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1   **Isolation of peripheral populations of Canada lynx**

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21

22 **ABSTRACT**

23 Landscape barriers to gene flow, such as rivers, can affect animal populations by limiting the

24 potential for rescue of these isolated populations. We tested the Riverine Barrier Hypothesis,

25 predicting that the St. Lawrence River in eastern Canada would cause genetic divergence of

26 Canada lynx (*Lynx canadensis*, Kerr, 1792) populations by restricting dispersal and gene flow.

27 We sampled 558 lynx from eastern Canada and genotyped these at 14 microsatellite loci. We

28 found 3 genetic clusters, defined by the St. Lawrence River and the Strait of Belle Isle, a

29 waterway separating Newfoundland from mainland Canada. These waterways were not

30 absolute barriers, however: we found 24 individuals that appeared to have crossed. Peripheral

31 populations of lynx are threatened in parts of Canada and the USA, and it is thought that these

32 populations are maintained by immigration from the core. Our findings suggest that in eastern

33 North America, rescue might be less likely because the St. Lawrence River restricts dispersal.

34 We found that ice cover was often sufficient to allow lynx to walk across the ice in winter. If

35 lynx used ice bridges in winter, climate warming could cause a reduction in the extent and

36 longevity of river and sea ice, further isolating these peripheral lynx populations.

37

40

41 **INTRODUCTION**

42 Landscape-scale impediments to dispersal, such as mountain ranges (e.g., Reding et al. 2013)

43 and roads (e.g., Epps et al. 2005), can cause a reduction in gene flow that can lead to reduced

44 genetic diversity and extirpation of isolated populations (O'Grady et al. 2006). At the leading

45 edge of a shifting species' distribution, landscape-scale barriers might limit opportunities for

46 the species' range to expand as optimal environmental conditions shift (Kerr and Packer 1998).

47 At the trailing range edge, landscape features that reduce gene flow could lead to reduced

48 genetic diversity (Koen et al. 2014*a*) and a reduced potential for already vulnerable populations

49 to adapt to changing environmental conditions (Pearson et al. 2009). Landscape barriers might

50 also prevent rescue of isolated populations by limiting immigration from core populations

51 (Adams et al. 2011).

52 The influence of rivers on dispersal, species distributions, and speciation has been of

53 interest for over a century (Wallace 1852; Grinnell 1914; Goldman 1937). The Riverine Barrier

54 Hypothesis (Wallace 1852; Ayres and Clutton-Brock 1992) posits that rivers can act as barriers

55 to dispersal and can limit species ranges. It follows that dispersal rates across rivers would be

56 inversely proportional to river width and flow rate. Despite the number of empirical tests,

57 support for this hypothesis is mixed (Colwell 2000). Rivers have been shown to impede

58 dispersal and gene flow of a diversity of terrestrial species, including reptiles (lizards: Lamborot

59 et al. 2003), birds (Hayes and Sewlal 2004; Voelker et al. 2013), and mammals (mustelids:

60 Garroway et al. 2011, primates: Ayres and Clutton-Brock 1992; Peres et al. 1996). There are also

61 examples of rivers that do not act as barriers (Patton et al. 1994; Fairley et al. 2002; Lougheed

62 et al. 1999; Côté et al. 2012). Equivocal support for the Riverine Barrier Hypothesis might be a

63   function of the ecology and dispersal abilities of the focal species in relation to the width and

64   flow rate of the river in question (Haffer 1997). In northern regions, seasonal river ice can

65   dampen the isolating effects of rivers by making otherwise isolated regions accessible to non-

66   hibernating terrestrial mammals via ice bridges (Jackson 1920; Banfield 1954; Fuller and

67   Robinson 1982*b*; Gaston et al. 2012).

68   The distribution of Canada lynx (*Lynx canadensis*, Kerr, 1792) across North America has

69   contracted since European settlement (Laliberte and Ripple 2004), and the southern extent of

70   the range has continued to contract northward in recent decades (Koen et al. 2014*a*). The

71   Canada lynx is federally listed as threatened in the conterminous USA (U.S. Fish and Wildlife

72   Service 2000) and provincially listed as endangered in New Brunswick (New Brunswick

73   Endangered Species Regulation 2013) and Nova Scotia, Canada (Parker 2001). The St. Lawrence

74   River is over 750 km long and 1 - 42 km wide, and runs through the southern extent of lynx

75   range in Quebec, Canada (Fig. 1). Lynx are known to swim across relatively narrow rivers (100 –

76   300 m; Feierabend and Kielland 2014), but previous research has suggested that lynx

77   movements are impeded by a river as wide as the St. Lawrence (Rueness et al. 2003). Lynx

78   populations at the southern extent of their range may be maintained or supplemented by

79   immigration from core populations (Schwartz et al. 2002), and in eastern North America this

80   would imply that lynx immigrate southward from north of the St. Lawrence River. Indeed, the

81   possibility of rescue of the threatened southern lynx populations via dispersal from core

82   populations is an important component of assessment and recovery plans for lynx (Ruediger et

83   al. 2000; Nordstrom 2005; Nova Scotia Lynx Recovery Team 2007). Thus, understanding the role

84   of the St. Lawrence River in shaping genetic structure of lynx is an important conservation goal

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85   as it could have implications for the recovery of peripheral lynx populations in eastern North

86   America. If the St. Lawrence River is acting as an impediment to lynx movements and gene flow,

87   then there should be genetic divergence between lynx populations on either side of the river.

88   We also estimated temporal trends in ice cover to assess whether an ice bridge across the St.

89   Lawrence River exists and could be crossed by lynx in winter. For context, we compared our

90   results to a known obstacle to lynx gene flow, the Strait of Belle Isle that separates

91   Newfoundland from mainland Labrador and Quebec, Canada.

92

93   **METHODS**

94   The St. Lawrence River in eastern North America links the Great Lakes to the Atlantic

95   Ocean. The river ranges from 1 km wide in the fluvial sections west of Montreal, Quebec, to an

96   average width of 17 km east of Quebec City, Quebec, and widening to an average of 42 km at

97   the lower estuary (Environment Canada 2013, Fig. 1). Portions of the St. Lawrence River freeze

98   periodically during the winter, and sea ice cover in the Gulf of the St. Lawrence varies annually

99   (Johnston et al. 2005). Variability in freezing is due to a combination of factors, including

100   ambient and water temperature, surface wind, water current, tidal flows, and the North

101   Atlantic Oscillation (NAO, Johnston et al. 2005; Fisheries and Oceans Canada 2012). The

102   Canadian Coast Guard uses icebreakers to keep a channel of the St. Lawrence River open during

103   the winter, from Montreal to Quebec City, for shipping and flood control (Fisheries and Oceans

104   Canada 2001; Dong 2011). For comparison, the Strait of Belle Isle, separating Newfoundland

105   from mainland Labrador and Quebec, Canada, is a 15 - 60 km wide waterway in the Gulf of the

106   St. Lawrence that usually freezes in winter (Fig 1., Fisheries and Oceans Canada 2012).

107 **Sample collection and genetic profiling**

108   We collected skin samples (2.5mm x 2.5mm) from the pelts of Canada lynx harvested in

109 Quebec, Labrador, and Newfoundland, Canada, from fur auctions between 2008 and 2011.

110 Furbearer harvesting in Quebec was reported by administrative units called Unités de Gestion

111 des Animaux à Fourrure (UGAF). Thus, we used the centroid of the UGAF as the sample location

112 (Fig. 1). The average size of the 58 UGAFs for which we had at least one lynx sample was 4356.8

113 (SD = 7818.0) km^2. We were able to categorize the Newfoundland and Labrador samples as

114 being harvested from mainland (Labrador) or island (Newfoundland) only. In 2010 we obtained

115 tissue samples of 15 incidental lynx mortalities in New Brunswick, Canada from the New

116 Brunswick museum. We grouped these samples into one site. As lynx in New Brunswick tended

117 to occur in the northwest of the province (Parker 2001), we used the centroid of this region as

