Delineating genetic groupings in continuously distributed species across largely homogeneous landscapes: a study of American black bears (*Ursus americanus*) in Ontario, Canada

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Abstract: There is a crucial need to understand the genetic consequences of landscape modifications on continuous populations that could become fragmented, and to evaluate the degree of differentiation of isolated populations that were historically part of the core. Using 15 microsatellite loci, we evaluated the genetic structure of American black bears (*Ursus americanus* Pallas, 1780) across a vast, contiguous Ontario landscape ($>1 \times 10^6$ km^2) that largely represents their pre-European settlement distribution. Because geographic barriers are absent, we predicted that isolation by distance would drive genetic structure. We identified three genetic clusters (Northwest, Southeast, and Bruce Peninsula) that were less differentiated than when assessed with mtDNA, suggesting the influence of male-biased dispersal on large-scale genetic differentiation. Isolation by distance ($r = 0.552$, $P = 0.001$) was supported by a weak, clinal variation between Northwest and Southeast, illustrating the challenges to delineate populations in wide-ranging taxa. The Bruce Peninsula cluster, confined to a small area under strong anthropogenic pressures, was more differentiated from neighbouring clusters ($F_{ST} > 0.13$, $P < 0.0001$), with a genetic diversity corresponding to disjunct populations of black bears. Our results could be used in landscape genetics models to project the evolution of population differentiation based on upcoming landscape modifications in northern regions of North America.

Key words: American black bear (*Ursus americanus*), cluster, gene flow, genetic structure, isolation by distance, male-biased dispersal, microsatellite, North America, spatial autocorrelation.

Résumé : La compréhension des conséquences génétiques des changements environnementaux pour les populations continues qui pourraient devenir fragmentées est d'une importance cruciale, tout comme l'évaluation du degré de différentiation des populations historiquement centrales. Grâce à 15 microsatellites, nous avons évalué la structure génétique de l'ours noir (*Ursus americanus* Pallas, 1780) dans un vaste paysage contigu ($>1 \times 10^6$ km^2) en Ontario représentant en bonne partie l'aire de répartition de l'espèce avant l'arrivée des Européens. Étant donné l'absence de barrières géographiques, nous anticipions que l'isolement par la distance influencerait la structure génétique. Nous avons identifié trois groupes génétiques (nord-ouest, sud-est et péninsule de Bruce) qui présentaient une différentiation moins importante que celle établie par analyse d'ADN mitochondrial, suggérant un effet de la dispersion des mâles sur la différentiation génétique à grande échelle. L'isolement par la distance ($r =$ 0,552, $P = 0,001$) a été confirmé par la variation génétique clinale entre les groupes du nord-ouest et du sud-est, illustrant les difficultés liées à la délimitation de populations de taxons à grande aire de répartition. Le groupe de la péninsule de Bruce, confiné à une petite région assujettie à de fortes pressions anthropiques, était plus différencié des groupes voisins ($F_{ST} > 0,13$, $P < 0,0001$), sa diversité génétique correspondant à celle de populations d'ours noirs isolées. Nos résultats pourraient être utilisés dans les modèles de génétique du paysage afin de prévoir l'évolution de la différentiation des populations en fonction des futurs changements environnementaux dans les régions nordiques de l'Amérique du Nord.

Mots-clés : ours noir (*Ursus americanus*), flux génétique, structure génétique, isolement par la distance, dispersion des mâles, microsatellite, Amérique du Nord, autocorrélation spatiale.

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Introduction

Over the last 500 years, many species have experienced range contractions and demographic declines as a consequence of habitat loss and landscape fragmentation (Laliberté and Ripple 2004; Wiegand et al. 2005). This has led to concerns regarding the maintenance of overall biodiversity (Fahrig 2003), as the continuity of a species' range affects contemporary levels of genetic diversity and differentiation, which, along with demographic processes, can be used as indicators of species persistence (Lande 1993; Young et al. 1996; Keyghobadi 2007). Indeed, small geographically isolated populations exhibit lower heterozygosity than continuous populations (Frankham 1997; Segelbacher et al. 2003; Höglund et al. 2007; Ohnishi et al. 2007; White and Searle 2007), and are also more likely to be subject to inbreeding depression (Frankham 1995; Keyghobadi 2007). Thus, research that identifies intraspecific genetic discontinuities and variation in genetic diversity is essential, as it allows for the delineation of population boundaries or management units, but also provides data that enable wildlife managers to assess population viability and implement strategies that target groups of conservation and evolutionary relevance (Schwartz et al. 2007). Overall, such studies give insight into the modifications of movement and migration patterns as a result of changes in landscape connectivity, thus enabling the comparison of historical movement patterns with more contemporary processes that arise as a consequence of habitat fragmentation (Schwartz et al. 2007).

The reduction in geographic range of many North American mammals following European settlement has resulted in contrasting contemporary distributions within species, such that both isolated and continuously distributed populations are now observed in wide-ranging species that were historically panmictic (e.g., gray wolf (*Canis lupus* L., 1758): Mech and Boitani 2008; American puma (*Puma concolor* (L., 1771)): Anderson 1983; wolverine (*Gulo gulo* (L., 1758)): Banci 1994; Canada lynx (*Lynx canadensis* Kerr, 1792): Koehler and Aubry 1994; American marten (*Martes americana* (Turton, 1806)): Gibilisco 1994; fisher (*Martes pennanti* (Erxleben, 1777)): Gibilisco 1994; American black bear (*Ursus americanus* Pallas, 1780): Vaughan and Pelton 1995). For this reason, studies are needed to identify baseline levels of gene flow expected in the absence of disturbance, relative to levels that are currently observed in more anthropogenically influenced regions of the continent where populations are more isolated. In this ecological context, methods for distinguishing subtle genetic delineations at fine scales within continuously distributed species are useful, as they allow a more precise understanding of population genetic structuring patterns, and therefore help identify the actions necessary to ensure persistence in the event of future habitat fragmentation (Schwartz and McKelvey 2009).

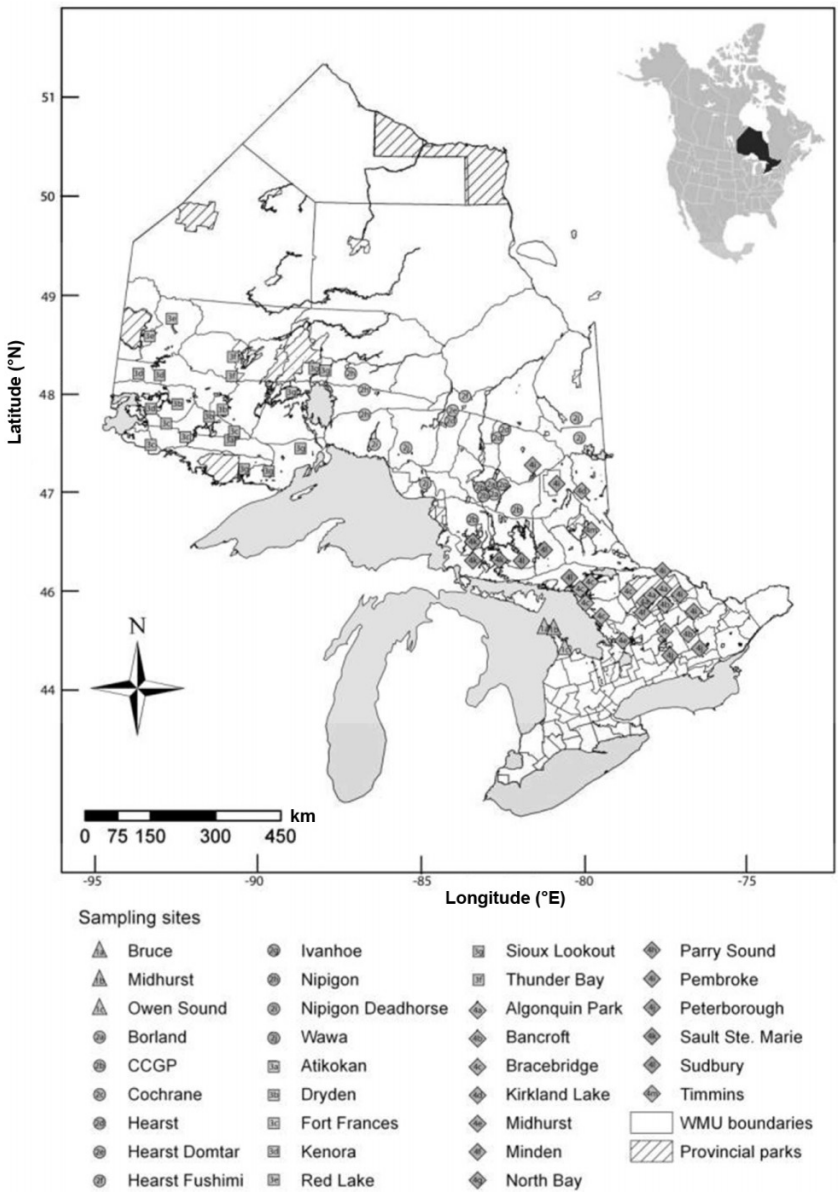
Although American black bears have extensive dispersal abilities (male dispersal ~200 km: Lee and Vaughan 2003; Rogers 1987), they display historical genetic signatures related to postglacial recolonization (Byun et al. 1997; Wooding and Ward 1997; Pelletier et al. 2011), similar to other species (Arbogast 1999; Aubry et al. 2009; Conroy and Cook 2000; Demboski et al. 1999; Demboski and Sullivan 2003). More contemporary genetic structure detected in black bears has been explained by physiogeographic features that decrease levels of gene flow (islands: Paetkau and Strobeck 1994; ice: Peacock et al. 2007; elevation: Cushman et al. 2006), or, in the southern portion of the continent, by population isolation resulting from habitat loss owing to anthropogenic activities (Warrillow et al. 2001; Boersen et al. 2003; Csiki et al. 2003; Triant et al. 2004; Dixon et al. 2006; Onorato et al. 2007).

