UNDERSTANDING HISTORICAL AND CONTEMPORARY GENE FLOW PATTERNS OF ONTARIO BLACK BEARS: TOWARDS REFINING MANAGEMENT STRATEGIES

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ABSTRACT

UNDERSTANDING HISTORICAL AND CONTEMPORARY GENE FLOW PATTERNS OF ONTARIO BLACK BEARS: TOWARDS REFINING MANAGEMENT STRATEGIES

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Consequences of habitat loss and fragmentation include smaller effective population sizes and decreased genetic diversity, factors that can undermine the long-term viability of large carnivores that were historically continuously distributed. I evaluated the historical and contemporary genetic structure and diversity of American black bears (*Ursus americanus*) in Ontario, where bear habitat is largely contiguous, except for southern regions that experience strong anthropogenic pressures. My objectives were to understand gene flow patterns in a natural system still largely reflective of pre-European settlement to provide context for the extent of genetic diversity loss in southern populations fragmented by anthropogenic influences. Phylogeographic analyses suggested that Ontario black bears belong to a widespread "continental" genetic group that further divides into 2 subgroups, likely reflecting separate recolonization routes around the Great Lakes following the Last Glacial Maximum. Population genetic analyses based on individual genotypes showed that Ontario black bears are structured into 3 contemporary genetic clusters. Two clusters, located in the Northwest (NW) and Southeast (SE), are geographically vast and genetically diverse. The third cluster is less diverse, and spatially restricted to the Bruce Peninsula (BP). Microsatellite analyses revealed that the NW and SE clusters are weakly differentiated from each other relative to

mitochondrial DNA findings, suggesting male-biased dispersal and isolation by distance across the province. I also conducted simulations to assess competing hypotheses that could explain the reduced genetic diversity on the BP, which supported a combination of low migration and recent demographic bottlenecks. I showed that management actions to increase genetic variation in BP black bears could include restoring landscape connectivity between BP and SE; however, the irreversible human footprint in the area makes regular translocations from SE individuals a more practical alternative. Overall, my work suggests that: 1) historical genetic processes in Ontario black bears were likely predominated by isolation by distance, 2) large mammalian carnivores such as black bears can become isolated and experience reduced diversity in only a few generations, and 3) maintaining connectivity in regions under increased anthropogenic pressures could prevent populations from becoming small and geographically and genetically isolated, and should be a priority for conserving healthy populations.

KEYWORDS: American black bear; bottleneck; carnivore; cluster; conservation genetics; female philopatry; gene flow; genetic structure; genetic restoration; isolation by distance; male-biased dispersal; microsatellite; mitochondrial DNA (mtDNA); North America; Ontario; phylogeography; translocation; *Ursus americanus*.

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CHAPTER 1

GENERAL INTRODUCTION

As global anthropogenic pressures increase, increasing levels of habitat loss and fragmentation threaten the long-term persistence of many species (Fahrig 2003). Indeed, small and geographically isolated populations have higher risks of extinction than large and continuously distributed populations. Reasons for this include higher probabilities of inbreeding (Frankham 1998; Wang et al. 1999; Spielman et al. 2004; Frankham 2005), possible loss of adaptive potential (Keller and Waller 2002; Keyghobadi 2007), and increased vulnerability to stochastic events (Lande 1993). Knowledge of historical migration patterns, as well as drivers of contemporary movement, demographic trends, and genetic structure, is therefore needed to help define lasting management and conservation actions to maintain species stability and overall biodiversity. Recent advances in molecular genetic techniques have provided insights into both historical and contemporary population processes for a broad range of species. For large mammalian carnivores especially, these techniques have allowed for minimally invasive field research based on fecal and hair samples to monitor species that are elusive or costly to trap (Taberlet et al. 1997; Kohn et al. 1999; Paetkau 2003; Schwartz et al. 2007). Data from such studies enable the implementation of management initiatives based on scientific data that could not have been gathered otherwise.

Range contractions in large carnivores

Across the world, the range of many carnivores has been greatly reduced compared to their historical distribution (Servheen 1990; Rodriguez and Delibes 2002; Luo et al. 2004; Dubach et al. 2005), leading to increased population isolation, smaller population sizes, and decreased genetic diversity. Large mammalian carnivores are a key component of ecosystem stability due to their role in trophic cascades (McLaren and Peterson 1994; Berger et al. 2001; Ripple and Beschta 2008), and are viewed as flagship species by the public. For these reasons, recovery plans have been implemented to prevent further population declines, and to help them regain levels of genetic diversity that would reduce concerns for their long-term persistence (Florida panther - Hedrick 1995; Scandinavian wolf - Vilà et al. 2003; Mexican wolf - Fredrickson et al. 2007; Louisiana black bear - Triant et al. 2004).

In North America, the most significant reductions have occurred in the southern portion of the continent in response to higher levels of human density. In this area, remnant large carnivore populations are now limited to isolated habitat pockets. In contrast, in the northern region, the current range of large carnivores mostly corresponds to their historical distribution (Laliberté and Ripple 2004). Because of this, continuously distributed northern populations can be used as references to assess the impacts and processes of landscape fragmentation, habitat loss, and decreased population size on conspecific populations that are, or will become, isolated. Such data provide context on the historical state of the now fragmented populations, and can thus inform future conservation and management initiatives in these regions. One important phase of this type of research is to determine how these unfragmented populations are genetically

structured. If they are panmictic in their natural state, population connectivity should be preserved to maintain genetic diversity. If, on the other hand, such populations correspond to numerous genetically diverse populations across their distribution, then fragmentation would not necessarily be detrimental (Theodorou et al. 2009), or its effects could be delayed (Richmond et al. 2009). Such knowledge is therefore important to establish proactive rather than reactive measures to maintain biodiversity in the face of the increased landscape fragmentation that may occur in currently undisturbed areas (Cardillo et al. 2006 and 2008).

Understanding gene flow patterns through molecular genetic techniques

Through molecular genetic techniques, scientists have been able to uncover and date historical processes such as bottlenecks, colonization, dispersal events, and vicariance (Arbogast 2001). Mitochondrial DNA (mtDNA), a maternally inherited marker, has helped describe events that have occurred before and around the Last Glacial Maximum (26,500–19,000 years B.P., Clark et al. 2009). Phylogeographic studies, which link biogeography and phylogenetics (Avise et al. 1987), have helped determine the location of several glacial refugia (Byun et al. 1997; Demboski et al. 1999; Byun et al. 1999). The existence of these refugia has since explained the presence of intraspecific genetic differentiation in populations with contemporary overlapping distributions (Wooding and Ward 1997; Taberlet et al. 1998; Arbogast 2007).

In addition to historical processes of differentiation, molecular genetic techniques can be used to test contemporary demographic and gene flow patterns. These methods involve the identification of recent population contractions, the delineation of contemporary population boundaries, the evaluation of population dynamics trends, and the identification of landscape features impeding gene flow. Over the last 3 decades, biparentally inherited neutral markers such as microsatellites have been used extensively to conduct this type of research. The popularity of these markers can be explained by their high variability, which allows them to be used to differentiate individuals by genotyping, and thus, to conduct analyses at the individual rather than at the population level. Through genetic information obtained at the individual level, it has become possible to estimate demographic information such as abundance and vital rates. In addition, it has also been possible to estimate heterozygosity and calculate allelic frequencies, factors that are indicators of genetic diversity (Schwartz et al. 2007). Along with demographic parameters, these measures provide an estimation of long-term persistence potential (Lande 1988 and 1993; Reed and Frankham 2003; Frankham 2005).

Methods such as genetic assignment tests, which carry out analyses based on genotypic information, have enabled researchers to determine if an individual sampled in one location originated from that location, or was a potential migrant. The first test developed by Paetkau et al. (1995), was based on allele frequencies. The theory was that an individual had a higher probability of sharing alleles with individuals of the population it was born in, rather than with individuals of the population it had migrated into. This method relied on the definition of populations prior to assigning individuals. Subsequently, non-spatial Bayesian assignment methods were developed (Pritchard et al. 2000; Dawson and Belkhir 2001; Corander et al. 2003; Falush et al. 2003). These

methods were based on a probabilistic approach, where individuals were assigned to an incremental number of possible populations (*K*) based on their genotypes, while allelic frequencies of each population were estimated. The main benefit of the Bayesian approach was that populations were not required to be defined *a priori*. In addition, it allowed the detection of subtle genetic divisions resulting from admixture, as well as the estimation of the most likely number of biological populations included in the empirical dataset. Bayesian assignment tests were then enhanced by the inclusion of spatial information into the modelling process (Guillot et al. 2005; François et al. 2006; Chen et al. 2007).

These genetic advancements have provided valuable benefits to the fields of conservation genetics and landscape genetics. In conservation genetics, assessments of gene flow and structuring patterns, combined with population dynamics analyses, enabled defining populations as Evolutionary Significant Units (Moritz 1994) or Management Units (Palsbøll 2007). In this field, genetic data can also be used to assess the need to supplement populations considered at risk (Hedrick 1995), predict the impacts of habitat restoration on genetic diversity (Larkin et al. 2004; Dixon et al. 2006), or determine the minimum population size at which a population could retain evolutionary potential and avoid extinction (Reed et al. 2003).

Landscape genetics is a combination of population genetics and landscape ecology (Manel et al. 2003) used to determine what landscape features can explain genetic differentiation between populations. Interest in this recent discipline stems from the ability to correlate contemporary anthropogenic influences on the landscape to specific genetic patterns observed in the wild. A major focus of this research has been on the consequences of habitat fragmentation and habitat loss on genetic differentiation between

populations. The reason for this is that species persistence depends on the capacity of individuals to reproduce and maintain genetic diversity both within and among populations (Fahrig 2003; Keyghobadi 2007). The fact that low genetic variability and inbreeding, as found in island populations (Frankham 1997), can also be observed in mainland populations as a result of limited movement of individuals between habitat patches, is one of the main issues facing biodiversity today (Keller and Waller 2002; Fahrig 2003; Keyghboadi 2007). In addition, due to species-specific ecological requirements, landscape features have different effects on animal movements, and thus gene flow. For these reasons, it is necessary to assess the impacts of spatial features on genetic patterns of various taxa. With this approach, it is possible to quantify the effects of habitat variations on animals movements (Cushman et al. 2006), identify populations that are potentially at risk of geographic and genetic isolation due to a lack of suitable habitat (Galindo et al. 2006), delineate corridors that would allow connectivity to be retained (Epps et al. 2007), or detect the presence of barriers to gene flow (McRae et al. 2005).

Overall, disciplines using molecular genetics give insights into the ecology and evolution of species. In addition, they make valuable contributions to inform management actions that would prevent wild populations from local extirpation, as they allow population trajectories to be predicted under alternative ecological scenarios of gene flow, population size, and habitat availability. These techniques can be applied to a broad range of taxa, and are especially useful for the conservation of species that require large geographic ranges, have low reproductive rates, and low population densities, as these factors increase the risk of extirpation (McKinney 1997; Purvis et al. 2000; Cardillo et al. 2005).

American black bears in North America and in Ontario

Demography and conservation status

American black bears (*Ursus americanus*) are widely distributed in North America, and demographic studies have shown contrasting densities across their range (0.03 individual/km^2 to 1.30 individual/km^2; Tredick and Vaughan 2009; Obbard et al. 2010). In Ontario, black bear densities calculated with open population models (Gardner et al. 2009) are within the range of other American black bear populations (Robinson et al. 2009; Clark et al. 2010; Gardner et al. 2010), with values ranging from 0.03 to 0.15 individual/km^2 in the boreal forest (Obbard et al. 2010), and 0.04 to 0.19 individual/km^2 in the Great Lakes St. Lawrence region (GLSLR; Howe et al. *unpublished*). It is not surprising that the lowest densities are found in the boreal forest, and the highest in the GLSLR, as habitat quality, known to be higher in the GLSLR (Obbard and Howe 2008), is negatively correlated with home range size (Jones and Pelton 2003; Koehler and Pierce 2003). In North America, female black bear annual survival rates in the range of 0.7 to 0.95 have been observed (Clark and Smith 1994; Sorensen and Powell 1998; Bales et al. 2005), which corresponds to the value estimated in Ontario (0.91; Obbard and Howe 2008). Population growth rates in black bears are most often positive (Yodzis and Kolenosky 1986; Bales et al. 2005), although negative values were detected in the White River National Wildlife Refuge (Arkansas, USA), and attributed to the effects of hunting pressure (Clark et al. 2010).

Generally low densities and growth rates, common in large mammalian carnivores, make isolated black bear populations susceptible to extirpation. This is concerning, as over the last 400 years, the geographic range of American black bears (est. 900,000 individuals; IUCN 2008) has reduced significantly despite their high dispersal abilities (Rogers 1987; Costello et al. 2008) and generalistic nature (Schoen 1990). This reduction is a consequence of post-settlement anthropogenic pressures, and as a result, their current range now only covers 69% of their historical distribution (Scheick et al. 2011– Fig. 1.1). Numerous isolated populations that are, or were, of conservation concern exist in the southern portion of the continent (Arkansas, Oklahoma, Louisiana, Florida, New Mexico, Texas, Mexico - Warrillow et al. 2001; Csiki et al. 2003; Triant et al. 2004; Dixon et al. 2007; Onorato et al. 2007; Scheick, *pers. comm.*). In the United States, American black bears only occupy 44% of their historical range, although sightings are now being recorded in previously unoccupied states (e.g., Iowa, Nebraska, South Dakota, North Dakota, Ohio – Scheick et al. 2011). In contrast, their distribution remains mostly contiguous in the northeastern and northwestern states of the United States as well as in Canada, where 95% of their historical range is still intact (Vaughan and Pelton 1995; Scheick et al. 2011 – Fig. 1.1). This large portion of undisturbed habitat, however, might become more fragmented with a northward human expansion (Cardillo et al. 2006 and 2008), and sources of human-induced mortality such as hunting (Obbard and Howe 2008; Clark et al. 2010), and vehicular traffic (Garrison et al. 2007; Ryan et al. 2007; Hostetler et al. 2009; McCown et al. 2009), might increase.

A significant proportion of American black bears can be found in Ontario, where numbers are estimated at 95,000 ± 10,000 bears (M.E Obbard, *unpublished data*). In this province, the landscape is largely contiguous, with no obvious ecological features that would impede dispersal such as wide rivers, mountains, or radical habitat change, except for the southern periphery, which is under high anthropogenic pressures (Statistics Canada 2002). As a consequence of these land-use patterns, Ontario black bears are continuously distributed, except for a small population located on the Bruce Peninsula (BP). This population is geographically isolated due to growing habitat fragmentation that prevents connectivity with adjacent populations located in the more disturbed southern region (Howe et al. 2007). The ecological context presented above shows that the Ontario landscape provides a study area where historical population processes that correspond to a natural, undisturbed state, are still likely at play, and can be directly compared to areas where fragmentation has increased following European settlement.

Genetic diversity and gene flow patterns

Phylogeographic analyses that evaluate historical patterns of differentiation have revealed that American black bears are historically divided into 2 phylogeographic clades, as observed in other North American species (Northern flying squirrel, *Glaucomys sabrinus*, Arbogast 1999; American marten, *Martes Americana*, Demboski et al. 1999; Canada lynx, *Lynx Canadensis*, Rueness et al. 2003; red fox, *Vulpes vulpes*; Aubry et al. 2009). In these species, a widespread, "continental" clade ranges from the eastern seaboard to the Rocky Mountains and Alaska, and a "coastal" clade runs along the Pacific Coast (Byun et al. 1997; Wooding and Ward 1997; Stone and Cook 2000; Peacock et al. 2007). In black bears, the continental clade has been suggested to be further divided into

2 subclades, although a lack of data from the mid-eastern part of their range led to the supposition that this weak genetic differentiation was spurious (Wooding and Ward 1997). Mitochondrial DNA data from the province of Ontario would be useful to fill this knowledge gap, and could reveal historical gene flow patterns previously undetected.

Contemporary population genetic studies have identified contrasting levels of genetic diversity and genetic differentiation across the range of American black bears. Genetic structuring of black bear populations has been explained by landspace features that prevent gene flow, such as islands (Paetkau and Strobeck 1994), ice (Peacock et al. 2007), elevation (Cushman et al. 2006), and geographic isolation resulting from habitat loss due 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). High levels of heterozygosity have been observed in populations that are either contiguous or connected to others via migration ($0.70 < H_O < 0.94$; Paetkau and Strobeck 1994; Csiki et al. 2003; Belant et al. 2005; Cushman et al. 2006; Robinson et al. 2007; Pelletier et al. 2012), whereas levels as low as 29% have been detected in isolated populations (Paetkau and Strobeck 1994; Warrillow et al. 2001; Csiki et al. 2003; Triant et al. 2004; Dixon et al. 2007; Onorato et al. 2007). Most of these isolated populations are either island populations (Paetkau and Strobeck 1994), or populations located in the southern periphery of the current distribution having lost diversity from a lack of connectivity with more contiguous segments (Onorato et al. 2004; Dixon et al. 2007). For the latter, conservation concerns exist, and federal-wide or state-wide conservation listings have been warranted (Louisiana black bear - Neal 1992; Florida black bear -Wooding 1993). In the case of Florida black bears, recent delisting at the state level was based on a steady increase in abundance over the past 3 decades, although a few subpopulations remain

listed (Florida Fish and Wildlife Conservation Commission 2011 and 2012). In Ontario, genetic variability levels observed in the undisturbed regions of the province could be compared to the ones observed in more fragmented areas. In addition, the demographic information available from the BP population (Howe et al. 2007) could be supplemented by a genetic assessment, to 1) detect genetic distinctiveness that could lead to its designation as a Management Unit, 2) evaluate the presence of inbreeding that can occur in small, isolated populations, and 3) inform the need for potential genetic restoration actions.

Objectives

The land-use patterns observed in Ontario, with higher habitat fragmentation in the South compared to the North (human density: 1 to < 50 individuals/km^2 at the southeastern periphery; < 0.4 individuals/km^2 in the rest of the province, above Lat. 45.5; Statistics Canada 2002), reflect, at a smaller scale, what is observed across the continent. As such, studying how genetic variation is distributed in Ontario black bears could provide insights into historical and contemporary processes that shape structuring patterns of other wide-ranging mammals in situations where the landscape is undisturbed or fragmented. Such research is useful to identify suitable actions that could be implemented before the detrimental consequences of geographic isolation, small population size, and reduced genetic diversity, could affect their populations. For this reason, Ontario black bears provide a useful biological model to identify and predict the impacts of processes that may reduce habitat connectivity between currently continuously distributed populations, and may result in negative effects on genetic diversity and long-term

persistence. This research would also provide context for the extent of population fragmentation of black bears in other regions of the continent, and could illustrate the natural processes driving gene flow prior to European settlement. Finally, these data would be useful to inform contemporary conservation and management plans for this species in Ontario, actions that may be similarly required for other black bear populations.

The majority of molecular studies of American black bears have focused on populations located in the western and southern portions of their current distribution (Byun et al. 1997; Wooding and Ward 1997; Stone and Cook 2000; Warrillow et al. 2001; Triant et al. 2004; Dixon et al. 2007; Onorato et al. 2007; Peacock et al. 2007; but see Paetkau and Strobeck 1994). Thus, the genetic information from Ontario black bears addresses a previous lack of data from a large portion of their mid-eastern range. In addition, the molecular and modeling techniques presented here can be applied to other species that may experience further range contractions and loss of habitat.

Specifically, I used a 315 base-pair fragment of the mtDNA control region, 15 microsatellite loci, and gender identification, to assess the genetic structure and genetic diversity of black bears across Ontario. Genetic data were primarily obtained from black bear hair samples collected from barbed wire hair traps (Woods et al. 1999) in most Ontario Wildlife Management Units (Obbard et al. 2010; Fig. 1.3). My goal was to use this large genetic dataset to generate a comprehensive understanding of historical and contemporary processes shaping genetic variation in a large mammalian carnivore whose range spans a vast landscape under contrasting levels of anthropogenic pressures. In Ontario, these pressures (e.g., human density, road density) are expected to increase in the

northern region, as governmental incentives have been created to encourage development (Growth Plan for Northern Ontario 2011).

I first identified historical gene flow patterns of Ontario black bears, and integrated the results obtained to range-wide information from previous studies (Chapter 2); I then uncovered more contemporary structuring patterns across the province, and compared them with historical genetic groupings (Chapter 3); finally, I identified the reasons for low genetic diversity in a small, geographically isolated population that represents a single genetic cluster, and predicted how the level of genetic variability would evolve if the population remained isolated or received translocated individuals (Chapter 4).

To identify both historical and contemporary processes of genetic differentiation, I conducted analyses in different frameworks. First, I took a phylogeographic approach to determine how Ontario black bears fit into the 2 historical clades previously identified by Wooding and Ward (1997), and which were hypothesized to have arisen due to isolation in different glacial refugia (Byun et al. 1997). As Ontario is located in the mid-eastern portion of North America, I hypothesized that mtDNA analyses would indicate that bears sampled in Ontario would belong to the widespread continental clade. My objective was also to test the hypothesis of genetic differentiation within this clade, as suggested by Wooding and Ward (1997), by adding samples from a previously unexamined portion of the range. The goal was to obtain a clearer picture of the events that shaped historical genetic variation in Ontario black bears. This was further augmented by comparing mitochondrial patterns of genetic differentiation to those obtained from nuclear data (microsatellite genotypes) at 8 Ontario sampling sites (Mills 2005) to identify patterns of sex-biased dispersal.

Second, I used a population genetic approach based on microsatellites to contrast the historical genetic results with more contemporary genetic information. Here, the goals were to: 1) identify small and large scale patterns of contemporary genetic structure with various techniques such as assignment tests and spatial autocorrelation, 2) determine if the mtDNA structure corresponded to that obtained from the faster evolving microsatellite markers, 3) explicitly test for sex-biased dispersal, and 4) explain the levels of contemporary differentiation observed between the genetic groupings detected in Ontario. The ultimate purpose of these analyses was to assess the limitations that researchers face when trying to delineate populations within wide-ranging, continuously distributed species. Based on black bears dispersal abilities, I hypothesized that in Ontario, individuals would be genetically structured by a pattern of isolation by distance, which would be illustrated by low levels of differentiation between sites, and significant spatial autocorrelation. To identify potential genetic clusters, I used both non-spatial and spatial Bayesian assignment tests. I also hypothesized that tests based on assignment indices would detect male-biased dispersal.

Third, I used an individual-based modeling approach to complement the empirical genetic data obtained through mtDNA and microsatellites, and used a predictive framework to assess the persistence potential of the isolated Bruce Peninsula (BP) black bear population. In Ontario, the BP population is both small and geographically isolated (Howe et al. 2007). As such, it is important to determine if further concerns regarding its persistence can be identified through genetic data, and if specific actions have to be implemented for conservation purposes. Based on results regarding the level of genetic diversity of this population (Pelletier et al. 2011 and 2012), I conducted simulations to 1) identify the potential reasons for low genetic variability, 2) predict its long-term viability,

and 3) determine the need for translocation efforts from individuals located in the contiguous portion of southeastern Ontario for genetic or demographic restoration actions. I hypothesized that BP black bears had undergone a recent reduction in population size as a consequence of large fires (Suffling et al. 1995) resulting in a demographic and genetic bottleneck, and conducted forward simulations to test for various scenarios of historical genetic drift, recently reduced migration, and population reduction. The information obtained is useful to understand the conditions under which other American black bear populations could become small and geographically and genetically isolated. In addition, this modeling approach can also be used to assess the probability of persistence of other wide-ranging species that will likely experience geographic isolation and decreased population sizes in a near future.

Overall, my thesis provides an integrative understanding of historical and contemporary gene flow patterns in a large mammalian carnivore known to be affected by human-related activities. In addition, it provides a general framework that can be applied to other wide-ranging species to predict the persistence potential of isolated populations, and of those that may become fragmented.

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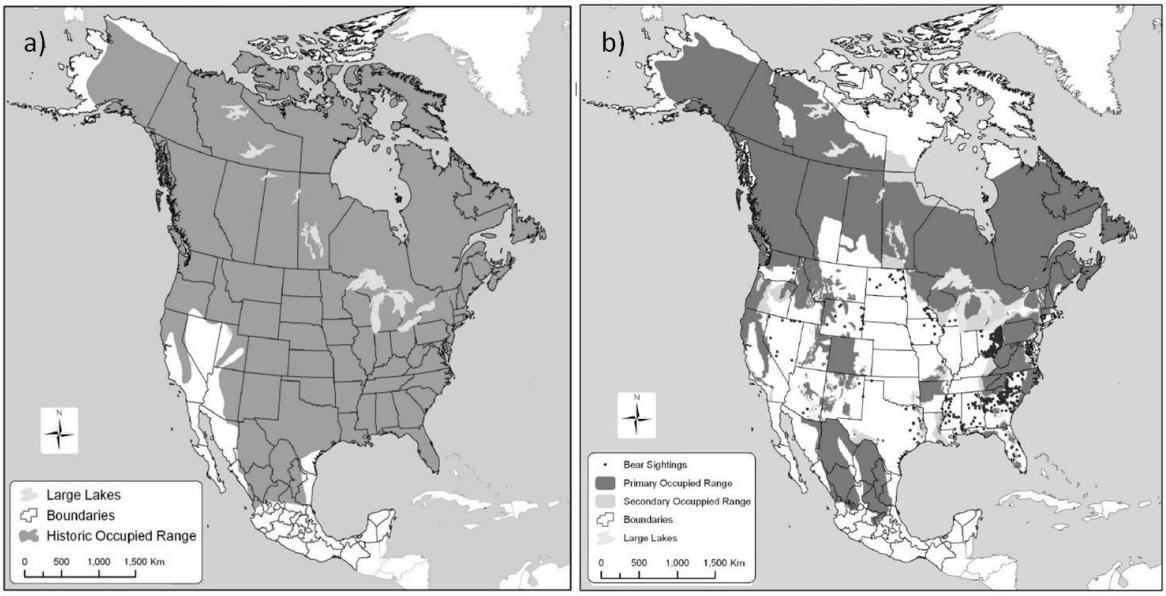

Fig. 1.1. a) Historical range of the American black bear (pre-European settlement); b) Current range of the American black bear (Vaughan and Pelton 1995; Scheick et al. 2011). *Reproduced with authorization from Scheick et al. (2011)*.

