Population subdivision and peripheral isolation in American badgers (*Taxidea taxus*) and implications for conservation planning in Canada

D.M. Ethier, A. Laflèche, B.J. Swanson, J.J. Nocera, and C.J. Kyle

Abstract: In Canada, three subspecies of American badgers (*Taxidea taxus* (Schreber, 1777)) traditionally are identified; two of which are listed as endangered because of their restricted geographic range and low population sizes. To verify their subspecific designations and genetic insularity, we analyzed mitochondrial control region sequences within and among badger subspecies (*Taxidea taxus jacksoni* Schantz, 1946, *Taxidea taxus jeffersonii* (Harlan, 1825), and *Taxidea taxus taxus* (Schreber, 1777)) from nine locations in Canada and bordering United States. Although subspecies designations were supported (a priori subspecific designations, $n = 3$, AMOVA: $F_{ST} = 0.40$, $p < 0.001$), insular populations also were found within subspecific ranges as shown by spatial analysis of molecular variation, which suggested that our sample set consisted of five genetic groups ($F_{ST} = 0.39$, $p < 0.001$). These five distinct groupings included the subdivision of *T. t. jeffersonii* on either side of the Selkirk Mountains, and of *T. t. jacksoni* in the western part of its range grouping more closely with *T. t. taxus* of Manitoba. These results indicate that endangered populations of badgers may be more segregated than previously identified using morphological characteristics as proxies for subspecific designation. These results have important implications for the conservation of badgers in Canada, particularly of the two endangered subspecies.

Key words: Taxidea taxus, American badger, mtDNA, subspecies, genetic diversity.

Résumé : Au Canada, trois sous-espèces de blaireaux d'Amérique (*Taxidea taxus* (Schreber, 1777)) sont habituellement identifiées, dont deux figurent sur la liste des espèces en voie de disparition en raison de leurs aires de répartition limitées et de la faible taille de leurs populations. Afin de vérifier les affectations et l'insularité génétique de ces sous-espèces, une analyse a été effectuée des séquences de région de contrôle de l'ADN mitochondrial au sein et entre des sous-espèces de blaireaux (*Taxidea taxus jacksoni* Schantz, 1946, *Taxidea taxus jeffersonii* (Harlan, 1825) et *Taxidea taxus taxus* (Schreber, 1777)) provenant de neuf localités au Canada et dans des États limitrophes des États-Unis. Si les résultats appuient la désignation des sous-espèces (résultats d'AMOVA pour $n = 3$ sous-espèces désignées a priori : $F_{ST} = 0{,}40$, $p < 0{,}001$), des populations insulaires ont également été cernées dans des aires sous-spécifiques, comme l'indique l'analyse spatiale de la variation moléculaire, qui suggère que l'ensemble d'échantillons comprenait cinq groupes génétiques distincts ($F_{ST} = 0{,}39$, $p < 0{,}001$). La distribution de ces cinq groupes comprend la subdivision de *T. t. jeffersonii* en deux sous-groupes, de part et d'autre des monts Selkirk, et de *T. t. jacksoni* dans la portion occidentale de son aire, où il rejoint plus étroitement *T. t. taxus* du Manitoba. Ces résultats indiquent que les populations de blaireaux en voie de disparition pourraient être plus ségrégées que ce que laissaient croire les caractéristiques morphologiques utilisées par le passé pour la désignation des sous-espèces. Ils sont également importants en ce qui concerne la conservation des blaireaux au Canada, particulièrement celle des deux sous-espèces en voie de disparition.

Mots-clés : Taxidea taxus, blaireau d'Amérique, ADNmt, sous-espèce, diversité génétique.

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Introduction

Genetic diversity is important for the maintenance of population viability and adaptive potential of species. Identifying genetically distinct populations is therefore essential to maintaining high levels of biodiversity and reducing the probability of extinction (Waples 1991). Populations of most species show some degree of genetic structuring, which may be related to a variety of factors. For example, geographic distribution, phlyogeography, life-history characteristics, and barriers to dispersal may contribute all, in part, to shaping genetically distinct populations (Balloux and Lugon-Moulin 2002). Understanding the degree to which each of these factors influence patterns of genetic exchange between groups is

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thus central in conservation biology, where reliable estimates of population differentiation and genetic isolation are needed for the protection of rare or endangered species.

American badgers (*Taxidea taxus* (Schreber, 1777)) are solitary semifossorial mustelids whose range spans much of central and western North America. In Canada, three extant subspecies of badger are recognized: *Taxidea taxus jeffersonii* (Harlan, 1825) in southeastern British Columbia, *Taxidea taxus taxus* (Schreber, 1777) in the Prairie Provinces, and *Taxidea taxus jacksoni* Schantz, 1946 in the Great Lakes region of Ontario (Long 1972; Fig. 1). Negative population trends caused by habitat fragmentation, loss of prey, road mortality, and persecution (Rahme et al. 1995; Newhouse and Kinley 2000; Scobie 2002; Apps et al. 2002) characterize the conservation status of badgers. As a result, *T. t. jacksoni* and *T. t. jeffersonii* have been recognized as being endangered in Canada (COSEWIC 2000) with fewer than 200 and 600 individuals remaining, respectively. Furthermore, *T. t. taxus* in Alberta is recognized as a sensitive species (Scobie 2002). In some habitat types (e.g., shrub–steppe), badgers occur at relatively high population densities for a medium-sized carnivore (0.38–5.0 badgers/km^2), with male home ranges overlapping those of several females during the breeding season (Lindzey 1971; Messick and Hornocker 1981; Goodrich and Buskirk 1998). At their range limits, badgers can occur at low, but variable, densities (Kinley and Newhouse 2008) and may exhibit source–sink population dynamics. Badgers typically disperse at 3–4 months of age (Lindzey 1982), when juvenile females can travel >50 km and males >110 km (Messick and Hornocker 1981). Given these life-history characteristics, genetic structure between badger populations may be low because a large effective population size prevents genetic drift, whereas dispersers prevent the loss of allelic variation between populations. Conversely, small isolated populations of badgers, which tend to occur at the periphery of the species range (e.g., Ontario and British Columbia), may be subject more to genetic drift, fixation of deleterious mutation, local adaptation, and local extirpation.