In contrast to the southern region of North America (United States, Mexico), black bears are mostly continuously distributed throughout the northern part of their range (Scheick et al. 2011). In Canada, 95% of the historic range is still occupied (Scheick et al. 2011). From the central to eastern region of Canada, habitat of black bears is presumed contiguous across an extensive area (~3×10^6 km^2), with no obvious barriers to movement such as large rivers, mountains, or radical habitat change. Unlike the eastern coastal region, this part of Canada generally lacks a pronounced human presence (mean human density <0.4 individual/km^2; Statistics Canada 2002), and as such may best represent the distribution of black bears prior to European settlement in eastern North America. Thus, we assume that the genetic structure currently detected among black bears from central to eastern Canada could be used as baseline data characterizing gene flow patterns when the species was largely panmictic. Such data could then be compared with what is observed in isolated populations located in regions with higher levels of anthropogenic activity (Csiki et al. 2003; Larkin et al. 2004; Dixon et al. 2006; Onorato et al. 2007; Van Den Bussche et al. 2009).

Our goal was to characterize the genetic structure of black bears in the absence of strong anthropogenic and physiogeographic influences across a wide geographic area (~1×10^6 km^2) that contains a large number of individuals (~95 000; M.E. Obbard unpublished data). By doing so, we looked to obtain reference levels of genetic differentiation characteristic of pre-18th century gene flow patterns. First, we hypothesized that dispersal abilities of black bears were likely to have erased postglacial historical influences previously detected in a mitochondrial DNA (mtDNA) study (Pelletier et al. 2011). We predicted that biparentally inherited markers such as microsatellites would illustrate a pattern of isolation by distance across regions, which would contrast with the moderate levels of differentiation found with mtDNA owing to postglacial recolonization patterns and female philopatry (Pelletier et al. 2011). Second, we hypothesized that weak genetic subdivisions would be more frequent in the southeastern region than in the central and northwest areas of Ontario, as high levels of anthropogenic activities occur in this area (human density from 1 to more than 50 individuals/km^2 in the southeastern periphery vs. <0.4 individuals/km^2 in most of the rest of the province; Statistics Canada 2002).

We collected hair samples from black bears at 61 locations across Ontario and profiled them based on 15 microsatellite loci (Fig. 1). Given the geographic extent of our study region and its largely continuous habitat, we used methods capable of identifying genetic clusters expected to be weakly differentiated and to detect isolation by distance (Wright 1943). First, we used two individual Bayesian clustering models to distinguish cryptic genetic discontinuities and identify genetic clus-

Fig. 1. Map of sampling sites in Ontario where hairs from American black bears (*Ursus americanus*) were collected for genetic analyses. The four different shapes for the various sampling sites represent the four clusters detected through previous mitochondrial DNA (mtDNA) analyses (Pelletier et al. 2011). Triangles represent the Bruce Peninsula cluster, diamonds represent the Southeast cluster, circles represent the Central cluster, and squares represent the Northwest cluster.



ters (Pritchard et al. 2000; Falush et al. 2003; François et al. 2006; Chen et al. 2007). Second, we used non-Bayesian techniques to assess more subtle levels of genetic variation at the southeastern periphery of the province (Mantel 1967; Hardy and Vekemans 1999; Diniz-Filho and De Campos Telles 2002; Kelly et al. 2010). Through this study, we aim to provide context on the extent of genetic isolation of more southerly populations that were once contiguous with the Ontario population. Such information could later be included in landscape change models to identify sites where connectivity and genetic diversity are likely to become lower and thus direct management and conservation strategies.

Materials and methods

Sampling

We collected samples between 1997 and 2009 as part of Ontario's Enhanced Black Bear Management Program to estimate bear densities and population trends throughout the province. We processed ~10 000 bear hair samples obtained from baited barbed wire hair traps (Woods et al. 1999) located at 61 sampling sites (Fig. 1). In addition, 120 samples were obtained opportunistically (live trapping, hunting, or road kills). All hair samples were stored dry in paper envelopes at room temperature until DNA extraction was performed.

DNA extraction

To allow for a high amplification success rate, we extracted DNA up to 2 months after collection of hair samples (Roon et al. 2003), during which samples were stored in paper in a cool, dry cabinet designed for this purpose. We extracted DNA from samples collected prior to 2004 using a DNeasy tissue extraction protocol (Qiagen, Mississauga, Ontario, Canada). For samples collected from 2004 to 2009, we followed a MagneSil paramagnetic bead automated DNA extraction procedure (Promega, Nepean, Ontario, Canada) using a P3 Evolution liquid handler (Perkin Elmer, Woodbridge, Ontario, Canada). For each individual sample, we suspended hairs in 180 μL of 1× lysis buffer (4 mol/L urea, 0.2 mol/L NaCl, 0.5% n-lauroyl sarcosine, 10 mmol/L CDTA (1,2-cyclohexanediamine), 0.1 mol/L Tris-HCL pH 8.0) (Applied Biosystems Inc., Burlington, Ontario, Canada). We then treated samples with 10 units of proteinase K (>600 U/mL, Qiagen) and incubated at 37 °C for 12 h. To minimize technical artefacts from low copy number DNA, we excluded all samples with <5 hairs with visible roots from analyses, with the vast majority (>90%) of samples consisting of 10–15 hairs with visible roots. Extracted DNA from the hairs was not directly quantified, but assessed relative to amplifications of diluted positive control DNA samples of 2 ng and 200 pg.

Microsatellite amplification

We amplified 15 microsatellite loci using multiplex polymerase chain reactions (PCRs). We used primers G10A, G10D, G10B, G10L, G10C, G10J, G10P, G10X, G10U, G10M (Paetkau and Strobeck 1994; Paetkau et al. 1995); G10H, UarMU59, UarMU05, UarMU50 (Taberlet et al. 1997), and MSUT-6 (Kitahara et al. 2000). For the primers presented in Taberlet et al. (1997), we used the external forward and internal reverse primers for *UarMU59*, the external forward and reverse primers for *UarMU50*, and the internal forward and external reverse for *UarMU05*. We determined gender via amplification of the *Amelogenin* gene using primers SE47 and SE48 (Ennis and Gallagher 1994). For the 15 microsatellites and the *Amelogenin* gene, we synthesized one primer of each pair with a fluorescent dye group, HEX, 6-FAM, or NED for subsequent detection and analysis on an ABI Prism 377 for pre-2004 samples, a MegaBACE 1000 (GE Healthcare, Piscataway, New Jersey, USA) for the 2004–2005 samples, and an ABI 3730 for the 2006–2009 samples. PCRs were pooled on two lanes on the DNA sequencers. Pooled reactions 1 (MP1) consisted of three PCR reactions: (1) multiplex of G10A, G10B, G10L, UarMU05, G10D; (2) multiplex of G10H, G10J; (3) Amelogenin. Pooled reactions 2 (MP2) consisted of four PCR reactions: (1) G10X, G10M; (2) G10U, G10C; (3) UarMU59; (4) UarMU50, G10P, MSUT-6. We performed all DNA amplifications in 10 μL consisting of 1× PCR buffer (Qiagen), 200 μmol/L dNTPs, 0.1–0.5 μmol/L forward and reverse primers, 1.0 μg of bovine serum albumin (DNase and RNase free, Amersham BioSciences Inc., Piscataway, New Jersey, USA), 0.5 units of *Taq* polymerase (5 U/μL) (Qiagen), and 4 μL of the eluted DNA. PCR conditions consisted of 5 min at 94 °C, then 31 cycles of 30 s at 94 °C, 45 s at 52 °C, and 2 min at 72 °C followed by a final cycle of 20 min at 72 °C. We added 0.5 μL of the pooled amplicons to 9.5 μL of HiDi formamide and ROX standard and ran on the automated sequencers.