Note: Further range fragmentation exists in Mexico, which is not indicated on the map (Scheick, *pers. comm.*).

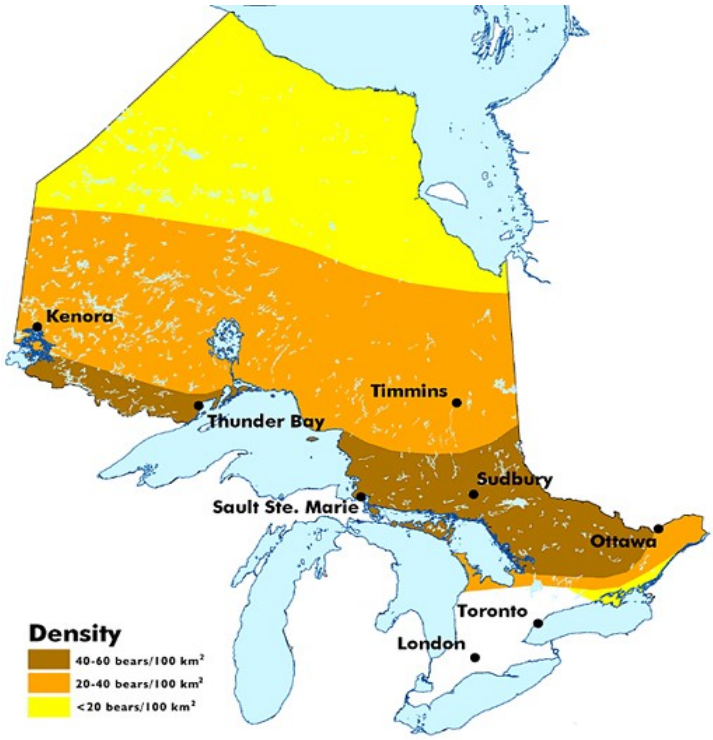

Fig. 1.2. Approximate delineation of density zones of American black bear in Ontario, Canada (for revised densities, see Obbard and Howe 2008; Howe et al. *in press*; Obbard et al. *unpublished*).

http://www.mnr.gov.on.ca/en/Business/Bearwise/2ColumnSubPage/STEL02_167695.html, MNR BearWise accessed 12/01/2013.

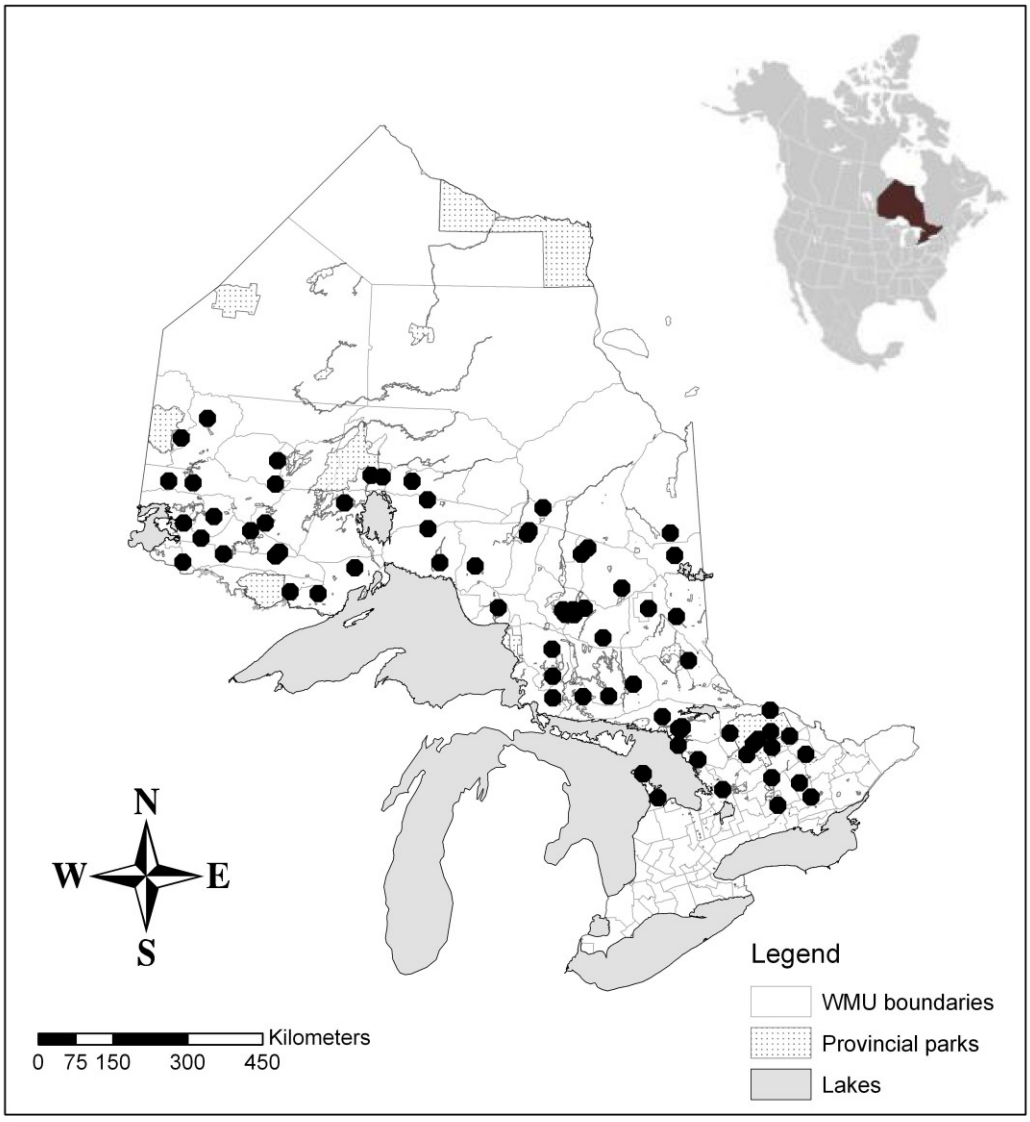

Fig. 1.3. Site locations (black dots) for black bears samples collected across Ontario, obtained from barbed wire hair traps.

CHAPTER 2

SMALL-SCALE GENETIC STRUCTURE OF AMERICAN BLACK BEARS ILLUSTRATES POTENTIAL POSTGLACIAL RECOLONIZATION ROUTES

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Abstract

In the absence of obvious barriers to dispersal, microsatellite studies of vagile mammalian carnivores frequently find panmictic-like genetic structure over wide scales, whereas high levels of differentiation at much finer scales are detected with mitochondrial DNA. Given the maternal inheritance of mtDNA, these differences are often attributed to male-biased dispersal and/or remnants of postglacial range expansion. Based on such contrasting results, it is not always clear how to delineate contemporary populations. We investigated the genetic structure of American black bears (*Ursus americanus*) over a wide geographic area (> 1,700 km) that has no obvious physiogeographic barriers to gene flow. We analyzed a 315-base pair fragment of the mtDNA control region from 660 individual bears from 23 regions of Ontario. Relative to black bear studies based on nuclear data, mitochondrial analyses revealed much stronger patterns of genetic structure among regions ($0.09 < F_{ST} < 0.44$), even at small scale intervals (< 150 km), which likely reflects strong female philopatry combined with male-biased dispersal. The patterns of genetic differentiation among regions were consistent with previously described historical patterns in black bears, specifically the division of the species into two phylogeographic clades (coastal and continental). We confirmed that further subdivision of the continental clade occurs in a region where obvious physiogeographic barriers do not exist. We postulate that this small-scale differentiation can be explained by residual patterns from postglacial recolonization routes on either side of the Great Lakes. We suggest that it was maintained through extreme female philopatry due to habitat saturation following the postglacial geographic expansion. Based on our results, we propose that a combination of several molecular markers can be useful in defining

population units for conservation and management decisions, rather than using only biparentally inherited microsatellites.

Keywords: American black bear, female philopatry, genetic structure, microsatellite, mtDNA, North America, Ontario, phylogeography, *Ursus americanus*.

Introduction

Understanding genetic structuring patterns of species increases our knowledge of their ecology and evolution, and helps inform conservation and management strategies directed toward maintaining stable populations. A problem that arises, however, is that research using combinations of neutral molecular marker types that have different rates of evolution and modes of inheritance can reveal contrasting patterns of genetic differentiation (Hellborg et al. 2002; Johnson et al. 2003; Brito 2007; Flanders et al. 2009). For this reason, studies that incorporate both biparentally inherited nuclear microsatellites and maternally inherited mitochondrial DNA are becoming increasingly important in describing population delineations of highly vagile species that have different male and female life histories (Chappell et al. 2004; Tomasik and Cook 2005).

In the absence of physiogeographic features that impede the movement of animals, contrasting levels of differentiation detected between microsatellite and mitochondrial DNA analyses have been explained by factors such as low dispersal distances, long-term isolation of historical lineages, cryptic boundaries, and sex-biased dispersal (Irwin 2002; Tomasik and Cook 2005). For large and mesocarnivores, for which topographic barriers to dispersal were perceived to be absent, microsatellite analyses have

shown panmictic structuring patterns, even across large distances (brown bear, *Ursus arctos*—Paetkau et al. 1998; lynx, *Lynx canadensis*—Schwartz et al. 2002; marten, *Martes americana*—Kyle and Strobeck 2003). At large scales, genetic differentiation can be explained by factors such as isolation by distance (wolf, *Canis lupus*—Geffen et al. 2004; puma, *Puma concolor*—McRae et al. 2005; marten—Broquet et al. 2006), or anthropogenic and natural influences acting as barriers to dispersal (wolverine, *Gulo gulo*—Kyle and Strobeck 2002; puma—McRae et al. 2005; bobcat, *Lynx rufus*—Millions and Swanson 2007). However, even over small distances across which no barriers exist, genetic structure can be observed in wide-ranging species (lynx—Rueness et al. 2003; black bear—*Ursus americanus*, Peacock et al. 2007). This suggests that factors such as the maintenance of historical lineages due to an intermediate level of dispersal (Peacock et al. 2007), or cryptic differentiation (Rueness et al. 2003), also play a role in contemporary structuring patterns that are not necessarily defined by the current landscape.

Mitochondrial DNA studies of North American taxa focusing on the identification of such historical lineages show that many species share similar patterns of genetic structure at the continental scale, reflecting common physiogeographic patterns (Byun et al. 1997; Wooding and Ward 1997; Arbogast 1999; Demboski et al. 1999; Conroy and Cook 2000; Demboski and Sullivan 2003; Aubry et al. 2009). Two main mtDNA clades are identified most often: a geographically restricted coastal clade, found along the North Pacific Coast, and a widespread continental clade. Because many species sharing this pattern of differentiation are associated with forest, the genetic division between these two main clades has been attributed to the existence of isolated forest refugia located on opposite sides of the continent during the last glaciation.

Although molecular studies focusing on North American species are numerous, few of them deal with highly vagile species found across extensive sampling areas free of physiogeographic barriers. For this reason, there is a lack of comprehensive studies of genetic differentiation focusing on taxa that are both continuously and widely distributed, although such research would provide a base of comparison for studies that identify genetic discontinuities in isolated and fragmented populations. The American black bear is no exception, as studies of this species focus mostly on isolated populations that have arisen from habitat loss and human-caused mortality in the southern part of its range (Vaughan and Pelton 1995), such as Florida (Dixon et al. 2006), Louisiana (Csiki et al. 2003; Larkin et al. 2004), Arkansas (Csiki et al. 2003; Van Den Bussche et al. 2009), or Mexico (Onorato et al. 2004), whereas few data exist on the core population that remains in the northern part of the distribution. Although capable of extensive dispersal movements of more than 200 km from their natal site (Rogers 1987; Lee and Vaughan 2003), American black bears show relatively high levels of genetic structuring across their range (Paetkau and Strobeck 1994; Csiki et al. 2003; Onorato et al. 2004). In this species, small-scale population divisions identified through genetic studies have been attributed to isolation by physiogeographic barriers (islands—Paetkau and Strobeck 1996; water bodies—Peacock et al. 2007; Robinson et al. 2007), geographic distance (Mills 2005), and landscape gradients (Cushman et al. 2006; Dixon et al. 2006).

At the continental scale, molecular studies of black bears have focused mostly on populations in the western and southern portions of the current distribution (Paetkau and Strobeck 1996; Byun et al. 1997; Wooding and Ward 1997; Stone and Cook 2000; Peacock et al. 2007; Van Den Bussche et al. 2009), resulting in a lack of data from the eastern part of the range (Fig. 2.1). Ontario comprises more than a third of the

contemporary range of black bears in eastern North America, and harbors about 100,000 individuals (M.E. Obbard, *pers. obs.*). With the exception of the far southern regions of the province, the landscape is largely contiguous, with no obvious physiographic features such as rivers, mountains, or drastic habitat change that would impede dispersal.

We analyzed mtDNA sequences of the control region of black bears obtained from hair samples of 660 individuals from 23 locations across Ontario (Fig. 2.2). Our first goal was to assess the mitochondrial genetic structure of black bears across a 1,700 km continuum in a landscape that is largely homogeneous. We hypothesized that within this continuous landscape, due to black bear male-biased dispersal combined with female philopatry (Rogers 1987; Lee and Vaughan 2003) and the maternal mode of inheritance of mtDNA, our results would show strong differentiation among regions, a pattern that would not be observed with biparentally inherited neutral markers (Johnson et al. 2003; Chappell et al. 2004; Tomasik and Cook 2005). Our second goal was to place the mtDNA results into a wider continental context by clarifying how Ontario black bears relate to other North American populations.

These results, in light of other microsatellite studies of black bears, can help determine if the observed differentiation is caused by contemporary factors that promote genetic structuring, such as territoriality and natal philopatry, or if it could be explained by historical events that illustrate the consequences of continental processes, such as long-term isolation and colonization. In addition to providing further insights into the ecology of black bears, our findings have implications regarding how data from several molecular markers with different underlying evolutionary histories can be assimilated and interpreted in the implementation of conservation and management plans for large carnivores.

Materials and Methods

Sample collection and DNA analysis

Between 1997 and 2007 black bear hair samples were collected along trap lines grouped in 23 sampling sites located across Ontario (Fig. 2.2). Samples were obtained both opportunistically (live trapping, hunting, or road kills) and from baited barbed wire hair traps (Woods et al. 1999). These procedures followed the animal care guidelines approved by the American Society of Mammalogists (Gannon et al. 2007).

For hair samples collected from 1997 to 2004, DNA extraction was performed using a modified version of the DNeasy tissue extraction protocol (Qiagen, Mississauga, Canada). For each individual sample, 10 to 15 hairs with visible roots were suspended in a solution containing 500 µl of 1X lysis buffer (Applied Biosystems, Inc., Streetsville, Canada) and incubated in 10 µl proteinase K (Qiagen) at 37^{o}C for 12 h. After incubation, standard Qiagen tissue extraction procedures were followed. Samples collected from 2004 to 2007 were extracted following a MagneSil paramagnetic bead automated DNA extraction procedure (Promega, Nepean, Canada) using a P3 Evolution (Perkin Elmer, Woodbridge, Canada) liquid handler, eluting in a final volume of 75 µl.

A 315–base pair (bp) fragment of the mtDNA black bear control region was amplified by polymerase chain reaction, using the primers H16498 (Ward et al. 1991) and L15997 (Wooding and Ward 1997). The sequences were obtained from black bears that had been identified individually based on 15 microsatellite loci (Mills 2005; C. J. Kyle and M.E. Obbard, *pers. obs.*) and gender analyses (primers S47 and S48; Ennis and Gallagher 1994). DNA amplification reactions contained 1X PCR buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.3 mg/mL Bovine Serum Albumin (BSA), 0.2 µM of each primer, 1U of

Taq DNA polymerase (Invitrogen Corp., Burlington, Canada), and 1 ng of DNA extract as a template. Amplification reactions were run on a Dyad Disciple Peltier Thermal Cycler (Bio-Rad Laboratories, Inc., Mississauga, Canada) programmed for an initial 5-min denaturation step at 94^{o}C, followed by 35 cycles of the following steps: denaturation at 94^{o}C for 1 min, annealing at 60^{o}C for 1 min, and extension at 72^{o}C for 1.5 min. The extension was completed after a final extension step at 60^{o}C for 45 min. Amplified products were separated and quantified via electrophoresis in 1.5% agarose gels. PCR products were purified using QIAquick Purification Kit (Qiagen) to remove excess primers and dNTPs. Forward and reverse sequences were obtained 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.).

To facilitate analyses the 23 sampled sites (Appendix I) were pooled into 4 geographic clusters (Bruce, Southeast, Central, and Northwest), based on both geographic proximity and microsatellite data that suggested weak genetic divisions between these broad geographic regions (Mills 2005).

Sequence analysis

We profiled 660 individuals for which mtDNA fragments were edited and aligned manually with MEGA 4.1 (Tamura et al. 2007) relative to previously identified haplotypes downloaded from GenBank (Appendix II). Sequences that did not align to previously identified haplotypes in the literature were considered new haplotypes only after resequencing with the reverse primer to confirm the sequence. All the sequences obtained in this study were submitted to GenBank (accession numbers GU724158 to GU724193).

Haplotype frequencies were calculated with FABOX (Villesen 2007), and levels of genetic diversity were estimated using ARLEQUIN 3.1 (Excoffier et al. 2005) by calculating haplotype diversity (*h*, the probability that 2 haplotypes drawn randomly from a population are different), nucleotide diversity (π, the mean number of pairwise differences per site between 2 sequences—Nei and Kumar 2000), and genetic divergence (F_{ST}—Weir and Hill 2002). Due to discrepancies in sample sizes between clusters (Bruce: n = 38 individuals; Southeast: n = 321; Central: n = 126; Northwest: n = 175), we conducted a rarefaction analysis with ADZE-1.0 (Szpiech et al. 2008) to standardize the levels of haplotypic diversity.

We tested for departure from the neutral model of evolution and population growth by computing Tajima's *D* (Tajima 1989), and Fu's *Fs* tests (Fu 1996) using ARLEQUIN 3.1 (1,000 permutations). The *D* test compares the number of segregating sites in the sample to the mean number of pairwise differences between haplotypes, whereas the *Fs* test determines the probability of obtaining the observed number of haplotypes given the observed average number of pairwise differences. To achieve an alpha of $P = 0.05$ for the rejection of the null hypothesis of neutrality *Fs* must be negative (indication of population expansion) and its *P*-value < 0.02.

Genetic structure

In our study design, where possible, we selected 30 individuals per sampling site to conduct the mtDNA analyses. We estimated the degree of differentiation among sampled sites, and among the broad geographic clusters, by calculating pairwise F_{ST} values (Weir and Cockerham 1984) in ARLEQUIN 3.1 (1,000 permutations, $P < 0.05$—Excoffier et al. 2005). To evaluate the optimal grouping pattern of the sampled sites

without *a priori* assumptions we conducted a spatial analysis of molecular variance (SAMOVA, 1,000 initial conditions—Dupanloup et al. 2002). Because of their geographic proximity, we grouped samples from Algonquin (n = 50) and Bracebridge (n = 5) to conduct this analysis. Of all our SAMOVA results (our sites divided into K = 2 to 15 groups), those that had the highest variance among clusters (F_{CT}) were reported (K = 2, 4, and 11). Two of these (K = 2 and 4) were compared to the results of an analysis of molecular variance (AMOVA—Excoffier et al. 1992) based on the geographic clusters determined *a priori*. Both SAMOVA and AMOVA comparisons examined the partitioning of genetic variation among clusters, among sampled sites within clusters, and within sampled sites.

Pairwise F_{ST} values were also used to perform Mantel tests (Mantel 1967) to establish whether the level of genetic differentiation was correlated with geographic distance between sampled sites (Wright 1943). In addition, a partial Mantel test was conducted to model a barrier to gene flow between the *a priori* defined geographic clusters. The additional matrix used to conduct this test was a binary function of presence (1) or absence (0) of a hypothetical barrier between sites. Through the Isolation By Distance Web Service version 3.14 (Jensen et al. 2005), we regressed the pairwise F_{ST} values between all the sampling sites against pairwise geographic distances (km) using 1,000 randomization steps. Geographic distances between each sampling location were obtained by plotting the samples in ArcGIS version 9.0 (Environmental Systems Research Institute, Inc., Toronto, Canada), and by calculating the distance between their centroids.

Phylogenetic analyses

The relationships between the haplotypes found in Ontario were estimated by creating a Median Joining Network (MJN—Bandelt et al. 1999) with the haplotypes that were observed in more than 5 individuals (or > 0.9%) across the entire data set, using the software NETWORK version 4.5 (Network 2008). The cluster differentiation found in Ontario black bears was then assessed with respect to the phylogeographic structure identified at the continental scale (Wooding and Ward 1997), by integrating sequences from Ontario with all available black bear haplotypes (Appendix II). As intraspecific haplotype differences can be low (e.g., only 1 nucleotide substitution), phylogenetic relationships can be represented accurately by a haplotype network (Posada and Crandall 2001). Therefore, we constructed a second MJN that included both the Ontario and all other North American sequences to clarify the relationships among haplotypes at the continental scale.

Results

Genetic diversity in Ontario

The analysis of the 315-bp fragment of the mtDNA control region obtained from the 660 black bear samples identified 36 haplotypes and 26 variable sites (Genbank accession numbers GU724158 to GU724193, Appendix II). Of these haplotypes, 11 were observed previously and 25 newly identified, of which 14 were identified only in a single individual (Appendix I, II). Eight haplotypes were observed in > 1 but < 6 individuals (relative frequency < 0.9%), and 5 (HAP1, 2, 5, 6, and 15) had a relative frequency > 10%.

The neutral model of evolution could not be rejected for any of the sampled sites using either *D* or *Fs*. The values for *D* ranged from 0.000 for the Bruce Peninsula National Park (BPNP; *P* = 1.000) to 20.974 (Bracebridge; *P* = 0.999), with an overall value of *D* = 9.594 (*P* = 0.926). Results from the *Fs* test ranged from 9.038 (Kenora; *P* = 0.895) to 0.256 (Borland+Ivanohe; *P* = 0.178), with an overall value of *Fs* = 3.437 (*P* = 0.591). Population specific F_{ST} values ranged from 0.281 (Red Lake) to 0.323 (BPNP).

Haplotypic diversity within sampled sites ranged from 0.419 (Fort Frances A) to 0.893 (Midhurst), with an overall haplotypic diversity of 0.691. Nucleotide diversity (π) within sampled sites ranged from 0.002 (BPNP) to 0.026 (Red Lake), with an overall nucleotide diversity of $\pi = 0.015$ (Appendix III).

When analyzing these mtDNA data based on the microsatellite genetic clusters delineated by Mills (2005), a high genetic diversity was detected in the Southeast (0.832) compared to the other clusters (Bruce: 0.501; Central: 0.680; Northwest: 0.753). The low haplotypic diversity found in the Bruce cluster was not a consequence of smaller sample size, because when standardized, its value remained low compared to the other clusters (Table 2.1).

Distribution of haplotypes in Ontario

Haplotypic distribution varied both among and within black bear clusters. Among clusters, strong differences in the frequency of the most common haplotypes were observed. The 2 predominant haplotypes in the Southeast cluster (HAP1 and HAP2, cluster frequencies = 26%) differed from the haplotype most frequently observed in the Central cluster (HAP5, cluster frequency = 54%) and from the one that was predominant in the Northwest cluster (HAP15, cluster frequency = 46%; Fig. 2.2). Only 2 haplotypes

were found in the isolated Bruce cluster. One of them was predominant in Central Ontario (HAP5) but had lower frequencies in the other main clusters, whereas the other was common in Southeast Ontario (HAP2) but found in very low frequencies in the Northwest and Central clusters (Appendix I; Fig. 2.2 and 2.3). Finally, among the 8 rare haplotypes other than singletons found in Ontario (relative frequency < 0.9%), 100% (n = 8) were restricted geographically to their respective cluster, and 62.5% (n = 5) were restricted to one sampling site within a cluster.

Genetic structure

Various levels of differentiation were observed among sampling sites, with pairwise F_{ST} values (Weir and Cockerham 1984) ranging from 0.849 (BPNP/Fort Frances A; $P = 0.000$) to -0.078 (Midhurst/Sioux Lookout; $P = 0.504$). The results indicated that BPNP bears showed a higher degree of genetic differentiation when compared to bears from the other sampling localities.

All of the geographic clusters were highly differentiated from each other, with the lowest level of divergence found between the Central and Southeast clusters ($F_{ST} = 0.120$; $P = 0.000$) and the remainder of the values ranging from 0.419 (Bruce/Northwest; $P = 0.000$) to 0.210 (Bruce/Central; $P = 0.000$; Table 2.2). In addition, the Northwest cluster was more differentiated from the Central cluster ($F_{ST} = 0.301$; $P = 0.000$) than the Bruce cluster.