Subspecies of badgers originally were described based on variation in morphological characteristics and geographic distribution (Long 1972). Such regional differences can reflect environmental epigenetic effects, or stem from limited genetic exchange. Morphometrics can be used to coarsely identify management units, but subspecific designations confirmed using molecular genetic approaches provide more insight into the evolutionary significance of these taxonomic divisions (O'Brien and Mayr 1991; O'Brien 1994). The objective of our research therefore was to assess the partitioning of mitochondrial DNA (mtDNA) control region variation to determine if the traditional subspecies hypothesis based on morphometrics is concordant with evolutionary lineages defined by mtDNA analysis. To accomplish this, we investigate the level of genetic diversity within and among the three traditionally defined subspecies, and describe the phylogenetic relationship of these populations. Identifying discrete populations and understanding their interrelationship will provide information needed for badger conservation planning in Canada.

Materials and methods

Sample collection

We collected blood, hair, and tissue samples from three morphologically recognized badger subspecies: *T. t. jacksoni* from southwestern Ontario ($n = 26$) and the Upper (UP; $n = 18$) and Lower (LP; $n = 18$) Peninsulas of Michigan; *T. t. jeffersonii* from the East Kootenay ($n = 46$) and Thompson–Okanagan ($n = 10$) regions of British Columbia; and *T. t. taxus* from Alberta ($n = 42$), north-central Montana ($n = 12$), southern Manitoba ($n = 56$), and Saskatchewan ($n = 27$) (Fig. 1; supplementary Table S1[1]). Samples were obtained from previous field studies in Ontario and British Columbia (see Kyle et al. 2004) or from fur harvests for samples from Manitoba, Saskatchewan, Alberta, and Michigan. We grouped samples, a priori, into the aforementioned units based on spatial proximity and unified ecological features such as mountain ranges, lakes, and rivers.

Laboratory procedure

We used a Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc.) to isolate genomic DNA, whereby conserved primers described by Delisle and Strobeck (2005) were used to amplify an 817 base pair (bp) segment of the mtDNA control region. The nucleotide sequences of the primers that we used were mtDloopU (5′-CTAACATGAATCGGAGGACAACCAG-3′) and mtDloopL.int2 (5′-TATGTCCTGCGACCATTGACT-3′). We selected the control region (d-loop) because the presence of conserved regulatory elements in combination with more rapidly evolving segments makes it useful for studies of microevolution (Tarr 1995). The mitochondrial genome is also phylogenetically informative as a result of maternal inheritance, lack of recombination, and relatively high variability (Hartl and Clark 1997).

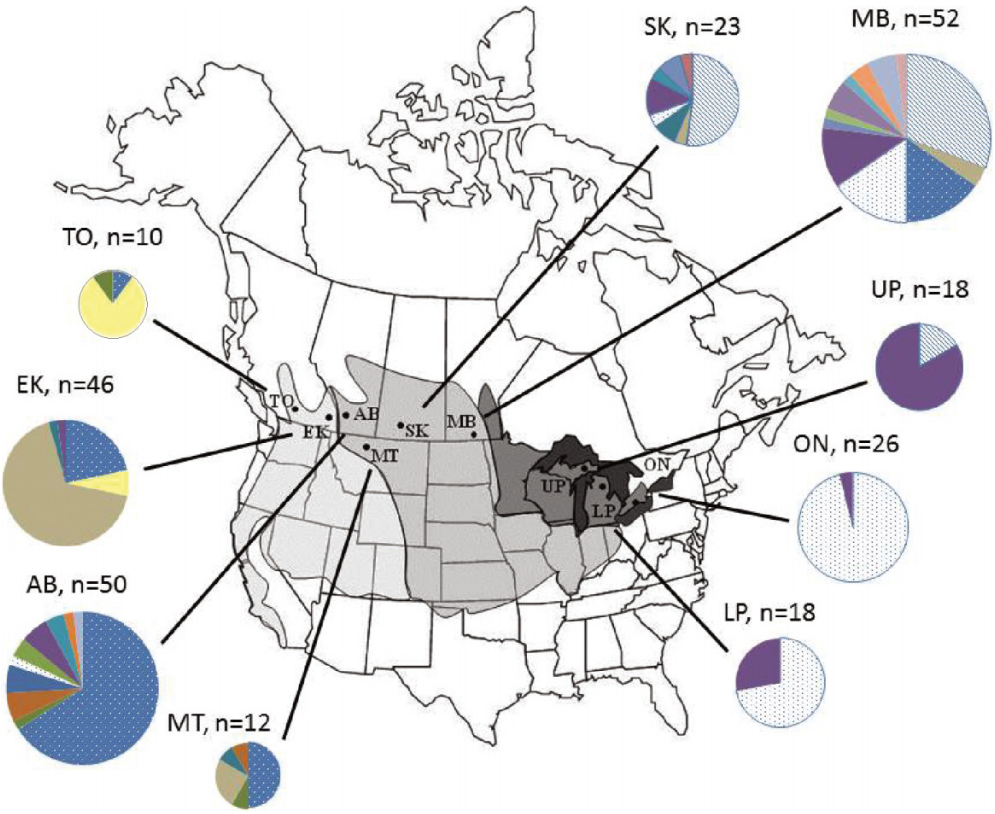
DNA amplifications were performed in 15 μL reactions containing 1 × buffer, 0.2 mmol/L dNTPs, 2.5 mmol/L $MgCl_2$, 0.2 mg/mL BSA, 0.33 mmol/L of each primer, 1U of *Taq* DNA polymerase (Invitrogen), and ~10 ng of DNA extract as template. Polymerase chain reaction (PCR) was performed in a Dyad Disciple Peltier Thermal Cycler (Bio-Rad Laboratories, Inc.) programmed for an initial 5 min denaturation step at 94 °C, followed by 35 cycles of the following steps: denaturation at 94 °C for 60 s, annealing at 60 °C for 60 s, and extension at 72 °C for 60 s, and a final extension step at 60 °C for 45 min. Each batch of PCR included an extraction blank and negative control (no template added). We separated and quantified amplified products via electrophoresis in 1.5% agarose gels stained with EtBr. PCR products were then purified using Exonuclease I and Antarctic Phosphatase Buffer (New England BioLabs) to remove any unincorporated primers and excess dNTPs. We obtained forward and reverse sequences by using BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, Inc.). Sequencing was performed on an automated DNA sequencer (ABI 3730; Applied Biosystems, Inc.).