Individual identification

Prior to assessing if samples collected from hair traps originated from the same individual, we used the following steps to validate our genotypes. Two technicians scored and verified all generated profiles. We removed all profiles that did not amplify >6/8 loci from MP1. We ran the remaining profiles (~80%) through the program GENECAP (Wilberg and Dreher 2004) to assess the grouping of genotypes for individual bear identification. All genotypes with >2 allele differences were deemed individual bears. All genotypes with 2 or fewer allele differences were reassessed to determine if the samples could be excluded as originating from the same individual, given the potential for genotype artefacts from low template DNA arising from noninvasive hair sampling (Taberlet et al. 1999). Specifically, we inspected allele morphology and quality in GENEMARKER (SoftGenetics, State College, Pennsylvania, USA) by (*i*) peak height (signal strength in RFU), and the potential for (*ii*) allelic dropout, (*iii*) poor-quality alleles not conforming to scoring criteria, (*iv*) preferential amplification, (*v*) incorrect stutter pattern, (*vi*) pull-up, and (*vii*) contamination.

A subset of all samples (30%) with the same genotype or two or fewer allele differences was ran at MP2 to ensure that the samples were not improperly pooled as individuals. All samples with unique genotypes at MP1 were also ran at MP2. Again, we used GENECAP to identify individual bears from these 15 microsatellite loci and the gender locus. There were no cases of samples considered to be individuals based on the initial seven microsatellite loci that were not also considered individual bears after amplification of the second set of pooled loci.

We used GENECAP (Wilberg and Dreher 2004) to evaluate the ability of our marker set to discriminate between individual bears by calculating the probability of randomly drawn individuals to have the same genotype (P_{id}), and the probability that full siblings would share the same genotype (P_{sib}) (Waits et al. 2001). In addition, we ran the "difference in capture history" test implemented in DROPOUT (McKelvey and Schwartz 2005) to determine if new individuals were detected after removal or addition of loci.

Assessment of genotyping error

Following individual identification, we divided genotypes into two categories using a subset of five sampling sites (Parry Sound, Pembroke, Hearst, Wawa, and Dryden) spread across our sampling region: (*i*) genotypes observed only once (single captures) and (*ii*) genotypes observed more than once. For this category, we recorded the number of allele call changes made by the technicians while grouping genotypes and compared it with the total number of loci scored to determine our base genotyping error (no. of loci scored = no. of samples analyzed × no. of loci).

After confirmation of our assessment of individuals through the analysis of MP1 and 30% of the samples at MP2, we assessed genotyping error rate (Er) by calculating, for each sampling site, Er = (total no. of changes at MP1 + total no. of changes at MP2) / (total no. of loci scored at MP1 + total no. of loci scored at MP2). We calculated mean error rate as $\overline{\mathrm{Er}} = (\sum_{i}^{s} \mathrm{Er}) / \text{total no. of sampling sites}$, where *i* is the first sampling site and *s* is the last sampling site.

Hardy–Weinberg equilibrium and linkage disequilibrium

We used GENEPOP version 4 (Rousset 2008) to evaluate deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD). We performed HWE exact tests, first using the probability option, followed by tests for heterozygote deficiency and excess. We used sequential Bonferroni correction to adjust α values for multiple comparisons among loci at multiple sampling sites ($P = 0.00005$) (Rice 1989). For LD, we set Markov chain parameters to dememorization number of 10 000, 1 000 batches, and 10 000 iterations for all tests. We used sequential Bonferroni correction to adjust α values for multiple comparisons among loci at multiple sampling sites ($P = 0.000008$). Locus *G10P* deviated significantly from HWE at 95% of the sampling sites owing to a lack of heterozygotes, so we removed it from our data set and further analyses. We re-ran calculations of HWE and LD without *G10P*, and adjusted α values for comparisons among a total of 61 sites and 14 loci ($P = 0.00006$ for HWE; $P = 0.000009$ for LD). We re-ran GENECAP (Wilberg and Dreher 2004) to check for duplicates without *G10P*.

Genetic clusters determined by Bayesian methods

We used the Bayesian clustering programs STRUCTURE version 2.3 (Pritchard et al. 2000; Falush et al. 2003) and TESS version 2.3 (François et al. 2006; Chen et al. 2007) to determine the optimal number of genetic groups, or clusters (K), in which to assign individuals based on their allele frequencies under the assumption of maximized HWE and minimized LD.

STRUCTURE implements an aspatial method that accounts for admixture in individuals (one genotype can originate from multiple clusters), and calculates the membership proportions (q) of each individual genotype to each of the inferred clusters. We used the F model that assumes admixture with correlated allele frequencies (Falush et al. 2003) and ran STRUCTURE five times at $K_{max} = 1$–15, with 200 000 burn-ins and 500 000 Markov chain Monte Carlo iterations. We estimated K for STRUCTURE clustering according to Evanno et al. (2005). To estimate cluster membership values and account for label switching, we ran 10 additional independent runs at the most probable K value and averaged the results in CLUMPP version 1.2 (Jakobsson and Rosenberg 2007). From the output given by CLUMPP, we assigned individuals into clusters based on four different cut-off membership values ($q = 0.6$, $q = 0.7$, $q = 0.8$, and $q = 0.9$). Each individual that had a membership value lower than q was left unassigned. Then, we visualized the clusters using DISTRUCT version 1.1 (Rosenberg 2004). To detect levels of potential genetic substructure that could have gone undetected in the broad analysis, we repeated this procedure within each of the clusters identified with STRUCTURE.

For each cut-off membership value, we compared the trade-off between the ability to detect genetic structure and the loss of data owing to the increasing number of unassigned individuals. Differences in genetic structure were determined through two χ^2 tests: one assessed the differences in proportions between all individuals (assigned to a cluster and unassigned) at each cut-off membership value. To control for the bias owing to the increase in unassigned individuals, the second test only compared the proportions of individuals assigned to a cluster at each q.

To account for the influence of geographic location when assessing genetic admixture levels, we used the program TESS (François et al. 2006), which assumes that spatially proximate individuals are more genetically similar than individuals located far from each other (Dirichlet distribution). TESS identifies genetic discontinuities in continuous populations and allows the user to visualize genetic clusters that may be overestimated or diminished by Bayesian clustering programs that are limited when genetic variation is continuously distributed along a cline (STRUCTURE version 2.3 and BAPS version 4.1; Corander and Marttinen 2006). We ran TESS five times under both the nonadmixture and the BYM admixture models (admixture parameter: $\alpha = 1$; Durand et al. 2009) at $K_{max} = 2$–15, with 20 000 burn-ins and 50 000 iterations. For the nonadmixture model, we used several values of the spatial interaction parameter ($\psi = 0$ aspatial, $\psi = 0.1$ low interaction, $\psi = 0.25$, $\psi = 0.6$, and $\psi = 1$ high interaction) to account for spatial connectivity in the genetic clustering. For the BYM admixture model, we used $\psi = 0.6$ and averaged the results of five runs of each K_{max} in CLUMPP. To choose the best K among the different values of K_{max} under both models, we plotted the mean of the deviance information criterion (DIC, a measure of both model fit and model complexity; Spiegelhalter et al. 2002) at each K_{max} against K_{max} and determined at which value of K_{max} the line graph started to plateau to select an interval of the most likely K. Finally, we used DISTRUCT to look at the barplots of each of those K values and selected the best one based on both the DIC value and the K value that had the most stable barplot. For the best selected K value in the BYM model, we used the output given by CLUMPP to interpolate the admixture coefficients on a map of Ontario with the kriging method provided in the R version 2.1.1 package "fields" (R Development Core Team 2006; Venables and Ripley 2002).