118 our site coordinates for New Brunswick (Fig. 1). The lynx from Quebec and Newfoundland and

119 Labrador presented by Row et al. (2012) are a subset of what we present here. Furthermore, all

120 lynx samples presented here are a subset of those reported in Koen et al. (2014*b*). We

121 measured pelt length to categorize individuals as adult or juvenile (Quinn and Gardner 1984;

122 Slough 1996).

123   We genotyped lynx at 14 microsatellite loci (Fca031, Fca035, Fca043, Fca077, Fca090,

124 Fca096, Fca441, Fca391, Fca559, Lc106, Lc109, Lc110, Lc111, Lc118) according to methods

125 described by Row et al. (2012). We manually scored allele sizes using Genemarker 1.7

126 (Softgenetics). All samples for both species were scored by the same individual using the same

127 criteria, and a second person independently scored a subset of the samples to ensure

128 consistency. We omitted samples that were missing alleles at ≥ 5 of 14 loci. We checked for

129   errors with software Microchecker 2.2.3 (van Oosterhout et al. 2004) and by examining

130   summary statistics with the adegenet package (version 1.4-2, Jombart 2008) in R (R

131   Development Core team 2014). To determine sex, we amplified the y-chromosome-specific Sry

132   locus and the Zfx fragment on the x-chromosome (Woods et al. 1999; Ortega et al. 2004;

133   Zigouris et al. 2012).

134   **Analysis of genetic data**

135   We grouped lynx samples into 5 sites based on geographic location: north of the St.

136   Lawrence River in Quebec (n = 331), south of the St. Lawrence River in Quebec (n = 165), New

137   Brunswick (n = 15), mainland Labrador (n = 18), and Newfoundland (n = 29). We used

138   Bonferroni-corrected (α = 0.0012) chi-square tests to determine whether allele frequencies

139   were in Hardy-Weinberg equilibrium (HWE), and estimated expected and observed

140   heterozygosity with the adegenet package (Jombart 2008) in R. We used the software Genepop

141   (web version 4.2, Raymond and Rousset 1995; Rousset 2008) to test for linkage disequilibrium

142   (Bonferroni-corrected; α = 0.0005). We used software HP-Rare 1.1 (Kalinowski 2005) to

143   estimate the number of alleles per locus (allelic richness), corrected for a sample size of 15 with

144   rarefaction, for our 5 sites. We estimated F_{IS} for each site, and pairwise D_{est} (Jost 2008) and F_{ST}

145   (Weir and Cockerham, 1984) between sites with the R package DiveRsity (Keenan et al. 2013),

146   with 95% confidence intervals on these estimates (999 bootstraps). For lynx sampled in

147   Quebec, we grouped samples as north or south of the St. Lawrence River and within groups,

148   calculated pairwise D_{est} and F_{ST} between UGAF administrative units. We grouped lynx sampled

149   from adjacent UGAFs to increase the sample size in each UGAF (north: 16 sites with an average

150   of 25.7 (SD=16.9) samples/site; south: 7 sites, with an average of 23.1 (SD=16.0) samples/site).

151   To further evaluate the genetic structure of lynx in our study area, we used an analysis of

152   molecular variance (AMOVA; Excoffier et al. 1992), with 5 sites nested within the three regions

153   delineated by waterways (south of the St. Lawrence River, north of the River, and

154   Newfoundland) with the poppr (Kamvar et al. 2014), adegenet (Jombert 2008), and ade4 (Dray

155   and Dufour 2007) packages in R. We used 999 permutations to assess statistical significance

156   with the ade4 and poppr packages, as described by Excoffier et al. (1992).

157   We used Bayesian clustering software (Structure version 2.3.4, Pritchard et al. 2000) to

158   identify genetic clusters. We ran 10 repetitions for each of K = {1, 2, 3, … , 9} with a burn-in of

159   500,000 Markov chain Monte Carlo iterations and followed by 1×10^6 iterations. We used an

160   admixture model without prior location information. We identified the most likely number of

161   genetic clusters with the Evanno method (Evanno et al. 2005) using software Structure

162   Harvester (Earl and vonHoldt 2012). We summarized the 10 replicates with software Clumpp

163   (Jakobsson and Rosenberg 2007) and visualized the results with software Distruct (Rosenberg

164   2004). We considered individuals to be admixed if they had $0.3 \geq Q \geq 0.7$, where Q represented

165   the proportion of an individual's genome assigned to a population (Pritchard et al. 2000). We

166   conducted a principal component analysis (PCA) of microsatellite genotypes with the ade4

167   package (Dray and Dufour 2007) in R. We used the PCA as a complementary analysis to

168   program Structure because unlike Structure, PCA does not rely on the assumption that

169   populations are in HWE. We note, however, that Structure appears to be robust to departures

170   from HWE (Hauser et al. 2006, Rodríguez-Ramilo et al. 2009). We estimated pairwise D_{est} (Jost

171   2008) and F_{ST} (Weir and Cockerham 1984) between clusters (in addition to between sites) with

172   the R package DiveRsity. For this analysis, we assigned admixed samples to the cluster that

173   accounted for >50% of its ancestry.

174   **Ice cover on the St. Lawrence River and Strait of Belle Isle**

175   We were interested in examining whether the St. Lawrence River and Strait of Belle Isle

176   froze in the winter such that lynx could walk across the ice. We analyzed weekly ice charts for

177   the Eastern Coast region from the Canadian Ice Service Archive (Meteorological Service of

178   Canada, Environment Canada) from Dec 1 – May 15, for each of 8 years (2004 - 2011) to assess

179   temporal trends in ice cover. We restricted our analysis to these years because ice charts prior

180   to 2004 with World Meteorological Organization colour coding were not available and our

181   samples were collected prior to 2011. From these data, we identified how many weeks (not

182   necessarily consecutive) that there was an ice bridge across the St. Lawrence River east of

183   Quebec City and across the narrow reaches of the Strait of Belle Isle near St. Anthony,

184   Newfoundland (Fig. 1). We defined an ice bridge as ice, connecting both banks of the river or

185   strait, with a concentration of ≥ 9. Ice concentration is the proportion of the water surface in a

186   defined area that is covered by ice, on a scale from 1 - 10 (Environment Canada 2005). We

187   considered grey ice (10 – 15 cm thick), grey-white ice (15 – 30 cm thick), and first year ice (> 30

188   cm thick) to be of sufficient thickness for crossing.

189

190   **RESULTS**

191   **Analysis of genetic data**

192   We successfully genotyped 558 lynx from Quebec (n = 331 north of the St. Lawrence River, n =

193   165 south of the river), New Brunswick (n = 15), Labrador (n = 18), and Newfoundland (n = 29;

194 Table 1). We omitted 7 samples from Quebec and 2 samples from Labrador because they were

195 missing alleles at ≥ 5 of 14 loci. Both Newfoundland lynx and Quebec lynx south of the St.

196 Lawrence River departed from HWE at 6 of 14 loci (NFLD: Lc111, Fca35, Lc109, Fca559, Lc106,

197 Fca77; QC south: Fca441, Fca96, Fca35, Lc106, Lc109, Lc110; $p < 0.0012$), whereas lynx north of

198 the river departed from HWE at one locus only (Fca96). The New Brunswick and Labrador sites

199 were in HWE at all loci. There was evidence of linkage disequilibrium for 4 pairs of loci (Fca96

200 and Fca559, Fca559 and Fca31, Fca31 and Fca441, and Fca391 and Fca110; $p < 0.0005$).