Sequence analysis

We performed sequencing in one direction providing 555 bp of high-quality sequence data. New variants and sin-

[1]Supplementary Table S1 is available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/z2012-029).

Fig. 1. Map of North America indicating sampling locations (●) and range distribution of American badger subspecies (*Taxidea taxus jeffersonii*, light grey; *Taxidea taxus taxus*, medium grey; *Taxidea taxus jacksoni*, dark grey). Mitochodrial DNA haplotype frequencies for each sampling location ($n = 9$) are illustrated with pie chats, where each colour (on the Web, shades of grey in print) corresponds to a different haplotypes and the size of the pie chat corresponds to sample size. EK, East Kootenay; TO, Thompson–Okanagan; MT, Montana; AB, Alberta; SK, Saskatchewan; MB, Manitoba; UP, Upper Peninsula of Michigan; LP, Lower Peninsula of Michigan; ON, Ontario.



gularly occurring haplotypes were confirmed by sequencing in both the forward and the reverse directions. We aligned sequences using the CLUSTAL W function in MEGA version 4.0 (Tamura et al. 2007) and we checked for errors manually. We used Arlequin version 3.11 (Excoffier et al. 2005) to calculate estimates of haplotype (h) and nucleotide (π) diversity for regional populations, subspecies, and for the entire data set, where h is the probability that any two randomly selected samples will not have the same haplotype and π is the mean number of nucleotide differences per site between two samples (Nei and Kumar 2000). To test for in situ population growth and neutrality, we applied Tajima's D and Fu's F_S test, where D is calculated to compare the number of segregating sites in relation to the mean number of nucleotide difference between DNA sequences (Tajima 1989) and F_S is the probability of exhibiting an excess of rare haplotypes compared with a neutral population (F_S should be considered significant if $p < 0.02$; Fu 1997). We permuted all calculations 10 000 times. Haplotype richness and number of haplotypes private to a population were calculated along with corrected estimates using ADZE version 1.0 (Szpiech et al. 2008), which employs a rarefaction approach to compensate for differences in sample sizes.

To test the subspecies hypothesis, as well as alternative scenarios, of badger genetic structure, we used analysis of molecular variance (AMOVA) and spatial AMOVA (SAMOVA). We used Arlequin to run the AMOVA to test a priori groupings based on morphological subspecies designations ($K = 3$) described by Long (1972). We used the program SAMOVA (Dupanloup et al. 2002) to find geographically continuous groups that are maximally differentiated, without the need to make prior assumptions about group structure. SAMOVA iteratively seeks for a user-defined number of groups (K) that maximizes the total genetic variance resulting from differences among groups (ϕ_{CT}) and minimizes the genetic variance shared between populations within groups (ϕ_{ST}). To estimate regional differences in genetic structure based on sampling locations ($K = 9$), we calculated population pairwise F_{ST} in Arlequin using 10 000 permutations under the hypothesis of no difference between regions. Significance levels for F_{ST} were determined using a conservative $\alpha \leq 0.01$ to compensate for familywise type I error. To test whether genetic structure (pairwise F_{ST} values) was a function of geographic distance, we ran a Mantel test (Mantel 1967) in Arlequin (10 000 iterations).

Sequences were analyzed using Modeltest version 3.7 (Posada and Crandall 1998) to determine the best model of nucleotide evolution. We selected the model by evaluating Akaike's information criterion (Akaike 1974; Posada and Buckley 2004). We then retrieved Bayesian estimations of phylogeny in BEAST version 1.6.1 (Drummond and Rambaut 2007) and constructed a phylogenetic network using the median-joining option (Bandelt et al. 1999) and maximum parsimony construction (Polzin and Daneschmand 2003) in Network version 4.5

(available from http://www.fluxus-engineering.com/sharenet.htm, accessed 7 February 2012). Wolverines (*Gulo gulo* (L., 1758)) (GenBank accession No. AM711901) and fishers (*Martes pennanti* (Erxleben, 1777)) (GenBank accession No. HQ705177) were selected as an out-group given their phylogenetic relationship with North American badgers (Delisle and Strobeck 2005).