Genetic diversity and level of differentiation

We calculated allele frequencies, observed heterozygosity (H_O), and expected heterozygosity (H_E) at each sampling site with Microsatellite Toolkit (Park 2001). Based on the results from the Bayesian analyses, we estimated the degree of differentiation between all pairs of genetic clusters by calculating pairwise F_{ST} values (Weir and Cockerham 1984) and Nei's standard genetic distance (D_S) (Nei 1978) in SPAGeDi version 1.3 (Hardy and Vekemans 2002).

To assess if relatedness between individuals at each sampling site could skew our results towards a higher level of genetic structure, we ran the program ML-RELATE to estimate putative pairwise relationships between individuals (unrelated, half-sibling, full sibling, or parent–offspring), as well as maximum likelihood estimates of relatedness (r) between all pairs of individuals (Kalinowski et al. 2006).

Isolation by distance and spatial distribution of alleles

We performed Mantel tests (Mantel 1967) in GENALEX version 6.3 (Peakall and Smouse 2006), by regressing pairwise genetic distance (expressed as $F_{ST}/(1 - F_{ST})$) and pairwise standard genetic distance (D_S) between all the sampling sites against pairwise geographic distances (km), using 999 randomization steps. We calculated geographic distances between each sampling location in SPAGeDi version 1.3. We

conducted a global test across the 61 sampling sites and then we conducted tests within each cluster.

To assess spatial genetic autocorrelation, we performed analyses in GENALEX version 6.3. As the maximum distance between two sites was 1462 km, we used 30 even distance classes of 50 km, for which we performed 999 permutations and 1000 bootstrap. The confidence intervals obtained allowed us to compare our results with the expectation of random distribution across our sampling area. To test for local patterns of genetic autocorrelation (e.g., neighbor mating; Schwartz and McKelvey 2009), we conducted analyses within each identified cluster.

We also assessed subtle levels of differentiation by a spatial analysis of shared alleles in SAshA version 1 (Kelly et al. 2010). This program identifies geographically restricted alleles by comparing geographic distances between shared alleles to distances expected for panmixia. We assessed the significance of the difference between the observed mean distance (OM) and the expected mean distance (EM) between shared alleles through 1000 permutations.

Results

Individual identification and error rate

We detected 2839 individuals in our data set. The probability of unrelated individuals sharing identical genotypes was $P_{id} = 2.4 \times 10^{-19}$ and the probability of full siblings sharing identical genotypes was $P_{sib} = 4.5 \times 10^{-7}$. Genotyping error across a subset of five sampling sites was low ($\overline{Er} = 2.18\%$).

"Difference in capture history" tests (McKelvey and Schwartz 2005) with 13/14 loci did not show evidence of additional individuals. This shows that for our complete data set of 14 loci, we have not generated a large number of false genotypes that could undermine our population genetics analyses.

Hardy–Weinberg equilibrium and linkage disequilibrium

Within sites, HWE was met at 91% of all loci/sites combinations ($n = 854$) and deviations from HWE were not consistent for a particular locus or sample site. Similarly, none of the LD tests indicated significant nonrandom associations of loci ($P < 0.000009$).

Genetic clusters determined by Bayesian methods

The aspatial algorithm implemented in STRUCTURE assigned individuals into three main genetic clusters (highest $\Delta K = 266.45$) geographically restricted to different regions of Ontario (Northwest, Southeast, and Bruce Peninsula; Fig. 2*a*). All of these clusters were subdivided into two clusters (Northwest: $\Delta K = 60.12$; Southeast: $\Delta K = 17.64$; Bruce Peninsula: $\Delta K = 541.11$) for a total of six clusters (Fig. 2*b*).

The TESS nonadmixture model suggested an optimal K value of $K = 5$, as it was the closest value to the K_{max} identified in the barplot given by DISTRUCT ($K_{max} = 6$) that had the lowest proportion of unassigned sites. For the BYM admixture model, although the DIC graph did not plateau, the values of DIC displayed a lower rate of decrease starting around $K_{max} = 7$. Similar to the model without admixture, the barplot given by DISTRUCT and the proportion of unassigned sites suggested $K = 5$. The clusters detected in both TESS models corresponded to the clusters previously identified in STRUCTURE, with two clusters being located in the Northwest (Northwest A and B), two in the Southeast (Southeast A and B), and one on the Bruce Peninsula (Figs. 2*c*, 3).

Despite an increase in the proportion of unassigned individuals from $q = 0.6$ to $q = 0.9$, the genetic structuring pattern stayed consistent for all membership cut-off values at both $K = 3$ ($\chi^2_{[6]} = 0.2485$, $P = 0.9997$) and $K = 5$ ($\chi^2_{[12]} = 10.298$, $P = 0.5898$) when we controlled for the number of unassigned individuals (Fig. 4). In the absence of control, differences were significant (for $K = 3$: $\chi^2_{[9]} = 27.0815$, $P = 0.0014$; for $K = 5$: $\chi^2_{[15]} = 77.6337$, $P < 0.0001$).

Genetic diversity and level of differentiation

Overall, we detected high levels of heterozygosity (mean $H_O = 0.7496 \pm 0.021$; mean $H_E = 0.7821 \pm 0.022$), as well as a high number of alleles per site (mean no. of alleles per sampling site $= 8.82 \pm 2.76$) (Table 1). Observed heterozygosity ranged from 0.4345 (Owen Sound) to 0.8187 (Sault Ste. Marie), whereas expected heterozygosity ranged from 0.4473 (Owen Sound) to 0.8224 (Atikokan). The mean number of alleles per site ranged from 2.21 (Owen Sound) to 11.21 (Algonquin) (Table 1).

All of the five genetic clusters identified in TESS were significantly, though weakly, differentiated from each other with the lowest level of divergence found between Southeast A and Southeast B ($F_{ST} = 0.0075$, $P < 0.0001$; Table 2) and the remainder of the values ranging from weakly differentiated (Northwest A and Southeast B: $F_{ST} = 0.0181$, $P < 0.0001$) to moderately differentiated (Northwest B and Bruce Peninsula: $F_{ST} = 0.1407$, $P < 0.0001$) (Table 2). Nei's standard genetic distance (D_S) followed the same trend as pairwise F_{ST} values (Mantel test of pairwise F_{ST} vs. pairwise D_S: $r = 0.998$, $P = 0.009$).

Relatedness among individuals was unlikely to influence estimated levels of genetic structure across our sampling range, because among all the putative relationships between individual pairs ($n = 4\,028\,541$), 88.3% had a genetic similarity corresponding to unrelated individuals, 11.3% to half-siblings, 0.29% to full siblings, and 0.06% to parent–offspring, with the mean maximum likelihood relatedness across all pairs of individuals being $r = 0.047$.

When we evaluated relatedness on the Bruce Peninsula only, we found that 74.9% of all pairs of individuals ($n = 9\,591$) were unrelated, 16.2% were half-siblings, 4.2% were full siblings, and 4.8% were parent–offspring. The mean maximum likelihood relatedness was high ($r = 0.407$).

Isolation by distance and spatial distribution of alleles

Mantel tests of pairwise F_{ST} vs. geographic distance showed significant isolation by distance among all sampling sites ($r = 0.161$, $P = 0.002$), which was supported more strongly when the Bruce Peninsula samples were removed from the analysis ($r = 0.552$, $P = 0.001$). Within the Northwest cluster, isolation by distance was also high and significant ($r = 0.490$, $P = 0.001$), though weaker but still significant within the Southeast cluster ($r = 0.255$, $P = 0.003$). All Mantel test results based on F_{ST} reflected the results obtained with D_S ($r = 0.577$, $P = 0.001$ across all sites).

Fig. 2. (*a*) Barplot representing $K_{max} = 3$ genetic clusters of American black bears (*Ursus americanus*) identified by STRUCTURE version 2.3 for $q = 0.6$ (blue, Northwest cluster; green, Southeast cluster; red, Bruce Peninsula cluster). (*b*) Barplots representing the subclusters found within the three main genetic clusters identified by STRUCTURE. (*c*) Barplot representing $K_{max} = 5$ genetic clusters identified by TESS version 2.3 (blue, Northwest A; light blue, Northwest B; light green, Southeast A; green, Southeast B; red, Bruce Peninsula).