201 Allelic richness and private allelic richness in Quebec south of the St. Lawrence River

202 were 16.6% and 67.5% lower than north of the river (Table 2). Allelic richness and private allelic

203 richness in Newfoundland were 38.8% and 51.8% lower than in Labrador (Table 2). Both F_{ST} and

204 D_{est} indicated high genetic differentiation on either side of the St. Lawrence River and the Strait

205 of Belle Isle relative to sites on the same side of the waterway (Table 3). There was greater

206 genetic differentiation between Newfoundland and Labrador than between populations north

207 and south of the St. Lawrence River (Table 3). Within Quebec, gene flow was relatively high

208 between lynx on the same side of the St. Lawrence River: F_{ST} and D_{est} between pairs of UGAFs

209 on the same side of the river were lower (Supplementary Tables 1 and 2) than the values we

210 observed for sites on opposite sides of the river (Table 3). We detected evidence of relatively

211 high inbreeding among lynx in Newfoundland (Table 2).

212 The likelihood values from our Structure analysis indicated two genetic clusters

213 (Supplementary Fig. 1) separating Newfoundland, Labrador, and Quebec (north of the St.

214 Lawrence River) from New Brunswick and Quebec (south of the river). Our PCA results (Fig. 2),

215 however, suggested 3 genetic clusters, grouping New Brunswick and Quebec (south of the

216   river) into one cluster, Labrador and Quebec (north of the river) into a second cluster, and

217   Newfoundland as a third cluster. F_{ST} and D_{est} values (Table 3) were consistent with the latter

218   finding that the St. Lawrence River and the Strait of Belle Isle are impediments to gene flow.

219   Furthermore, the 3 clusters themselves were genetically differentiated (Supplementary Table

220   3). Our AMOVA showed that that while the majority (88.9%) of the variation was within sites (Φ

221   = 0.110, p<0.001), a significant proportion (10.4%) of the genetic variation was partitioned

222   among regions separated by waterways (i.e., south of the St. Lawrence River, north of the River,

223   and Newfoundland; Φ = 0.104, p<0.001), with less variation attributed to sites nested within

224   regions (0.7%, Φ = 0.007, p = 0.042). Visual inspection of our Structure plot also suggested 3

225   genetic clusters (Fig. 3), and although this is not demonstrated by our likelihood values

226   (Supplementary Fig. 1b), it does agree with our PCA, AMOVA, F_{ST} and D_{est} results, and also with

227   findings from Row et al. (2012) that lynx in Newfoundland are a separate genetic cluster from

228   mainland lynx. As such, and as suggested by Evanno et al. (2005), we have used the weight-of-

229   evidence to interpret our results as three genetic clusters.

230   **Dispersal across the St. Lawrence River**. We found 9 (2.7%) lynx (7 adult males, 2 adult

231   females) north of the St. Lawrence River that clustered with lynx south of the river. Likewise,

232   we found 9 (5.4%) lynx (5 adult males, 3 adult females) south of the river that clustered with

233   lynx north of the river: one of these (male of unknown age) was sampled in New Brunswick. We

234   found 1 (6.7%) lynx (adult of unknown sex) in Labrador that clustered with lynx south of the St.

235   Lawrence River (Fig. 4). All of these individuals were likely first generation dispersers (0.1 > Q >

236   0.90). We identified the same 19 individuals with both program Structure and PCA. We

237   identified 4 admixed lynx (sharing DNA between north and south clusters): 2 (adult males) were

238   found north of the river and 2 were found south of the river in Quebec (adult male) and New

239   Brunswick (female of unknown age) (Fig. 4).

240   **Dispersal across the Strait of Belle Isle.** We found 4 (13.8%) lynx (1 adult male, 2 adult females,

241   1 adult of unknown sex) in Newfoundland that clustered with lynx from Labrador and Quebec

242   (north of river). We also found 1 (0.6%) lynx (adult female) south of the St. Lawrence River in

243   Quebec that was assigned to the Newfoundland cluster (Fig. 4). We estimated that this

244   individual was a first-generation disperser (Q = 0.996), and although we do not know its travel

245   route, the lynx likely crossed both the Strait of Belle Isle and the St. Lawrence River. We

246   identified the same 5 individuals as dispersers with both program Structure and PCA. We found

247   one lynx (female of unknown age) north of the St. Lawrence River in Quebec that was admixed,

248   sharing DNA from the north cluster and Newfoundland (Fig. 4).

249   **Ice cover on the St. Lawrence River and Strait of Belle Isle**

250   Between 2004 and 2011, there was an ice bridge across the St. Lawrence River east of Quebec

251   City every year (Table 4). The number of weeks that an ice bridge was present varied across

252   years, and those weeks were not necessarily consecutive. When an ice bridge was present

253   across the St. Lawrence River, it tended to be composed of relatively thin grey (10 - 15 cm thick)

254   or grey-white (15 – 30 cm thick) ice. There was also an ice bridge across the Strait of Belle Isle

255   every year: it tended to form later in the winter, but was present for longer (Table 4). The ice

256   bridge tended to be composed of thin (30 – 70 cm thick), medium (70 – 120 cm thick), and thick

257   (>120 cm thick) first year ice.

258

259   **DISCUSSION**

260 The St. Lawrence River appeared to pose an impediment to lynx dispersal and gene flow: we

261 found genetic clustering on either side of the river, as demonstrated by Bayesian clustering,

262 PCA, AMOVA, and pairwise differentiation metrics, supporting the Riverine Barrier Hypothesis.

263 We found only 4 admixed animals, further corroborating that the river restricts lynx gene flow.

264 The St. Lawrence River and Strait of Belle Isle are not absolute barriers, however – we found 24

265 adult lynx that crossed these waterways. A prediction of the Riverine Barrier Hypothesis is that

266 wider segments of the river near the mouth represent a stronger barrier than narrower

267 segments near the headwater. Although we do not know where along the bank lynx crossed

268 the St. Lawrence River, our results lend some support for this prediction: 13 of the 19 first

269 generation river-crossers were sampled closer to the headwater than the mouth of the river

270 (Fig. 4). The admixed lynx that we sampled must have been the offspring of a river-crossing

271 disperser and an individual that did not cross the river, suggesting that river crossing by lynx has

272 occurred over several generations.

273 We found greater genetic structure between Newfoundland and Labrador than

274 between the north and south of the St. Lawrence River, implying that the Strait of Belle Isle

275 restricts lynx gene flow more so than the St. Lawrence River does. Previous research has shown

276 that lynx across Canada have relatively low genetic structure owing to high gene flow and the

277 ability of lynx to disperse long distances (Schwartz et al. 2002; Campbell and Strobeck 2006;

278 Row et al. 2012). Our findings add to our understanding of the population structure of this

279 putatively vagile and panmictic species. A comparison of F_{ST} values suggests that the St.

280 Lawrence River ($F_{ST} = 0.053$) and the Strait of Belle Isle ($F_{ST} = 0.179$) pose a greater impediment

281 to lynx dispersal than does the Rocky Mountains in western Canada ($F_{ST} \approx 0.016$; Rueness et al.

282   2003). Likewise, the F_{ST} that we observed in lynx on either side of the St. Lawrence River was an

283   order of magnitude higher than that observed across the entire continent (F_{ST} = 0.007 Alaska to

284   Quebec, Row et al. 2012). The striking genetic structure in this otherwise vagile species

285   underlines the importance of waterways in shaping the past and future genetic composition of

286   the lynx.

287   We do not know whether the lynx in our study walked across the ice in winter or swam

288   across during ice-free seasons. Lynx can swim across rivers: Feierabend and Kielland (2014)

289   observed 2 lynx repeatedly crossing an unfrozen, 100 - 300 m-wide glacial river in air

290   temperatures of -27°C. The width of the St. Lawrence River ranges from <1 km west of

291   Montreal to >42 km at the river's mouth. It is possible that lynx swim across the narrower

292   sections of the St. Lawrence River, but it seems less likely that lynx would swim across the 15 –

293   60 km wide Strait of Belle Isle. Our main objective in assessing ice cover, however, was not to

294   determine whether lynx walked or swam across the waterways, but simply to evaluate whether

295   walking was typically possible. The extent of ice cover on the St. Lawrence River was variable

296   within and between years and a channel through much of the river is kept open with

297   icebreakers. Coyotes (*Canis latrans*, Say, 1823) and red fox (*Vulpes vulpes*, L., 1758) will readily

298   cross river ice once shipping lanes have refrozen (Fuller and Robinson 1982*a*), and it is also

299   possible that lynx walked across river ice on the St. Lawrence despite the periodically open

300   channel. The thickness and extent of ice cover on the Strait of Belle Isle tended to be greater

301   than that of the St. Lawrence River, thus it is possible that lynx walked across the 15 – 60 km of

302   sea ice; lynx have been shown to cross up to 50 km of sea ice in the Arctic (Gaston et al. 2012).