Results

From the 255 badger samples analysed, we identified 20 haplotypes with a total of 16 variable sites: 15 transitions and a 1 base deletion (GenBank accession Nos. GU901415–GU901669). Six of the haplotypes were found in more than one subspecies range; one was distributed universally across all subspecies in Canada (HAP10), four occurred in *T. t. jeffersonii* and *T. t. taxus* (HAP1, HAP3, HAP4, HAP5), and one was shared by *T. t. jacksoni* and *T. t. taxus* (HAP8) (Fig. 1). Both haplotype and nucleotide diversities varied across regions and subspecies ranges (Tables 1*a*–1*d*). Global haplotype diversity was high (0.82 ± 0.01), whereas global nucleotide diversity was relatively low (0.006 ± 0.004) (Table 1*d*). Peripheral badger populations displayed depressed levels of haplotype and nucleotide diversities, with Ontario ranking lowest (haplotype: 0.08 ± 0.07; nucleotide: <0.001). Among subspecies, *T. t. taxus* presented higher levels of haplotype (0.74 ± 0.04) and nucleotide diversity (0.006 ± 0.004), relative to *T. t. jeffersonii* (haplotype: 0.55 ± 0.07; nucleotide: 0.005 ± 0.003) and *T. t. jacksoni* (haplotype: 0.52 ± 0.04; nucleotide: 0.002 ± 0.001) (Table 1*b*). Neutrality tests using Tajima's D and Fu's F_S were nonsignificant for the global test (Table 1*d*), which implies that there were no selective pressures acting on the species with regards to the mtDNA control region. When populations were considered independently, there was a significantly negative Tajima's D for the Thompson–Okanagan region (Tajima's D = –1.87, $p < 0.01$; Tables 1*a*, 1*c*), suggesting that this population may have recently undergone expansion and (or) experienced positive selection (Tajima 1989). Adjusted measures of total allelic and private allelic richness were generally lower than raw estimates (Tables 1*a*–1*d*). Overall patterns between raw and adjusted values were consistent, with badgers from the Prairie region (Alberta, Saskatchewan, Montana, and Manitoba) having the greatest amount of haplotype (range: 4.045–6.088) and private haplotype richness (range: 0.783–2.443) adjusted for sample size. Badgers from Ontario and the UP of Michigan had the lowest levels of adjusted haplotype richness (range: 1.385–1.931) and lacked private haplotypes.

AMOVA lent support to the traditionally defined subspecies structure (F_{ST} = 0.40, $p < 0.001$; Table 2). Interpopulation variation was relatively high for a carnivore with moderate dispersal abilities at 17.9%, which suggests limited gene flow between subspecies. SAMOVA identified $K = 5$ as the optimal number of genetic groups from the nine regions sampled, based on the degree of change in ϕ_{CT} (Table 2). This group configuration also resulted in a significant relationship as determined by AMOVA (F_{ST} = 0.39, $p < 0.001$; Table 2). The SAMOVA explains considerably more of the interpopulation variation (39.0%) than the traditionally defined subspecies, suggesting a higher degree of regional subdivision than described previously. The groups proposed by SAMOVA are fairly analogous to those defined by subspecies, with some notable differences, including the subdivision of *T. t. jeffersonii* on either side of the Selkirk Mountains and *T. t. jacksoni* from the UP of Michigan grouping more closely with *T. t. taxus* of Manitoba (Table 2). Higher levels of haplotype and nucleotide diversity were observed in the prairie badgers, relative to the peripheral populations (Table 1*c*). Pairwise F_{ST} values further support the assertion of strong genetic subdivision in American badgers (0.35 ± 0.23, mean ± SD). All regions were genetically differentiated, with exceptions noted in Table 3. The isolation by distance model (IBD) was supported by the Mantel test ($p <$ 0.001, $R = 0.67$).

The Hasegawa, Kishino, and Yano (HKY; Hasegawa et al. 1985) (A: 0.28; C: 0.25; G: 0.17; T: 0.30) evolutionary model best fit the empirical sequence data according to AIC, with 91% invariable sites and a gamma distribution shape parameter of 0.69. Bayesian and maximum parsimony analyses recovered concordant phylogenetic lineages among the studied badger populations. These lineages displayed a high degree of nodal support based on posterior probabilities (Fig. 2) and were separated by ≥6 mutational steps (Fig. 3). We inferred that all haplotypes were derived from one of two ancestral haplotypes (lineage I: HAP10; lineage II: HAP1). These lineages are not entirely concordant with the subspecies ranges, where clades are formed of a mixture of individuals from each subspecies, where lineage I was composed of 75% *T. t jeffersonii*, 44% *T. t. taxus*, and 95% *T. t. jacksoni*. HAP10 was dominant in eastern badger populations of *T. t. jacksoni*, whereas HAP1 predominated in *T. t. taxus* and *T. t. jeffersonii* from the East Kootenay (Fig. 3). Divergence estimates for American badger haplotypes also support the notion of two phylogenetically separate lineages (Fig. 2).

Discussion

Using mtDNA analysis, we show that American badgers in Canada are more segregated than previously identified using morphometrics. In contrast to previous estimates, we detected five genetic groups across the badger's Canadian range. We determined that the endangered *T. t. jeffersonii* is subdivided into two groups separated by the Selkirk Mountains. Likewise, the range of the endangered *T. t. jacksoni* is likely smaller than estimated previously; the population in the UP of Michigan was formerly thought to be composed of *T. t. jacksoni*, but our data suggest that it groups more closely with *T. t. taxus* in Manitoba. We discuss the analytical designations for each of these three subspecies below, and place this into a broader phylogeographic perspective with conservation implications.

Taxidea taxus jeffersonii

SAMOVA revealed that badgers from the Thompson–Okanagan and East Kootenay regions are genetically distinct (F_{ST} = 0.53, $p < 0.001$), which is in contrast to the previous designation of a single group (based on morphometry; Long 1972). This segregation is likely because gene flow is restricted by the Selkirk Mountains (Kyle et al. 2004). Therefore, the Thompson–Okanagan group is geographically isolated from other badger subspecies in Canada, whereas

Table 1. Haplotypes and nucleotide diversities, measures of haplotype richness (corrected to sample size $g = 10$), Tajima's D, and Fu's F_S values for each (*a*) regional sampling location, (b) traditionally defined American badger (*Taxidea taxus*) subspecies, (*c*) groupings based on spatial analysis of molecular variance (SAMOVA), and (*d*) the entire data set.