The SAMOVA result that had the highest variance among groups was $K = 2$ ($F_{CT} = 0.343$, $P = 0.000$), which separated Dryden, Fort Frances A, and Thunder Bay (all included in the Northwest geographic cluster) from all the other sampled sites in Ontario. $K = 4$ also had a high variance among groups ($F_{CT} = 0.311$, $P = 0.000$), and grouped

Dryden/Fort Frances A/Thunder Bay, Kenora/Fort Frances B (also included in the Northwest geographic cluster) together, Timmins (Southeast cluster) on its own, and the rest of the Ontario localities (Table 2.3). At $K = 11$ ($F_{CT} = 0.306$, $P = 0.000$), all the sampled sites that were grouped into the Central cluster stayed together (Bor+Iv/CCGP/Hearst/Nipigon), as did most of the sites grouped into the Southeast (Sudbury/Bancroft/North Bay/Sault Ste. Marie, Midhurst/Parry Sound, and Algonquin_Bracebridge/Pembroke) and Northwest (Dryden/Fort Frances A/Thunder Bay, and Kenora/Fort Frances B) clusters (Table 2.3). The corresponding AMOVA based on geographic clusters ($K = 2$ corresponding to Northwest versus the other clusters, $K = 4$ to all the geographic clusters) also indicated genetic differentiation, but at lower levels than SAMOVA ($F_{CT} = 0.278$ and 0.239, respectively). Both SAMOVA and AMOVA demonstrated that a substantial portion of the mtDNA genetic variability was found among groups, whereas the differences among sites within groups accounted for less variation. Variation within sites, on the other hand, accounted for the major part of the observed variation.

The results of the Mantel test showed that the genetic differentiation between sampling sites across Ontario could partly be explained by isolation by distance, as the correlation between geographic and genetic distances was significant ($r = 0.315$, $P = 0.002$). Isolation by distance was supported more strongly when the BPNP samples were removed from the analysis ($r = 0.347$, $P = 0.002$). However, significance decreased when both BPNP and the Northwest samples were removed ($r = 0.287$, $P = 0.005$) and was absent when only the Northwest samples were removed ($r = 0.09$, $P = 0.256$).

Phylogenetic analyses

The MJN of the haplotypes found in Ontario showed that the most frequent haplotypes identified were all located in the trunk of the network (Fig. 2.3). The MJN of the sequences found across the continent, including the samples we obtained in Ontario, showed two genetically distinct groups, one largely restricted to the Pacific Northwest region and highly divergent from the other North American haplotypes (10 mutational steps), and a second one encompassing the rest of the continent, which corresponded to the two clades identified by Wooding and Ward (1997). Within the widespread continental clade we found a geographical distinction between a subclade running along the Eastern seaboard of North America and another one found in western Canadian provinces and American states (Fig. 2.1). This intraclade divergence was detected because 2 haplotypes that were almost exclusively restricted to the northwestern cluster of Ontario (HAP15 and HAP24) also were found in other western Canadian provinces and American states, but not anywhere else along the eastern side of the continent (Fig. 2.3).

Discussion

Although many studies investigate genetic structuring patterns of fragmented populations to better inform conservation and management initiatives, only a few focus on genetic variation across largely homogeneous landscapes. Such studies are useful because they add context about the state of fragmented populations by showing how genetic variation is distributed in the absence of ecological or anthropogenic disturbance. In addition, they help make inferences about how fast continuously distributed populations can be subjected to extirpation in case of isolation. Our study used an

extensive data set to describe black bear mtDNA genetic structure across a presumed continuous landscape. Relative to black bear microsatellite data also obtained in Ontario, which detected F_{ST} values illustrating a weak structure for this marker (pairwise F_{ST} < 0.02 between the non-isolated geographic clusters—Mills 2005), the values detected by our mtDNA analyses revealed a structure that was defined more strongly for this type of marker. This discrepancy in the levels of structuring detected with mtDNA and microsatellite suggests male-biased dispersal, as seen in other species (Chappell et al. 2004; Johnson et al. 2003; Tomasik and Cook 2005). In addition, integrating the Ontario haplotypes into a network that included other North American sequences showed that historical remnants of phylogeographic isolation were still observed in black bears at restricted spatial scales and were most likely maintained by sex-biased dispersal.

Our mtDNA analyses detected differences in haplotypic composition among Ontario regions and a geographic restriction of haplotypes. The most frequent haplotype found in the Northwest (HAP15) was not shared with any other clusters, and the dominant haplotypes in the Central (HAP5 and HAP6) and Southeast (HAP1 and HAP2) clusters were seldom found in northwestern Ontario (Fig. 2.2). HAP24, which was close to HAP15 on the network, but less frequent, was also restricted to the northwestern cluster in Ontario. In addition, of the 36 haplotypes found in Ontario, 8 were found in only one region, even when we excluded the singletons, illustrating a high proportion of private haplotypes in the province.

The SAMOVA results did not reveal genetic structuring that corresponded completely to the *a priori* defined geographic clusters. The SAMOVA provided plausible results at $K = 2$, $K = 4$, and $K = 11$, for which the variance among groups was maximized, but the variance among populations within groups was minimized. At $K = 2$, the

SAMOVA grouped only 3 of the 8 Northwest sampled sites together. This was not expected, but could be explained by the fact that these sites are the ones that have the lowest HAP5 frequency compared to the rest of the Northwest populations. At $K = 4$, two additional Northwest sites were grouped together (Kenora/Fort Frances B), both of which had a high HAP15 frequency and a higher HAP5 frequency than the first group of localities that was pulled from this cluster. In addition, a site from the Southeast cluster, Timmins, formed a group on its own, which was explained by the fact that the highest HAP18 frequency (F = 36%) was detected at this location. At $K = 11$, all the sampled sites that were grouped *a priori* into the defined Central cluster stayed together, and most of the sites grouped into the Southeast and Northwest clusters also grouped together. In addition to Timmins and BPNP (Southeast), the Northwest sites that had the lowest HAP15 frequencies remained separate (Fort Frances C, Sioux Lookout, Red Lake; Table 2.3). These differences between the grouping patterns likely illustrate genetic structuring occurring at different geographic scales. At the largest scale ($K = 2$), sampled sites from the Northwest cluster of Ontario are separating from the others, and at the smallest scale ($K = 11$), further divisions appear within clusters. Despite this substructuring pattern, the clusters boundaries that were defined *a priori* are still present overall.

In addition to the SAMOVA results, the separation of the Northwest sites from the rest of Ontario was supported by the Mantel test, as the significance of the correlation between geographic and genetic distances decreased when these populations were excluded. This suggests that the high differentiation between the Northwest and the other clusters might have skewed the results towards supporting isolation by distance across our sampling study. Because Bruce bears were also highly differentiated from the rest of the clusters, and had a level of genetic diversity that was much lower, with only two

haplotypes detected among 38 individuals (HAP2 and HAP5), we conducted a Mantel test that excluded them in addition to the Northwest sites. With only the Central and Southeast populations, the correlation between geographic and genetic distance was detected only at the threshold of significance, which is concordant with the lower F_{ST} values observed between these clusters.

Previous findings that expressed a conservation concern for Bruce black bears due to their geographic isolation from the rest of the Ontario individuals (Howe et al. 2008) were supported by the genetic results of the present study. Bruce bears were highly differentiated from the others, and had a lower haplotypic diversity, a pattern that could be explained by isolation by fire events during the last 150 years (M.E. Obbard, *pers. obs.*). Because the 2 haplotypes found within the Bruce cluster are common in the rest of the province, and because no unique genetic haplotypes were found in Bruce black bears, we conclude that they do not form an evolutionary unit, although the assessment of more contemporary genetic markers may later indicate that they could be defined as a Designatable Unit (COSEWIC 2005).

In addition to these different levels of structuring, contrasting levels of differentiation were detected between microsatellite (Mills 2005) and mitochondrial analyses. Excluding the Bruce, F_{ST} values based on microsatellites illustrated subtle levels of genetic structure ($0.008 < F_{ST} < 0.140$), whereas values based on mtDNA illustrated stronger levels of differentiation ($0.120 < F_{ST} < 0.419$), even at geographic distances as small as 150 km. For example, although the Northwest cluster of Ontario was not isolated geographically from the other clusters (Fig. 2.2), pairwise F_{ST} values showed that it was strongly differentiated from them. Such results, combined with the absence of topographic barrier to dispersal across the sampling area, have been explained by low

effective population sizes, low dispersal distances, long-term isolation of lineages, cryptic barriers (Irwin 2002), or sex-biased dispersal (Tomasik and Cook 2005). Male black bears are known for their long distance dispersal capabilities (Rogers 1987). In addition, total abundance of black bears is reasonably high in Ontario (approximately 100,000 individuals; M.E. Obbard, *pers. obs.*). These factors suggest that Ontario black bears may be at equilibrium, and hence, we would expect genetic drift to have little impact on them. Our results do not support a panmictic structure in Ontario black bears, and show discrepancies in differentiation levels between genetic markers. The most likely explanation for these discrepancies is a combination of male-mediated gene flow and female natal philopatry, which supports our prediction, as well as previous studies that detected those patterns in black bears (Rogers 1987; Onorato et al. 2007; Costello et al. 2008).

In black bears and other taxa found in North America, such as northern flying squirrels (*Glaucomys sabrinus*), red foxes (*Vulpes vulpes*), long tailed voles (*Microtus longicaudus*), American pine martens (*Martes americana*), and yellow pine chipmunks (*Tamias amoenus*), 2 main historical lineages were identified, a continental one and a coastal one (Byun et al. 1997; Wooding and Ward 1997; Arbogast 1999; Demboski et al. 1999; Conroy and Cook 2000; Demboski and Sullivan 2003; Aubry et al. 2009). Their origin has been suggested to derive from several isolated refugia during the last glacial maximum (LGM) along the coasts of the North Pacific and East Atlantic; however, the exact locations of these refugia remain unclear. In black bears, the continental lineage extends from Alaska southward to New Mexico and eastward to Newfoundland and Florida, and the coastal one extends from Alaska to California, and also occurs in British Columbia, Alberta, and Montana (Byun et al. 1997; Wooding and Ward 1997; Stone and

Cook 2000; Peacock et al. 2007). In addition to this continental/coastal divergence, Wooding and Ward (1997) found a low east/west genetic differentiation within the continental clade, and suggested that the disjunct distribution of these two potential subclades was due to a lack of samples from the central part of North America (e.g., no samples from Ontario, Manitoba, or Michigan). This intraclade subdivision is subtle (Wooding and Ward 1997), and it cannot be explained by a prominent physiogeographic feature such as an isolated glacial refugia.

Our samples, collected on a 1,700 km continuum across Ontario, allowed us to fill this sampling gap that existed in the mideastern portion of the black bears' range, and to subsequently put our results into a broader continental context. Our second network including sequences from Ontario and sequences from the rest of the North American continent showed that HAP15 and HAP24 (both mostly restricted to the Northwest cluster of Ontario) were restricted to the mideastern to western part of the black bear's range (Alberta, Alaska, British Columbia, Manitoba, Montana, New Mexico, Ontario, and Utah). In contrast, HAP1 and HAP2 (both mostly restricted to the southeastern portion of Ontario) were restricted to the eastern part of their range (Florida, Louisiana, Mexico-Texas, New Brunswick, and Ontario; Fig. 2.3). In addition, the Ontario Northwest cluster was strongly differentiated from the Central cluster. This geographic restriction of haplotypes, and this high level of genetic differentiation detected at a very small scale in Ontario, suggest that black bears located on the western (Northwest cluster of Ontario) and eastern (Central and Southeast clusters of Ontario) sides of the province belong to the western and eastern North American continental subclades, respectively. This clade subdivison was also supported by the significant partial Mantel test that modeled a barrier to gene flow between the Northwest and the rest of the Ontario populations (partial r =

0.255, $P = 0.007$). This pattern of geographic distribution of genetic types shows that the disjunct distribution previously identified by Wooding and Ward (1997) was not only due to a lack of sampling in the central part of the North America, as our results still identify the eastern/western subdivision of the continental clade (Fig. 2.3) at a very small geographic scale.

For black bears located in the southwestern region of North America, barriers to gene flow were suggested to be driving this type of differentiation (Onorato et al. 2004). The presence of a physiogeographic barrier represented by the Chihuahan desert, which restricts gene flow between the sites of Mexico-Texas and New Mexico, could have helped maintain a high level of differentiation between black bear populations (Onorato et al. 2007). However, the absence of topographic barriers to long-distance dispersal on the eastern side of the continent, and at a smaller scale, between the differentiated Central and Northwest clusters of Ontario, seems to rule out a structure linked to long-lasting landscape features. This finding supports the results from Peacock et al. (2007), who suggested that clusters are not necessarily defined by physical barriers. Given the evolutionary rate of the control region of mtDNA in American black bears (~0.028 substitutions per site per million years—Wooding and Ward 1997), historical factors are likely driving such a differentiation pattern. This continental clade subdivision likely has occurred over a much more restricted length of time than the coastal/continental clade division, because it is not strong enough to suggest isolated glacial refugia on the eastern side of the Rockies. Rather, the shape of our network, with a few ancestral haplotypes (HAP1, HAP15) having many recent derivatives, suggests range expansion (Avise 2000). Because this east/west subdivision of the continental clade seems to follow the pattern of the retreat of the last ice sheet (Adams and Faure 1997), we suggest that it exists because

after departing from an ancestral population located in the main continental refugium during the late Pleistocene, black bears followed two opposite recolonization routes on either side of the receding ice sheet. Due to rapid geographic expansion following the melting ice, the two subclades met in northern Ontario. The habitat at the contact zone between the two subclades likely became saturated, inhibiting future female migration. Thus, the historical genetic structure that arose during the postglacial recolonization of North America, which was first due to isolation by distance after the postglacial range expansion, could have been maintained subsequently by female philopatry and male-biased dispersal, resulting in the observed contemporary clusters. That the Mexico-Texas haplotypes are closely related to haplotypes from the eastern North American subclade, whereas those from New Mexico are more closely related to sequences from the western subclade, further confirms our proposition of two recolonization routes. It also supports the second long-distance colonization hypothesis proposed by Onorato et al. (2004) suggesting that dispersal of black bears from the eastern United States lead to their current distribution in the Mexico-Texas region.

Our sampling across Ontario allowed us to detect the continental clade subdivision at a small geographic scale (150 km between the two subclades). In studies for which samples were collected at longer distance intervals, this differentiation was observed at a more intermediate scale, even with markers that have a higher rate of evolution than mtDNA, such as microsatellites (lynx, F_{ST} = 0.0622, P = 0.01—Rueness et al. 2003; piping plovers*, Charadrius melodus*, F_{ST} = 0.473, P<0.000—Miller et al. 2009). In these studies it was suggested that this differentiation could be caused by contemporary rather than historical factors, and that structuring patterns were influenced by climate variations through habitat and breeding site choice. In lynx, the cryptic division was suggested to be

due to opposite effects of the North Atlantic Oscillation (NAO) on the snow conditions of different climatic regions, which would affect hunting abilities and habitat choice of lynx, because individuals would stay on one specific side of this NAO line because of habitat familiarity, which would lead to genetic structuring despite the absence of barrier to gene flow (Stenseth et al. 1999, 2004). For the piping plover (Miller et al. 2009) the genetic structure was explained by differential levels of breeding-site fidelity due to opposite flooding conditions in the neighboring regions. The location of this NAO line, which marks the division between the Continental and Atlantic climatic regions (Stenseth et al. 1999), corresponds to where we identified the cryptic genetic subdivision of the wide continental clade in black bears (Fig. 2.1). We cannot envision how differential climatic conditions could maintain such small scale differentiation for black bears, but these findings in other species warrant further investigation, at least to verify if males disperse more likely within clusters, as opposed to between them.

In addition to future research aspects, we suggest that future conservation and management decisions for mammalian carnivores, and especially ones that are known to have differential male and female dispersal patterns, are made based on genetic information that uses both microsatellites and mtDNA. As shown here, microsatellites are not fully informative when historical lineages are maintained contemporarily by dispersal patterns, and management decisions solely based on microsatellites can lead to changes in the genetic composition of populations. In Arkansas, for example, the genetic composition of populations that belonged historically to the Continental Eastern subclade changed into a Continental Western subclade type after they received translocated individuals from Manitoba and Minnesota (Van Den Bussche et al. 2009). The mitochondrial genome has also highly functional fragments (Ballard and Whitlock 2004;

Rutledge et al. 2010) in addition to the neutral control region. If the variation of neutral fragments reflects that of functional fragments, not accounting for variation in mtDNA could negatively impact management actions that focus on recovery of populations.

To complement this study we suggest gathering more data from potential secondary contact zones between the two continental subclades and examining functional markers to look for possible local adaptive responses. The field of ecological genomics, for example, would allow us to identify the genes that are involved in the various responses to differential environmental conditions. Future studies of North American forest species whose distribution is similar to the one of black bears should focus on explaining the small-scale intralineage diversification on the eastern side of the continent, because it could lead to new findings on the influence of both contemporary and historical forces on the dynamics of species diversification. At the local scale, we showed that the genetic structure of Ontario black bears reflects their historical differentiation levels in the absence of barriers to gene flow. Such information can be used as a baseline to quantify the amount of disturbance in the currently isolated North American black bear populations.

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Fig. 2.1. Map of sampling locations of black bears, including our Ontario sites and sites from other studies across North America. The Coastal phylogeographic clade is represented by dark gray triangles and the Continental clade by circles. The circles representing the two continental subclades are respectively black (Continental Western subclade), and light gray (Continental Eastern subclade). The circles that are both dark gray and black, located in Montana, represent sites where bears from both the Coastal and Continental clades were found. The circle that is both light gray and black, located in

Oklahoma, represents a site where bears from both the Continental Western and Continental Eastern subclades were found. Based on the information provided by the Median Joining Network (Fig. 2.3), the Ontario samples were attributed to each of the Continental subclades: the Northwest cluster belongs to the Continental Western subclade and the other Ontario clusters belong to the Continental Eastern subclade.

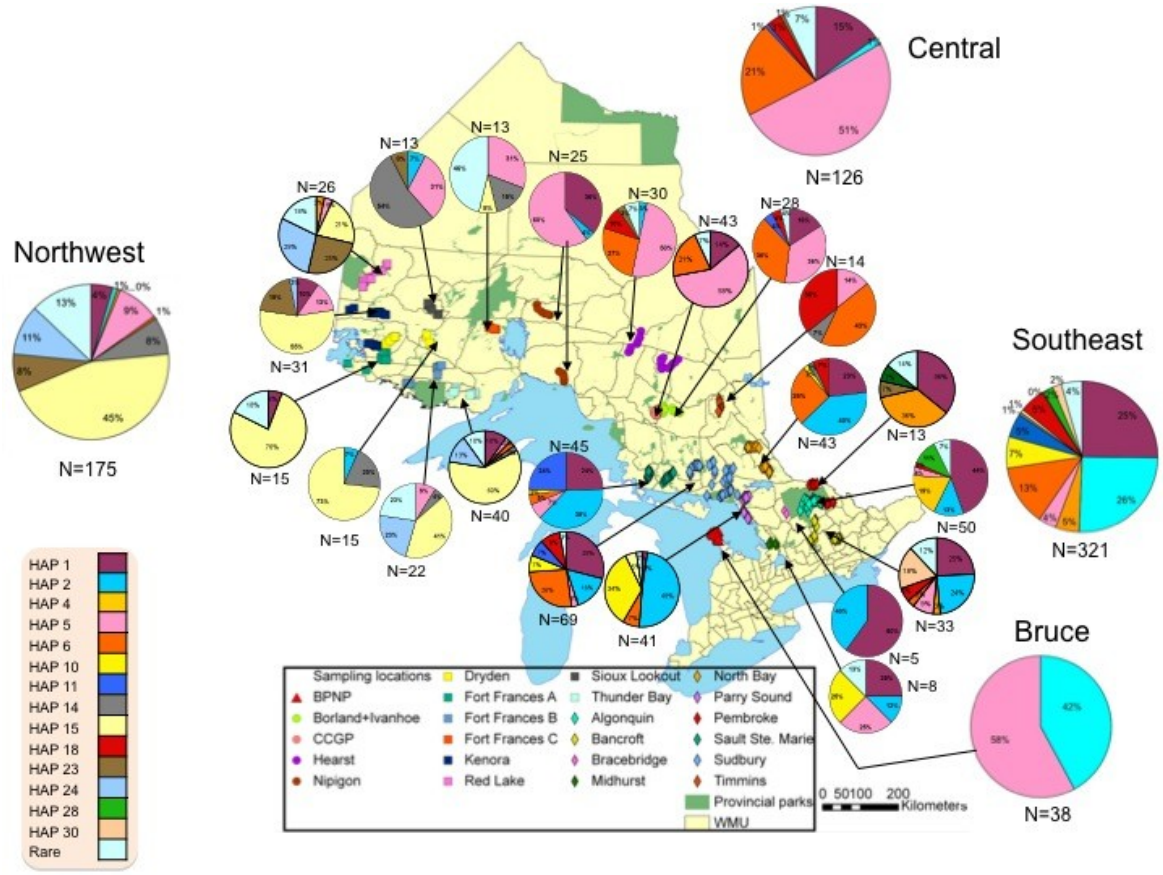

Fig. 2.2. Frequencies of haplotypes represented > 5 times in the 23 sampling locations distributed across Ontario (small pies) and grouped into 4 geographic clusters (large pies). Triangles represent the sampling sites in the Bruce cluster, diamonds represent the sites in the Southeast cluster, circles represent the sites in the Central cluster, and squares represent the sites in the Northwest cluster.

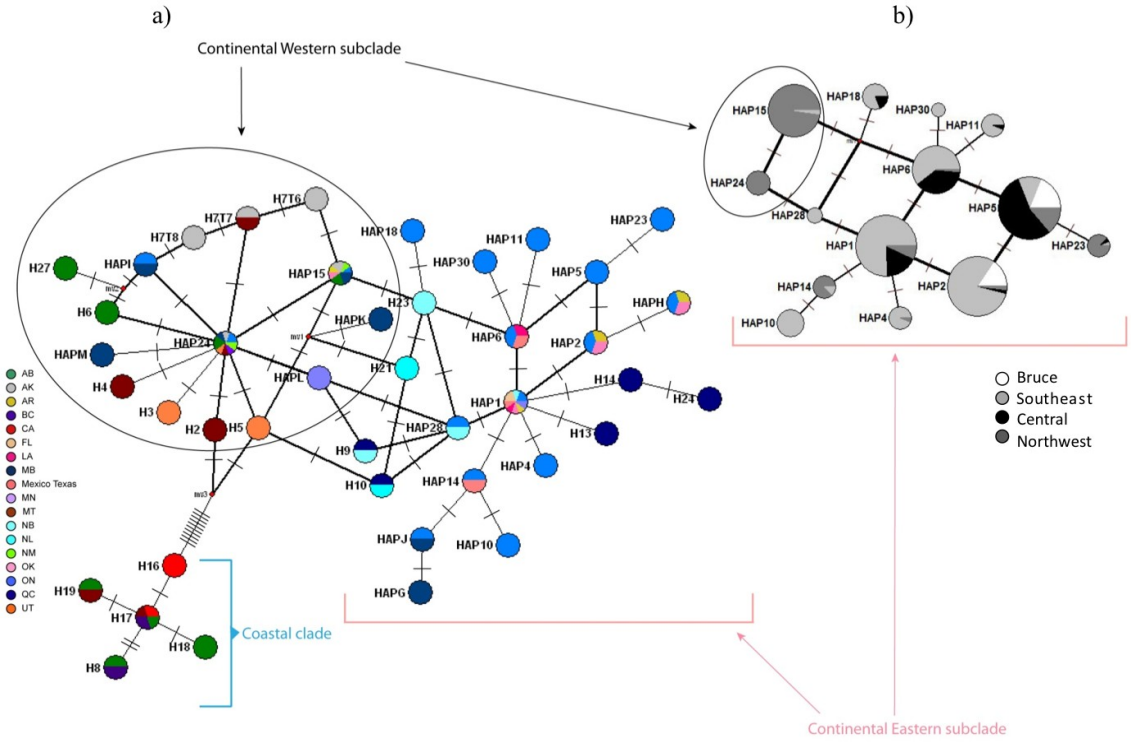

Fig. 2.3. a) Median Joining Network including sequences from sites located across North America, obtained from GenBank (see Appendix III for citations), and the Ontario sequences that appeared > 5 times in the data set. Each color represents the sites where the respective sequences were found (states or provinces), and the slash marks represent the genetic distance (number of base pair differences) between each haplotype. Circle size is not proportional to the frequency of the haplotypes. The haplotypes within the black circle belong to the Continental Western subclade identified in black bears (Wooding and Ward 1997), whereas the rest of the sequences belong to its Continental Eastern subclade, with the exception of H8, H16, H17, H18 and H19, which belong to the Coastal clade of black bears. The red dot is a median vector, which is a potential common ancestor between 2 haplotypes. b) Ontario Median Joining Network showing the relationships between the most frequent haplotypes from our study area, based on 315-bp fragments of

the mitochondrial control region of black bears. Circle size is proportional to the total number of individuals sharing each haplotype, and slices are proportional to the number of individuals per cluster carrying a particular haplotype. The Bruce cluster is shown in white, the Southeast cluster in light gray, the Central cluster in black and the Northwest cluster in dark gray. The most frequent haplotypes (HAP15 and HAP24) in the Northwest cluster belong to the Western Continental clade subdivision, whereas all the other Ontario sequences are found in the Eastern Continental clade subdivision.

Table 2.1. Comparison of observed and standardized haplotypic diversity (obtained with the rarefaction analysis conducted with ADZE-1.0— Szpiech et al. 2008) in each cluster.

	Sample size	# of haplotypes	Standardized # of haplotypes	# of private haplotypes	Standardized # of private haplotypes
Bruce	38	2	2	0	0
Southeast	321	21	10.16	10	4.64
Central	126	13	7.27	6	2.52
Northwest	175	18	9.95	8	6.49

Table 2.2. Pairwise comparison of mtDNA genetic differentiation (F_{ST} values) for American black bears (*Ursus americanus*) between the geographical regions of Ontario. Pairwise F_{ST} values are located below the diagonal (*italics*) for microsatellites (Mills 2005) and above the diagonal for mtDNA. Significant ($P < 0.05$) values are indicated as *. All *P*-values for the mtDNA data were $P = 0.000$. Sampling sites are mapped in Fig. 2.2.