303   The timing of juvenile lynx dispersal can be variable: Poole (1997) found that lynx kittens

304   generally dispersed between March and November. In addition to juvenile dispersal, adult lynx

305   make long-distance exploratory movements at various times of the year (Squires and Laurion

306   2000; Squires and Oakleaf 2005; Moen et al. 2010). Lynx are obligate predators of the

307   snowshoe hare (*Lepus americanus*, Erxleben, 1777) and are known to exhibit a 10-year cyclic

308   fluctuation with hares (Elton and Nicholson 1942). Several studies have found dispersal rates of

309   adult lynx to be highest following hare population declines (Ward and Krebs 1985; Slough and

310   Mowat 1996; Poole 1997). In nearby central Ontario, hare population abundance peaked in

311   2007 and reached a low in 2013 (Ontario Ministry of Natural Resources, unpublished data). The

312   adult lynx that crossed the river did so before our sampling occurred (beginning in 2008), and

313   might have been moving in response to the concomitant decline in hare populations. This

314   speculation could explain our finding of few admixed individuals - the influx of first generation

315   dispersers was recent (in response to the recent hare decline).

316   Narrower segments of the St. Lawrence River in southern Ontario are permeable to

317   movement by other mid-sized carnivores. Carr et al. (2007) showed that the St. Lawrence River

318   has not impeded fisher (*Pekania (Martes) pennanti*, Erxleben, 1777) range expansion from the

319   Adirondack region of New York, USA, into eastern Ontario, Canada. Likewise, Cullingham et al.

320   (2009) showed that the St. Lawrence River has allowed gene flow of raccoons (*Procyon lotor*,

321   (L., 1758), and thus did not stop the spread of the raccoon rabies virus from New York into

322   southeastern Ontario, Canada. The Strait of Belle Isle appears to be less permeable to

323   terrestrial mammalian dispersers. It is thought that black bears (*Ursus americanus hamiltoni,*

324   Cameron, 1957; Paetkau and Strobeck 1996; Marshall et al. 2011) colonized Newfoundland

325 from Labrador across the Strait of Belle Isle, but such migration events by bears are rare

326 (Paetkau and Strobeck 1996). Recent occurrences of wolves (*Canis lupus*, L., 1758) on

327 Newfoundland are thought to be migrants from Labrador (Government of Newfoundland and

328 Labrador 2012), and recent outbreaks of rabies on the island of Newfoundland suggest

329 immigration of arctic (*Alopex lagopus*, L., 1758) or red (*V. vulpes*) fox from Labrador or Quebec

330 (Nadin-Davis et al. 2008). Lynx in Newfoundland are morphologically (Saunders 1964; van Zyll

331 de Jong 1975; Khidas et al. 2013) and genetically (Row et al. 2012) distinct from mainland lynx

332 populations. We found that lynx cross the Strait of Belle Isle from mainland (Labrador and/or

333 Quebec) to Newfoundland and vice versa – this is one of few contemporary examples of mid-

334 sized carnivores crossing the Strait of Belle Isle.

335 Peripheral populations of lynx are already vulnerable because less suitable

336 environmental conditions tend to be correlated with low gene flow and low genetic diversity

337 (Koen et al. 2014*a*). Similarly, we found that lynx south of the St. Lawrence River and on the

338 island of Newfoundland have relatively low neutral allelic richness. If there is a correlation

339 between neutral and adaptive genetic variation, our results could indicate that these peripheral

340 lynx populations are less likely to adapt to changing environmental conditions.

341 It is expected that climate change will further limit the distribution of lynx in eastern

342 North America (Carroll 2007). Climate change is also expected to cause a northward shift in

343 bobcat (*L. rufus*, Schreber, 1777) distribution (Anderson and Lovallo 2003; Roberts and

344 Crimmins 2010), increasing the area of sympatry of lynx and bobcat. This interspecies range

345 overlap will threaten lynx population persistence at their southern range extent through

346 competition (Peers et al. 2013) and hybridization (Schwartz et al. 2004; Homyack et al. 2008;

347   Koen et al. 2014*b*). If the St. Lawrence River impedes bobcat gene flow as it does lynx, it is

348   possible that the river might protect the core of lynx range north of the river by limiting

349   northward range expansion of bobcats from south of the river. It is unclear whether climate

350   warming will reduce the likelihood of ice bridges forming across the river because ice formation

351   in the Gulf of the St. Lawrence is a function of not just temperature, but also wind, water

352   current, tidal flow, and the NAO (Johnston et al 2005; Fisheries and Oceans Canada 2012).

353   Between 1969 and 2002, however, there was a 20 - 40% reduction in sea ice cover during the

354   spring thaw in the Gulf of the St. Lawrence (Johnston et al. 2005).

355   The St. Lawrence River and the Strait of Belle Isle have important roles in shaping the

356   future distribution of lynx in eastern North America. Lynx populations south of the St. Lawrence

357   River - in New Brunswick, Nova Scotia, and the northeastern United States - are already

358   classified as threatened or provincially endangered. It is thought that peripheral populations of

359   lynx are maintained by immigration of lynx from the core of the range (Schwartz et al. 2002).

360   We showed that the St. Lawrence River and the Strait of Belle Isle act as impediments to gene

361   flow, isolating these populations from the range core. Thus, rescue of these isolated

362   populations by dispersers is less likely than previously thought. If individual lynx are crossing

363   the St. Lawrence River and the Strait of Belle Isle in the winter by walking across the ice, climate

364   warming could reduce the duration and extent of ice bridges across the waterways, further

365   isolating these peripheral lynx populations.

366

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376 **REFERENCES**

377 Adams, J.R., Vucetich, L.M., Hedrick, P.W., Peterson, R.O., and Vucetich, J.A. 2011. Genomic

378   sweep and potential genetic rescue during limiting environmental conditions in an

379   isolated wolf population. Proc. R. Soc. B Biol. Sci., **278**(1723): 3336–3344.

380   doi:10.1098/rspb.2011.0261.

381 Anderson, E.M., and Lovallo M.J. 2003. Bobcat and lynx. *In* Wild mammals of North America

382   Volume II. *Edited by* G.A. Feldhamer and B. Thompson. Johns Hopkins University Press,

383   Baltimore, Maryland. pp. 758–786.

384 Ayres, J.M., and Clutton-Brock, T.H. 1992. River boundaries and species range size in Amazonian

385   primates. Am. Nat. **140**(3): 531–537.

386 Banfield, A.W.F. 1954. The role of ice in the distribution of mammals. J. Mammal. **35**(1): 104–

387   107.

388 Campbell, V., and Strobeck, C. 2006. Fine-scale genetic structure and dispersal in Canada lynx

389   (*Lynx canadensis*) within Alberta, Canada. Can. J. Zool. **84**(8): 1112–1119.

390   doi:10.1139/z06-099.

391 Carmichael L.E., Clark, W., and Strobeck, C. 2000. Development and characterization of

392   microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. Mol. Ecol.

393   **9**(12): 2197–2198. doi:10.1046/j.1365-294X.2000.105323.x.

394 Carr, D., Bowman, J., Kyle, C.J., Tully, S.M., Koen, E.L., Robitaille, J.-F. and Wilson P.J. 2007.

395   Rapid homogenization of multiple sources: genetic structure of a recolonizing

396   population of fishers. J. Wildl. Manage. **71**(6): 1853–1861. doi:10.2193/2006-274.