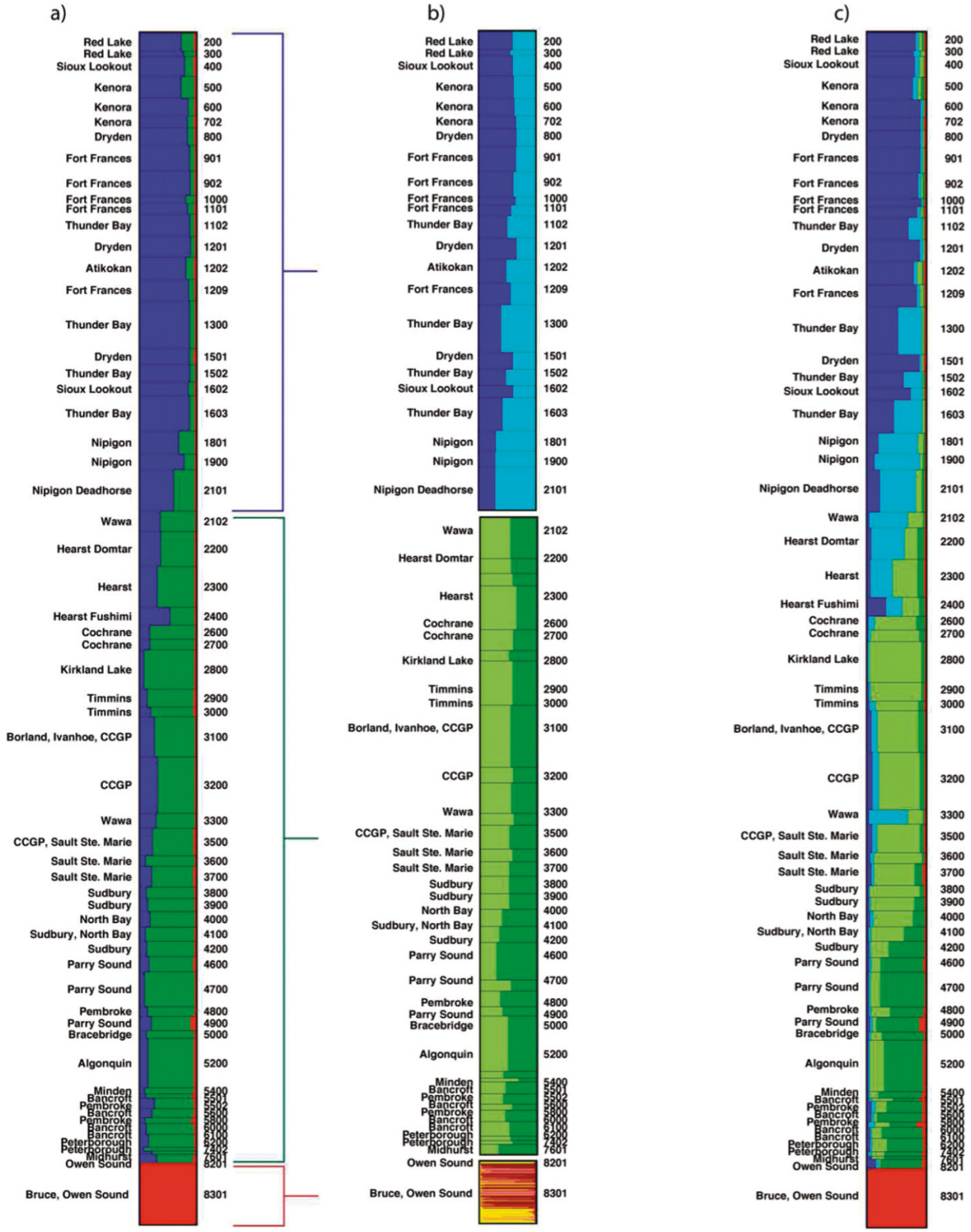
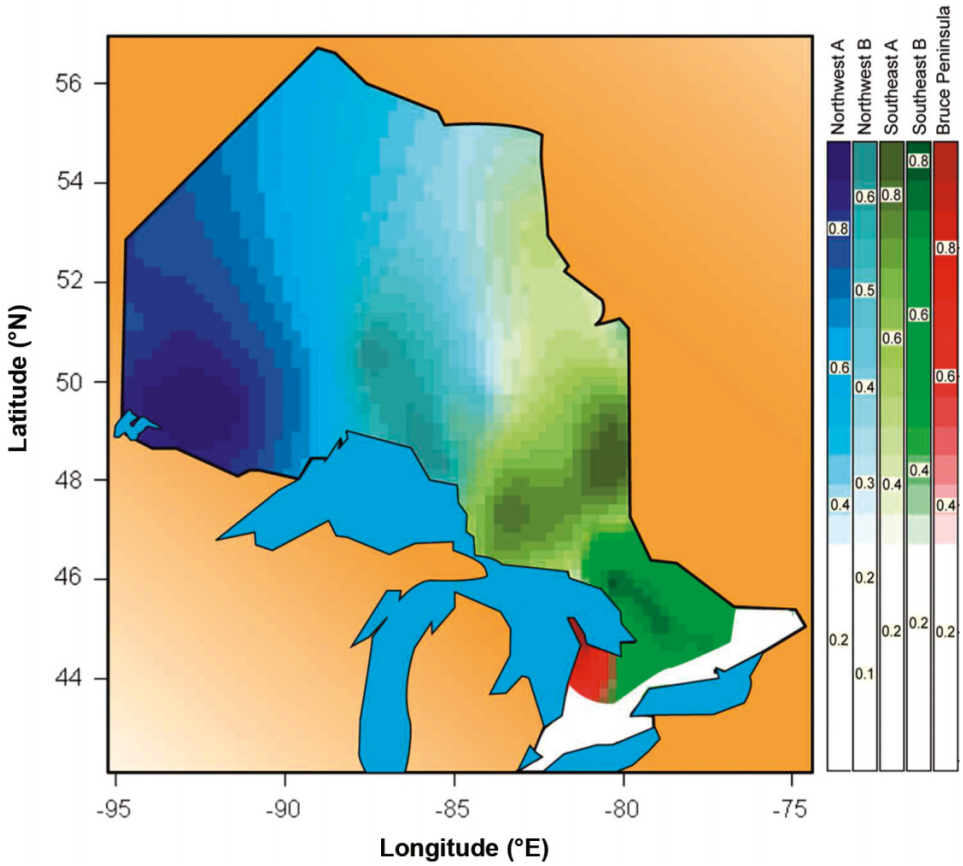


Fig. 3. Interpolated map of posterior membership coefficients in the $K_{max} = 5$ genetic clusters of American black bears (*Ursus americanus*) identified by TESS version 2.3 (blue, Northwest A; light blue, Northwest B; light green, Southeast A; green, Southeast B; red, Bruce Peninsula).



Spatial autocorrelation analyses revealed that the correlation between geographic distance and genetic distance became null at the distance class 500–550 km. From distance classes 50 to 450 km, the correlation was positive and significant ($0.001 < P < 0.036$), and from the distance classes 600 to 1450 km, the correlation was negative and significant ($0.008 < P < 0.023$) (Figs. 5*a*–5*c*).

The difference in overall expected vs. observed geographic distances between shared alleles, although small, was significant ($P < 0.001$). Alleles were found more closely together (OM = 509.8 km) than expected under random distribution (EM = 517.4 km), but overall, there was a slow decrease in the frequency of shared alleles with increasing distance.