	Northwest	Central	Southeast	Bruce
Northwest	-	0.301*	0.266*	0.419*
Central	*0.013*	-	0.120*	0.210*
Southeast	*0.020*	*0.008*	-	0.328*
Bruce	*0.131**	*0.129**	*0.140**	-

Table 2.3. Results of analysis of molecular variance (AMOVA), and spatial analysis of molecular variance (SAMOVA; *italics*) are indicated for each of the number of groups (*K*) into which the black bear sampling sites were pooled. Results include degrees of freedom (d.f.), percentage of variance, and fixation indices. Significant ($P < 0.05$) values are indicated as *. All *P*-values were $P = 0.000$.

	K = 2			*K* = 4			*K* = 11		
Source of variation	d.f	% of variance	Fixation indices	d.f	% of variance	Fixation indices	d.f	% of variance	Fixation indices
Among groups (F_{CT})	1	27.78/*34.31*	0.278*/*0.343**	3	23.89/*31.12*	0.239*/*0.311**	10	*30.57*	*0.306**
Among populations within groups (F_{SC})	20	14.28/*14.88*	0.198*/*0.227**	18	11.97/*12.49*	0.157*/*0.181**	11	*1.75*	*0.025**
Within populations (F_{ST})	638	57.94/*50.81*	0.421*/*0.492**	638	64.14/*56.39*	0.359*/*0.436**	638	*67.68*	*0.323**

Appendix I. Distribution of the 36 mtDNA haplotypes at 23 sampling sites of American black bears (*Ursus americanus*) across Ontario, and measures of their absolute and relative frequencies. (°) indicates the haplotypes that occurred only once in the complete data set and were not inluded in the analyses, and (*) indicates the haplotypes that occurred < 6 times in the data set and were excluded from data set 2. Sampling sites are mapped in Fig. 2.2.

Cluster	Sampling site	# H	HAP1	HAP2	HAP4	HAP5	HAP6	HAP7°	HAP8*	HAP9°	HAP10	HAP11	HAP12°	HAP13°	HAP14
Bruce	BPNP	2	.	16	.	22	.	.	.	.	.	.	.	.	.
Southeast	Algonquin	7	24	7	10	2	.	.	.	.	.	.	.	.	.
Southeast	Sudbury	9	20	11	.	2	18	.	.	.	5	5	.	.	.
Southeast	Pembroke	6	5	.	5	.	.	.	.	.	.	.	.	.	.
Southeast	Parry Sound	6	1	20	.	.	3	.	.	.	14	.	.	.	.
Southeast	Bracebridge	2	3	2	.	.	.	.	.	.	.	.	.	.	.
Southeast	Midhurst	5	2	1	.	2	.	.	.	.	2	.	.	.	.
Southeast	Bancroft	10	8	8	1	3	1	.	.	.	.	.	.	.	.
Southeast	North Bay	6	10	17	.	.	11	.	.	.	1	.	.	.	1

Southeast	Timmins	4	.	.	.	2	6	.	.	.	.	.	.	.	1
Southeast	Sault Ste. Marie	6	11	17	.	3	2	.	.	.	1	11	.	.	.
Central	Bor+Iv	9	4	.	.	9	9	1	1	1	.	1	1	.	.
Central	CCGP	6	6	.	.	25	9	.	1	.	.	.	.	1	.
Central	Nipigon	3	9	1	.	15	.	.	.	.	.	.	.	.	.
Central	Hearst	6	.	1	.	15	8	.	.	.	.	.	.	.	.
Northwest	Kenora	5	3	.	.	4	.	.	.	.	.	.	.	.	.
Northwest	Dryden	3	.	1	.	.	.	.	.	.	.	.	.	.	3
Northwest	Fort Frances A	4	1	.	.	.	.	.	.	.	.	.	.	.	.
Northwest	Fort Frances B	6	.	.	.	2	.	.	.	.	.	.	.	.	1
Northwest	Fort Frances C	5	.	.	.	4	.	.	.	.	.	.	.	.	2
Northwest	Sioux Lookout	4	.	1	.	4	.	.	.	.	.	.	.	.	7
Northwest	Thunder Bay	8	4	.	.	1	1	.	.	.	.	.	.	.	1
Northwest	Red Lake	8		.	1	1	.	.	.	.	.	.	.	.	.
TOTAL			111	103	17	116	68	1	2	1	23	17	1	1	16
Relative frequency (total)			0.168	0.156	0.026	0.176	0.103	0.002	0.003	0.002	0.035	0.026	0.002	0.002	0.024

Appendix I. (*Continued*)

Cluster	Sampling site	# H	HAP15	HAP16°	HAP18	HAP19°	HAP20°	HAP21*	HAP22*	HAP23	HAP24	HAP25*	HAP26°	HAP27°	HAP28	HAP29°
Bruce	BPNP	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Southeast	Algonquin	7	.	.	1	.	.	.	.	.	.	.	.	.	6	.
Southeast	Sudbury	9	.	.	6	.	.	.	.	.	.	.	.	.	.	1
Southeast	Pembroke	6	.	.	.	.	1	.	.	1	.	.	.	.	1	.
Southeast	Parry Sound	6	2	.	.	1	.	.	.	.	.	.	.	.	.	.
Southeast	Bracebridge	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Southeast	Midhurst	5	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Southeast	Bancroft	10	.	.	2	.	.	.	.	.	.	.	.	.	.	.
Southeast	North Bay	6	.	.	3	.	.	.	.	.	.	.	.	.	.	.
Southeast	Timmins	4	.	.	5	.	.	.	.	.	.	.	.	.	.	.
Southeast	Sault Ste. Marie	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Central	Bor+Iv	9	.	.	1	.	.	.	.	.	.	.	.	.	.	.

Central	CCGP	6	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Central	Nipigon	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Central	Hearst	6	.	.	3	.	.	.	.	1	.	2	.	.	.	.
Northwest	Kenora	5	17	.	.	.	.	.	.	6	1	.	.	.	.	.
Northwest	Dryden	3	11	.	.	.	.	.	.	.	.	.	.	.	.	.
Northwest	Fort Frances A	4	13	1	.	.	.	.	.	.	.	.	.	.	.	.
Northwest	Fort Frances B	6	9	.	.	.	.	.	1	.	5	.	.	.	.	.
Northwest	Fort Frances C	5	1	.	.	.	.	3	3	.	.	.	.	.	.	.
Northwest	Sioux Lookout	4	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Northwest	Thunder Bay	8	24	.	.	.	.	.	.	.	5	.	.	.	.	.
Northwest	Red Lake	8	6	.	.	.	.	.	.	7	8	.	.	1	.	.
TOTAL			83	1	21	1	1	3	4	16	19	3	1	1	7	1
Relative frequency (total)			0.126	0.002	0.032	0.002	0.002	0.005	0.006	0.024	0.029	0.005	0.002	0.002	0.011	0.002

Appendix I. (*Continued*)

Cluster	Sampling site	# H	HAP30	HAP31*	HAP32°	HAP33*	HAP34°	HAP35°	HAP36*	HAP37*	HAP38°	*n*
Bruce	BPNP	2	.	.	.	.	.	.	.	.	.	38
Southeast	Algonquin	7	.	.	.	.	.	.	.	.	.	50
Southeast	Sudbury	9	.	.	.	.	.	1	.	.	.	69
Southeast	Pembroke	6	.	.	.	.	.	.	.	.	.	13
Southeast	Parry Sound	6	.	.	.	.	.	.	.	.	.	41
Southeast	Bracebridge	2	.	.	.	.	.	.	.	.	.	5
Southeast	Midhurst	5	.	.	.	.	.	.	.	.	.	8
Southeast	Bancroft	10	6	2	1	.	1	.	.	.	.	33
Southeast	North Bay	6	.	.	.	.	.	.	.	.	.	43
Southeast	Timmins	4	.	.	.	.	.	.	.	.	.	14
Southeast	Sault Ste. Marie	6	.	.	.	.	.	.	.	.	.	45
Central	Bor+Iv	9	.	.	.	.	.	.	.	.	.	28

Central	CCGP	6	.	.	.	.	.	.	.	.	.	43
Central	Nipigon	3	.	.	.	.	.	.	.	.	.	25
Central	Hearst	6	.	.	.	.	.	.	.	.	.	30
Northwest	Kenora	5	.	.	.	.	.	.	.	.	.	31
Northwest	Dryden	3	.	.	.	.	.	.	.	.	.	15
Northwest	Fort Frances A	4	.	.	.	.	.	.	.	.	.	15
Northwest	Fort Frances B	6	.	.	.	4	.	.	.	.	.	22
Northwest	Fort Frances C	5	.	.	.	.	.	.	.	.	.	13
Northwest	Sioux Lookout	4	.	.	.	.	.	.	.	.	.	13
Northwest	Thunder Bay	8	.	.	.	.	.	.	3	.	1	40
Northwest	Red Lake	8	.	.	.	.	.	.	.	2	.	26
TOTAL			6	2	1	4	1	1	3	2	1	660
Relative frequency (total)			0.009	0.003	0.002	0.006	0.002	0.002	0.005	0.003	0.002	

Appendix II. Locations, authors, and GenBanK accession numbers of all the haplotypes used in this study.

HAP	Location	Authors	GenBanK
HAP1	FL MexicoTexas NB ON	Wooding and Ward (1997); Onorato et al. (2004)	AF012319; AY334364; GU724158
HAP2	ON		GU724159
HAP4	ON		GU724160
HAP5	ON		GU724161
HAP6	ON MexicoTexas	Onorato et al. (2004)	AY334365; GU724162
HAP7	ON		GU724163
HAP8	ON		GU724164
HAP9	ON		GU724165
HAP10	ON		GU724166
HAP11	ON		GU724167
HAP12	ON		GU724168

HAP13	ON		GU724169
HAP14	ON MexicoTexas	Onorato et al. (2004)	AY334363; GU724170
HAP15	ALB AK NM ON	Paetkau and Strobeck (1996); Onorato et al. (2004); Robinson et al. (2007)	U34264; AY334367; EF198771; GU724171
HAP16	ON		GU724172
HAP18	ON		GU724173
HAP19	ON		GU724174
HAP20	ON		GU724175
HAP21	ON		GU724176
HAP22	ON		GU724177
HAP23	ON		GU724178
HAP24	AK ALB BC MONT NM ON UT	Paetkau and Strobeck (1996); Wooding and Ward (1997); Onorato et al. (2004); Robinson et al., (2007)	U34265; AF012305; AY334366; EF198812; GU724179
HAP25	ON		GU724180

HAP26	ON		GU724181
HAP27	ON		GU724182
HAP28	NB ON	Paetkau and Strobeck (1996); Wooding and Ward (1997)	U34261; AF012312; GU724183
HAP29	ON		GU724184
HAP30	ON		GU724185
HAP31	ON QB	Wooding and Ward (1997)	AF012316; GU724186
HAP32	ON		GU724187
HAP33	ON		GU724188
HAP34	ON		GU724189
HAP35	ON		GU724190
HAP36	ON		GU724191
HAP37	ON		GU724192
HAP38	ON		GU724193
H2	MONT	Wooding and Ward (1997)	AF012306

H3	UT	Wooding and Ward (1997)	AF012307
H4	MONT	Wooding and Ward (1997)	AF012308
H5	UT	Wooding and Ward (1997)	AF012309
H6	ALB	Wooding and Ward (1997)	AF012310
H7t7	AK MONT	Robinson et al. (2007)	EF198815
H8	ALB BC	Robinson et al. (2007)	EF198844
H9	NB QB	Robinson et al. (2007)	EF198862
H10	NF QB	Paetkau and Strobeck (1996)	U34267
H13	QB	Wooding and Ward (1997)	AF012313
H14	QB	Wooding and Ward (1997)	AF012314
H16	CA	Wooding and Ward (1997)	AF012317
H17	ALB BC CA MONT	Wooding and Ward (1997)	AF012318
H18	ALB	Wooding and Ward (1997)	AF012320
H19	ALB MONT	Wooding and Ward (1997)	AF012321
H21	NF QB	Wooding and Ward (1997)	AF012322

H23	NB	Wooding and Ward (1997)	AF012323
H24	QB	Paetkau and Strobeck (1996)	U34260
H27	ALB	Paetkau and Strobeck (1996)	U34262
H7t6	AK	Paetkau and Strobeck (1996)	U34263
H7t8	AK	Paetkau and Strobeck (1996)	U34266

Appendix III. Measures of neutrality (Tajima and Fu's tests), nucleotide (π) and haplotype (h) diversity and their standard deviations (*SD* π and *SD* h), and sampling site specific F_{ST} for 23 sampling sites of American black bears (*Ursus americanus*) across Ontario. Sampling sites are mapped in Fig.1 and 2.

		Neutrality			
Sampling site	Sample size	Tajima's *D*	*D P*-value	Fu's *Fs*	*Fs P*-value
BPNP (Bruce Peninsula National Park)	38	0.000	1.000	1.784	0.509
Algonquin	50	6.978	0.915	3.077	0.478
Sudbury	69	5.539	0.867	2.782	0.424
Bracebridge	5	20.974	0.999	3.142	0.796
Midhurst	8	13.032	0.950	0.732	0.276
Pembroke	13	7.580	0.872	1.620	0.395
Parry Sound	41	5.684	0.866	4.278	0.626
Bancroft	33	5.791	0.830	1.220	0.280
North Bay	43	7.713	0.935	3.632	0.581

Timmins	14	14.115	0.996	4.706	0.836
Sault Ste. Marie	45	13.103	0.997	4.507	0.635
Bor+Iv (Borland+Ivanhoe)	28	2.602	0.487	0.256	0.178
CCGP (Chapleau Crown Game Preserve)	43	6.811	0.929	1.786	0.431
Nipigon	25	11.042	0.980	4.615	0.768
Hearst	30	6.846	0.893	3.648	0.622
Kenora	31	16.764	0.999	9.038	0.895
Dryden	15	10.258	0.966	6.366	0.910
Fort Frances A	15	9.066	0.941	4.174	0.764
Fort Frances B	22	14.043	0.995	4.570	0.731
Fort Frances C	13	10.306	0.953	3.094	0.671
Sioux Lookout	13	9.940	0.952	3.086	0.690
Thunder Bay	40	8.916	0.968	2.728	0.456
Red Lake	26	13.557	0.998	4.207	0.650
TOTAL	660	9.594	0.926	3.437	0.591

Appendix III. (*Continued*)

		Nucleotide diversity		Haplotype diversity		
Sampling site	Sample size	π	*SD* π	h	*SD* h	F_{ST}
BPNP (Bruce Peninsula National Park)	38	0.002	0.002	0.501	0.031	0.323
Algonquin	50	0.012	0.007	0.746	0.045	0.305
Sudbury	69	0.014	0.008	0.815	0.024	0.301
Bracebridge	5	0.008	0.006	0.600	0.175	0.314
Midhurst	8	0.015	0.010	0.893	0.086	0.302
Pembroke	13	0.016	0.010	0.780	0.081	0.299
Parry Sound	41	0.014	0.008	0.652	0.051	0.302
Bancroft	33	0.018	0.010	0.856	0.033	0.295
North Bay	43	0.012	0.007	0.735	0.036	0.305
Timmins	14	0.018	0.010	0.714	0.079	0.296
Sault Ste. Marie	45	0.014	0.008	0.748	0.034	0.302
Bor+Iv (Borland+Ivanhoe)	28	0.013	0.008	0.794	0.050	0.303

CCGP (Chapleau Crown Game Preserve)	43	0.008	0.005	0.611	0.067	0.312
Nipigon	25	0.008	0.005	0.530	0.064	0.312
Hearst	30	0.015	0.008	0.685	0.067	0.301
Kenora	31	0.025	0.013	0.656	0.076	0.283
Dryden	15	0.015	0.009	0.448	0.135	0.300
Fort Frances A	15	0.013	0.008	0.419	0.141	0.303
Fort Frances B	22	0.022	0.012	0.771	0.062	0.289
Fort Frances C	13	0.019	0.011	0.833	0.060	0.294
Sioux Lookout	13	0.013	0.008	0.654	0.106	0.305
Thunder Bay	40	0.016	0.009	0.622	0.081	0.299
Red Lake	26	0.026	0.014	0.825	0.039	0.281
TOTAL	660	0.015	0.008	0.691	0.071	/

CHAPTER 3

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

Evaluating the degree of differentiation of isolated populations that were historically continuously distributed can help understand the genetic consequences that landscape modifications may have on populations that could become fragmented. Using 15 microsatellite loci, we evaluated the genetic structure of American black bears (*Ursus americanus*) across a vast, contiguous Ontario landscape (> 1×10^6 km^2) that likely represents their pre-European settlement distribution. Since geographic barriers are largely absent in the region under study, we predicted that isolation by distance would drive genetic structure. We identified 3 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 black bear populations. The reasons for this low genetic diversity are not yet clear, and should be determined to assess if this is a consequence of human activities. Most of the large scale genetic signals detected in this study likely represent black bear gene flow patterns under undisturbed conditions. As such, these data could be used as references in models that project the evolution of population differentiation based on upcoming landscape modifications in northern regions of North America.

Keywords: American black bear; cluster; gene flow; genetic structure; Isolation by distance; male-biased dispersal; microsatellite; North America; spatial autocorrelation; *Ursus americanus*

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. These data, along with demographic information, 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; Hoglund et al. 2007; Ohnishi et al. 2007; White and Searle 2007), and are also more likely to be subject to inbreeding (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*—Mech and Boitani 2008; American puma, *Puma concolor*—Anderson 1983; wolverine, *Gulo gulo*—Banci 1994; Canada lynx, *Lynx canadensis*—Koehler and Aubry 1994; American marten, *Martes americana*—Gibilisco 1994; fisher, *Martes pennanti*—Gibilisco 1994; American black bear, *Ursus americanus*—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. Indeed, such methods allow for 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 prevent 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 due 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, black bear habitat 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 provinces, this part of Canada generally lacks a pronounced human presence (average 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 to what is observed in isolated populations located in regions with higher levels of anthropogenic activity (Csiki et al. 2003; Dixon et al. 2006; Larkin et al. 2004; Onorato et al. 2007; Van Den Bussche et al. 2009).

Our goal was to characterise black bear genetic structure 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-18^{th} 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, which would contrast with the moderate levels of differentiation found with mtDNA due to postglacial recolonization patterns and male-biased dispersal (Pelletier et al. 2011). Here, we also explicitly tested for sex-biased dispersal, and hypothesized that comparison between genetic indices of males and females would indicate that males are the dispersing sex, and females the philopatric sex. 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 black bear hair samples at 61 locations across Ontario, and profiled them at 15 microsatellite loci and the Amelogenin locus for gender (Fig. 3.1). 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 clusters (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 aimed to provide context on the extent of genetic isolation of more southerly populations that were once contiguous with the Ontario population. Our goal was also to provide a reference of black bear gene flow patterns under undisturbed conditions, data which may be later included in landscape change models to identify sites where connectivity and genetic diversity are likely to become lower, and thus inform management strategies that aim to maintain population stability.

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. 3.1). 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 no more than 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, Canada). For samples collected from 2004 to 2009, we followed a MagneSil paramagnetic bead automated DNA extraction procedure (Promega, Nepean, Canada) using a P3 Evolution liquid handler (Perkin Elmer, Woodbridge, Canada). For each individual sample, we suspended hairs in 180 ul of 1 X lysis buffer (4 M urea, 0.2 M NaCl, 0.5% n-lauroyl sarcosine, 10mM CDTA (1,2-cyclohexanediamine), 0.1 M Tris-HCL pH 8.0)(Applied Biosystems Inc., Burlington, Canada). We then treated samples with 10 units of proteinase K (> 600 U/ml, Qiagen), and incubated at 37^{o}C for 12 hours. 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 composed 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 2ng and 200pg.

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 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, 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 3 PCR reactions: i) multiplex of G10A, G10B, G10L, MU05, G10D; ii) multiplex of G10H, G10J; iii) Amelogenin. Pooled reactions 2 (MP2) consisted of 4 PCR reactions: i) G10X, G10M; ii) G10U, G10C; iii) MU59; iv) MU50, G10P, MSUT6. We performed all DNA amplifications in 10 ul consisting of 1X PCR buffer (Qiagen), 200 uM dNTPs, 0.1-0.5 uM forward and reverse primers, 1.0ug of bovine serum albumin (DNAse and RNAse free, Amersham BioSciences Inc., Piscataway, USA), 0.5 units of Taq polymerase (5U/ul)(Qiagen), and 4ul of the eluted DNA. PCR conditions consisted of 5 minutes at 94^{o}C, then 31 cycles of 30 seconds at 94^{o}C, 45 seconds at 52^{o}C, and 2 minutes at 72^{o}C followed by a final cycle of 20 minutes at 72^{o}C. We added 0.5 ul of the pooled amplicons to 9.5uL of HiDi formamide and ROX standard and run 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 non-invasive hair sampling (Taberlet et al. 1999). Specifically, we inspected allele morphology and quality in GENEMARKER (SoftGenetics, State College, PA, 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 2 or fewer allele differences, were run at MP2 to ensure that the samples were not improperly pooled as individuals. All samples with unique genotypes at MP1 were also run 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 as individuals based on the initial 7 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 to assess genotyping error using a subset of 5 sampling sites (Parry Sound, Pembroke, Hearst, Wawa, and Dryden) spread across our sampling region: i) genotypes observed only once (single captures); ii) genotypes observed more than once. For the latter category, we recorded the number of allele call changes made by the technicians while grouping genotypes, and compared it to 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: $\overline{Er} = \sum_{i}^{s} Er$ / 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 v4 (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 due to a lack of heterozygotes, so we removed it from our dataset 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 2.3 (Pritchard et al. 2000; Falush et al. 2003) and TESS 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 5 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 1.2 (Jakobsson and Rosenberg 2007). To test for the effect of number of individuals on genetic structuring patterns, we assigned individuals into clusters based on 4 different cut-off membership values (q = 0.6, q = 0.7, q = 0.8, and q = 0.9) from the output given by CLUMPP. Each individual that had a membership value lower than q was left unassigned. Then, we visualized the clusters using DISTRUCT 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 due to the increasing number of unassigned individuals. Differences in genetic structure were determined through two χ^2 tests: one assessed the differences in proportions for all the individuals (assigned to a cluster and unassigned) at each cut-off membership value. To control for the bias due 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 2.3 and BAPS 4.1 - Corander et al. 2006). We ran TESS 5 times under both the non-admixture 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 non-admixture 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 5 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.2.1.1 package "fields" (R 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), pairwise R_{ST} values (measure of differentiation based on allele size; Hardy et al. 2003), and Nei's standard genetic distance, D_S, (Nei 1978) in SPAGeDi 1.3 (Hardy and Vekemans 2002). We also used SPAGeDi to assess the influence of mutations and migration on observed patterns of genetic structure. This was conducted by permuting R_{ST} among alleles, at each locus. Non significance of this test suggests a higher contribution of migration than mutations to genetic differentiation, and thus, that F_{ST} is a more appropriate descriptor of gene flow (Hardy et al. 2003).

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 MLRelate 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).

Sex-biased dispersal

We used FSTAT 2.93 (Goudet 2001) to test for male-biased dispersal and female philopatry. To do so, F_{IS}, F_{ST}, the mean assignment index (mAI), and the variance of the assignment index (vAI), were compared between males (n = 1,377) and females (n = 1,077), using a randomization procedure (10,000 replicates – Goudet et al. 2002).

Expectations are that the dispersing sex should show a positive F_{IS} from a heterozygote deficiency due to sampling sites harboring both immigrants and residents. Thus, F_{IS} should be higher, and positive, in the dispersing sex. F_{ST}, on the other hand, is expected to be lower in the dispersing sex as a result of homogeneization of allelic frequencies between sites. As a result of dispersal, the probability of correctly assigning an individual from the dispersing sex to its population of origin (*m*AI) should be lower than that of the philopatric sex. Finally, a higher variance should arise when assigning individuals from the dispersing sex to their population of origin, compared to the philopatric sex.

Isolation by distance and spatial distribution of alleles

We performed Mantel tests (Mantel 1967) in GENALEX 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 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 6.3. As the maximum distance between two sites was 1,462 km, we used 30 even distance classes of 50 km, for which we performed 999 permutations and 1,000 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.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 1,000 permutations.

Results

Individual identification and error rate

We detected 2,839 individuals in our dataset. The probability of unrelated individuals sharing identical genotypes was $P_{id} = 2.4\times10^{-19}$, and the probability of full siblings sharing identical genotypes was $P_{sib} = 4.5\times10^{-7}$. Genotyping error across a subset of 5 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, along with our estimated genotyping error rate, shows that for our complete dataset of 14 loci, we did not generate a large number of false genotypes ($n = 62$) that could undermine our population genetic 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 non-random associations of loci (P < 0.000009).

Genetic clusters determined by Bayesian methods

The aspatial algorithm implemented in STRUCTURE assigned individuals into 3 main genetic clusters (highest ΔK = 266.45) geographically restricted to different regions of Ontario (Northwest, Southeast, and Bruce Peninsula; Fig. 3.2a). When analyzed one by one, all of these clusters were further divided into 2 subclusters (Northwest A / Northwest B: ΔK = 60.12; Southeast A / Southeast B: ΔK = 17.64; Bruce Peninsula A Bruce Peninsula B: ΔK = 541.11), for a total of 6 clusters (Fig. 3.2b).

The TESS non-admixture 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 2 clusters being located in the Northwest (Northwest A and B), 2 in the Southeast (Southeast A and B), and one on the Bruce Peninsula (Fig. 3.2c and Fig. 3.3).

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 $K = 3$ ($\chi^2 = 0.2485$, d.f. = 6, $P = 0.9997$) and $K = 5$ ($\chi^2 = 10.298$, d.f. = 12, $P = 0.5898$) when we controlled for the number of unassigned individuals (Fig. 3.4). In the absence of control, differences were significant (for $K = 3$: $\chi^2 = 27.0815$, d.f. = 9, $P = 0.0014$; for $K = 5$: $\chi^2 = 77.6337$, d.f. = 15, $P < 0.0001$).