397   Carroll, C. 2007. Interacting effects of climate change, landscape conversion, and harvest on

398   carnivore populations at the range margin: marten and lynx in the northern

399   Appalachians. Conserv. Biol. **21**(4): 1092–1104. doi:10.1111/j.1523-1739.2007.00719.x.

400   Colwell, R.K. 2000. A barrier runs through it… or maybe just a river. Proc. Natl. Acad. Sci. U.S.A.

401   **97**(25): 13470–13472. doi:10.1073/pnas.250497697.

402   Côté, H., Garant, D., Robert, K., Mainguy, J., and Pelletier, F. 2012. Genetic structure and rabies

403   spread potential in raccoons: the role of landscape barriers and sex-biased dispersal.

404   Evol. Appl. **5**(4): 393–404. doi:10.1111/j.1752-4571.2012.00238.x.

405   Cullingham, C.I., Kyle, C.J., Pond, B.A., Rees, E.E., and White, B.N. 2009. Differential permeability

406   of rivers to raccoon gene flow corresponds to rabies incidence in Ontario, Canada. Mol.

407   Ecol. **18**(1): 43–53. doi:10.1111/j.1365-294X.2008.03989.x.

408   Dong, N. 2011. Border ice processes on the Saint Lawrence River. M.Sc. thesis, Département de

409   Génie Civil, Université Laval, Quebec City, QC, Canada.

410   Dray, S. and Dufour, A.B. 2007. The ade4 package: implementing the duality diagram for

411   ecologists. Journal of Statistical Software, **22**(4): 1–20.

412   Earl, D.A. and vonHoldt, B.M. 2012. Structure harvester: a website and program for visualizing

413   Structure output and implementing the Evanno method. Con. Gen. Res, **4**(2), 359-361.

414   doi:10.1007/s12686-011-9548-7

415   Elton, C., and Nicholson, M. 1942. The ten-year cycle in numbers of the lynx in Canada. J.

416   Animal Ecol. **11**(2): 215-244.

417   Environment Canada. 2005. Manual of standard procedures for observing and reporting ice

418   conditions (MANICE). Meteorological Service of Canada. Available from

419   http://www.ec.gc.ca/glaces-ice/default.asp?lang=En&n=4FF82CBD-1 [accessed 11

420   August 2014].

421   Environment Canada. 2013. Hydrography of the St. Lawrence River. Available from

422   http://www.ec.gc.ca/stl/default.asp?lang=En&n=59C4915D-1 [accessed 11 August

423   2014].

424   Epps, C.W., Palsbøll, P.J., Wehausen, J.D., Roderick, G.K., Ramey, R.R., and McCullough, D.R.

425   2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert

426   bighorn sheep. Ecol. Lett. **8**(10): 1029–1038. doi: 10.1111/j.1461-0248.2005.00804.x.

427   Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from

428   metric distances among DNA haplotypes: application to human mitochondrial DNA

429   restriction data. Genetics, **131**: 479–491.

430   Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals

431   using the software STRUCTURE: a simulation study. Mol. Ecol. **14**(8): 2611-2620.

432   doi:10.1111/j.1365-294X.2005.02553.x

433   Fairley, T.L., Povoa, M.M., and Conn, J.E. 2002. Evaluation of the Amazon River delta as a barrier

434   to gene flow for the regional malaria vector, *Anopheles aquasalis* (Diptera: Culicidae) in

435   northeastern Brazil. J. Med. Entomol. **39**(6): 861–869.

436   doi:http://dx.doi.org/10.1603/0022-2585-39.6.861.

437   Feierabend, D., and Kielland, K. 2014. Multiple crossings of a large glacial river by Canada lynx

438   (*Lynx canadensis*). Can. Field-Nat. **128**(1): 80–83.

439   Fisheries and Oceans Canada. 2001. Icebreaking operations levels of service. Available from

440   http://www.ccg-gcc.gc.ca/eng/CCG/Ice_Service_Standards [accessed 11 August 2014].

441   Fisheries and Oceans Canada. 2012. Ice climatology and environmental conditions. *In*: Ice

442   navigation in Canadian waters. Icebreaking Program, Maritime Services Canadian Coast

443   Guard, Fisheries and Oceans Canada. Cat. No. Fs154-31/2012E-PDF.

444   Fuller, T.K., and Robinson, W.L. 1982*a*. Some effects of winter shipping on movements of

445   mammals across river ice. Wildl. Soc. Bull. **10**(2): 156–160.

446   Fuller, T.K., and Robinson, W.L. 1982*b*. Winter movements of mammals across a large northern

447   river. J. Mammal. **63**(3): 506–510.

448   Garroway, C.J., Bowman, J., and Wilson, P.J. 2011. Using a genetic network to parameterize a

449   landscape resistance surface for fishers, *Martes pennanti*. Mol. Ecol. **20**(19): 3978–3988.

450   doi:10.1111/j.1365-294X.2011.05243.x.

451   Gaston, A.J., Gavrilo, M., and Eberl, C. 2012. Ice bridging as a dispersal mechanism for Arctic

452   terrestrial vertebrates and the possible consequences of reduced sea ice cover.

453   Biodiversity, **13**(3-4): 182–190. doi:10.1080/14888386.2012.719177.

454   Goldman, E.A. 1937. The Colorado River as a barrier in mammalian distribution. J.

455   Mammal. **18**(4): 427–435.

456   Grinnell, J. 1914. An account of the mammals and birds of the lower Colorado Valley: With

457   especial reference to the distributional problems presented (Vol. 12, No. 4). University

458   of California Press.

459   Government of Newfoundland and Labrador. 2012. News release: Genetic retesting of DNA

460   confirms second wolf on island of Newfoundland. Available at

461   http://www.releases.gov.nl.ca/releases/2012/env/0823n04.htm [accessed 11 August

462   2014].

463   Haffer, J.R. 1997. Alternative models of vertebrate speciation in Amazonia: an overview.

464   Biodivers. Conserv. **6**: 451–476. doi: 10.1023/A:1018320925954.

465   Hauser, L., Seamons, T.R., Dauer, M., Naish, K.A., and Quinn, T.P. 2006. An empirical verification

466   of population assignment methods by marking and parentage data: hatchery and wild

467   steelhead (*Oncorhynchus mykiss*) in Forks Creek, Washington, USA. Mol. Ecol. **15**(11):

468   3157-3173. doi:10.1111/j.1365-294X.2006.03017.x.

469   Hayes, F.E., and Sewlal, J.A.N. 2004. The Amazon River as a dispersal barrier to passerine birds:

470   effects of river width, habitat and taxonomy. J. Biogeogr. **31**(11): 1809–1818. doi:

471   10.1111/j.1365-2699.2004.01139.x.

472   Homyack, J.A., Vashon, J.H., Libby, C., Lindquist, E.L., Loch, S., McAlpine, D.F., Pilgrim, K.L., and

473   Schwartz, M.K. 2008. Canada lynx-bobcat (*Lynx canadensis* × *L. rufus*) hybrids at the

474   southern periphery of lynx range in Maine, Minnesota and New Brunswick. Am. Midl.

475   Nat. **159**(2): 504–508. doi:http://dx.doi.org/10.1674/0003-

476   0031(2008)159[504:CLLCLR]2.0.CO;2.

477   IUCN. 2013. IUCN Red List of Threatened Species. Version 2013.2. www.iucnredlist.org.

478   Downloaded on 09 April 2014.

479   Jackson, H.H.T. 1920. An apparent effect of winter inactivity upon distribution of mammals. J.

480   Mammal. **1**(2): 58–64.

481   Jakobsson, M., and Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation

482   program for dealing with label switching and multimodality in analysis of population

483   structure. Bioinformatics, **23**(14): 1801–1806. doi:10.1093/bioinformatics/btm233.

