250

years of range expansion (table 1), where the genetic boundary occurs at least 30 km west of the Niagara River (figure 2). Also in NIA, genetic homogenization develops in scenarios with two genetic clusters after 2000 years. At this point, there is an equal likelihood of individuals being unassigned or assigned to one of two clusters (table 2).

Among all regions, allelic richness in both source populations (NIA_{origin} and STL_{origin}) are most similar to each other and similarly decreases over time at a comparable rate (figure 2). The NIA_{constriction} is the only other region that experiences a monotonic decline in allelic richness. In the founded populations, NIA_{destination}, allelic richness increases from 75 to 150 years and then declines from 250 years. This trend is similar for STL_{destination}, but diversity in this region is greater and does not decrease until 800 years.

4. DISCUSSION

We undertook a landscape genetic approach to investigate NIA and STL population structures because we hypothesized that factors influencing their structures are also affecting rabies disease spread. By simulating colonization events in these landscapes, in the absence of a river barrier effect, we can assess how landscape configuration affects population

Table 1. STRUCTURE results from sampling the NIA landscape 250 years after colonization: number of individuals unassigned (U) and assigned (C) to clusters with a probability of ownership of 0.8 more. ($K=2$ is most likely because the mean of the estimated logarithms of probability of data ($\ln \Pr(X|K)$) across $K=1-5$ has the greatest rate of change (ΔK) at $K=2$, as calculated following Evanno *et al.* (2005). Values are means from five runs of the ORM with identical starting conditions.)

K	mean $\ln \Pr(X K)$ ($\pm$ s.d.)	ΔK	U	mean number individuals ($\pm$ s.d.)				
				$C1$	$C2$	$C3$	$C4$	$C5$
1	-92 674.7 ($\pm$ 218.2)	0.00	0 ($\pm$ 0)	2255 ($\pm$ 0)	—	—	—	—
2	-87 411.6 ($\pm$ 232.6)	22.57	134 ($\pm$ 16)	1550 ($\pm$ 12)	571 ($\pm$ 6)	—	—	—
3	-87 400.1 ($\pm$ 278.7)	0.80	1097 ($\pm$ 68)	542 ($\pm$ 6)	277 ($\pm$ 27)	339 ($\pm$ 65)	—	—
4	-87 611.1 ($\pm$ 221.7)	1.90	1471 ($\pm$ 97)	511 ($\pm$ 11)	57 ($\pm$ 46)	65 ($\pm$ 48)	151 ($\pm$ 9)	—
5	-88 244.1 ($\pm$ 614.1)	—	1636 ($\pm$ 19)	457 ($\pm$ 46)	0 ($\pm$ 1)	2 ($\pm$ 4)	32 ($\pm$ 40)	128 ($\pm$ 17)

Figure 2. Allelic richness for NIA and STL landscapes at approximately 100-year intervals over 1000 years of colonization. Allelic richness is calculated from raccoons sampled for regions: NIA_{origin} ($n=1491$, filled circles); NIA_{constriction} ($n=278$, filled triangles); NIA_{destination} ($n=486$, filled squares); STL_{origin} ($n=550$, open squares); and STL_{destination} ($n=550$, open circles).

genetic structure over time. In our simulations, $K=2$ is the most likely genetic structure in NIA, and it persists for at least 1000 years; whereas in STL, only one genetic cluster is evident through time. The NIA structure arises from the newly founded population being dominated by the genetic material of the colonizers. Expanding populations are typically lower in density along their periphery, facilitating genetic drift to fix or lose alleles, and thus increasing the likelihood of genetic differentiation from the source population (Excoffier & Ray 2008). The landscape constriction in NIA is literally a spatial bottleneck accentuating founder effects. For both NIA_{destination} and STL_{destination}, allelic richness initially increased as colonizers filled the region; however, the landscape constriction reduced the rate at which colonizers entered NIA_{destination}. Consequently, colonizers had a smaller influence on the NIA_{destination} population over time; with fewer colonizers adding to genetic diversity, genetic drift would have a stronger relative influence on the population, as indicated by earlier declines in allelic richness in NIA_{destination} than STL_{destination}.

Landscape configuration has been found to be a significant factor structuring populations in other theoretical and empirical systems (Biek *et al.* 2007; McRae & Beier 2007). We suggest that it affects

Table 2. STRUCTURE results from testing for $K=2$ by analysing 2255 raccoons in NIA and 560 raccoons in STL that were evenly sampled across both model landscapes. (Estimated logarithms of probability of data (est $\ln \text{prob}$) are shown, as well as the number of individuals unassigned and assigned (spatially congruent with source and destination regions) with a probability of ownership ≥ 0.8 . Values are means from five runs of the ORM with identical starting conditions.)