Genetic diversity and level of differentiation

Overall, we detected high levels of heterozygosity (mean $H_O = 0.75$ +/- 0.02; mean $H_E = 0.78$ +/- 0.02), as well as high number of alleles per site (mean no. of alleles per sampling site = 8.82 +/- 2.76) (Table 3.1). Observed heterozygosity ranged from 0.43 (Owen Sound) to 0.82 (Sault Ste. Marie), whereas expected heterozygosity ranged from 0.45 (Owen Sound) to 0.82 (Atikokan). The mean number of alleles per site ranged from 2.21 (Owen Sound) to 11.21 (Algonquin – Table 3.1).

The R_{ST} permutation test did not detect any significant contribution of allele size to genetic differentiation between the 5 genetic clusters identified in TESS. As such, oly differentiation levels based on F_{ST} are reported. All of these 5 genetic clusters 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 3.2) and the remainder of the values ranging from weakly differentiated ($F_{ST} = 0.0181$ - Northwest A and Southeast B; $P < 0.0001$) to moderately differentiated ($F_{ST} = 0.1407$ - Northwest B and Bruce Peninsula; $P < 0.0001$; Table 3.2). 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, as 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 average 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 average maximum likelihood relatedness was high ($r = 0.407$). For this cluster, $P_{sib} = 1\times10^{-5}$, and $P_{id} = 7.8\times10^{-10}$.

Sex-biased dispersal

All of the indices used to identify sex-biased dispersal, indicated a significant difference between males and females. F_{IS} was higher in males than females ($F_{IS\text{-}Males} = 0.0425$; $F_{IS\text{-}Females} = 0.0268$; $P = 0.0078$), and F_{ST} was lower in males than females ($F_{ST\text{-}Males} = 0.0239$; $F_{ST\text{-}Females} = 0.0365$; $P = 0.0001$). The assignment index was negative in males, and positive in females ($mAI_{Males} = -0.491$; $mAI_{Females} = 0.665$; $P = 0.0001$), and its variance higher in males ($vAI_{Males} = 18.25$; $vAI_{Females} = 16.26$; $P = 0.0212$).

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).

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 1,450 km, the correlation was negative and significant ($0.008 < P < 0.023$; Fig. 3.5).

The difference in overall expected versus 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 describe 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; Peacok et al. 2007). However, there is a lack of studies focusing on genetic structure across landscapes that still mostly correspond to this species pre-European settlement distribution, while including non-natural influences at their 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 black bear populations. 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 black bear genetic clusters 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 3.1). These results fall within the range of 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 bear (*Ursus arctos*) (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 3 main genetic clusters, 2 genetically diverse and weakly differentiated from each other, located in the Northwest and Southeast regions 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. 3.2, 3.3), suggesting that the Northwest sites differ from the Southeast sites despite a low level of differentiation between them (Table 3.2).

We expected high population admixture levels due 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 4 membership cut-off values ($q = 0.6$, $q = 0.7$, $q = 0.8$, $q = 0.9$ - Fig. 3.4), 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 differentiated 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 ($r = 0.552$, $P = 0.001$ without Bruce Peninsula) 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 (Fig. 3.2 and 3.3) is mostly driven by isolation by distance.

In this study, a clear pattern of male-biased dispersal was dectected by various population genetic indices (Goudet et al. 2002). On the other hand, no clear signal of female philopatry was detected, although field observations from several studies have indicated this pattern in this species (Rogers 1987; Lee and Vaughan 2003). Although capable of extensive movements, males have not been shown to move further than 200 km from their natal site (Rogers 1987). Thus, in addition to isolation by distance, we suggest that the geographic limits to which males travel when reaching the subadult stage (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. 3.5) could be a reflection of dispersal events over multiple generations. Indeed, the average 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 3 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 in the southeastern region of the province, gene flow and effective

population size are high enough to mitigate the effects of habitat fragmentation. 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 southeastern 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 genetic 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 which 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 due to geographic isolation induced by the shape of the Peninsula after the colonization of this area by black bears; ii) genetic drift due 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.

The level of diversity on the Bruce Peninsula is consistent with what has been observed in genetically depauperate black bear populations 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. 2008; 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 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 due to its geographic isolation.

Although lower levels of genetic variation have been found in other isolated black bear populations (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 black bear habitat is under pressure from development (Obbard et al. 2010). Considering their high level of relatedness (r = 0.407), black bears in this area could suffer from inbreeding, which may 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 dataset across such a large geographic area. When put in context of other black bear genetic research, our results show that Ontario black bears (with the exception of the Bruce Peninsula) may be used as a reference that corresponds to the levels of genetic diversity and structure that should be observed among intact black bear populations that 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, black bear populations 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 3 main genetic groups in Ontario, which mostly correspond to the mtDNA clusters (Pelletier et al. 2011), can help refine management decisions in this province.

Due to 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 that 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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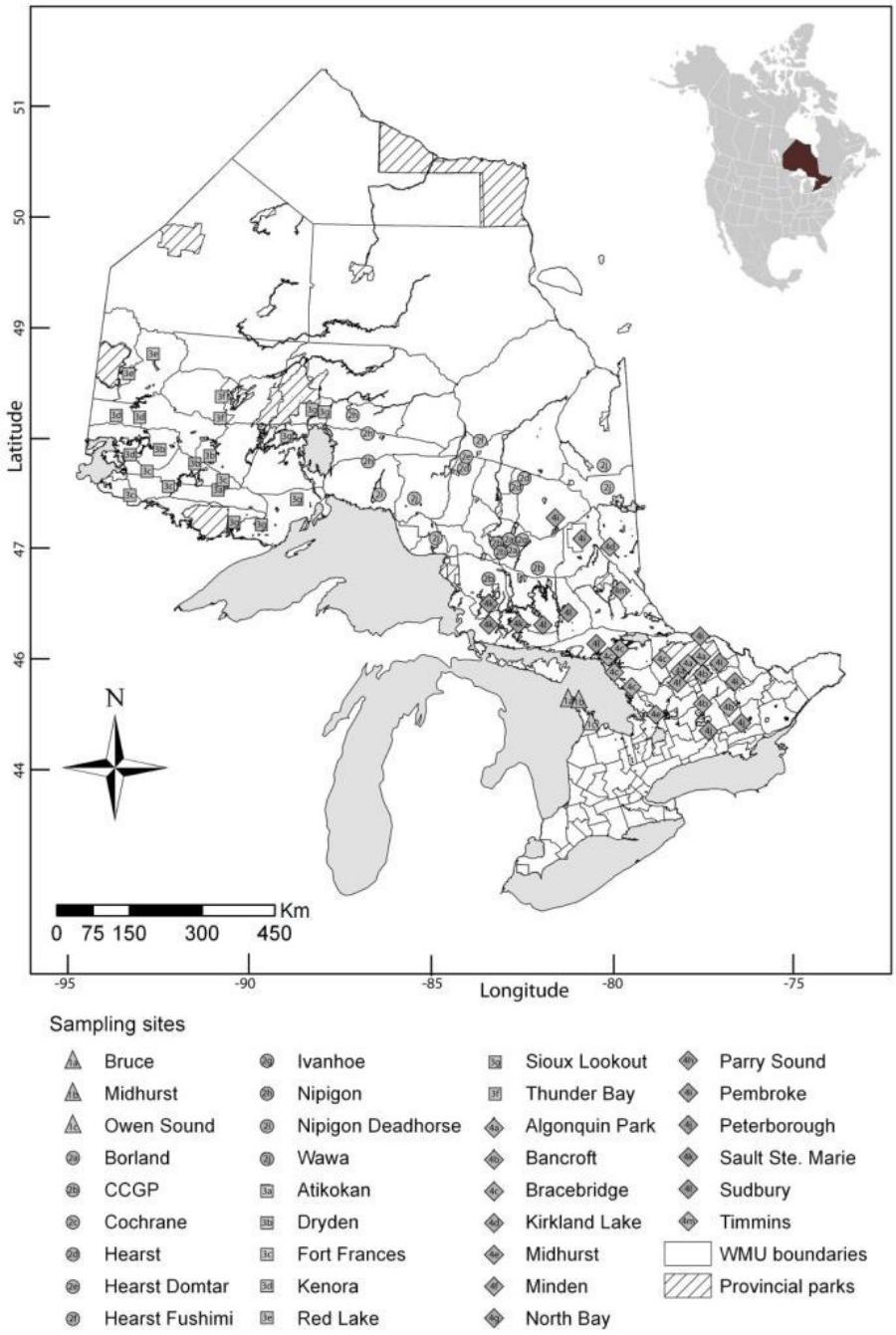

Fig. 3.1. Map of sampling sites in Ontario where black bear (*Ursus americanus*) hairs were collected for genetic analyses. The 4 different shapes for the various sampling sites represent the 4 clusters detected through previous mtDNA analyses (Pelletier et al. 2011). Triangles represent the Bruce Peninsula cluster, diamonds represent the Southeast cluster, circles the Central cluster, and squares the Northwest cluster.

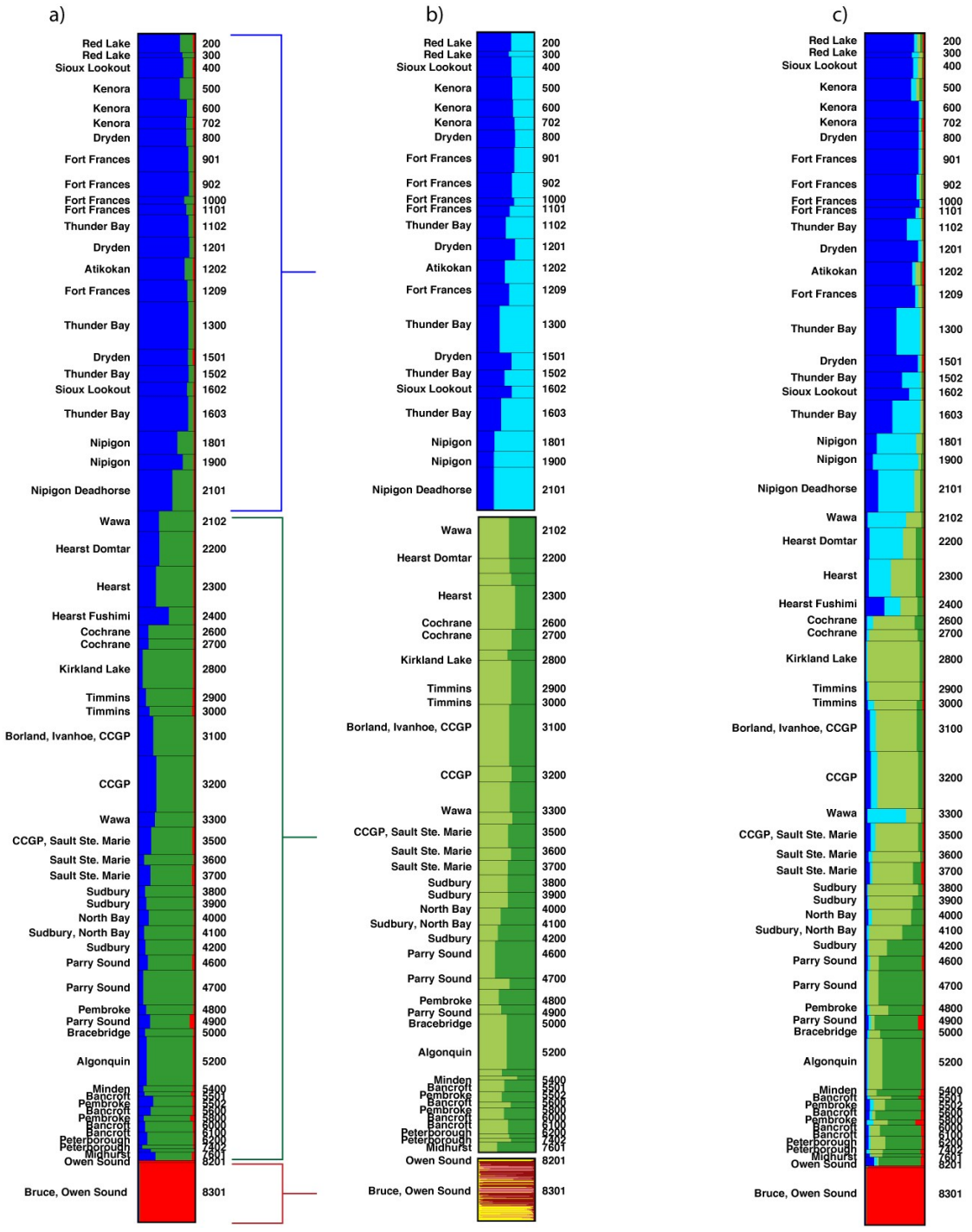

Fig. 3.2. a) Barplot representing K_{max} = 3 black bear (*Ursus americanus*) genetic clusters identified by STRUCTURE 2.3 for q = 0.6. Blue represents the Northwest cluster, green the Southeast cluster, and red the Bruce Peninsula cluster. b) Barplots representing the

subclusters found within the 3 main genetic clusters identified by STRUCTURE. c) Barplot representing K_{max} = 5 genetic clusters identified by TESS 2.3 (Northwest A-blue, Northwest B-light blue, Southeast A-light green, Southeast B-green, and Bruce Peninsula-red).

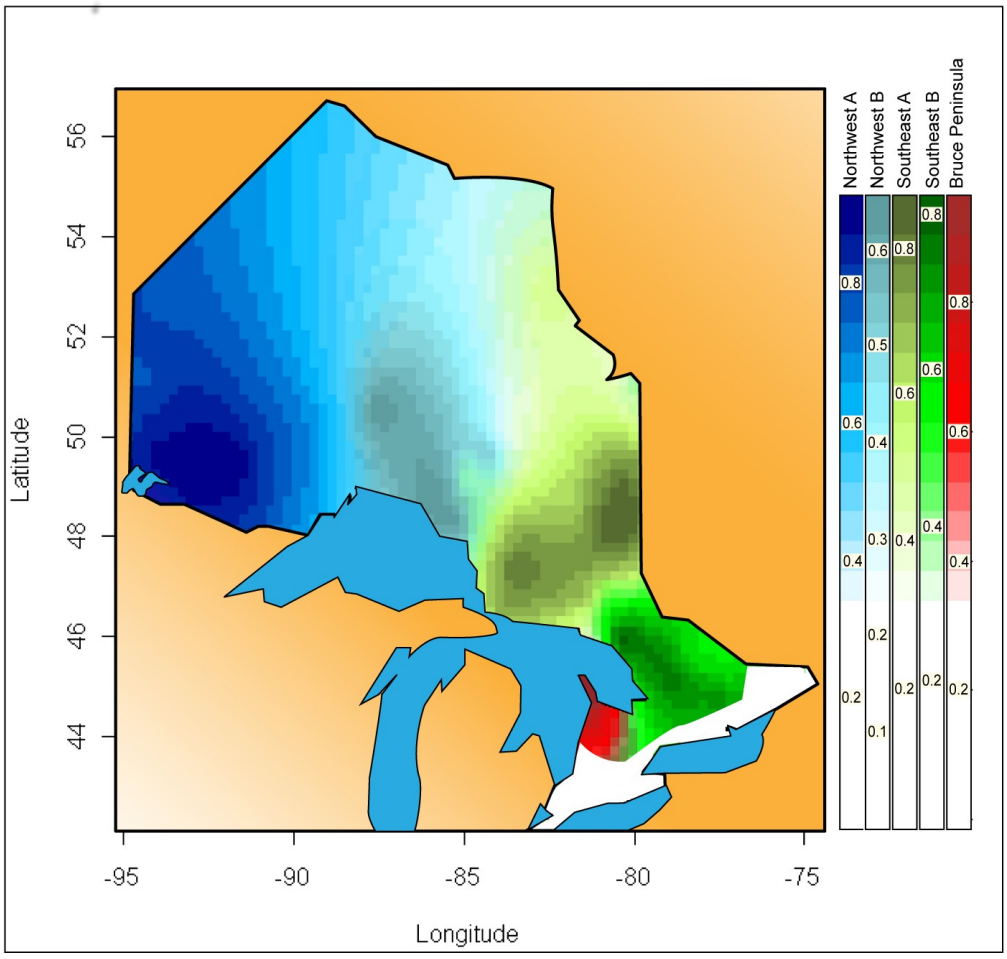

Fig. 3.3. Interpolated map of posterior membership coefficients in the K_{max} = 5 black bear (*Ursus americanus*) genetic clusters identified by TESS 2.3 (Northwest A-blue, Northwest B-light blue, Southeast A-light green, Southeast B-green, and Bruce Peninsula-red).

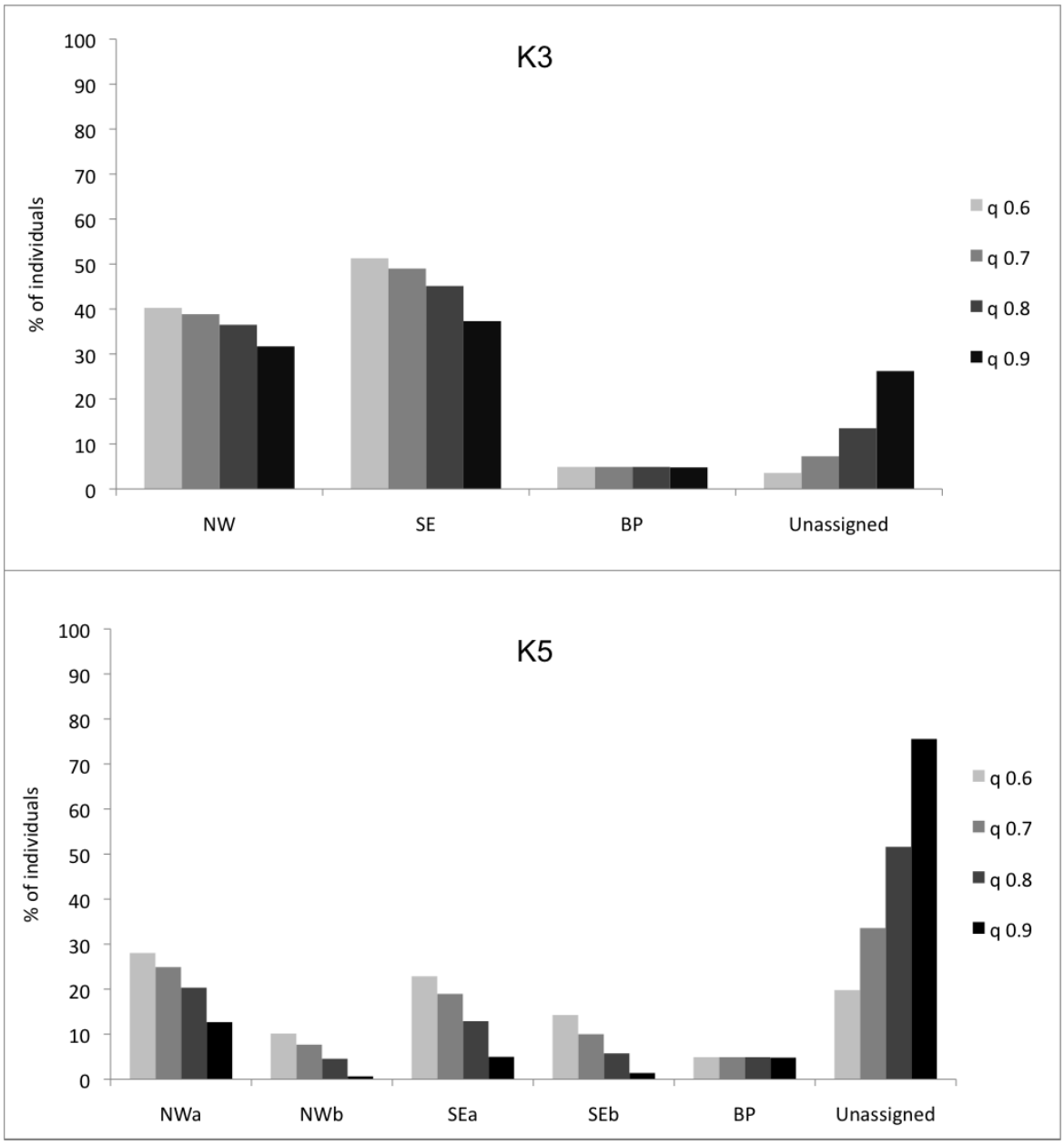

Fig. 3.4. Histogram of the percentage of individuals assigned to the various clusters at *K* = 3 and *K* = 5, for different cluster membership values (*q* = 0.6, 0.7, 0.8, and 0.9).

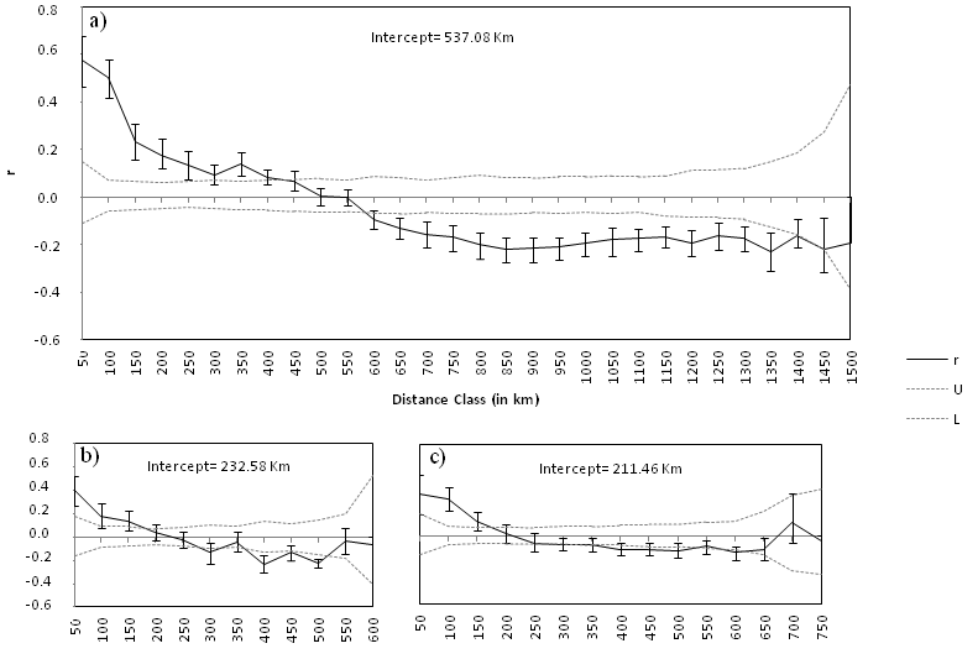

Fig. 3.5. Spatial autocorrelation correlograms drawn in GENALEX 6.3 (Peakall and Smouse 2006) of (a) our entire Ontario sampling area, (b) the sites located within the Northwest cluster identified by Bayesian clustering algorithms, and (c) the sites located within the Southeast cluster identified by Bayesian clustering algorithms. r (full line) is the correlation coefficient between genetic differentiation and the geographic distance. The 95% confidence interval is represented by dashed lines (upper and lower bounds – U and L, respectively) 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 black bears (*Ursus americanus*) with geographic distance.

Table 3.1. Descriptive statistics per site where we collected American black bear (*Ursus americanus*) genetic samples (number of bears (*n*), expected heterozygosity (HE), observed heterozygosity (HO), mean number of alleles (No. alleles), and their respective standard deviation (SD)), including site locations within Wildlife Management Units (WMU).

WMU	Site	*n*	HE	SD (HE)	HO	SD (HO)	No. alleles	SD (No. alleles)
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
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

Table 3.2. Genetic differentiation levels among the 5 American black bear (*Ursus americanus*) genetic clusters identified in TESS 2.3 for q = 0.6, evaluated through pairwise F_{ST} values (sampling sites are mapped in Fig. 3.1). For all the comparisons, *P*-values were significant, with $P < 0.0001$.

	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	-

CHAPTER 4

DETERMINING CAUSES OF GENETIC DIFFERENTIATION IN AN ISOLATED LARGE CARNIVORE (*URSUS AMERICANUS*) POPULATION TO INFORM POTENTIAL CONSERVATION ACTIONS

CITATION:

Pelletier, A., Obbard, M.E., Harnden, M., Howe, E.J., Burrows, F.G., White, B.N., and Kyle, C.J. 2013. Determining causes of genetic differentiation in an isolated large carnivore (*Ursus americanus*) population to inform potential conservation actions. *In prep*.

Abstract

Most conservation studies focus on both geographically and genetically isolated populations, as these can have high extinction risks. However, the identification of isolation is not sufficient to determine extinction probabilities, as local extirpation also depends on the processes (contemporary, historical, natural or anthropogenic) that induced this situation. To inform future conservation initiatives in currently widely distributed mammals, it would thus be useful to understand the reasons for genetic distinctiveness in populations that are adjacent to continuously distributed populations. In Ontario, a black bear population with reduced genetic diversity, located on the Bruce Peninsula (BP), is next to a continuum of populations, making the processes that led to its reduced variability unclear. As such, this population provides a biological model to evaluate alternative hypotheses that may explain contemporary reduced diversity in large carnivores. We conducted forward simulations to test for 1) genetic drift following the colonization of the BP after the Last Glacial Maximum; 2) a recent bottleneck associated with forest fires; 3) reduced migration between BP and southeastern individuals due to loss of habitat following European settlement; 4) a combination of a recent population crash and reduced migration. We also performed simulations to assess the need for genetic restoration actions through translocation efforts. Our results suggest that a recent demographic bottleneck involving 2 drops in population size, associated with reduced migration into the BP, led to current levels of genetic differentiation and diversity. Results also suggest that under geographic isolation, BP black bears could retain at least 80% of their current diversity over the next 100 years. Further, a single translocation effort would only help increase genetic diversity on the short-term. We conclude that

management actions could be warranted if reduced genetic diversity or inbreeding started to negatively affect the fitness of BP black bears. Although landscape management to restore migration would be the most effective method to enhance long-term genetic diversity and prevent inbreeding, regular translocations of bears from southeastern Ontario would be more feasible, as the region has been irreversibly modified by anthropogenic influences that represent strong barriers to gene flow.