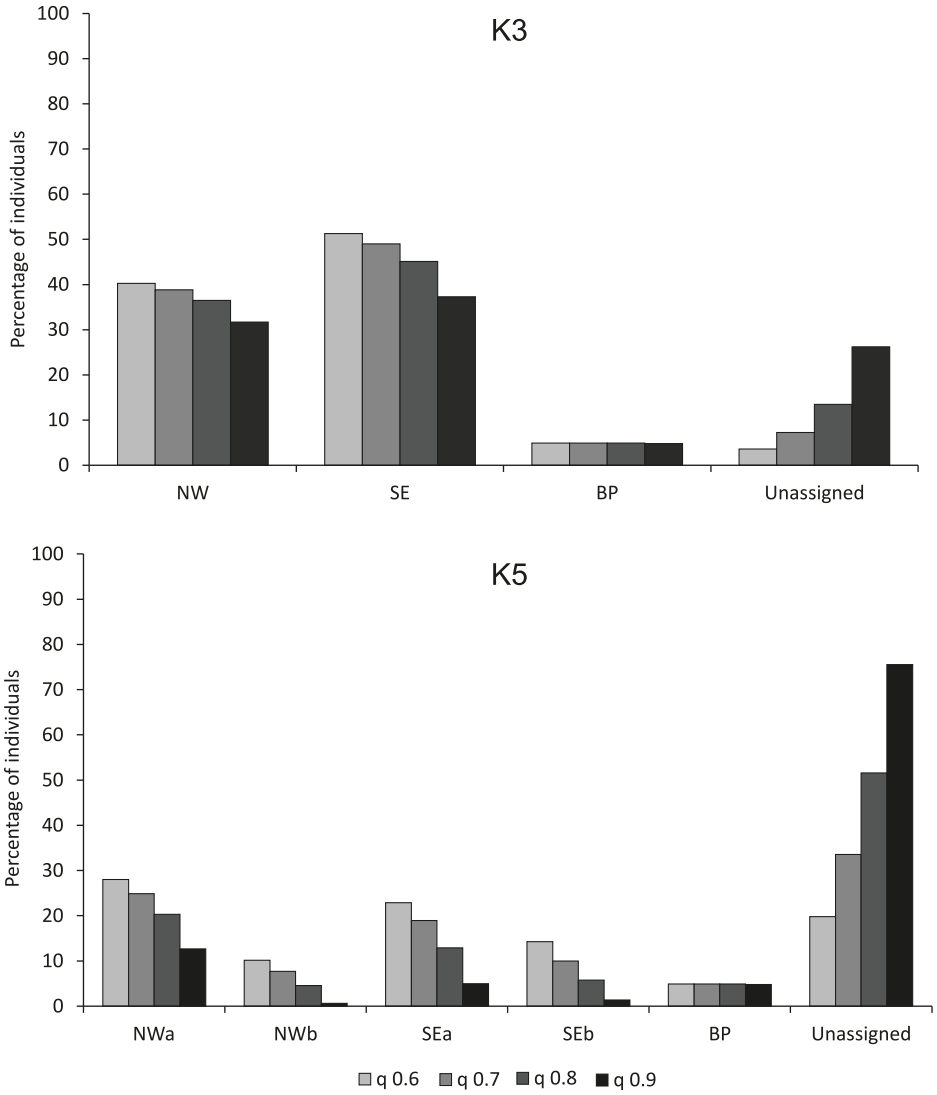
Discussion

Various studies of American black bear described the effects of geographic isolation, bottlenecks, and anthropogenic features on genetic differentiation (Paetkau and Strobeck 1994; Warrillow et al. 2001; Boersen et al. 2003; Csiki et al. 2003; Triant et al. 2004; Cushman et al. 2006; Dixon et al. 2006; Onorato et al. 2007; Peacock et al. 2007); however, few have focused on genetic structure across a mostly contiguous landscape that still corresponds with the species pre-European settlement distribution, with non-natural influences located only at the periphery. Yet, research in such systems is useful to identify large-scale genetic processes and assess the degree of fragmentation of populations that have low genetic variation and are now isolated from the larger continuum of populations of black bears. However, delineating clear population boundaries is difficult when the landscape is contiguous and the species is widely distributed. Here, we used a suite of tools to show that defining genetic clusters of black bears is still possible and appropriate despite their weak and clinal spatial genetic variation, even if clear population limits are absent.

Genetic clusters in Ontario

Our study of Ontario black bears revealed contrasting levels of contemporary genetic diversity and differentiation across a mostly intact landscape. As expected for large populations that share high levels of gene flow, we observed high levels of genetic variability (mean $H_O = 0.7496$; mean $H_E = 0.7821$) and allelic diversity (mean no. of alleles per sampling site = 8.82) (Table 1). These results fall within the range of what has been found in other genetically healthy populations of black bears in North America (Paetkau and Strobeck 1994), as well as other wide-ranging, long-lived mammals such as Canada lynx (Schwartz et al. 2003) and brown bears (*Ursus arctos* L., 1758) (Tammeleht et al. 2010). Despite evidence for high levels of gene flow, Bayesian clustering analyses determined that black bears in our study area were genetically structured into three main genetic clusters: two genetically diverse and weakly differentiated from each other, located in the Northwest and Southeast re-

Fig. 4. Histogram of the percentage of individual American black bears (*Ursus americanus*) assigned to the various clusters at $K = 3$ and $K = 5$, for different cluster membership values ($q = 0.6$, 0.7, 0.8, and 0.9).



gions of the province ($F_{ST} = 0.013$, $P < 0.0001$), and one located on the Bruce Peninsula that was isolated and more strongly differentiated from the other clusters ($F_{ST} > 0.13$, $P < 0.0001$). This clustering pattern is generally consistent with results from mtDNA markers in Ontario (Pelletier et al. 2011) and is also supported by the fact that our error rate would have led to only 62 genotyping errors, which would not affect the overall structure detected here. In addition to these main clusters, both mtDNA and microsatellite analyses detected further genetic subdivisions, suggesting that genetic structuring, although weak, also occurs at smaller geographic scales (Pelletier et al. 2011). Beyond the identification of genetic clusters, our nuclear DNA results showed a clinal pattern of genetic differentiation as a consequence of a slow change in allele frequencies from the Northwest to the Southeast (Figs. 2*a*–2*c*, 3), suggesting that the Northwest sites differ from the Southeast sites despite a low level of differentiation between them (Table 2).

We expected high population admixture levels owing to the high dispersal abilities of black bears and their continuous distribution in Ontario. Thus, as an alternative to using one arbitrary membership cut-off value (q), we used different q values to better support genetic clusters. Broad genetic structure patterns were consistent across all four membership cut-off values ($q = 0.6$, $q = 0.7$, $q = 0.8$, $q = 0.9$) (Fig. 4), illustrating that for weakly differentiated, wide-ranging species, choosing a low threshold to delineate genetic groupings can also be appropriate. Indeed, including individuals with low membership values leads to lower genetic differentiation among clusters and could result in the failure to detect existing patterns. Thus, when weak clusters or clinal structure are identified at such low cut-off values, and are further supported by higher thresholds, it suggests that the pattern observed is not an artefact but accurately reflects the spatial changes in genetic variation, despite the fact that actual populations cannot be clearly defined. In such cases, we suggest that using the genetic structure observed to make management and conservation decisions would still be suitable.

Drivers of genetic structure

American black bears are a vagile species, continuously distributed across much of Ontario, and are weakly differenti-

Table 1. Descriptive statistics per site where we collected genetic samples (number of bears (*n*), expected heterozygosity (H_e), observed heterozygosity (H_o), and the mean number of alleles and their respective standard deviations (SD)) from American black bears (*Ursus americanus*), including site locations within wildlife management units (WMU).