year	est $\ln \text{prob}$	unassigned	NIA _{origin}	NIA _{destination}
NIA				
150	-88 021.1	96	1622	537
200	-87 881.7	100	1602	553
250	-87 123.5	156	1533	561
450	-84 093.9	302	1329	624
650	-79 595.1	337	1204	714
750	-77 078.8	335	1176	744
850	-74 056.1	427	1099	729
950	-71 359.5	426	1090	739
1200	-64 045.6	380	1063	812
1300	-61 107.6	435	999	821
1400	-59 837.3	546	880	829
1600	-56 115.6	599	847	809
1800	-54 070.1	668	831	756
1900	-52 496.1	708	781	766
2000	-51 251.9	784	761	710
2100	-50 424.2	800	767	688
2200	-48 879.6	683	843	729
year	est $\ln \text{prob}$	unassigned	STL _{origin}	STL _{destination}
STL				
100	-18 099.7	379	93	87
200	-18 346.2	437	65	59
300	-18 466.8	466	47	47
400	-19 546.4	472	45	43
500	-18 659.2	460	52	49
600	-18 866.1	453	54	53
700	-18 503.6	461	47	52
800	-18 311.1	387	86	87
900	-18 071.1	446	57	57
1000	-18 007.3	486	38	37

rabies spread in NIA by reducing the rate at which infected raccoons enter Ontario below a level necessary to establish a rabies outbreak. We are not implying that the Niagara River or rabies control strategies have no effect on preventing disease spread into NIA, Ontario; however, the Canada–USA landscape constriction in NIA is an additional factor keeping NIA Ontario disease-free.

In simulation modelling, the quality of model output depends on appropriate representation of system processes. ORM has been validated (Rees 2008; Rees *et al.* 2008a), so we are confident of its ability to represent raccoon ecology and genetics. Our approach demonstrates the value of using landscape genetics to understand disease spread. The flexible structure of the ORM enables testing of factors hypothesized to affect animal movements, gene flow, and hence, the spread of infectious disease, from which we conclude that the narrowing of land in NIA is an important factor reducing the risk of raccoon rabies spread into NIA, Ontario.

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- Archie, E. A., Luikart, G. & Ezenwa, V. O. 2009 Infecting epidemiology with genetics: a new frontier in disease ecology. *Trends Ecol. Evol.* **24**, 21–30. (doi:10.1016/j.tree.2008.08.008)
- Avise, J. A. 2004 *Molecular markers, natural history, and evolution*, 2nd edn. Sunderland, MA: Sinauer Associates, Inc.
- Biek, R., Henderson, C. J., Waller, L. A., Rupprecht, C. E. & Real, L. A. 2007 A high-resolution genetic signature of demographic and spatial expansion in epizootic rabies virus. *Proc. Natl Acad. Sci. USA* **104**, 7993–7998. (doi:10.1073/pnas.0700741104)
- Cullingham, C. I., Kyle, C. J., Pond, B. A., Rees, E. E. & White, B. N. 2009 Differential permeability of rivers to raccoon gene flow corresponds to rabies incidence in Ontario, Canada. *Mol. Ecol.* **18**, 43–53. (doi:10.1111/j.1365-294X.2008.03989.x)
- Evanno, G., Regnaut, S. & Goudet, J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620. (doi:10.1111/j.1365-294X.2005.02553.x)
- Excoffier, L. & Ray, N. 2008 Surfing during population expansions promotes genetic revolutions and structuration. *Trends Ecol. Evol.* **23**, 347–351. (doi:10.1016/j.tree.2008.04.004)
- Kalinowski, S. T., Taper, M. L. & Marshall, T. C. 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**, 1099–1106. (doi:10.1111/j.1365-294X.2007.03089.x)
- McRae, B. H. & Beier, P. 2007 Circuit theory predicts gene flow in plant and animal populations. *Proc. Natl Acad. Sci. USA* **104**, 19 885–19 890. (doi:10.1073/pnas.0706568104)
- Pritchard, J. K., Stephens, M. & Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
- Rees, E. E. 2008 Approaches to modelling raccoon rabies. PhD thesis, Trent University, Canada.
- Rees, E. E., Pond, B. A., Cullingham, C. I., Tinline, R. R., Ball, D., Kyle, C. J. & White, B. N. 2008a Assessing a landscape barrier using genetic simulation modelling: implications for raccoon rabies management. *Prev. Vet. Med.* **86**, 107–123. (doi:10.1016/j.prevetmed.2008.03.007)
- Rees, E. E., Pond, B. A., Phillips, J. R. & Murray, D. L. 2008b Raccoon ecology database: a resource for population dynamics modelling and meta-analysis. *Ecol. Inform.* **3**, 87–96. (doi:10.1016/j.ecoinf.2008.01.002)
- Rosatte, R. C. 2000 Management of raccoons (*Procyon lotor*) in Ontario, Canada: do human intervention and disease have significant impact on raccoon populations? *Mammalia* **64**, 369–390.
- Trigger, B. G., Washburn, W. E., Salomon, F., Adams, R. E. W., Schwartz, S. B. & MacLeod, M. J. 2000 *The Cambridge history of the native peoples of the Americas*. Cambridge, UK: Cambridge University Press.
- Wandeler, A. & Salsberg, E. 1999 Raccoon rabies in eastern Ontario. *Can. Vet. J.* **40**, 731–731.
- Winkler, W. & Jenkins, S. 1991 Raccoon rabies. In *The natural history of rabies*, 2nd edn (ed. G. M. Baer), pp. 240–325. Boca Raton, FL: CRC Press.