Keywords: American black bear; bottleneck; carnivore; conservation genetics; extirpation; gene flow; genetic rescue; inbreeding; microsatellite; North America; simulation; translocation; *Ursus americanus.*

Introduction

Over the last century, North American carnivores have experienced range contractions as a result of harvest pressures, habitat loss, fragmentation, and subsequent geographic isolation (Laliberté and Ripple 2004), leading to reduced effective population size and genetic diversity in several regions of the continent (Fahrig 2003; Wiegand et al. 2005). The long-term persistence of these fragmented segments may be threatened, as small isolated populations have higher extirpation risks than populations that are more continuous (Frankham 1995; Frankham 1997; Lande 1993; Keyghobadi 2007). In the southern portion of North America, several wide-ranging carnivores are now of conservation concern due to the aforementioned processes (e.g., American puma – *Puma concolor* – Anderson 1983; American marten – *Martes Americana* – Gibilisco 1994; fisher – *Martes pennanti* – Gibilisco 1994; American black bear – *Ursus americanus* –

Vaughan and Pelton 1995; gray wolf – *Canis lupus* – Mech 1995; brown bear – *Ursus arctos* – Paetkau et al. 1998a), despite the fact that some of them are now recolonizing areas where they were formerly extirpated (Scheick et al. 2011; LaRue et al. 2012). In more northern regions of the continent (northern United States and Canada), most of these species are still widely and continuously distributed, although the presence of population segments displaying genetic structure not fully explained by isolation by distance has been noticed (e.g., fisher – Kyle et al. 2001; wolverine – *Gulo gulo* – Kyle and Strobeck 2002, Zigouris et al. 2012; American puma – *Puma concolor* – McRae et al. 2005; artic fox – *Vulpes lagopus* –Norén et al. 2011; American black bear – Pelletier et al. 2012). The existence of such structured populations has been explained by population peripherality (Schwartz et al. 2003; Zigouris et al. 2012), restriction of movement within preferred habitat types (Sacks et al. 2004; Schwartz et al. 2009), habitat features limiting dispersal (McRae et al. 2005), and historical colonization events (Wisely et al. 2004; McRae et al. 2005). Preventing these northern populations from becoming as geographically and genetically fragmented as southern conspecifics has become important for the maintenance of species stability and overall biodiversity, particularly with the growing environmental pressures and habitat loss caused by human activities. To do so, identifying the factors that led to low genetic diversity in genetically disjunct northern populations is necessary, as local extirpation risks are linked to the timescales and the nature of the processes that result in isolation.

Through forward time simulations and coalescence-based analyses, it is possible to distinguish between contemporary and historical processes that explain the level of genetic diversity observed in these populations, as well as their level of differentiation from the neighboring core populations (Balloux 2001; Kuo and Janzen 2003; Currat et al. 2004; Cornuet et al. 2008; Cornuet et al. 2010). Genetic signals induced by demographic bottlenecks can also be identified through the observation of a heterozygosity excess at polymorphic loci (Cornuet and Luikart 1996; Piry et al. 1999), or through a decrease in the total number of alleles relative to the range in allele size (Garza and Williamson 2001). Thus, these methods allow to differentiate a lack of genetic diversity resulting from historical colonization events, which would not necessarily warrant conservation concerns, from a lack of diversity that results from more recent non-natural events, which could cause conservation concerns and lead to the implementation of appropriate management actions.

Studies of fragmented populations that are located in the geographic vicinity of intact segments can be used to identify the processes that can induce genetic isolation, as well as the timescales on which such isolation can occur. Despite this, many conservation studies of vagile carnivores focus on population segments that are fragmented and distant from the core (e.g., fisher – Aubry and Lewis 2003; wolverine – Cegelski et al. 2003; Florida black bear – Dixon et al. 2006; Florida panther – Culver et al. 2008). For this reason, it is difficult to understand the context of such isolation, as these populations cannot be directly compared to contiguous segments that reflect a more undisturbed state.

Among species of large carnivores, the American black bear provides a useful biological model to identify the processes that can lead to genetic distinctiveness in population segments that are adjacent to continuously distributed populations. First, this species is widely distributed across North America (Scheick et al. 2011), and genetic diversity and differentiation levels have been shown to be influenced by landscape features (Cushman et al. 2006; Dixon et al. 2007), or island biogeography (Paetkau and Strobeck 1994 and 1996). Second, populations located in the southern portion of the continent are fragmented and display low genetic diversity ($0.29 < H_O < 0.56$; Paetkau and Strobeck 1994; Warrillow et al. 2001; Triant et al. 2004; Dixon et al. 2007; Onorato et al. 2007), whereas the majority of northern populations are either continuously distributed, or highly connected to mainland populations, as well as genetically diverse ($0.70 < H_O < 0.94$; Paetkau and Strobeck 1994; Belant et al. 2005; Robinson et al. 2007; Pelletier et al. 2012). Third, this northern region harbors a few genetically distinct populations located in the vicinity of the continuous core (Robinson et al. 2007; Pelletier et al. 2012). Studying these populations would help to 1) understand the processes that led to this genetic distinctiveness, 2) determine if contemporary or historical processes are involved, and 3) inform potential conservation initiatives to prevent future fragmentation and genetic isolation of currently continuous segments.

In Ontario (Canada), black bears are continuously distributed across a largely intact landscape, except for the southern periphery where anthropogenic influences are high (Statistics Canada 2002). In this province, black bears are genetically diverse across a large geographic area, although a population located on the Bruce Peninsula (BP) is under growing anthropogenic influences that likely prevent migration (Howe et al. 2007),

is genetically differentiated from the core (Pelletier et al. 2011 and 2012), shows a high level of relatedness among individuals (Pelletier et al. 2012), and displays a level of diversity comparable to threatened southern populations (Paetkau and Strobeck 1994; Warrillow et al. 2001; Triant et al. 2004; Onorato et al. 2007). The lack of diversity in these latter populations has been attributed to bottlenecks (Boersen et al. 2003; Triant et al. 2004), and/or geographic isolation over long periods of time (Paetkau and Strobeck 1994; Warrillow et al 2001; Onorato et al 2007; Brown et al. 2009).

The contrasting levels of land-use observed in Ontario, with higher habitat fragmentation in the south than in the north, reflect, at a smaller scale, what is currently observed across the continent. Because of this, identifying the reasons for the lack of diversity of the BP population could provide insights into the future state of other vagile species currently continuously distributed in northern North America. Indeed, this part of the continent is considered to be a hotspot of latent extinction risk due to future human expansion, and is expected to experience increased levels of habitat fragmentation (Cardillo et al. 2006 and 2008). In Ontario specifically, governmental incentives have been implemented to develop the northern portion of the province (Growth Plan for Northern Ontario 2011). As such, there is a possibility that an increase in the number of populations with characteristics comparable to BP black bears may occur in this currently undisturbed region.

Concerns exist regarding the long-term persistence of BP black bears, due to their small population size (220 to 660 individuals; Howe et al. 2007), and their on-going geographic isolation. This isolation is caused by increasing pressures from development on essential bear habitat in the region (Obbard et al. 2010a). In addition, microsatellite

analyses have detected higher levels of divergence between BP black bears and black bears from other regions of Ontario ($0.123 < F_{ST} < 0.141$), compared to observations from the rest of the province (Pelletier et al. 2012), and only 2 mitochondrial DNA haplotypes were identified on the BP of the 36 present in Ontario (Pelletier et al. 2011). Further, the level of relatedness among BP individuals is high (Pelletier et al. 2012), which, along with the reduced population size, could indicate a future risk of inbreeding (Frankham 1995; Frankham 1997).

Several causes could explain the BP population's genetic distinctiveness. First, its low diversity could be due to a historical, postglacial colonization event that could have resulted in a founder effect followed by genetic drift due to geographic isolation. In this case, the population could maintain itself in the future at a constant, low level of genetic diversity, as suggested for Kodiak brown bears (Paetkau et al. 1998a; Paetkau et al. 1998b) and Scandinavian wolverines (Walker et al. 2001). Second, BP black bears could have experienced a recent demographic bottleneck, which could have resulted in the observed genetic signal. Large fires linked to agricultural activities occurred on the BP in the early 1900s (Suffling et al. 1995). Such fires could have both increased the mortality of black bears on the Peninsula, and prevented movement between the Peninsula and surroundings areas by destroying a large portion of their habitat. Third, the separation of the BP from the broader Ontario population, as a result of human-induced landscape fragmentation following European settlement, could have led to the current reduced genetic diversity through sustained isolation (Pelletier et al. 2012). Finally, a combination of a recent population crash and recovery, followed by a lack of migration due to human influences, could also explain the structuring of the BP population from the rest of the

province. Given these possibilities to explain the lack of genetic diversity and the genetic isolation of this population, additional management actions beyond those proposed by Howe et al. (2007) may be considered to ensure the long-term persistence of BP black bears.

To inform such management initiatives, we tested these alternative hypotheses to identify the processes explaining BP black bears reduced genetic diversity and high differentiation with other Ontario populations. First, we investigated the potential reasons for genetic distinctiveness through forward simulations. These methods allow to model the history of a population from past to present. Variables such as population size, mating system, migration rate, and mutation process, can be easily parameterized, as opposed to coalescent based analyses, which are more limited when attempting to simulate complex evolutionary trajectories (Carvajal-Rodriguez 2008; Arenas 2012). In this study, we tested for several possible scenarios: 1) a founder effect following the colonization of the BP by black bears after the Last Glacial Maximum; 2) a recent genetic bottleneck due to forest fires on the BP 100 years ago; 3) reduced migration between BP and mainland individuals due to increased anthropogenic influences following European settlement; 4) a combination of a recent population crash and reduced migration with the mainland. We conducted simulations of each scenario with EASYPOP (Balloux 2001) and BottleSim (Kuo and Janzen 2003). Then, we compared the genetic structure and diversity obtained in the simulated datasets with our empirical dataset from the southeastern portion of the province, which likely represents the undisturbed, initial state of what BP black bears were prior to the colonization of Ontario that followed the LGM (Pelletier et al. 2012). Second, as an alternative to forward simulations, we tested the bottleneck hypothesis by

determining the presence of a genetic signal that could have been induced by a recent reduction in population size in BP black bears (Piry et al. 1999; Garza and Williamson 2001).

To further investigate the conservation status of the BP population based on genetic data, we also conducted simulations to determine if 1) at a constant population size over the next 500 years, the BP population could maintain its current level of diversity, and 2) translocations of individuals from southeastern Ontario into the BP could result in an increase in diversity that would help reach a level similar to the one currently observed in the rest of the province.

Materials and Methods

Study area

The Bruce Peninsula (1,100 km^2; geographical center: Latitude: 44.84154 / Longitude: -81.2296) is part of the Niagara escarpment in southwestern Ontario, and separates Lake Huron from Georgian Bay (Fig. 4.1). The BP is mostly composed of private properties, agricultural lands, urban areas, and hiking trails, and has a human density estimated from 10 to 64 pers./km^2 (Statistics Canada 2002). In the northern portion, where Bruce Peninsula National Park (BPNP – 154 km^2) is located (Parks Canada 1997), the habitat is mainly undisturbed (Moreland 1996). In contrast, in the southern portion of the peninsula, high levels of urban development likely impede the movement of black bears between the mainland and the BP (Howe et al. 2007). These distinct levels of development in the northern and southern portions of the BP are reflected in differences in vegetation composition. Species linked to agricultural activity

are prominent in southern regions, whereas northern regions of BP include conifer, mixed conifer-deciduous, and small patches of deciduous forests (Kaiser 1995; Suffling et al. 1995; Young et al. 1996; Coady 2005). The presence of both coniferous and deciduous species on the peninsula provides hard and soft mast for black bears, which are essential elements to their various seasonal diets (Young et al. 1996; Coady 2005).

Sampling

Between 1997 and 2009, we collected black bear hair samples obtained from baited barbed wire hair traps (Woods et al. 1999) across Ontario. This sampling effort was conducted as part of the Ontario's Enhanced Black Bear Management Program to estimate bear densities and population dynamics throughout the province (Obbard et al. 2010b; Howe et al. *in press*). In this study, we focused on the sampling sites located on the BP and 9 surrounding southeastern sites (Fig. 4.1). All hair samples were stored dry in paper envelopes at room temperature until DNA analyses (mtDNA sequencing, individual microsatellite genotyping, gender determination, and estimation of genotyping error) were performed, following the conditions described in Pelletier et al. (2011 and 2012). Our dataset included 139 individuals from the BP and 647 individuals from the adjacent southeastern sites previously genotyped in Pelletier et al. (2012) through the analysis of 15 microsatellite loci (Paetkau and Strobeck 1994; Taberlet et al. 1997; Kitahara et al. 2000).

Forward time simulations

We conducted forward simulations to differentiate between 5 alternative scenarios to explain the low genetic diversity of BP black bears relative to immediately adjacent southeastern (SE) populations (Fig. 4.2). To determine if reduced migration, sudden reductions in population size, or both, could have played a role, we calculated the number of alleles (Na), effective number of alleles (Na_e), observed heterozygosity (H_O), and expected heterozygosity (H_E) in GENALEX 6.4 (Peakall and Smouse 2006), for both our empirical data and each simulation output. We assessed the significance of differences in genetic diversity by comparing the range of each simulated value to our empirical data.

Scenario 1 corresponded to the colonization of the BP by black bears following the Last Glacial Maximum (LGM), and various levels of migration between BP and SE. Scenario 2 focused on genetic drift as a primary driver, with colonization of the BP following the LGM, but with very little to no migration in and out of the BP following colonization (Fig. 4.2). Scenario 3 corresponded to a genetic bottleneck caused by a recent population crash followed by population recovery. Scenario 4 corresponded to a slow decline in diversity from a lack of migration between BP and SE as a consequence of human influences on the BP over the last 400 years. Scenario 5 combined the reduced migration induced by human activities with the recent population crash (Fig. 4.2). For comparison purposes, we also assessed the number of generations required for our initial populations to lose their initial genetic diversity and reach equilibrium. To do so, we ran the simulation corresponding to the null hypothesis of scenario 1 (see below) for 20,000 generations.

-Historical migration

We ran scenarios 1 and 2 in EASYPOP (Balloux 2001) with 2 simulated populations corresponding to black bears found on the BP and in SE. SE sampling sites surrounding the BP were modeled as one panmictic population based on genotypic data that showed that those sites grouped as one genetic cluster (Pelletier et al. 2012). We used 3 alternative initial population sizes for each simulated population based on black bear density levels from eastern Ontario (0.2 bear/km^2; 0.4 bear/km^2; 0.6 bear/km^2 - Yodzis and Kolenosky 1986; for revised density estimates, see Howe et al. *in press*), as a starting point for each scenario. BP surface area is 1,100 km^2, with alternative population sizes at N_{BP} = 220, 440, and 660 (Howe et al. 2007). For SE, we assumed an undisturbed, still fully forested landscape with a surface of bear habitat of 11,000 km^2, and obtained N_{SE} = 2,200, 4,400, and 6,600 individuals. Both populations included equal sex ratios based on data previously obtained in this region (Kolenosky 1990; Howe et al. 2007).

For **scenario 1**, we used 3 alternative migration processes between BP and SE:

Null hypothesis (50% of male migration; 50% of female migration). Here, we assumed that male migration was constrained by the amount of female migration. Males were not expected to disperse into new habitat where females are absent, because movement can be influenced by female availability (Klatt and Ritchison 1994; Alberts and Altmann 1995).

Saturation hypothesis (90% of male migration; 50% of female migration). Here, we assumed a high migration rate for both males and females. In brown bears, it has been shown that a surface of suitable habitat as large as 24,000km^2 can become fully occupied

within a time span of 25 to 104 years (Pyare et al. 2004). The dispersal abilities of black bears (Rogers 1987; Lee and Vaughan 2003) could also lead them to colonize free habitat within a short timespan. We presumed that as free habitat was being colonized by black bears, latecomers were arriving in areas of high densities, leading to high dispersal rates even for females, who could have used this strategy to avoid competition for resources (Clutton-Brock and Lukas 2012). Although postglaciation migration rates for males and females are unknown, we assumed that the proportion of dispersing females was much lower than for males due to the strong pattern of male-biased dispersal observed in mammalian species (Greenwood 1980; Lee and Vaughan 2003; Costello et al. 2008; Costello 2010).

Female philopatry hypothesis (90% of male migration; 3% of female migration). Here, we assumed a situation close to what is currently observed in other black bear populations, with high male dispersal and low female dispersal (Rogers 1987; Schwartz and Franzmann 1992; Lee and Vaughan 2003; Costello 2010; Pelletier et al. 2011).

For **scenario 2a,** we used various low parameters of migration to model genetic drift:

Reduced movement hypothesis (10% of male migration and 3% of female migration). We assumed a reduced migration rate for males, but still much higher than for females. Indeed, studies of black bears in New Mexico and Québec suggest that males can show reduced levels of migration under various density conditions, although females remain mainly philopatric (Costello et al. 2008; Roy et al. 2012).

Extremely reduced movement hypothesis (3% of male migration and 3% of female migration). We assumed that male migration was as low as female migration. Although such a low level of male migration has not been reported in American black bear, it has been shown that when population density is well below carrying capacity, some males can stay within a 6 km distance of full siblings, parents, or offsprings. A possible reason for this is that the low competition level might drive subadults to establish a home range near their natal site, rather than dispersing (Costello et al. 2008).

No migration hypothesis. We assumed that both males and females remained in their area of origin. This modeled a system driven solely by genetic drift, and provided reference diversity measures for complete isolation.

Under **scenario 2b**, we further explored the parameter space of historically reduced migration around the one-migrant rule (Wright 1931; Mills and Allendorf 1996) with:

0.1 migrant per generation hypothesis (male migration only). This rate has been suggested for populations of fishers located at 2 extremities of a peninsula (Wisely et al. 2004), although it resulted in a higher level of differentiation than currently observed between BP and SE (Pelletier et al. 2012).

0.2 migrant per generation hypothesis (male migration only).

0.5 migrant per generation hypothesis (male migration only).

1 migrant per generation hypothesis (male migration only).

2 migrants per generation hypothesis (male migration only).

10 migrants per generation hypothesis (male migration only).

We set our genetic parameters based on previous microsatellite data from Pelletier et al. (2012), which included genetic analyses of Ontario black bears over 15 microsatellite loci. As one locus (G10P) was not in HWE, it was excluded from our analyses. The number of alleles per locus in this dataset varied from 10 to 27 (mean = 14.43). Based on this, we ran our simulations with 14 loci and 15 allelic states. We used free recombination between loci, a mutation rate (μ) of $2*10^{-4}$ to fall within the mutation rate of mammals for these types of loci (Ellegren 1995; Crawford and Cuthbertson 1996), a single-step mutation model, and we set our initial population with maximum genetic variability to perform our simulations. To run our model, our simulated panmictic population was required to have an initial genetic diversity similar to the one empirically observed in the SE genetic cluster (Pelletier et al. 2012). We used the results from the null hypothesis of scenario 1 to assess at which point in time the population reached a similar level of genetic variation (800 generations). Based on this, each EASYPOP simulation was run for an additional 800 generations.

We estimated bear generation time intervals from demographic data recorded in a protected forested area located in our southeastern genetic cluster (Algonquin Park – Obbard et al. *unpublished data*). Generation time was calculated as the sum of the earliest reproduction of a female (5 years), and age of latest reproduction of a female (15 years) divided by 2, giving an estimate of 10 years. We simulated 10 replicates of scenarios 1 and 2 under the 3 alternative density levels for 1,200 and 400 generations, for a total of 72 situations (12 migration situations * 3 population densities * 2 timescales). The alternative number of generations enabled us to model bear colonization from the time at which vegetation came back across the Great Lakes (12,000 years ago – Adams and

Faure 1997), and from the time boreal vegetation was present in Northern Canada (4,000 years ago – Adams and Faure 1997).

As our goal was to simulate one population that differentiates itself over time due to various migration and demographic factors, we needed to ensure that our 2 model populations represented one panmictic unit at the beginning of each simulation. To do so, we used STRUCTURE 2.3 (Pritchard et al. 2000; Falush et al. 2003) to assess the level of structure between our 2 initial populations. For each of our simulations, we randomly picked 3 EASYPOP outputs (out of 10) and evaluated the genetic structure between the individuals at the first generation. STRUCTURE groups individuals into specific genetic clusters based on the membership proportions (q) of each individual genotype to each inferred cluster. The algorithm accounts for admixture in individuals (one genotype can originate from multiple clusters). We used the F-model that assumes admixture with correlated allele frequencies (Falush et al. 2003), and ran STRUCTURE 5 times at K_{max} = 1–5, with 100,000 burn-ins and 500,000 Markov Chain Monte-Carlo iterations. We estimated the optimal number of clusters K through StructureHarvester (Earl and vonHoldt 2012), which implements the Evanno method (Evanno et al. 2005).

We also conducted a sensitivity analysis to evaluate how our various migration, density and timescale parameters affected the genetic structuring pattern between individuals. For each of our 72 situations, we picked the 3 EASYPOP outputs (out of 10) that had the lowest, highest, and medium level of observed heterozygosity at the last generation. For each of those 3 outputs, we randomly picked 100 males and 100 females from Population 1 (simulated BP) and 100 males and 100 females from Population 2

(simulated SE). We then evaluated the genetic structure between the individuals of the last simulated generation from Population 1 and 2 in STRUCTURE.

-Recent demographic bottleneck

BottleSim (Kuo and Janzen 2003) is a program that simulates population bottlenecks by implementing an overlapping generation model in which the user can vary the population size over time. In BottleSim, we used our empirical data from the SE sites surrounding the BP as our genotypic input file. This allowed the program to set the initial parameters of genetic diversity based on our previous study (Pelletier et al. 2012) to perform the analyses for **scenario 3**.

In 1903 and 1908, large fires occurred on the BP (Suffling et al. 1995). These fires are considered to have resulted in high direct and indirect mortality for BP black bears, however, they did not affect the same areas of the BP in both years (Suffling et al. 1995). Based on the amount of bear habitat damaged by each fire (Suffling et al. 1995), we conducted 10 iterations of scenario 3 under alternative rates of population declines and recovery. We used 2 sudden drops in population size 5 years apart to reflect the occurrence of each fire. For the first drop, representing the smaller of the two fires, we used 3 alternative mortality proportions (10%, 20%, and 40%). For the second drop, which represented a larger fire, we used 90% and 70% mortality based on the population size estimated 5 years following the first fire. These alternative mortality proportions allowed us to obtain results under both a conservative and a more drastic situation. We modeled population recovery through 2 alternative growth rates (a 10% growth rate, which corresponds to highly productive black bear populations in the Great Lakes St.

Lawrence region, and a 5% growth rate, which corresponds to regulated, hunted populations (Howe *pers. comm*.; Kolenosky 1990).

Based on an age distribution estimated from survival and reproductive information for black bears in Ontario (longevity: 20 years; age of reproductive maturation: 5 years; frequency of production of litters: once every 2 years - Yodzis and Kolenosky 1986; Kolenosky 1990; Obbard and Howe 2008), as well as reproductive data from the BP (Obbard et al. *unpublished data*), we used a 75% of generation overlap (75% of individuals were assigned a random age value, while all the other 25% had their age set to zero in year 0). We used a dioecious reproductive system (males and females were differentiated) with random mating. We ran our simulations over a period of 120 years, and induced the first drop in population size at year 13, and the second at year 18 to correspond to the 1903 and 1908 fires. For comparison purposes, we also set null conditions, with a constant population size over the same period of time. As the recovery rate was not affected by the initial population size (N_{BP} = 220, 440, or 660) due to our use of a constant growth rate following the population decline (Figure not shown), we reported results for a starting population size of 220 individuals.

-Reduced migration due to anthropogenic influences on the landscape

Scenario 4 corresponded to a low genetic diversity due to highly reduced migration between BP and SE as a consequence of human influences such as agriculture, industrial practices, and urban development over the last 400 years (Butzer 1992; Muller and Middleton 1994; Elliott 1998). In EASYPOP, we used the same population and

genetic parameters as for scenarios 1 and 2, and simulated the following alternative migration rates between BP and SE:

0.1 migrant per generation hypothesis (male migration only).

0.2 migrant per generation hypothesis (male migration only).

0.5 migrant per generation hypothesis (male migration only). This level of migration is not considered high enough to maintain genetic diversity between 2 populations (Wright 1931). We are assuming that BP black bears were part of the large continuum of Ontario populations, and as such, they were historically as genetically diverse as the surrounding SE populations. Under these circumstances, such a low level of gene flow could explain the difference observed between BP and SE individuals (H_E = 0.55 and 0.81, respectively; allelic richness = 4 and 13.4, respectively).

1 migrant per generation hypothesis (male migration only). This level of migration has been identified as the minimum at which negative effects of drift can be avoided (Wright 1931). However, it has been shown that allelic diversity as low as 2.25, and H_E as low as 0.27, can occur for populations that have this level of migration, and which have only been isolated for a short period of time (Dixon et al. 2007).

2 migrants per generation hypothesis (male migration only). This level of migration corresponds to what has been observed between BP and SE in a previous study (F_{ST} = 0.129; Nm = 1.89 - Pelletier et al. 2012).

10 migrants per generation hypothesis (male migration only). This level of migration has been suggested to maintain genetic variation between populations, even when the island model of migration is not fully respected (Mills and Allendorf 1996). Still, this number represents a lower amount of migrants than what is observed between

the Southeast and Northwest sites, where no landscape barrier to movement exists (F_{ST} = 0.013; Nm = 18.5 - Pelletier et al. 2012), and as such corresponds to reduced migration relative to the historical context.