			H_e		H_o		No. of alleles	
WMU	Site	*n*	Mean	SD	Mean	SD	Mean	SD
2	Red Lake	44	0.792	0.016	0.769	0.017	9.43	2.50
3	Red Lake	13	0.793	0.019	0.754	0.033	6.71	1.86
4	Sioux Lookout	49	0.763	0.027	0.681	0.018	9.43	3.06
5	Kenora	54	0.791	0.017	0.732	0.016	9.64	3.27
6	Kenora	42	0.796	0.012	0.755	0.018	8.64	2.44
7b	Kenora	30	0.773	0.018	0.725	0.022	7.86	2.03
8	Dryden	42	0.775	0.012	0.687	0.019	8.71	2.20
9a	Fort Frances	61	0.791	0.018	0.767	0.015	9.21	3.09
9b	Fort Frances	60	0.797	0.014	0.789	0.014	9.50	3.11
10	Fort Frances	19	0.790	0.017	0.730	0.028	7.71	1.77
11a	Fort Frances	26	0.800	0.011	0.808	0.021	8.57	2.24
11b	Thunder Bay	53	0.787	0.016	0.775	0.015	9.29	2.20
12a	Dryden	51	0.777	0.017	0.780	0.016	9.00	2.91
12b	Atikokan	56	0.822	0.012	0.777	0.015	10.79	2.26
12ab	Fort Frances	53	0.802	0.014	0.789	0.015	8.71	2.49
13	Thunder Bay	113	0.805	0.010	0.787	0.010	10.79	4.15
15a	Dryden	41	0.788	0.018	0.742	0.018	9.07	2.92
15b	Thunder Bay	38	0.794	0.018	0.717	0.020	9.14	2.96
16b	Sioux Lookout	29	0.788	0.019	0.750	0.022	8.57	2.31
16c	Thunder Bay	79	0.799	0.021	0.777	0.013	10.57	3.27
18a	Nipigon	49	0.814	0.017	0.813	0.015	9.79	3.33
19	Nipigon	38	0.790	0.019	0.771	0.018	8.79	2.55
21a	Nipigon Deadhorse	101	0.816	0.014	0.756	0.012	10.93	3.50
21b	Wawa	37	0.788	0.021	0.761	0.019	9.29	3.22
22	Hearst Domtar	75	0.805	0.021	0.793	0.013	10.50	3.63
23	Hearst	90	0.808	0.021	0.762	0.012	10.64	3.46
24	Hearst Fushimi	45	0.809	0.015	0.763	0.017	9.50	3.06
26	Cochrane	33	0.803	0.020	0.751	0.020	9.21	2.86
27	Cochrane	27	0.793	0.022	0.733	0.023	8.43	2.28
28	Kirkland Lake	97	0.805	0.020	0.808	0.011	10.64	3.50
29	Timmins	45	0.815	0.020	0.731	0.018	9.71	2.97
30	Timmins	23	0.791	0.025	0.750	0.024	8.43	2.41
31	Borland, Ivanhoe, CCGP	98	0.809	0.023	0.754	0.012	11.14	4.07
32	CCGP	137	0.808	0.024	0.779	0.010	11.64	4.40
33	Wawa	34	0.788	0.023	0.757	0.020	9.14	3.25
35	CCGP, Sault Ste. Marie	68	0.809	0.021	0.798	0.013	10.07	3.58
36	Sault Ste. Marie	26	0.815	0.022	0.819	0.020	8.71	2.49
37	Sault Ste. Marie	52	0.779	0.025	0.765	0.016	9.79	3.47
38	Sudbury	28	0.800	0.025	0.786	0.021	8.29	2.20
39	Sudbury	32	0.814	0.022	0.744	0.021	9.64	3.15
40	North Bay	38	0.793	0.022	0.810	0.017	9.57	3.30
41	Sudbury, North Bay	35	0.786	0.028	0.784	0.019	8.93	2.37
42	Sudbury	38	0.800	0.021	0.760	0.019	9.71	3.54
46	Parry Sound	35	0.776	0.028	0.745	0.020	8.79	2.75
47	Parry Sound	83	0.795	0.022	0.766	0.013	10.43	3.94
48	Pembroke	24	0.802	0.023	0.771	0.023	8.71	2.67
49	Parry Sound	35	0.775	0.020	0.783	0.019	8.64	2.50
50	Bracebridge	20	0.802	0.019	0.762	0.026	8.14	2.74
52	Algonquin	122	0.802	0.020	0.791	0.010	11.21	4.14
54	Minden	15	0.784	0.041	0.767	0.029	7.86	2.07
55a	Bancroft	8	0.775	0.033	0.712	0.045	5.50	1.70
55b	Pembroke	27	0.782	0.020	0.718	0.024	8.43	3.20
56	Bancroft	22	0.789	0.016	0.775	0.024	8.00	1.88

Table 1 (*concluded*).

			H_e		H_o		No. of alleles	
WMU	Site	*n*	Mean	SD	Mean	SD	Mean	SD
58	Pembroke	13	0.738	0.041	0.676	0.036	6.29	2.27
60	Bancroft	26	0.773	0.020	0.755	0.023	8.29	1.94
61	Bancroft	32	0.787	0.020	0.732	0.021	8.14	2.66
62	Peterborough	10	0.752	0.049	0.783	0.037	6.07	2.16
74b	Peterborough	8	0.773	0.025	0.760	0.041	5.64	1.74
76a	Midhurst	21	0.739	0.041	0.614	0.029	7.21	2.67
82a	Owen Sound	4	0.447	0.065	0.435	0.067	2.21	0.70
83a	Bruce, Owen Sound	135	0.558	0.046	0.549	0.012	4.64	1.08
	Total	2839	0.782	0.022	0.750	0.021	8.82	2.76

ated spatially. As such, additional analyses were required to clarify the genetic groupings detected by clustering algorithms (Hardy and Vekemans 1999; Diniz-Filho and De Campos Telles 2002; Schwartz and McKelvey 2009). Here, we detected a significant increase in genetic differentiation with geographic distance through Mantel tests ($r = 0.552$, $P = 0.001$) and spatial autocorrelation analyses ($0.001 < P < 0.036$ from 50 to 450 km). In addition, the spatial analysis of shared alleles showed that the frequency of common alleles decreased slowly as geographic distance between sites increased ($P < 0.001$). Together, these results suggest that the clinal structuring pattern of black bears (Figs. 2*a*–2*c*, 3) is mostly driven by isolation by distance.

In addition to isolation by distance, we suggest that the geographic limits to which males travel when reaching maturity (Rogers 1987; Lee and Vaughan 2003) could also explain a proportion of the genetic differentiation between Northwest and Southeast. The fact that sites become genetically independent at a distance of 550 km (Fig. 5) could be a reflection of dispersal events over multiple generations. Indeed, the mean distance to which males disperse is much lower than the distance required to cross one of these large clusters. Thus, the division between postglacial lineages, located around the 550 km distance class (Pelletier et al. 2011), could be maintained at the contemporary time scale, while displaying lower divergence values relative to the results obtained with historical markers.

Our prediction that anthropogenic activities would lead to additional genetic divisions was not supported for Ontario black bears. Within each of the three large clusters identified at a coarse geographic scale, subtle genetic divisions were detected, although the level of differentiation between the Southeast subdivisions was lower than the ones observed between the Northwest subdivisions (Northwest A – Northwest B: $F_{ST} = 0.01$, $P < 0.0001$ vs. Southeast A – Southeast B: $F_{ST} = 0.007$, $P < 0.0001$), despite the higher anthropogenic pressure that exists in the south of the province (Statistics Canada 2002). This suggests that there is enough gene flow from the core to undermine any genetic drift of bears located at the periphery of their current Ontario distribution, where anthropogenic pressures are higher.

For the isolated, less diverse Bruce Peninsula cluster, the influence of human activities could be an additional process maintaining differentiation through the prevention of gene flow between mainland individuals and individuals from the Peninsula. However, the existence of genetic substructure within this cluster is likely due to the presence of different family groups ($r = 0.407$), and not of contrasting levels of human influences.

The overall pattern of genetic structure detected here, with portions of the population showing evidence of large-scale gene flow, whereas others are isolated, corresponds to what has been observed at a much larger scale in black bears, as well as other widely distributed North American mammals (e.g., wolverine: Kyle and Strobeck 2001; Canada lynx: Schwartz et al. 2003; brown bear: Paetkau et al. 1997; American puma: McRae et al. 2005; American marten: Kyle et al. 2000). The fact that the situation for American black bears in Ontario reflects patterns currently observed at the continental scale illustrates the importance of population genetics studies in wide-ranging species. Such studies can be conducted to compare the fate of isolated fragments relative to the core population, and their results can be used in combination with demographic data to make informed management and conservation decisions for current and future fragmented populations.