484   Johnston, D.W., Friedlaender, A.S., Torres, L.G., and Lavigne, D.M. 2005. Variation in sea ice

485   cover on the east coast of Canada from 1969 to 2002: climate variability and

486   implications for harp and hooded seals. Climate Res. **29**(3): 209–222.

487   Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers.

488   Bioinformatics, **24**(11): 1403–1405. doi:10.1093/bioinformatics/btn129.

489   Jost, L. 2008. G_{ST} and its relatives do not measure differentiation. Mol. Ecol. **17**(18): 4015–4026.

490   Doi:10.1111/j.1365-294X.2008.03887.x.

491   Kalinowski, S.T. 2005. HP-Rare: a computer program for performing rarefaction on measures of

492   allelic diversity. Mol. Ecol. Notes, **5**(1): 187–189. doi:10.1111/j.1471-8286.2004.00845.x.

493   Kamvar, Z.N., Tabima, J.F., and Grünwald, N.J. 2014. Poppr: an R package for genetic analysis of

494   populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281

495   http://dx.doi.org/10.7717/peerj.281.

496   Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., and Prodöhl, P.A., 2013. diveRsity: An R

497   package for the estimation of population genetics parameters and their associated

498   errors. Methods Ecol. Evol. **4**(8): 782–788. doi: 10.1111/2041-210X.12067.

499   Kerr, J., and Packer, L. 1998. The impact of climate change on mammal diversity in Canada.

500   Environ. Monit. Assess. **49**(2-3): 263–270. doi:10.1023/A:1005846910199.

501   Khidas, K., Duhaime, J., and Huynh, H.M. 2013. Morphological divergence of continental and

502   island populations of Canada lynx. Northeast. Nat. **20**(4): 587–608.

503   doi:http://dx.doi.org/10.1656/045.020.0413.

504   Koen, E.L., Bowman, J., Murray, D.L., and Wilson, P.J. 2014*a.* Climate change reduces genetic

505   diversity of Canada lynx at the trailing range edge. Ecography, **37**(8): 754–762. doi:

506   10.1111/j.1600-0587.2013.00629.x.

507   Koen E.L., Bowman, J., Lalor, J.L., and Wilson, P.J. 2014*b*. Continental-scale assessment of the

508   hybrid zone between bobcat and Canada lynx. Biol. Conserv. 178:107-115.

509   Laliberte, A.S. and Ripple, W.J. 2004. Range contractions of North American carnivores and

510   ungulates. Bioscience, **54**(2): 123–138. doi:10.1641/0006-

511   3568(2004)054[0123:RCONAC]2.0.C.

512   Lamborot, M., Eaton, L., and Carrasco, B.A. 2003. The Aconcagua River as another barrier to

513   *Liolaemus monticola* (Sauria: Iguanidae) chromosomal races of central Chile. Revista

514   Chilena de Historia Natural, **76:** 23–34.

515   Lougheed, S.C., Gascon, C., Jones, D.A., Bogart, J.P., and Boag, P.T. 1999. Ridges and rivers: a

516   test of competing hypotheses of Amazonian diversification using a dart-poison frog

517   (*Epipedobates femoralis*). Proc. R. Soc. Lond. B Biol. Sci. **266**(1431): 1829–1835.

518   doi:10.1098/rspb.1999.0853

519   Marshall, H.D., Yaskowiak, E.S., Dyke, C., and Perry, E.A. 2011. Microsatellite population

520   structure of Newfoundland black bears (*Ursus americanus hamiltoni*). Can. J. Zool. **89**(9):

521   831–839. doi: 10.1139/z11-056.

522   Menotti-Raymond, M., David, V.A., Lyons, L.A., Schaffer, A.A., Tomlin, J.F., Hutton, M.K., and

523   O'Brien, S.J. 1999. A genetic linkage map of microsatellites in the domestic cat (*Felis

524   catus*). Genomics, **57**(1): 9–23. doi:10.1006/geno.1999.5743.

525 Moen, R., Terwilliger, L., Dohmen, A.R., and Catton, S.C. 2010. Habitat and road use by Canada

526   lynx making long-distance movements. Duluth Center for Water and Environment,

527   Natural Resources Research Institute.

528 Nadin-Davis, S., Muldoon, F., Whitney, H., and Wandeler, A.I. 2008. Origins of the rabies viruses

529   associated with an outbreak in Newfoundland during 2002-2003. J. Wildl. Dis. **44**(1): 86–

530   98. doi:http://dx.doi.org/10.7589/0090-3558-44.1.86.

531 New Brunswick Endangered Species Regulation. 2013. NB Reg 96-26. Available from

532   http://canlii.ca/t/521zl [accessed 11 August 2014].

533 Nordstrom, L. 2005. Recovery outline: contiguous United States distinct population segment of

534   the Canada lynx. US Fish and Wildlife Service, Helena, USA.

535 Nova Scotia Lynx Recovery Team. 2007. Provincial Recovery Plan for the Canada Lynx (*Lynx*

536   *canadensis*), Nova Scotia.

537 O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D., Tonkyn, D.W., and Frankham, R. 2006.

538   Realistic levels of inbreeding depression strongly affect extinction risk in wild

539   populations. Biol. Conserv. **133**(1): 42–51. doi: 10.1016/j.biocon.2006.05.016.

540 Ortega, J., Franco, M., Adams, B.A., Ralls, K., and Maldonado, J.E. 2004. A reliable, non-invasive

541   method for sex determination in the endangered San Joaquin kit fox (*Vulpes macrotis*

542   *mutica*) and other canids. Conserv. Genet. **5**(5): 715–718. doi:10.1007/s10592-004-1862-

543   0.

544 Paetkau, D., and Strobeck, C. 1996. Mitochondrial DNA and the phylogeography of

545   Newfoundland black bears. Can. J. Zool. **74**(1): 192–196. doi:10.1139/z96-023.

546   Parker, G. 2001. Status report on the Canada lynx in Nova Scotia. Nova Scotia Species at Risk

547   Working Group, Sackville, NB.

548   Patton, J.L., Da Silva, M.N.F., and Malcolm, J.R. 1994. Gene genealogy and differentiation

549   among arboreal spiny rats (Rodentia: Echimyidae) of the Amazon basin: a test of the

550   riverine barrier hypothesis. Evolution, **48**(4): 1314–1323.

551   Pearson, G.A., Lago-Leston, A., and Mota, C. 2009. Frayed at the edges: selective pressure and

552   adaptive response to abiotic stressors are mismatched in low diversity edge

553   populations. J. Ecol. **97**(3): 450–462. doi:10.1111/j.1365-2745.2009.01481.x.

554   Peers, M.J.L., Thornton, D.H., and Murray, D.L. 2013. Evidence for large-scale effects of

555   competition: niche displacement in Canada lynx and bobcat. Proc. R. Soc. Lond. B Biol.

556   Sci. **280**: 20132495. doi:10.1098/rspb.2013.2495.

557   Peres, C.A., Patton, J.L., and da Silva, N.F. 1996. Riverine barriers and gene flow in Amazonian

558   saddle-back tamarins. Folia Primatol. **67**(3): 113–124. doi:10.1159/000157213.

559   Poole, K.G. 1997. Dispersal patterns of lynx in the Northwest Territories. J. Wildl. Manage.

560   **61**(2): 497–505.

561   Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using

562   multilocus genotype data. Genetics, **155**(2): 945–959.

563   Quinn, N.W., and Gardner, J.F. 1984. Relationships of age and sex to lynx pelt characteristics. J.

564   Wildl. Manage. **48**(3): 953–956.

565   R Development Core Team. 2014. R: A language and environment for statistical computing. R

566   Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-

567   project.org [accessed 11 August 2014]

568   Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for

569   exact tests and ecumenicism. J. Hered. **86**(3): 248–249

570   Reding, D.M., Carter, C.E., Hiller, T.L., and Clark, W.R. 2013. Using population genetics for

571   management of bobcats in Oregon. Wildl. Soc. Bull. **37**(2): 342–351. doi:

572   10.1002/wsb.243.