No migration hypothesis. We assumed that both males and females remained in their area of origin due to a complete barrier to movement induced by human activities between BP and SE.

Ten replicates of each hypothesis were ran under the 3 alternative density levels for 40 generations, for a total of 21 situations (7 migration situations * 3 population densities * 1 timescale). As in scenarios 1 and 2, we also ran STRUCTURE at the first and last generations of the simulations for scenario 4.

-Reduced migration due to anthropogenic influences on the landscape and demographic bottleneck

We used the EASYPOP outputs from the smallest population of scenario 4 as starting points to perform the simulations corresponding to **scenario 5** in BottleSim. This allowed BottleSim to start with an initial genetic diversity that represented a low diversity resulting from recently reduced migration between BP and SE. We used the same BottleSim parameters as for scenario 3 to model the population decline and recovery.

Effective population size

We used the heterozygosity-based method to obtain an estimation of long-term effective population size (Ne_L) on the BP. As this method is highly sensitive to the mutation process, we calculated Ne_L for our BP samples (n = 139) under both the Infinite Allele Model (IAM, where each new mutation creates a new allele), and the Stepwise Mutation Model (SMM, where the number of alleles can stay constant, increase, or decrease at each new mutation). Under the IAM, the relationship between heterozygosity and Ne_L can be calculated as: $H_E = 4Ne_L\ \mu/(1+4Ne_L\mu)$, where μ represents the mutation rate; as such, $Ne_L = H_E/4\mu(1 - H_E)$ (Crow and Kimura 1970). Under the SMM, Ne_L can be calculated as: $H_E = 1-(1+8Ne_L\mu)^{-1/2}$; as such, $Ne_L = [1/(1 -H_E)^2 - 1]/8\mu$ (Ohta and Kimura 1973).

We estimated contemporary Ne (Ne_C) based on a single temporal sample. We performed the linkage disequilibrium method implemented in the program LDNe 1.31 (Waples and Do 2008). This method allows to calculate the effective number of breeding adults based on one temporal sample based on Burrow's composite measure of disequilibrium (D*) (Campton 1987). An estimation of the mean squared correlation of allele frequencies ($r^{\wedge 2}$) is estimated for each pair of loci, and this measure is then used to calculate Ne_C (Hill 1981). It is assumed that small populations accumulate more disequilibrium than large populations over time (Hill 1981; Bartley et al. 1992). This method has the advantages of not underestimating Ne_C when samples are separated by only one generation, as opposed to temporal methods (Waples 1989; Tallmon et al. 2004), and of being robust to reduction in population size (Waples 2005). In addition, the correction factor used in LDNe (Waples 2006) allows for an elimination of bias that

exists when sample size is lower than the true Ne_C (England et al. 2006). Another benefit of the linkage disequilibrium method is that under our set of circumstances (number of samples available = 139; 220 < population size < 660 – Howe et al. 2007, and number of loci = 14) the assessment of Ne_C is precise, as its variance is low when the sample size is small and the number of loci high (Waples and Do 2010).

For comparison purposes, we also calculated Ne_C over one black bear generation (10 years) through 2 temporal methods implemented in NeEstimator (Peel et al. 2004). Individuals identified before 1999 were assigned to generation zero, while individuals identified since 2009 were assigned to generation 1. The first method, a moment based approach, estimates Ne_C based on changes in allele frequencies across generations (Waples 1989). The second method is a Bayesian approach based on coalescence, implemented in TM3 (Berthier et al. 2002).

Bottleneck analysis based on allelic data

The genetic signature of a recent bottleneck can be identified through heterozygosity excesses at polymorphic loci (Cornuet and Luikart 1996), or through a decrease in the total number of alleles relative to the range in allele size (M-ratio; Garza and Williamson 2001). We used both methods to assess the presence of a bottleneck in BP black bears. First, we used the program Bottleneck (Piry et al. 1999) to compare observed heterozygosity of our BP samples (H_E) with heterozygosity expected at mutation-drift equilibrium (H_{EQ}). This method is based on the theory that a bottleneck induces a reduction in allelic diversity at a faster rate than the reduction in heterozygosity. Thus, in the case of a recent reduction in effective population size, H_E should be

significantly larger than H_{EQ} (evidence of heterozygosity excess). We ran the program under the IAM, the SMM, and the Two-Phase Model (TPM, for which there is a certain proportion of both single-step and multi-step mutations, which is a more realistic model than the IAM and the SMM - Di Rienzo et al. 1994). For the TPM, we used a range of proportion of multi-step mutations (p_g) from 10% to 90%, in 20% increments (the recommended value for p_g is 10% in Piry et al. 1999, and 22% in Peery et al. 2012), although we can note that for microsatellite data, the SSM or TPM with p_g = 90% are usually more appropriate (Piry et al. 1999). The mean size of multi-step mutations (δ_g) was set at 3.1, leading to a variance among multi-step mutations (σ^2_g) equal to 12 (see Williamson-Natesan 2005 for the calculation of σ^2_g). We also ran Bottleneck with δ_g = 3.5, leading to an σ^2_g of 16, as recommended in Peery et al. (2012). As both our number of loci (l = 14) and number of individuals (n = 139) were high, we ran the program for 10,000 iterations to obtain precise estimates of H_{EQ}. The comparison between H_E and H_{EQ} was conducted with the Wilcoxon signed rank test, as it is both powerful and highly robust when fewer than 20 loci are available (Piry et al. 1999).

Second, we calculated the observed M-ratio (M) for our empirical BP data through M_P_Val, and determined its significance by comparing it to a critical value (Mc), calculated from hypothetical populations in mutation-drift equilibrium through Critical_M (Garza and Williamson 2001). The M-ratio evaluates the presence of a bottleneck by contrasting the number of possible allelic states at each locus to the number of allelic states actually present at each locus. Due to genetic drift inducing a loss of rare alleles, a bottlenecked population should have missing allelic states, whereas a population that did not experience a bottleneck should have all the possible allelic states present. In

this context, the M-ratio is calculated as M = k/r, where k is the number of alleles, and r is the range in allele size measured in repeat units. During a bottleneck, k decreases faster than r due to the loss of rare alleles, and thus, a bottleneck is identified when the observed M-ratio is lower than its critical value (Mc). The observed M-ratio is expected to be higher than 0.8 in populations that have not suffered a bottleneck, and is expected to be lower than 0.7 for populations that experienced a reduction in size (Garza and Williamson 2001).

Mc was first calculated for each possible BP population size (N_{BP} = 220, 440, and 660) suggested by Howe et al. (2007). As the sex-ratio in eastern Ontario black bears is not significantly different from 1:1 (Kolenosky 1990), N_{BP} was considered the effective population size. Based on a mutation rate of $2*10^{-4}$, our 3 pre-bottleneck θ were calculated as: $\theta = 4 \times 2*10^{-4} \times N_{BP}$. Thus, $\theta_{220} = 0.176$, $\theta_{440} = 0.352$, and $\theta_{660} = 0.528$. We parameterized a conservative mutation model with $\delta_g = 3.1$, and $p_g = 10\%$ (Garza and Williamson 2001), and we also used $\delta_g = 3.5$ and $p_g = 20\%$ (Peery et al. 2012). Using the same mutation parameters, we also calculated Mc based on the effective population size obtained through the linkage disequilibrium method, as well as both temporal methods (Ne_C).

Future of the BP population – effect of population size and translocations

To determine if translocations of SE individuals into the BP could positively affect genetic diversity over the next generations, we used the information provided by our BP samples to set our initial genetic parameters in BottleSim. We ran a simulation at a constant population size for 500 years based on the same population parameters as

scenario 3 (recent bottleneck). Then, we assessed the effect of translocations of SE individuals into the BP on the evolution of genetic diversity parameters (Na, Na_e, H_O, and H_E). Here, we replaced genotypes of BP individuals incrementally (n = 1; 5; 10; 20; 50; 100) by genotypes from SE individuals to set our parameters of initial diversity. We then ran the program with a constant population size for 500 years. Finally, we conducted the same simulations to determine the effect of both translocations and increase in population size (10 individuals every 50 years) on the parameters of genetic diversity over the next 500 years.

Results

It took approximately 10,000 generations for the 2 initial simulated populations representing BP and SE to lose their maximal level of genetic diversity and reach equilibrium under a situation of complete genetic mixing. Mean equilibrium values for Na and H_O were 4.456 (SD = 0.35), and 0.517 (SD = 0.02), respectively. Under the same set of conditions, it took 800 generations to reach a heterozygosity level of 0.8 (SD = 0.02) and a mean allelic diversity of 11.156 (SD = 0.32), which is comparable to what is observed with microsatellites in most contiguous, stable mammalian populations, and similar to what is currently observed in the southeastern portion of Ontario (Pelletier et al. 2012).

Forward time simulations

-Historical migration

For scenarios 1 (high migration) and 2a (reduced migration), STRUCTURE results from our simulated datasets did not show the presence of clear genetic clusters delineating BP from SE, except in the case of complete absence of migration, for which K = 2 across all densities and generation times. For the remaining scenarios, K did not plateau consistently across the 3 EASYPOP outputs that had the same parameter set, and ΔK values were both low and close to each other from K = 1-5. For each of these simulations, however, all individuals had an equal probability ($1/K$) of being assigned to each potential cluster, suggesting an absence of differentiation between the 2 populations after 1,200 and 400 generations. Except in the absence of migration, for which lower levels of diversity were detected compared to what was observed on the BP, all situations showed higher levels of diversity than our empirical data (Appendix; histograms not shown). As such, simulation results were refuted by our empirical data.

Under scenario 2b (one-migrant rule), STRUCTURE identified 2 clusters for all densities and generation times. All individuals, except for the initial proportion of migrants determined for each simulation, assigned to their original cluster for a minimum cluster membership level of 75%. Diversity measures that were not significantly different from what was observed on the BP were found between 0.5 migrants per generation over 400 generations, and 1 to 2 migrants per generation over 1,200 generations. It is only within this range of values that the simulation results were supported by our empirical data. At 0.2 migrants per generation, diversity measures were lower than what was observed on the BP, and they were higher at 10 migrants per generation (Appendix)**.**

-Recent bottleneck (demographic data)

Results from the scenario 3 simulations showed higher levels of diversity than our empirical BP data in all situations (Appendix), refuting this hypothesis as the sole factor causing low diversity in BP black bears.

-Recently reduced migration

Under scenario 4 (one-migrant rule), STRUCTURE identified 2 main clusters for all densities and migration rates. The highest proportion of unassigned individuals, (2% for a cluster membership level of 70%) was reached for 10 migrants per generation. For all situations, all individuals (except for the initial proportion of migrants determined at the beginning) were assigned to their original cluster at the end of the simulations, for a minimum cluster membership level of 75%. Although the alternative density levels did not change the results on the number of clusters, ΔK decreased as population size increased, and was lowest for 10 migrants per generation and 0.6 bears per km^2 (ΔK = 228.27 at K = 2). At 0.5 migrant per generation, diversity measures of the simulated isolated population were similar to what was empirically observed on the BP. It is only for this value that the simulation results were supported by our empirical data, as simulated diversity measures were lower at 0.2 migrant per generation, and higher at 2 migrants per generation (Appendix).

-Recently reduced migration and demographic bottleneck

The situations of scenario 5 (combination of scenarios 3 and 4) that were supported by our empirical BP data corresponded to a combination of 1 migrant per generation over 40 generations, and all bottleneck scenarios for which the second mortality drop was at 70%. For this level of migration, situations including a second drop at 90% also approached empirical data from the BP, although the observed number of alleles of the simulated BP was always lower than the empirical data. Another situation that was supported corresponded to 2 migrants per generation, a first mortality drop at 10% followed by a second one at 90%, and a growth rate of 10%. For this level of migration, other situations involving a second drop at 90% resulted in diversity levels similar to the ones seen on the BP for 3 measures out of 4 (Appendix).

Effective population size

Based on a mutation rate of $2*10^{-4}$, Ne_L for the BP population ranged from 1,527 under the IAM, to 2,461 under the SMM. As described in Waples and Do (2010), our estimate of Ne_C based on the linkage disequilibrium method with jackknifing was biased upward when P-crit allowed the inclusion of singletons. Thus, to balance the tradeoff existing between precision and bias in this method, we selected an estimate of Ne_C where the lowest allele frequencies included were equal or above 0.02. As such, Ne_C ranged from 12.6 to 18.5 (Table 4.1), which is much lower than the estimated population size on the BP (N_{BP} = 220, 440, and 660 - Howe et al. 2007).

Moment based approaches based on 2 temporal samples resulted in a higher Ne_C than obtained through a single temporal sample (Ne_C = 56.2; CI = [44.6-69.3]). Results from the Bayesian coalescence approach, on the other hand, were much closer to what was obtained through the linkage disequilibrium method (Ne_C = 22.8; CI = [22.5-23.0]).

Recent bottleneck (allelic data)

Bottleneck analyses for BP black bears did not detect the presence of a significant heterozygosity excess under the SMM nor the TPM up to a 70% proportion of multi-step mutations. From 50% of multi-step mutations under the TPM, to the IAM, heterozygosity excess was significant (Table 4.2). Finally, allele frequency distributions did not illustrate the presence of a mode shift, which can illustrate a bottleneck. In contrast, our M-ratio test detected a bottleneck in BP black bears. The observed M was 0.699, which was always lower than Mc obtained from various values of θ, δ_g, and p_g (Table 4.3), suggesting a reduced population size.

Future of the BP population – effect of population size and translocations

Translocations improved the initial diversity of the BP population, which increased with the number of SE individuals brought in (Fig. 4.3). However, for these translocations to help BP black bears reach a level of genetic diversity similar to SE, about 100 individuals from SE would have to be introduced into BP (Fig. 4.3).

With 5 to 10 individuals translocated, the increase in diversity was more apparent for the number of alleles than for the heterozygosity (Fig. 4.3), although this diversity was lost quickly, even when translocations were combined with an increase in population size (Fig. 4.3). Translocating 20 SE individuals, on the other hand, allowed for genetic diversity to be retained much longer despite geographic isolation (Fig. 4.3). Without translocations, the BP population was able to retain 86% of its allelic diversity and 97% of its initial heterozygosity over a 100-year period, and 64% of its allelic diversity and 81% of its initial heterozygosity over a 500-year period (Fig. 4.3).

Discussion

Wide-ranging large carnivores can be strongly influenced by lack of connectivity and habitat loss (Fahrig 2003; Wiegand et al. 2005). In some cases, geographic and genetic isolation may lead to loss of fitness (Roelke et al. 1993; Hedrick and Fedrickson 2010), although for some populations, survival is not negatively affected despite highly reduced genetic diversity (Paetkau et al. 1998b). To assess if management actions should be implemented to restore genetic diversity in populations that show reduced variability, identifying the processes that led to such a pattern is advisable, as some situations would induce conservation concerns, whereas others would not. In this study, we used forward simulations to determine if non-natural influences could have shaped the genetic differences observed between an isolated Ontario black bear population with reduced genetic diversity (BP), and the highly diverse neighboring core population that represents a historical, less disturbed state (SE). We suggest that although the genetic diversity of BP black bears is not threatened on the long-term, the high level of relatedness among BP

individuals (Pelletier et al. 2012), and the small size of the population, which is under increased habitat pressures (Howe et al. 2007), warrant conservation concern. What is currently observed in BP black bears may represent a possible future for localized subsets of the genetically diverse core population, especially if anticipated habitat fragmentation and increased mortality occur due to human development (Cardillo et al. 2006 and 2008).

Reasons for the low diversity on the BP

Genetic diversity can be influenced by biogeographic processes, such as isolation in glacial refugia (Waits et al. 1998), and by factors that occur on a more contemporary timescale, such as overharvesting (Allendorf et al. 2008), or landscape fragmentation (Fahrig 2003; Keyghobadi 2007). In several large carnivore species, both historical and contemporary reduced gene flow have been shown to lead to high levels of differentiation between populations, and low genetic diversity within isolated fragments (Paetkau and Strobeck 1996; Cegelski et al. 2003). In our study, historical migration rates modeled in scenarios 1 (historically high migration) and 2a (historically reduced migration) were too high to lead to the genetic differentiation observed between BP and SE. Simulations from scenario 2b (one-migrant rule), however, identified a small range of historical reduced migration levels that resulted in diversity measures similar to the ones found on the BP (0.5 migrant per generation over 400 generations to 2 migrants per generation over 1,200 generations). Simulations that modeled contemporary reduced migration (scenario 4 – one-migrant rule) between BP and SE identified only one level (0.5 migrant per generation) that could explain the reduced diversity of BP black bears (Appendix). Low diversity caused by recent anthropogenic influences has also been suggested in Florida

black bears, for which reduced migration was most likely followed by inbreeding (Dixon et al. 2007). Inbreeding may also represent a risk for BP black bears, as they display a high level of relatedness (Pelletier et al. 2012), and have a small population size (Howe et al. 2007).

In addition to reduced gene flow, rapid declines in population size can also have detrimental effects on populations' genetic diversity and fitness, which has been shown in species such as greater prairie chickens (Bouzat et al. 1998) and Florida panthers (Culver et al. 2008). In the case of BP black bears, the genetic signal of reduced diversity (Pelletier et al. 2011 and 2012) is unlikely to have been solely caused by a recent bottleneck, as none of the simulations from scenario 3 resulted in a diversity similar to the level currently observed. Results from scenario 5 (combination of one-migrant rule and recent bottleneck), however, suggest that several combinations of recently reduced migration followed by 2 sudden drops in population size at 5 years apart could explain the observed pattern (Appendix). The plausibility of a bottleneck in BP black bears was also supported by the M-ratio test, although this signal was not detected by heterozygosity excess tests.

The contrasting results obtained by bottleneck tests based on genetic data are similar to what has been found in other studies, with the M-ratio test performing better than the heterozygosity excess test (McEachern et al. 2011; Sastre et al. 2011). In addition, the fact that the allelic diversity was reduced in BP black bears, whereas the heterozygosity did not show the same trend, suggests a higher sensitivity of allelic richness to bottlenecks (Nei et al. 1975). As a result of the differential sensitivity of these 2 diversity measures, recently bottlenecked populations can show high heterozygosity,

while displaying low allelic richness (Nei et al. 1975). Finally, it is possible that the M-ratio test performed better due to the demographic bottleneck lasting for several generations (Williamson-Natesan 2005), before the BP population recovered its initial size, as observed in our simulations. Indeed, as opposed to the M-ratio test, the heterozygosity excess test is only able to detect a bottleneck a short time after it occurred (Cornuet and Luikart 1996; Peery et al. 2012).

Among all the alternative scenarios that were supported by our simulations, the hypothesis of a combination of recently reduced migration and demographic bottleneck caused by successive fires (scenario 5) seemed the most plausible to explain the reduced diversity of the BP population. As shown by our rejection of scenarios 1 and 2a, the BP population could not have reached its current level of diversity through genetic drift if historical migration was high. Indeed, under complete genetic mixing (null hypothesis under scenario 1), 10,000 generations would be required to drop from the high, initial level of polymorphism to the one currently observed on the BP, a timeline that is not in accordance with the recolonization of North America by wildlife after the last glaciations (only 4,000 years ago; Adam and Faure 1997). If migration was highly reduced, on the other hand, rates from 0.5 migrant per generation over 400 generations to 2 migrants per generation over 1,200 generations could explain the genetic diversity observed on the BP. This represents a reasonable range, as a lower migration rate (e.g., 0.2 migrant per generation) over the same period of time would induce a lower diversity than observed on the BP (Appendix). Even though this range of highly reduced historical gene flow was supported by our simulations, it is unlikely that the migration rate of black bears was this low during the last 4,000 years. Considering the dispersal abilities of this species (males

can travel over distances up to 200 km – Lee and Vaughan 2003; Rogers 1987), we suggest that the impact of forest clearings resulting from croplands grown by first nations, located along the coast of Georgian Bay (Fig. 4.1), was not sufficient for the migration rate to reach such a low level, as the rest of the habitat was still forested (Day 1953; Munoz and Gajewski 2010). The contrasting levels of diversity between BP and SE may also be indicative of a source-sink relationship. Indeed, based on the estimation of the number of immigrants, Nm (Nm = [(1/F_{ST})-1]/4, where N is the local population size, and m the proportion of immigrants – Wright 1951), a higher number of migrants from SE (source) into BP (sink) is expected. However, human encroachment into bear habitat likely prevents such movements (Howe et al. 2007; Obbard et al. 2010a), and no migration event from SE into the BP was detected to confirm it. Finally, although landscape constriction has been shown to reduce gene flow and promote genetic differentiation in raccoons as a consequence of a spatial bottleneck (Rees et al. 2009), over such a long period of time, the ability of black bears to travel long distances would likely have mitigated a potential founder effect, and resulted in population homogeneization. This is further supported by the geographical proximity of BP black bears to other populations. Additional genetic data (allelic richness; heterozygosity; haplotypic diversity) from black bears inhabiting the islands located in the vicinity of the BP, along with models simulating colonization events of Ontario, could help confirm if the reduced diversity is a consequence of historical or contemporary processes. Such data could also determine if landscape configuration and home range selection dynamics could have influenced genetic diversity and genetic differentiation between BP and SE.

Future of the BP population – effect of constant population size and translocations

Our simulations suggest that for BP black bears to regain levels of genetic diversity similar to what is observed in southeastern Ontario, it would require supplementing the BP population with a high number of individuals from SE. Our models also suggest that BP black bears may have experienced recently reduced migration combined with bottlenecks, resulting in decreased levels of genetic variability, which could lead to lower evolutionary potential (Reed and Frankham 2003; Frankham 2005). Despite this, genetic diversity measures do not suggest that the BP population is in critical condition, as it could retain a high proportion of both its current allelic diversity and its current heterozygosity over the next 100 years without the help of translocations (Fig. 4.3). Nevertheless, the level of relatedness among individuals is high (Pelletier et al. 2012), and even at carrying capacity, the size of the BP population will likely remain small due to the reduced surface of bear habitat in this region (Howe et al. 2007). In this situation, if no gene flow were to occur between BP and SE individuals over a sufficient period of time, the probability for BP black bears to become inbred and lose more diversity would increase (Frankham 1995; Frankham 1997). Indeed, based on the relationship between the inbreeding coefficient and the effective population size, our Ne_C estimates indicate that inbreeding could increase from 1 to 3% per generation in BP black bears. Inbreeding can have deleterious effects on population survival (Frankham 1995), and in some cases, genetic rescue efforts have to be conducted to ensure population persistence (Pimm et al. 2006; Fredrickson et al. 2007). As such, we suggest that the BP population be closely monitored to detect this type of genetic signal, so that appropriate

management actions can be implemented before the deleterious consequences of low genetic variability and potential inbreeding take effect.

An action that would help avoid inbreeding would be to supplement the BP population with individuals from SE to increase genetic diversity. To achieve this, regular translocations of 5 to 10 SE individuals could be conducted, as this new diversity would be lost quickly if translocations were not maintained. Due to the cost of translocations, bringing 20 SE individuals into the BP at once could also be considered, as it would increase the population diversity to a significantly higher level (Fig. 4.3). If such actions were to be implemented, caution would have to be taken to avoid outbreeding (Weeks et al. 2011), although it is unlikely to occur as it is improbable that BP and SE individuals are locally adapted. In addition, the possible spread of diseases following translocations would also need to be considered (Cunningham 1996).

Translocation efforts have been successful in Greater prairie chickens (Bouzat et al. 2009) and Florida panthers (Pimm et al. 2006). For BP black bears, conducting regular supplementation actions would allow diversity to be retained on the long-term if the BP population was to remain geographically isolated. Thus, similarly to Bouzat et al. (2009), we suggest that only conducting translocations might not be the best method to enhance the genetic diversity of the BP population, if such enhancement was required. It would be more efficient to conserve or enhance natural corridors between BP and SE to allow the population to remain connected to the mainland over the long-term. The proposition of protecting bear habitat around the Peninsula, along with reducing non-natural mortality and harvest levels, was also brought forward in the demographic study conducted by Howe et al. (2007) as an efficient solution to maintain the long-term persistence of BP

black bears. Our genetic results suggest that allowing for the possibility of a continuous intake of migrants, spread over several years, to come into the BP, would allow the population to retain a higher level of diversity than if the population was to remain isolated. Indeed, under isolation, the diversity brought in by the new migrants would be lost after a short period of time (Fig. 4.3). Here, we show that the level of diversity retained at a constant population size without translocations is still acceptable even after 300 years under isolation. However, for conservation purposes, a safe option would be to prevent the diversity from decreasing further, and retain the diversity brought in by the individuals from SE. The continuous intake of migrants suggested above would help achieve this, and would also help prevent inbreeding.

Conclusion

Both genetics and demographic methods supported a bottleneck in combination with recently reduced migration to explain the reduced genetic diversity of BP black bears. Thus, our results provide additional information to the demographic data that suggested that BP black bears were of conservation concern (Howe et al. 2007). BP black bears have an intermediate level of polymorphism (lower than contiguous, but higher than insular populations - Paetkau and Strobeck 1994; Warrillow et al. 2001; Belant et al. 2005; Triant et al. 2004; Dixon et al. 2007; Onorato et al. 2007; Robinson et al. 2007; Pelletier et al. 2012), and a low probability of losing more genetic diversity over the next 100 years in the absence of catastrophic events such as the fires that occurred in this region in the early 1900s (Fig. 4.3). Despite this, a risk of inbreeding exists in this

population, based on relatedness and population size estimates (Pelletier et al. 2012; Howe et al. 2007).