Subspecies	Location	N	Haplotype diversity (±variance)	Nucleotide diversity (±variance)	Haplotype richness	Haplotype richness ($g = 10$)	No. of private haplotypes	No. of private haplotypes ($g = 10$)	Tajima's D	Fu's F_S
(*a*) Regional sampling locations ($K = 9$).										
T. t. jeffersonii	EK	46	0.504±0.073	0.005±0.003	5	2.902	0	0.014	0.95	3.30
T. t. jeffersonii	TO	10	0.378±0.181	0.003±0.002	3	3.000	0	0.604	–1.87*	1.45
T. t. taxus	MT	12	0.727±0.113	0.007±0.004	5	4.500	0	0.783	1.18	1.42
T. t. taxus	AB	42	0.612±0.086	0.005±0.003	10	4.045	1	1.014	–0.50	–1.03
T. t. taxus	SK	27	0.644±0.100	0.005±0.003	8	4.474	0	1.171	–0.08	–0.10
T. t. taxus	MB	56	0.834±0.033	0.006±0.004	12	6.088	3	2.443	0.00	–0.75
T. t. jacksoni	UP	18	0.294±0.119	0.003±0.002	2	1.931	0	0.000	0.04	3.99
T. t. jacksoni	LP	18	0.425±0.099	0.001±0.001	2	1.993	0	0.000	0.87	1.04
T. t. jacksoni	ON	26	0.077±0.070	0.000±0.000	2	1.385	0	0.000	–1.16	–1.09
(*b*) Traditionally defined subspecies ($K = 3$).										
T. t. jeffersonii	EK, TO	56	0.553±0.068	0.005±0.003	5	6.000	1	1.600	1.35	2.75
T. t. taxus	MT,AB, SK, MB	137	0.740±0.038	0.006±0.004	19	13.791	13	8.285	0.17	–2.27
T. t. jacksoni	UP, LP, ON	62	0.516±0.043	0.002±0.001	3	3.000	0	0.003	–0.63	2.13
(*c*) SAMOVA population groupings ($K = 5$).										
T. t. jeffersonii	EK	46	0.504±0.073	0.005±0.003	5	2.902	0	0.593	0.95	3.29
T. t. jeffersonii	TO	10	0.378±0.181	0.003±0.002	3	3.000	0	1.257	–1.87*	1.45
T. t. taxus	MT, AB, SK	89	0.641±0.061	0.006±0.003	13	4.401	5	1.608	–0.17	–1.84
T. t. taxus/*T. t. jacksoni*	MB, UP	66	0.805±0.027	0.006±0.003	12	5.388	5	2.194	–0.20	–0.74
T. t. jacksoni	LP, ON	44	0.241±0.076	0.000±0.000	2	1.809	0	0.177	0.07	0.55
(*d*) Global model.										
		255	0.823±0.014	0.006±0.004					0.48	–1.95

Note: EK, East Kootenay; TO, Thompson–Okanagan; MT, Montana; AB, Alberta; SK, Saskatchewan; MB, Manitoba; UP, Upper Peninsula of Michigan; LP, Lower Peninsula of Michigan; ON, Ontario. *, $p < 0.001$.

Table 2. Comparison of explained variance calculated using analysis of molecular variance (AMOVA) for the three traditionally defined American badger (*Taxidea taxus*) subspecies, and the optimal group selection using spatial analysis of molecular variance (SAMOVA) (K = 1–5).

	Percent variation						
K	Among regions	Among populations within regions	Within populations	F_{SC}	F_{ST}	F_{CT}	Population subdivison
ANOVA traditionally defined subspecies							
3	17.85	21.73	60.42	0.26	0.39	0.18	ON, LP, UP; TO, EK; AB, MT, SK, MB
SAMOVA							
1							ON, LP, TO, AB, MT, SK, MB, UP, EK
2	33.25	14.83	51.93	0.22	0.48	0.33	ON, LP; TO, AB, MT, SK, MB, UP, EK
3	34.76	12.31	52.93	0.19	0.47	0.35	ON, LP; TO; AB, MT, SK, MB, UP, EK
4	34.21	4.68	61.11	0.07	0.39	0.34	ON, LP; TO; AB, MT, SK; MB, UP, EK
5	35.34	0.85	63.28	0.13	0.36	0.35	ON, LP; TO; AB, MT, SK; MP, UP; EK

Note: All reported values are significant at $p < 0.01$. Population groupings for the traditionally defined subspecies are shown relative to the population groupings calculated with SAMOVA. Population abbreviations defined in Tables 1*a*–1*d*.

Table 3. Pairwise estimates of population genetic distance (F_{ST}; above diagonal), mean geographic distance (km; below diagonal) and sample size (n; diagonal) among nine American badger (*Taxidea taxus*) populations in Canada and the United States.

	EK	TO	MT	AB	SK	MB	UP	LP	ON
EK	46	0.53*	0.14	0.33*	0.32*	0.12*	0.15*	0.38*	0.49*
TO	306	10	0.27	0.224*	0.20*	0.40*	0.69*	0.87*	0.49*
MT	488	776	12	0.022	0.01	0.05	0.24*	0.55*	0.68*
AB	151	444	419	50	–0.02	0.16*	0.38*	0.59*	0.66*
SK	592	863	534	441	27	0.17*	0.38*	0.60*	0.69*
MB	1273	1576	911	1132	772	48	0.04	0.24*	0.34*
UP	2288	2593	1882	2151	1787	1021	18	0.39*	0.62*
LP	2394	2699	1982	2258	1898	1131	113	18	0.18
ON	2820	3125	2390	2688	2340	1570	563	450	26

Note: Significant F_{ST} values (*) calculated with 10 100 permutations. Population abbreviations defined in Tables 1*a*–1*d*.