573   Roberts, N.M., and Crimmins, S.M. 2010. Bobcat population status and management in North

574   America: evidence of large-scale population increase. J. Fish Wildl. Manage. **1**(2): 169–

575   174. doi:http://dx.doi.org/10.3996/122009-JFWM-026.

576   Rodríguez-Ramilo, S.T., Toro, M.A., and Fernández, J. 2009. Assessing population genetic

577   structure via the maximisation of genetic distance. Genet. Sel. Evol. **41**(1): 49.

578   doi:10.1186/1297-9686-41-49

579   Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population structure.

580   Mol. Ecol. Notes, **4**(1): 137–138. doi:10.1046/j.1471-8286.2003.00566.x.

581   Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for

582   Windows and Linux. Mol. Ecol. Resour. **8**(1): 103–106. doi:10.1111/j.1471-

583   8286.2007.01931.x.

584   Row, J.R., Gomez, C., Koen, E.L., Bowman, J., Murray, D.L., and Wilson, P.J. 2012. Dispersal

585   promotes high gene flow among Canada lynx populations across North America.

586   Conserv. Genet. **13**(5): 1259–1268. doi:10.1007/s10592-012-0369-3.

587   Row, J.R., Wilson, P.J., Gomez, C., Koen, E.L., Bowman, J., Thornton, D., and Murray, D.L. 2014.

588   The subtle role of climate change on population genetic structure in Canada lynx. Global

589   Change Biol. **20**(7): 2076–2086. doi:10.1111/gcb.12526.

590 Ruediger, B., Claar, J., Gniadek, S., Holt, B., Lewis, L., Mighton, S., Rinaldi, T. Trick, J., Vandehrey,

591   A., Wahl, F., Warren, N., Wenger, D. and Williamson, A. 2000. Canada lynx conservation

592   assessment and strategy. USDA Forest Service, USDI Fish and Wildlife Service, USDI

593   Bureau of Land Management, and USDI National Park Service, Missoula, MT, USA.

594 Rueness, E.K., Stenseth, N.C., O'Donoghue, M., Boutin, S., Ellegren, H., and Jakobsen, K.S. 2003.

595   Ecological and genetic spatial structuring in the Canadian lynx. Nature, **425**(6953): 69–

596   72. doi:10.1038/nature01942.

597 Saunders Jr, J.K. 1964. Physical characteristics of the Newfoundland lynx. J. Mammal. **45**(1): 36–

598   47.

599 Schwartz, M.K., Mills, L.S., McKelvey, K.S., Ruggiero, L.F., and Allendorf, F.W. 2002. DNA reveals

600   high dispersal synchronizing the population dynamics of Canada lynx. Nature,

601   **415**(6871): 520–522. doi:10.1038/415520a.

602 Schwartz, M.K., Pilgrim, K.L., McKelvey, K.S., Lindquist, E.L., Claar, J.J., Loch, S., and Ruggiero, L.

603   F. 2004. Hybridization between Canada lynx and bobcats: genetic results and

604   management implications. Conserv. Genet. **5**(3): 349–355.

605   doi:10.1023/B:COGE.0000031141.47148.8b.

606 Slough, B.G. 1996. Estimating lynx population age ratio with pelt-length data. Wildl. Soc. Bull.

607   **24**: 495–499.

608 Slough, B.G., and Mowat, G. 1996. Lynx population dynamics in an untrapped refugium. J. Wildl.

609   Manage. **60**(4): 946–961.

610 Squires, J.R., and Laurion, T. 2000. Lynx home range and movements in Montana and Wyoming:

611   preliminary results. *In* Ecology and Conservation of Lynx in the United States. *Edited by*

612   L.F. Ruggiero, K.B. Aubry, S.W. Buskirk, G.M. Koehler, C.J. Krebs, K.S. McKelvey, and J.R.

613   Squires. University Press of Colorado, Boulder, USA. pp. 337–349.

614   Squires, J.R., and Oakleaf, R. 2005. Movements of a male Canada lynx crossing the greater

615   Yellowstone area, including highways. Northwest Sci. **79**(2-3): 196–201.

616   U.S. Fish and Wildlife Service. 2000. Endangered and threatened wildlife and plants;

617   determination of threatened status for the contiguous U.S. distinct population segment

618   of the Canada lynx and related rule; final rule. Federal Register. **65**(58): 16052–16086.

619   Van Oosterhout, C., Hutchinson, W.F., Wills, D.P., and Shipley, P. 2004. MICROCHECKER:

620   software for identifying and correcting genotyping errors in microsatellite data. Mol.

621   Ecol. Notes, 4(3): 535–538.

622   van Zyll de Jong, C.G. 1975. Differentiation of the Canada lynx, *Felis* (Lynx) *canadensis*

623   *subsolana*, in Newfoundland. Can. J. Zool. **53**(6): 699–705. doi:10.1139/z75-085.

624   Voelker, G., Marks, B.D., Kahindo, C., A'genonga, U., Bapeamoni, F., Duffie, L.E., Huntley J.W.,

625   Mulotwa, E., Rosenbaum, S.A., and Light, J.E. 2013. River barriers and cryptic

626   biodiversity in an evolutionary museum. Ecol. Evol. **3**(3): 536–545.

627   doi:10.1002/ece3.482.

628   Wallace, A.R. 1852. On the monkeys of the Amazon. Proc. Zool. Soc. Lond. **20**: 107–110.

629   Ward, R.M., and Krebs, C.J. 1985. Behavioural responses of lynx to declining snowshoe hare

630   abundance. Can. J. Zool. **63**(12): 2817–2824. doi:10.1139/z85-421.

631   Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population

632   structure. Evolution, **38**(6): 1358–1370.

633   Woods, J.G., Paetkau, D., Lewis, D., McLellan, B.N., Proctor, M., and Strobeck, C. 1999. Genetic

634   tagging of free-ranging black and brown bears. Wildl. Soc. Bull. **27**(3): 616–627.

635   Zigouris J., Dawson, F.N., Bowman, J., Gillett, R.M., Schaefer, J.A., and Kyle, C.J. 2012. Genetic

636   isolation of wolverine (*Gulo gulo*) populations at the eastern periphery of their North

637   American distribution. Conserv. Genet. **13**(6): 1543–1559. doi:10.1007/s10592-012-

638   0399-x.

639

640   Table 1. Summary statistics for 14 microsatellite loci used to genotype Canada lynx (*Lynx*

641   *canadensis*) from Quebec (*n* = 496), New Brunswick (*n* = 15), Labrador (*n* = 18), and

642   Newfoundland (*n* = 29).

Locus ID[a]	No. alleles	H_o	H_e
Fca31	8	0.689	0.740
Fca35	21	0.760	0.873
Fca391	7	0.705	0.741
Fca43	6	0.620	0.632
Fca441	7	0.720	0.771
Fca559	18	0.826	0.874
Fca77	7	0.667	0.723
Fca90	6	0.428	0.482
FCA96	9	0.743	0.801
Lc106	8	0.640	0.710
Lc109	8	0.720	0.826
Lc110	9	0.740	0.812
Lc111	8	0.689	0.724
Lc118	8	0.725	0.735

643   [a] Locus names beginning with Lc were developed from *Lynx canadensis* (Carmichael et al. 2000)

644   and locus names beginning with Fca were developed from *Felis catus* (Menotti-Raymond et al.

645   1999).

646   Table 2. Allelic richness[a] and private allelic richness[b] of 558 Canada lynx (*Lynx canadensis*)

647   grouped by sample location[c] and corrected for a sample size of 15 using rarefaction, and

648   inbreeding coefficient estimates (F_{is})[d].