Situation on the Bruce Peninsula

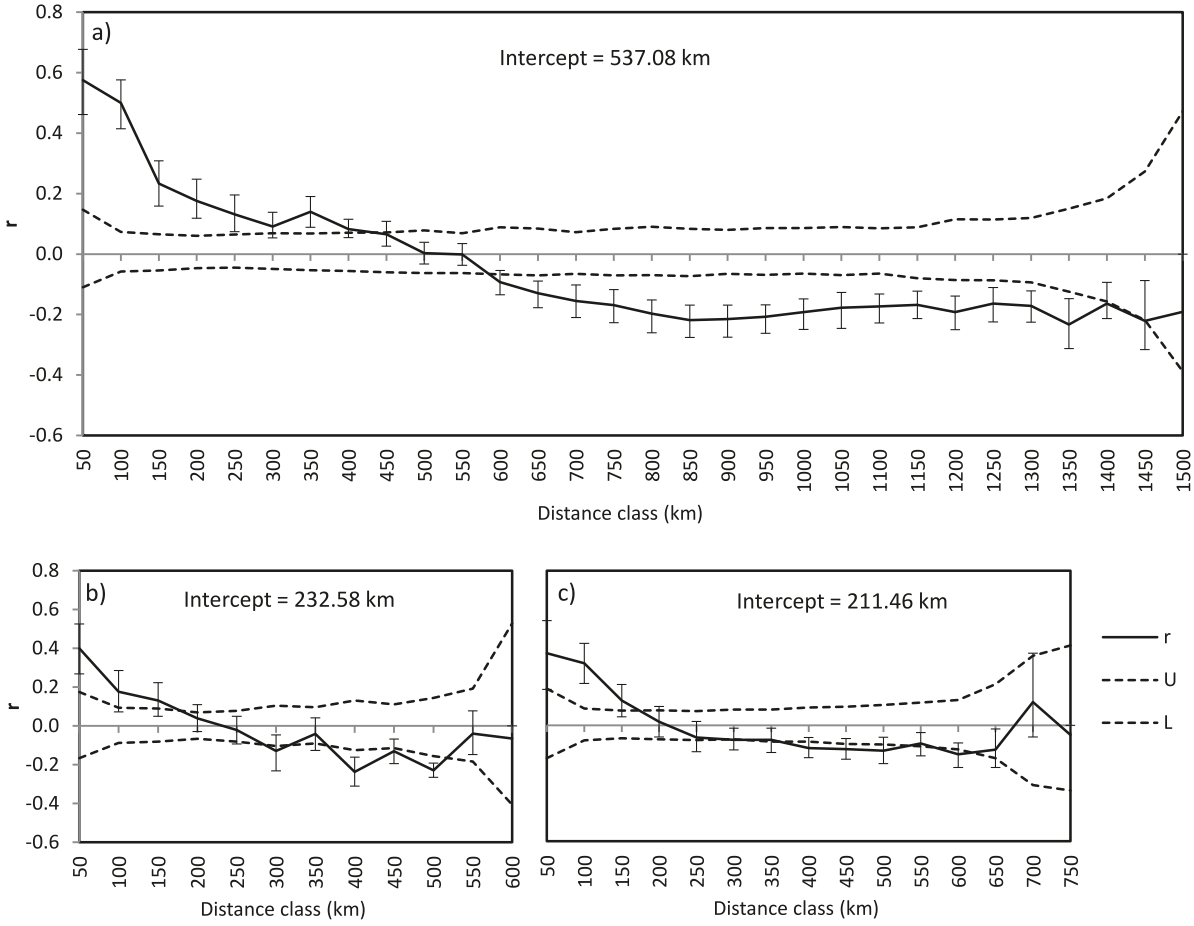
An exception to the clinal structure observed in black bears across the province was the Bruce Peninsula. None of the bears sampled on the Bruce Peninsula could be assigned to any other cluster identified in our analysis, and all of the individuals assigned to this cluster had a membership coefficient higher than 90%, except one individual for with $q = 74\%$. These results suggest that little gene flow occurs between Bruce Peninsula black bears and black bears found in other areas of southeastern Ontario, in contrast to what we detected in the rest of the province. The low level of genetic diversity detected on the Bruce Peninsula ($H_O = 0.5458$; $H_E = 0.5569$) also suggests a lack of gene flow, which could be due to several factors: (*i*) historical genetic drift owing to geographic isolation induced by the shape of the Peninsula after the colonization of this area by black bears; (*ii*) genetic drift owing to high road and settlement densities and intensive agricultural land along the southern edge of the Peninsula that have been preventing immigration of mainland individuals following the European settlement; (*iii*) recent genetic and demographic bottleneck. Overall, this lack of diversity confirms previous demographic and mtDNA research conducted in this area (Howe et al. 2007; Pelletier et al. 2011) and supports the fact that the Bruce Peninsula cluster could be considered a subpopulation.

Table 2. Genetic differentiation levels among the five genetic clusters of American black bears (*Ursus americanus*) identified in TESS version 2.3 for $q = 0.6$, evaluated through pairwise F_{ST} values (sampling sites are mapped in Fig. 1).

	Northwest A	Northwest B	Southeast A	Southeast B	Bruce Peninsula
Northwest A	—				
Northwest B	0.010	—			
Southeast A	0.017	0.011	—		
Southeast B	0.018	0.015	0.007	—	
Bruce Peninsula	0.133	0.141	0.123	0.127	—

Note: For all the comparisons, P values were significant ($P < 0.0001$).

Fig. 5. Spatial autocorrelation correlograms drawn in GENALEX version 6.3 (Peakall and Smouse 2006) of (*a*) our entire Ontario sampling area, (*b*) the sites located within the Northwest cluster identified by Bayesian clustering algorithms, and (*c*) the sites located within the Southeast cluster identified by Bayesian clustering algorithms. The *r* (solid line) is the correlation coefficient between genetic differentiation and geographic distance. The 95% confidence interval is represented by broken lines (upper (U) and lower (L) bounds) to compare our results with a random distribution. The bootstrapped 95% confidence error bars around *r* are also displayed. In all cases, there is a decline in the genetic correlation of American black bears (*Ursus americanus*) with geographic distance.



The level of diversity on the Bruce Peninsula is consistent with what has been observed in genetically depauperate populations of black bears located in the southern portion of the continent ($0.38 < H_O < 0.56$; Warrillow et al. 2001; Triant et al. 2004; Onorato et al. 2007). Such low diversity has been explained by the effects of bottlenecks (e.g., Tensas River, Louisiana: Boersen et al. 2003; coastal Louisiana: Triant et al. 2004) or geographic isolation (Paetkau and Strobeck 1994; Ohnishi et al. 2007; Brown et al. 2009), and we suggest that black bears experienced similar influences on the Bruce Peninsula. It is possible that extensive human disturbance related to logging and agriculture beginning in the 1870s, especially the use of fire to help clear the land, may have impacted bear density in this area. Particularly large fires in 1903 and 1908 destroyed much of the forested land in the northern two-thirds of the Peninsula (Suffling et al. 1995), and may have caused a dramatic and sudden decline in the number of bears. This documented dramatic ecological perturbation, in addition to the lack of diversity at both nuclear and mitochondrial markers (Bruce Peninsula black bears have only 2 mtDNA haplotypes out of the 36 that occur in Ontario; Pelletier et al. 2011), suggest that a bottleneck

might have occurred which the population has not been able to recover from genetically owing to its geographic isolation.

Although lower levels of genetic variation have been found in other isolated populations of black bears (Paetkau and Strobeck 1994; Csiki et al. 2003; Dixon et al. 2006), the lack of diversity in Bruce Peninsula black bears is of concern. Indeed, the population size there is low to ensure future population persistence (Howe et al. 2007), and important habitat of black bears is under pressure from development (Obbard et al. 2010). Thus, black bears in this area could suffer from inbreeding depression in subsequent years, which would impede the population's survival (Frankham 1995; Frankham 1997; Keyghobadi 2007). Consequently, there is a need for further research to clearly identify the reasons for the low heterozygosity found in Bruce Peninsula black bears and to evaluate possible mechanisms for the population to regain a level of genetic diversity that would be similar to more continuous populations located in the core of their distribution. Since Bruce Peninsula black bears still share common alleles with the other clusters, we recommend that future modeling analyses assess the effect of translocations from Southeast individuals into the Bruce Peninsula on genetic variation in the event of a restocking effort.

Our study is one of few genetic studies of a wide-ranging mammal that was conducted on such an extensive data set across such a large geographic area. When put in the context of other genetic research on black bears, our results show that Ontario black bears (with the exception of the Bruce Peninsula) may be used as a reference that corresponds with the levels of genetic diversity and structure that should be observed among intact populations of black bears which share high levels of gene flow. The level of differentiation observed between the Bruce Peninsula and the other clusters shows that despite the ability of individuals to disperse across long distances, populations of black bears can be significantly differentiated from the core when isolated. The remaining Ontario black bears seem to be weakly structured by isolation by distance combined with male-biased dispersal.

As expected, the differentiation between clusters was higher in mtDNA (Pelletier et al. 2011) relative to microsatellites, for which genetic structure across the province was subtle and weak, as can be anticipated for continuously distributed species. Because this contemporary genetic variation was clinal, and no abrupt break was detected, clearly delineating where one cluster started and the other ended was challenging. Still, the fact that microsatellites identified three main genetic groups in Ontario, which mostly correspond with the mtDNA clusters (Pelletier et al. 2011), can help refine management decisions in this province.

As a result of the suggested rapid rate of loss of genetic diversity in the Bruce Peninsula black bears, and the increasing influence of human activities on previously undisturbed landscapes, we suggest studies that examine wide-ranging species focus on modeling the impact of future landscape and climate changes on the population dynamics, genetic structure, and diversity of populations. In the event of such models finding an increase in the number of isolated fragments within species, leading to concerns regarding the vital rates and genetic health of populations, preventative measures could be taken to identify, conserve, and manage continuous landscape networks. This would promote high levels of genetic diversity and higher population sizes through the maintenance of connected sites at the continental scale and prevent northern populations from becoming as isolated as those observed in the southern portions of the continent.

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