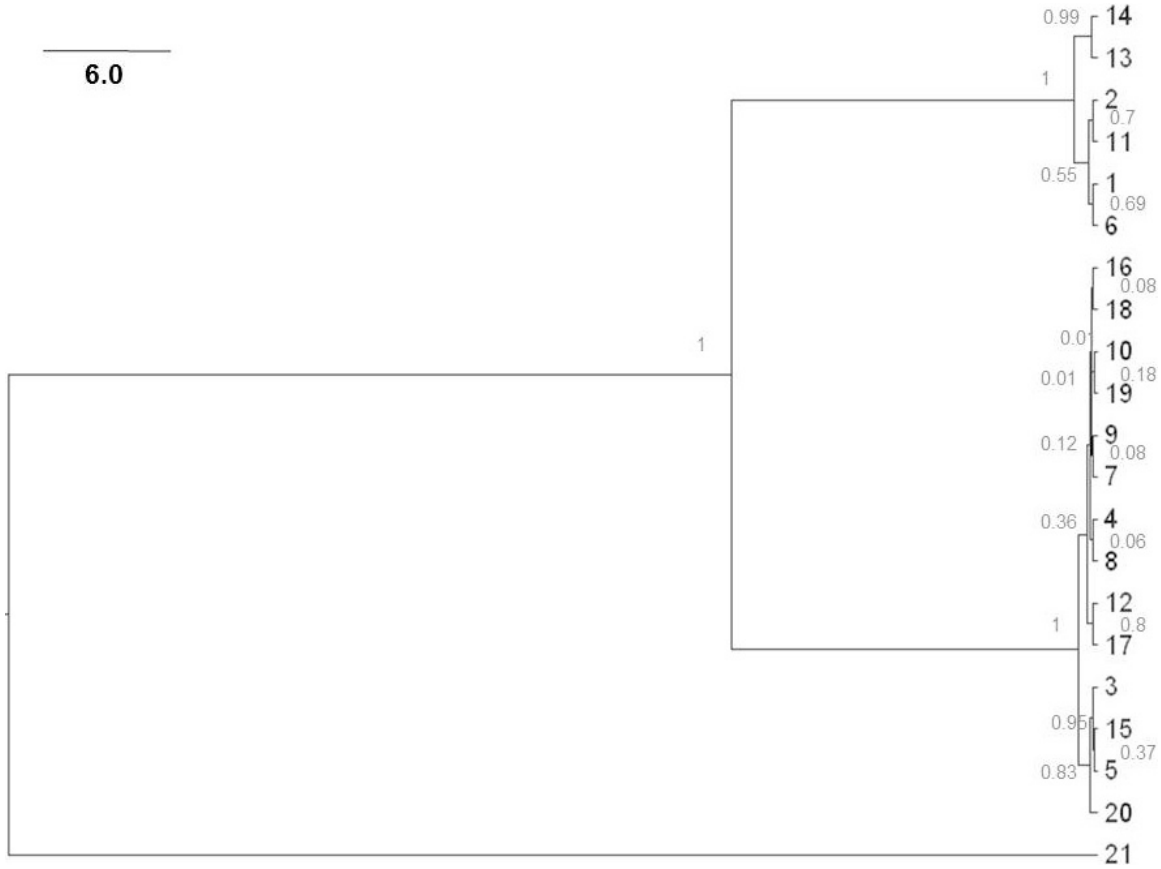
the East Kootenay region likely represents a contact zone between *T. t. jeffersoni* and *T. t. taxus*, as evidenced by the relative increase in levels of genetic variation (see also Kyle et al. 2004). Although the Rocky Mountains between the East Kootenay and *T. t. taxus* regions may reduce gene flow, they do not act as an impermeable barrier. The predominant haplotype occurring in the East Kootenay region (HAP4; 67%) also occurs in neighbouring populations of *T. t. taxus* (Montana, 26%; Saskatchewan, 4%; Manitoba, 4%), but not in the Thompson–Okanagan region. These findings suggest that female badgers may facilitate some genetic exchange between the East Kootenay region and the neighbouring *T. t. taxus* populations. It is important to note that from 2002 to 2004, 12 adult and 4 juvenile badgers (10 male, 6 female) were translocated from northwestern Montana into the East Kootenay region (Kinley and Newhouse 2008), as gene flow did not appear to be impaired between these regions, nor was there any indication of strong patterns of genetic structure within the region (Kyle et al. 2004); a result also supported here ($F_{ST} = 0.14$, $p = 0.03$). The samples used for our analysis pre-date the aided recovery program. We therefore cannot comment on the current genetic status of the East Kootenay badgers following population augmentation.

Genetic variation in the Thompson–Okanagan region was lower than in the East Kootenay region (Tables 1*a*–1*d*), and the haplotype structure was distinct (Fig. 3), supporting the suggestion that the Thompson–Okanagan badgers form an insular population (Kyle et al. 2004). The insularity of this population is expected because habitat in the *T. t. jeffersonii* range is less contiguous than those elsewhere (Apps et al. 2002). Given that badgers in the Thompson–Okanagan region are on the northern periphery of the subspecies range and are genetically segregated from neighbouring populations, they may be more susceptible to stochastic events that may promote local extirpation (Hanski 1999).

Taxidea taxus taxus

Badgers from northeastern Montana, Alberta, Saskatchewan, and Manitoba displayed the highest levels of haplotype and nucleotide diversity in all sampling localities (Tables 1*a*–1*d*), as suggested by Kyle et al. (2004). This is likely due to the absence of strong barriers to dispersal in the prairies (i.e., no mountain ranges, low road density, and relatively contiguous habitat) and substantial dispersal abilities of juvenile badgers (Messick and Hornocker 1981). SAMOVA divided *T. t. taxus* into two subgroups: west (Montana, Alberta, Saskatchewan) and east (Manitoba, UP) (Table 2). The inclusion of the UP of Michigan badgers with those from Manitoba was unanticipated but practicable given the geographic proximity of these groups and the lack of obvious physiographic barriers to dispersal between them. It is, however, less clear why Manitoba badgers are genetically differentiated from

Fig. 2. Bayesian phylogenetic reconstruction of American badger (*Taxidea taxus*) haplotypes (1–20), with divergence diagram (upper left) rooted with wolverines (*Gulo gulo*) (21). Internode labels represent the posterior probabilities of a particular branch. Scale bar in divergence tree in millions of years.