	Allelic richness	Private allelic richness	F_{is}		
			Estimate	95% CI (lower)	95% CI (upper)
QC north	5.19	0.40	0.021	0.005	0.037
QC south	4.33	0.13	0.051	0.022	0.079
NB	4.32	0.20	-0.016	-0.134	0.084
LAB	5.47	0.56	-0.027	-0.102	0.044
NFLD	3.35	0.27	0.127	0.033	0.222

649   [a] Average number of alleles per locus

650   [b] Average number of alleles per locus that are unique to a site

651   [c] We grouped lynx by harvest location: north of the St. Lawrence River in Quebec (QC north; *n* =

652   331), south of the St. Lawrence River in Quebec (QC south; *n* = 165), New Brunswick (NB; *n* =

653   15), Labrador mainland (LAB; *n* = 18), and Newfoundland (NFLD; *n* = 29).

654   [d] F_{IS} and 95% confidence limits (999 bootstraps) estimated with the R package diversity (Keenan

655   et al. 2013)

656 Table 3. Pairwise F_{ST} (Weir and Cockerham 1994; lower) and D_{est} (Jost 2008; upper), with 95%

657 confidence intervals in brackets, of 558 Canada lynx (*Lynx canadensis*) samples in Canada,

658 grouped by sample location[a].

	QC north	QC south	NB	LAB	NFLD
QC north		0.109 (0.093-0.126)	0.100 (0.068-0.156)	0.020 (0-0.062)	0.177 (0.130-0.218)
QC south	0.053 (0.045-0.060)		0.016 (0-0.052)	0.105 (0.057-0.160)	0.196 (0.145-0.242)
NB	0.045 (0.028-0.068)	0.006 (0-0.029)		0.101 (0.044-0.169)	0.193 (0.130-0.263)
LAB	0.005 (0-0.022)	0.049 (0.028-0.074)	0.046 (0.014-0.082)		0.177 (0.102-0.256)
NFLD	0.154 (0.121-0.181)	0.220 (0.186-0.248)	0.239 (0.188-0.289)	0.179 (0.125-0.230)	

659 [a] We grouped lynx by harvest location: north of the St. Lawrence River in Quebec (QC north; *n* =

660 331), south of the St. Lawrence River in Quebec (QC south; *n* = 165), New Brunswick (NB; *n* =

661 15), Labrador mainland (LAB; *n* = 18), and Newfoundland (NFLD; *n* = 29).

662 Table 4. Presence of an ice bridge[a] across the St. Lawrence River east of Quebec City, Quebec[b]

663 or across the Strait of Belle Isle between Newfoundland and mainland Canada.

Season[c]	St. Lawrence River			Strait of Belle Isle		
	No. weeks[d]	Date of first bridge	Date of last bridge	No. weeks[d]	Date of first bridge	Date of last bridge
2004	6	12/01/2004	01/03/2004	10	10/02/2004	26/04/2004
2005	8	13/12/2004	14/03/2005	10	17/01/2005	28/03/2005
2006	3	26/12/2005	20/02/2006	10	23/01/2006	03/04/2006
2007	3	12/02/2007	19/03/2007	15	29/01/2007	08/05/2007
2008	10	17/12/2007	24/03/2008	12	14/01/2008	28/04/2008
2009	8	22/12/2008	09/03/2009	13	19/01/2009	20/04/2009
2010	5	21/12/2009	01/02/2010	3	8/02/2010	26/04/2010
2011	8	27/12/2010	14/03/2011	3	21/02/2011	28/03/2011

664 [a] We defined an ice bridge as ice (concentration ≥9 and thickness >10cm) connecting both

665 banks of the waterway

666 [b] Data are from weekly ice charts obtained from the Canadian Ice Service Archive

667 (Meteorological Service of Canada, Environment Canada)

668 [c] We defined a season as 1 Dec – 15 May (24 weeks). For example, 2004 corresponds to 1 Dec

669 2003 – 15 May 2004

670 [d] The number of weeks (not necessarily consecutive) during the season that there was an ice
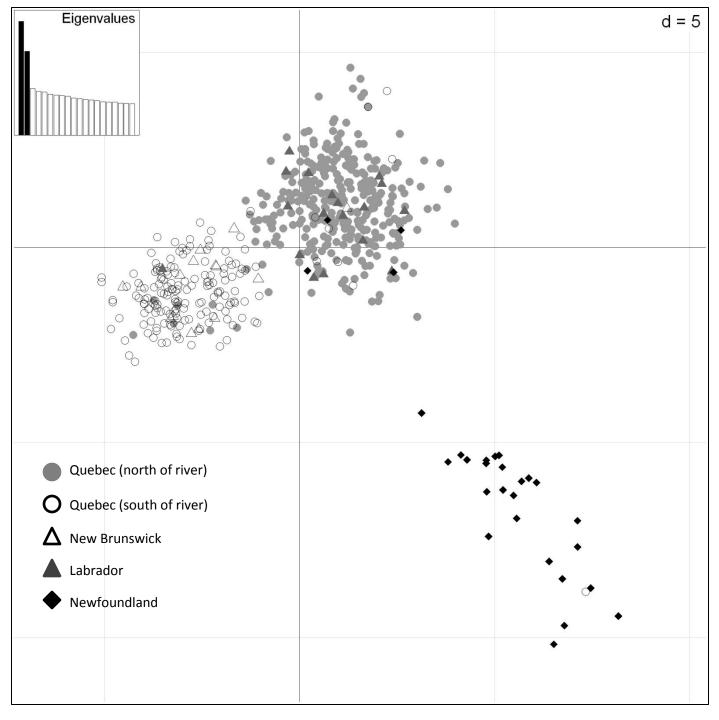
671 bridge

672   Figure 1. Location of 558 Canada lynx (*Lynx canadensis*) samples, with circle radius representing

673   the number of samples collected within each harvest unit (Unités de Gestion des Animaux à

674   Fourrure in Quebec) or province. "QC north" indicates the region of Quebec that is north of the

675   St. Lawrence River, and "QC south" indicates the region of Quebec that is south of the St.

676   Lawrence River. "M" and "QC" represent the location of Montreal and Quebec City,

677   respectively, on the St. Lawrence River. "A" represents St. Anthony on the Strait of Belle Isle.

678   The inset map indicates the study area and current distribution of Canada lynx in North America

679   (grey), reproduced with permission (IUCN 2013). NFLD = Newfoundland, NS = Nova Scotia, NB =

680   New Brunswick, ME = Maine, NH = New Hampshire, VT = Vermont, NY = New York.

681

682   Figure 2. Plot of principal component axes 1 and 2, showing genetic clustering of 558 Canada

683   lynx (*Lynx canadensis*) found north (QC north) and south (QC south) of the St. Lawrence River in

684   Quebec, New Brunswick, Labrador, and Newfoundland, Canada. Symbols represent sample

685   locations.

686

687   Figure 3. Structure plot (Pritchard et al. 2000), representing the proportion of an individual's

688   genome assigned to one of three populations, based on 10 replicates. Individual lynx (*Lynx*

689   *canadensis*) are grouped based on sample site (QC north = north of the St. Lawrence River in

690   Quebec, QC south = south of the river, NB = New Brunswick, LAB = Labrador, and NFL =

691   Newfoundland), and shading represents cluster assignment.

692

693   Figure 4. Locations of 558 Canada lynx (*Lynx canadensis*) samples representing three genetic

694   clusters: north of the St. Lawrence River (dark grey: Quebec and Labrador), south of the St.

695   Lawrence River (light grey: Quebec and New Brunswick), and Newfoundland (black diamonds),

696   with admixed individuals (0.3 ≤ Q ≤ 0.7) represented by X. We randomly located samples within

697   the respective harvest management units or near the centroid of Newfoundland and Labrador

698   for presentation.

Labrador

Quebec

Ontario

QC north

QC

M

QC south

NB

ME

NS

VT

NY

NH

A

St. Lawrence R.

Strait of
Belle Isle

NFLD

No. samples

· 1 - 5

· 6 - 10

● 11 - 15

● 16 - 20

● 21 - 25

● 26 - 50

0   500 km

QC (north)   QC (south)   NB LAB NFL