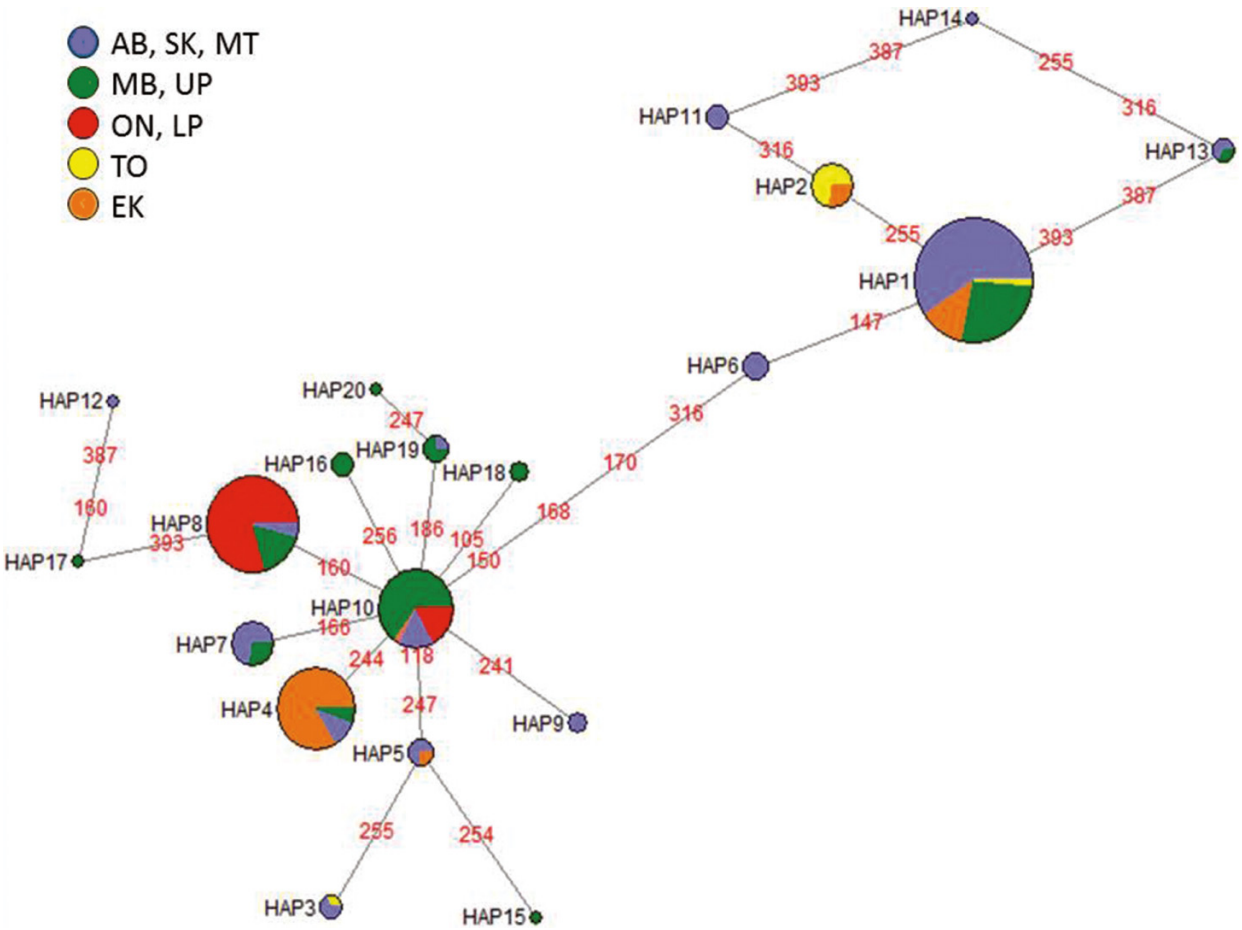
Saskatchewan (F_{ST} = 0.15, $p < 0.001$) and Alberta (F_{ST} = 0.16, $p < 0.001$), but not Montana (F_{ST} = 0.05, $p = 0.11$). In the Canadian prairies, *T. t. taxus* is at the northern periphery of the subspecies range and has experienced dramatic changes in habitat availability owing to agricultural and rural development in the last century, as well as substantial population decline owing to harvesting pressure (Scobie 2002). Although badgers in Alberta have experienced northward range expansion as habitat became available through aspen clearing, fire suppression in the south has enabled woody vegetation to thrive (Scobie 2002), thus limiting badger habitat and potentially restricting connectivity to neighbouring badger populations. This would imply that badgers in Alberta and Saskatchewan may be somewhat genetically isolated from the more contiguous core *T. t. taxus* populations residing in the United States prairies. Results may also be a consequence of low sample size and high degree of allelic diversity in Alberta, Saskatchewan, and Manitoba, leading to spurious structure. Investigation of other populations of *T. t. taxus*, particularly in the north-central United States, is needed to determine if genetic variation and gene flow are maintained throughout the distribution of this subspecies, and where genetic differences exist between core and peripheral populations.

Taxidea taxus jacksoni

The genetic structuring in eastern badger populations was not consistent with the original subspecies designation. Long (1972) defined the *T. t. jacksoni* subspecies based on 29 voucher specimens of known locality: Minnesota (n = 8), Wisconsin (n = 12), Michigan (UP; n = 3), Indiana (n = 1), and Ohio (n = 5). No specimens from either Ontario or the LP of Michigan were included in the original subspecies assessment. SAMOVA identified badgers from Ontario and the LP as being genetically distinct from the UP of Michigan. This result implies that the Strait of Mackinac, an 8 km wide channel separating the LP and UP of Michigan, likely functions as a significant barrier to dispersal for badgers. Other carnivores, including bobcats (*Lynx rufus* (Schreber, 1777)), also have displayed genetic isolation on either side of the Strait (Millions and Swanson 2007). The St. Clair River, a 0.5 km wide channel separating southwestern Ontario from the LP of Michigan, appears to have had a less substantial impact on dispersal of badgers between these regions. In all cases, sampled *T. t. jacksoni* populations display the lowest observed levels of genetic variation and approach genetic fixation in all regions (Tables 1*a*–1*d*), likely a result of isolation, demographic decline, and genetic drift.

Currently, it is difficult to ascertain how eastern badger subspecies are structured, and where subspecific boundaries might be drawn based on genetic differentiation. From our study, it is apparent that badgers from the UP are genetically isolated from badgers in the LP and that the traditionally defined *T. t. jacksoni* subspecies may be more segregated and geographically restricted than previously thought. UP badgers were not genetically distinct from Manitoba (F_{ST} = 0.04, p =

Fig. 3. Phylogenetic network using maximum parsimony construction for 20 American badger (*Taxidea taxus*) mitochondrial DNA haplotypes. Size of the symbol corresponds to the number of individuals belonging to a particular haplotype, whereas colour (on the Web, shades of grey in print) indicates the region defined by the spatial analysis of molecular variance (SAMOVA) ($K = 5$) in which the haplotypes occur. Population abbreviations defined in Fig. 1.



0.08), suggesting that the subspecific designation of this population should be *T. t. taxus* rather than *T. t. jacksoni*. This result could have considerable implications for the critically endangered *T. t. jacksoni* populations residing in Ontario. If badgers from the UP are *T. t. taxus*, then it would be reasonable to assume that badgers from Wisconsin, Minnesota, and the northwestern corner of Ontario are *T. t. taxus* as well, further reducing the estimated range of *T. t. jacksoni* in Ontario and all of North America. Genetic isolation and a low effective population size of *T. t. jacksoni*, particularly in Ontario, puts this peripheral populations of badgers at an elevated risk of local extirpation. Further sampling in the eastern United States is needed to better elucidate the relative effects of natural and anthropogenic barriers to dispersal and how this affects the genetic structuring of this subspecies.

Phylogeography

We observed significant geographic substructure with regards to haplotype frequencies (Tables 1*a*–1*d*) indicating limited genetic exchange among regions despite the dispersal ability of badgers and an absence of obvious physiogeographic barriers in many regions. The lack of deep phylogenetic structure noted here has been seen in other carnivores across large geographic ranges, including raccoons (*Procyon lotor* (L., 1758)) (Cullingham et al. 2008), fishers (Drew et al. 2003), and wolverines (Tomasik and Cook 2005).

Historic events, such as glaciation, may have been responsible for lineage formation in badgers noted in this study. Pleistocene records indicate that badgers occurred as far east as Pennsylvania and Maryland (Long 1972). Badgers and other prairie taxa are purported to have invaded the east at least twice during pre-Wisconsin glaciations (Long 1972). Ancestral *T. t. jacksoni* likely occupied sandy areas scattered through the coniferous forests of the Great Lakes region following glacial retreat, experiencing moderate levels of genetic exchange from neighbouring regions. Following the recolonization in the west by *T. t. jeffersonii*, the Rocky Mountains formed a substantial barrier to genetic exchange, forming the deep phylogenetic divide that we see in the Thompson–Okanagan region. Relatively continuous habitat in the Prairie Provinces promoted high levels of genetic exchange within *T. t. taxus* and moderate exchange with neighbouring subspecies. The occurrence of widely distributed haplotypes suggests historic population admixture as a result of dispersing individuals or possible recolonization from multiple sources after glaciation. Evidence presented here would also suggest that movement between regions, particularly in peripheral populations, has been restricted more re-

cently, likely owing in part to human-mediated habitat fragmentation.

Subspecific designation

Isolation of populations into disjointed refugia accentuates genetic divergence through genetic drift, especially if population size is small. Our AMOVA show significant genetic divergence between regionally sampled badger populations. Peripheral populations of *T. t. jacksoni* and *T. t. jeffersoni* appear to be isolated from core populations to varying degrees. In the west, the Selkirk Mountains function as physiogeographic barriers to dispersal, whereas barriers in the east are less obvious. Badgers of the *T. t. jacksoni* group likely occupy fragmented refugia corresponding to habitat requirements (e.g., soil type) and prey availability, and are in part maintained by geographic distance between fragments and physical barriers including lakes and larger rivers (e.g., Strait of Mackinac). Our analysis supports isolation by distance. However, highly significant F_{ST} values between adjacent populations make it equally likely that geographic barriers prevent movement. Given the scale at which samples were collected and the physiogeographic barriers between regions (e.g., mountains), these data do not support or refute either hypothesis. The suppression of genetic exchange is likely then exacerbated by anthropogenic habitat fragmentation. Limited gene flow from *T. t. taxus* to peripheral populations also makes dispersal-mediated demographic rescue unlikely. These populations consequentially may experience different selective pressures that enhance genetic divergence, or through genetic drift cause genetic fixation. Demographic estimates correlate strongly with genetic diversity estimates, suggesting that recent demographic declines may have resulted in loss of genetic diversity. This raises questions concerning genetic discontinuities in the prairie provinces, particularly along the northern range limit of *T. t. taxus*. The use of additional nuclear markers across a broader geographic area is needed to better explore the roles of vicariance, dispersal, and refugia in structuring genetic diversity in regions. Our mtDNA analysis suggests low to moderate levels of female-mediated gene flow between populations, and relatively high levels of historic connectivity, with the exception of the Thompson–Okanagan badger population. Unique haplotypes in this region and deep phylogenetic differentiation suggest prolonged physiographic isolation in Canada.

Although phenotypic variation has been used extensively to classify subspecies, defining biological groups for conservation consideration should be verified with molecular genetic approaches. A better understanding of how landscape features influence population genetic structure is needed by correlating various genetic markers with landscape and environmental features. Our results will allow better descriptions of how natural and human-mediated barriers to dispersal affect genetic structure of North American badger populations. Further studies that include genetic samples from a broader geographic area, particularly within the more southerly portion of the range, will enable researchers to better resolve genetic discontinuities in American badgers which will help guide conservation planning for this species.

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