Anthropogenic activities inducing habitat loss and fragmentation are increasing, and moving northward (Cardillo et al. 2006 and 2008; Growth Plan for Northern Ontario 2011). This, with the addition of climate change influencing resource availability, suggests that caution still has to be taken when implementing management strategies, as black bears located in the northern portion of the range could become more geographically isolated over the next 200 years. Such isolation could increase the probability of demographic bottlenecks, and in the event of landscape fragmentation, these isolated populations may experience higher mortality rates as a result of hunting and vehicular traffic. In addition, they could lose genetic diversity due to a lack of migration routes impeding gene flow. As such, we suggest that the future of black bears, based on both the genetic and ecological context, is fully linked with landscape management and the maintenance of corridors that would allow for population connectivity to be retained, a solution already put forward by Howe et al. (2007). From a genetic standpoint, conserving connectivity could mitigate the effects of reduced population size, and prevent inbreeding. Because of this, we recommend that large-scale landscape management be implemented as new infrastructures are being built in the northern fringe of the black bear range, so that large networks of forested land can be conserved before connectivity between black bear populations is lost. However, the economic and ecological contexts present on the Bruce Peninsula might not allow for such landscape management actions. Thus, supplementing the population with 20 SE individuals at once could be used as an alternative to boost the genetic diversity enough

so that it could be retained on an long enough period of time to shape a more long-term management plan.

Overall, our study allowed us to estimate the migration rates and timeframes necessary for populations to go from high to intermediate levels of diversity. In the current context of increased habitat loss and fragmentation, we provide an approach that can be applied to other vagile species to determine at which point concerns regarding the persistence of isolated populations should be raised. This type of research is integral to the maintenance of biodiversity, as it can inform long-term and large-scale management and conservation plans for populations that are becoming increasingly small and fragmented, and that may be at risk of inbreeding.

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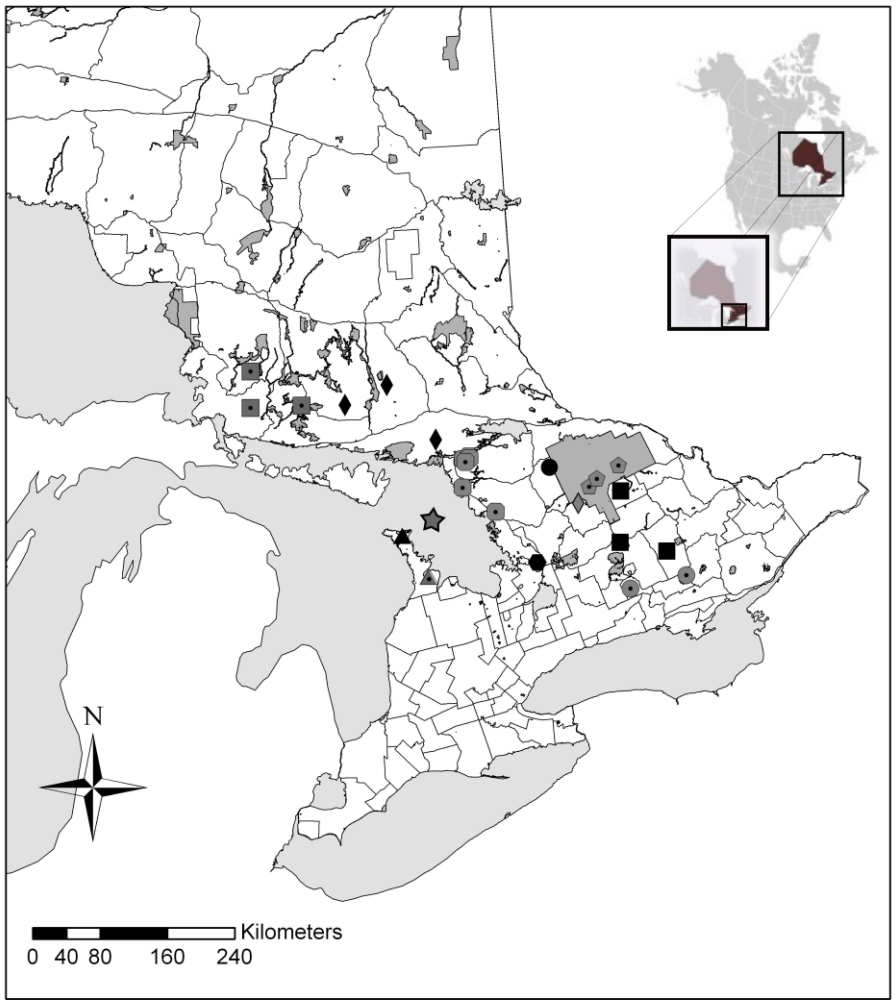


Fig. 4.1. Map of southeastern Ontario sites at which American black bears (*Ursus americanus*) hair samples were collected. Triangles represent the Bruce Peninsula sites (BP), while the rest of the sites belong to the southeastern population continuum (SE). The star indicates Georgian Bay.

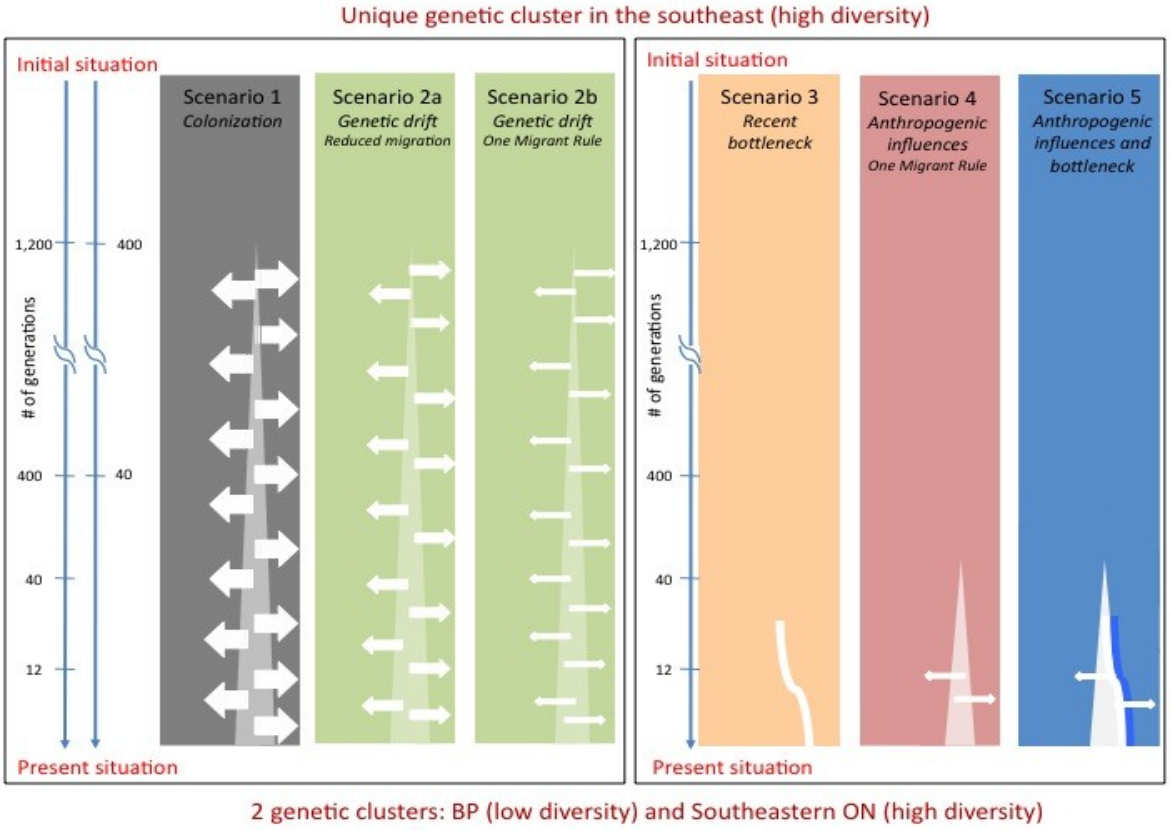
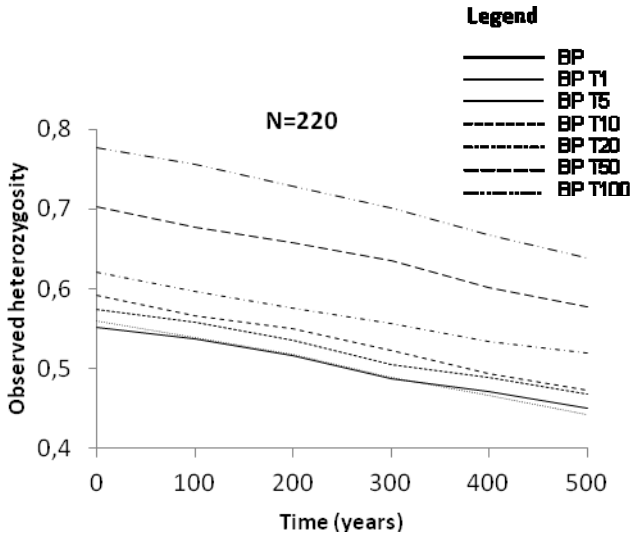
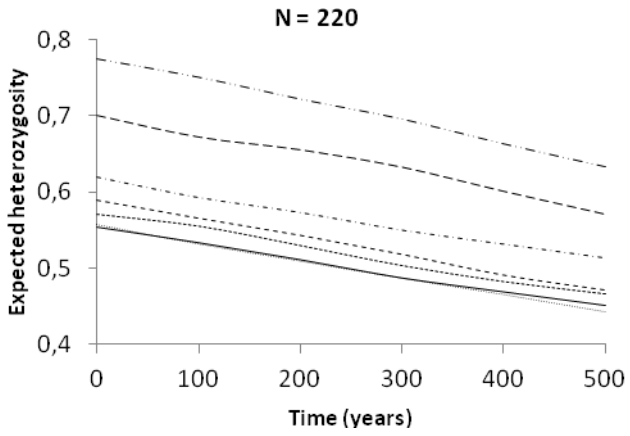
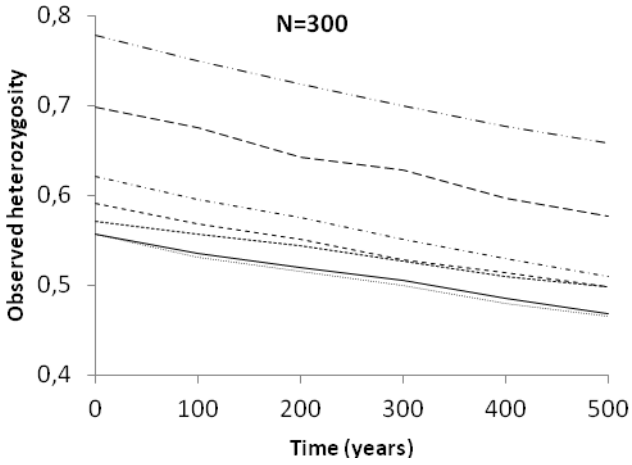
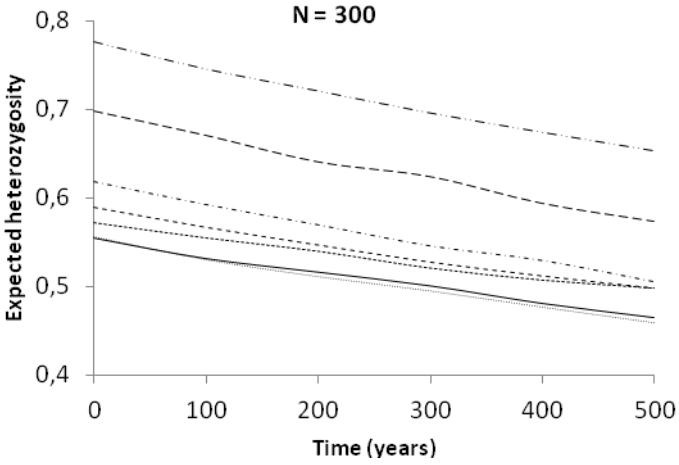


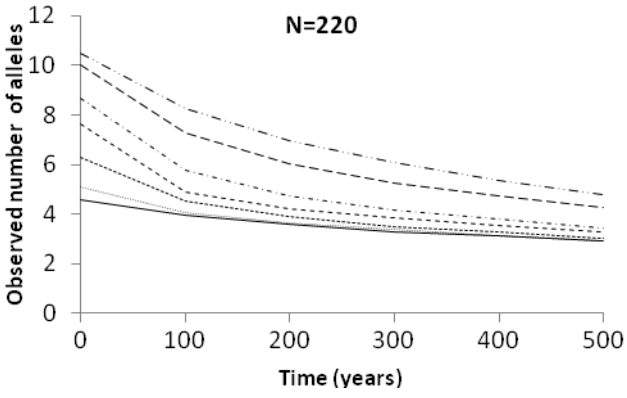
Fig. 4.2. Alternative scenarios tested to understand the reasons for the genetic differentiation and reduced genetic diversity of black bears located on the Bruce Peninsula (BP). Arrow width represents various levels of migration between sites from Southeastern Ontario and the BP. Two time scales are shown for scenarios 1, 2a, and 2b, as dual simulations were conducted for 1,200 and 400 generations for these scenarios.

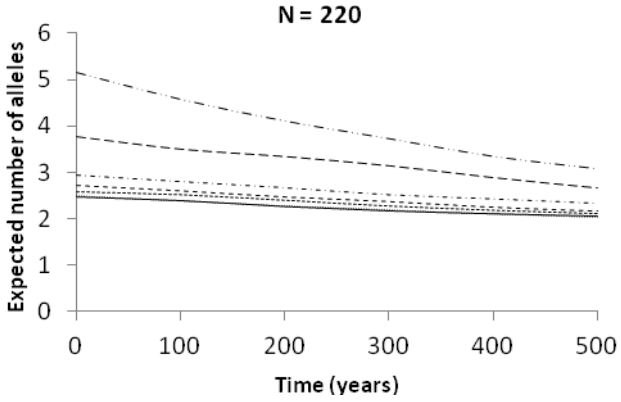

Legend
BP
BP T1
BP T5
BP T10
BP T20
BP T50
BP T100
N=220
Observed heterozygosity
Time (years)

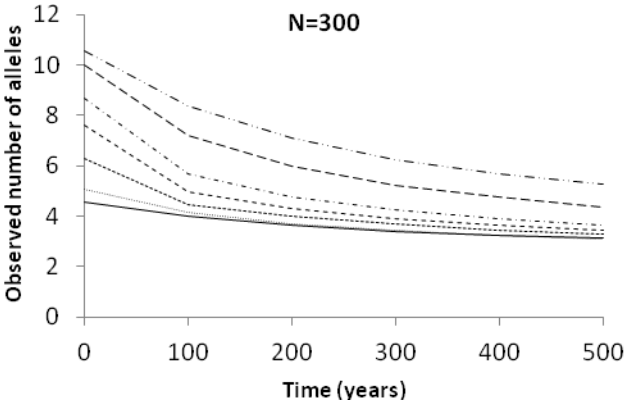

N = 220
Expected heterozygosity
Time (years)


N=300
Observed heterozygosity
Time (years)


N = 300
Expected heterozygosity
Time (years)


N=220
Observed number of alleles
Time (years)


N = 220
Expected number of alleles
Time (years)


N=300
Observed number of alleles
Time (years)

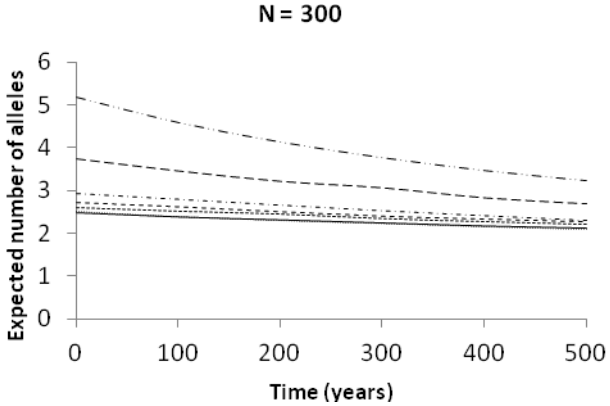

N = 300
Expected number of alleles
Time (years)

Fig. 4.3. Levels of genetic diversity (observed number of alleles, expected number of alleles, observed heterozygosity, and expected heterozygosity) over a 500-year period under varying translocation scenarios simulated in BottleSim. N = 220 represents a constant population size over the time period at the beginning of which translocations are conducted, and N = 300 represents a population for which both translocations and an increase in population size occur (increments of 10 individuals every 50 years). T1 to T100 correspond to the number of SE individuals translocated into the BP at year 0 of the simulations. In these simulations, translocated individuals are replacing the corresponding number of BP individuals.

Table 4.1. Estimation of contemporary effective population size (Ne_C) of BP and SE, at various sample sizes (n) for BP. Analyses were based on the linkage disequilibrium method implemented in the program LDNe 1.31 (Waples and Do 2008). P-crit represents the minimum level of allele frequency included in the analysis.

P-crit	*Harmonic Mean Sample Size*	Independent Comparisons	Overall r^2	Expected r^2	Estimated Ne_C^	95% CIs for Ne_C^	
						Parametric*	JackKnife on Loci*
BP							
n=139							
0.05	*134.7*	738	0.0264	0.0076	15.4	[12.9-18.1]	[12.6-18.5]
0.02	*135*	1060	0.0264	0.0076	15.3	[13.2-17.6]	[12.6-18.4]
0.01	*135.1*	1152	0.0238	0.0076	18.3	[15.9-20.9]	[15.2-21.8]
n=20							
0.05	*19.3*	772	0.0740	0.0606	21.1	[12.4-44.9]	[11.4-54.6]
0.02	*19.4*	808	0.0742	0.0605	20.7	[12.4-42.8]	[11.9-47.1]
0.01	*19.4*	808	0.0742	0.0605	20.7	[12.4-42.8]	[11.9-47.1]
n=50							
0.05	*49*	804	0.0392	0.0217	16.8	[13.0-21.7]	[12.5-22.6]
0.02	*49.1*	970	0.0369	0.0217	19.6	[15.4-25.3]	[15.5-25.1]
0.01	*49.1*	970	0.0359	0.0217	21.1	[16.5-27.4]	[16.6-27.2]
n=100							
0.05	*97.7*	733	0.0269	0.0106	18	[14.8-21.9]	[14.5-22.3]
0.02	*97.9*	929	0.0277	0.0105	17.1	[14.4-20.2]	[14.0-20.7]

0.01	*98*	971	0.0261	0.0105	19	[16.0-22.6]	[15.9-22.7]
SE							
n=647							
0.05	*620.7*	3145	0.0022	0.0016	560.8	[470.2-682.6]	[461.9-698.1]
0.02	*618.8*	6960	0.0021	0.0016	722.1	[625.3-846.6]	[602.4-887.2]
0.01	*618.9*	8808	0.0021	0.0016	762.4	[666.9-883.3]	[645.0-920.7]

*The jacknife method uses a correction factor to avoid the overestimation of the number of independent comparisons. Overall, this method performs better than the parametric method (Waples and Do 2008).

Table 4.2. Results of bottleneck heterozygosity excess tests based on allelic data for our BP samples. Three mutation models were used (the IAM infinite allele model, the TPM two-phase model from 10% to 90% of single step mutations, and the SMM stepwise mutation model). The expected number of loci with heterozygosity excess, the number of loci with heterozygosity deficiency vs. excess, the probability that a bottleneck was detected (Wilcoxon test P-values for one-tailed probabilities for heterozygosity excess), and the presence or absence of a mode-shift are provided. P-values less than 0.05 are indicated in bold. For comparison purposes, 2 parameters were used for δ_g (the mean size of multi-step mutations), following the recommendation of Piry et al. (1999) and Peery et al. (2012).

	Expected number of loci with heterozygosity excess	# loci with heterozygosity deficiency vs. excess	Probability excess (Wilcoxon test)	Mode shift
$\delta_g = 3.1$ - $\sigma^2_g = 12$				
IAM	7.68	2:12	**0.003**	
TPM-10%	7.93	2:12	**0.015**	
TPM-30%	8	4:10	**0.034**	no
TPM-50%	8.11	5:9	**0.045**	

TPM-70%	8.22	5:9	0.097	
TPM-90%	8.25	6:8	0.548	
SMM	8.28	6:8	0.821	
$\delta_g = 3.5$ - $\sigma^2_g = 16$				
IAM	7.68	2:12	**0.003**	
TPM-10%	7.88	2:12	**0.015**	
TPM-30%	7.96	3:11	**0.021**	
TPM-50%	8.08	5:9	**0.045**	no
TPM-70%	8.2	5:9	0.086	
TPM-90%	8.24	6:8	0.524	
SMM	8.28	6:8	0.821	

Table 4.3. Results of M-ratio analyses based on effective population sizes calculated from i) demographic data (N_{BP} = 220, 440, and 660) and ii) genetic data based on single and multiple temporal samples (Ne_C = 15.4, 22.8, and 56.2), with a mutation rate of μ = 0.0002. Mc represents the value above which 95% of the observed M-ratio (M, the number of alleles divided by the range in allele size) should be found, while P is the proportion of replicates found below the observed M-ratio. Estimation of Mc is based on θ, calculated as θ = 4Neμ. For each of our effective population size estimates, 10,000 iterations were conducted, with δ_g = 3.1 and 3.5 (the mean size of multi-step mutations), and p_g = 0.1 and 0.2 (the proportion of multi-step mutations), as suggested in Piry et al. (1999) and Peery et al. (2012). The observed M-ratio for our BP dataset, averaged over 14 loci, was M = 0.699.

	Ne	# loci	θ	δ_g	p_g	Mc	P
Estimations based on bear densities	220	14	0.176	3.1	0.1	0.866	0.0000
					0.2	0.798	0.0012
				3.5	0.1	0.855	0.0000
					0.2	0.777	0.0024
	440	14	0.352	3.1	0.1	0.855	0.0000
					0.2	0.783	0.0026
				3.5	0.1	0.839	0.0001
					0.2	0.755	0.0058
	660	14	0.528	3.1	0.1	0.846	0.0000
					0.2	0.771	0.0020
				3.5	0.1	0.823	0.0001
					0.2	0.740	0.0109

Estimations based on genetic estimates	15.4	14	0.012	3.1	0.1	0.881	0.0000
					0.2	0.814	0.0005
				3.5	0.1	0.869	0.0000
					0.2	0.799	0.0006
	22.8	14	0.018	3.1	0.1	0.879	0.0000
					0.2	0.814	0.0002
				3.5	0.1	0.869	0.0000
					0.2	0.799	0.0016
	56.2	14	0.045	3.1	0.1	0.878	0.0000
					0.2	0.807	0.0002
				3.5	0.1	0.865	0.0000
					0.2	0.795	0.0010

Appendix. Comparison of diversity measures between simulated data based on competing hypotheses (scenarios 1 to 5) and BP empirical data (number of observed alleles, Na ; effective number of alleles, Na_e ; observed heterozygosity, H_O ; expected heterozygosity, H_E). Significant differences were assessed by determining if the values (+/- standard error) overlapped each other (yes = overlap / no significant difference with empirical data; high = simulated data higher than empirical data; low = simulated data lower than empirical data). Histograms of comparisons are not shown. Diversity measures for SE sites surrounding the BP are provided for comparison purposes. The following abbreviations were used: M (male migration), F (female migration), GR (growth rate), Ma (proportion of population lost due to first fire), Mb (proportion of population lost due to second fire).

		n	# of generations	Na	Na_e	H_O	H_E
	SE	647	/	13.357	5.915	0.767	0.807
	BP	139	/	4.643	2.492	0.546	0.555
Historical migration							
	Colonization						
Scenario 1	Null hypothesis: 50% M; 50% F	220	400	high	high	high	high
		220	1200	high	high	high	high
	Saturation hypothesis: 90% M;	220	400	high	high	high	high
	50% F	220	1200	high	high	high	high
	Female philopatry hypothesis:	220	400	high	high	high	high
	90% M; 3% F	220	1200	high	high	high	high

	Reduced migration - Drift						
Scenario 2a	Reduced movement hypothesis:	220	400	high	high	high	high
	10% M; 3% F	220	1200	high	high	high	high
	Extremely reduced movement	220	400	high	high	high	high
	hypothesis: 3% M; 3% F	220	1200	high	high	high	yes
	No migration hypothesis: 0% M;	220	400	low	low	low	low
	0% F	220	1200	low	low	low	low
	Reduced migration - One-Migrant-Rule						
Scenario 2b	0.1 migrant per generation	220	400	low	low	low	low
		220	1200	low	low	low	low
	0.2 migrant per generation	220	400	low	low	low	low
		220	1200	**yes**	low	**yes**	low
	0.5 migrant per generation	220	400	**yes**	**yes**	**yes**	**yes**
		220	1200	yes	yes	yes	low
	1 migrant per generation	220	400	high	high	high	**yes**
		220	1200	**yes**	**yes**	**yes**	**yes**
	2 migrant per generation	220	400	high	high	high	high
		220	1200	**yes**	**yes**	**yes**	**yes**
	10 migrant per generation	220	400	high	high	high	high
		220	1200	high	high	high	high
Demographic bottleneck							
Scenario 3	Null hypothesis: constant population size	220	12	high	high	high	high
	Hypothesis 1a: Ma = 10%; Mb = 90%; GR = 5%	220	12	high	high	high	high

	Hypothesis 1b: Ma = 10%; Mb = 90%; GR = 10%	220	12	high	high	high	high
	Hypothesis 2a: Ma = 20%; Mb = 90%; GR = 5%	220	12	high	high	high	high
	Hypothesis 2b: Ma = 20%; Mb = 90%; GR = 10%	220	12	high	high	high	high
	Hypothesis 3a: Ma = 40%; Mb = 90%; GR = 5%	220	12	high	high	high	high
	Hypothesis 3b: Ma = 40%; Mb = 90%; GR = 10%	220	12	high	high	high	high
	Hypothesis 4a: Ma = 10%; Mb = 70%; GR = 5%	220	12	high	high	high	high
	Hypothesis 4b: Ma = 10%; Mb = 70%; GR = 10%	220	12	high	high	high	high
	Hypothesis 5a: Ma = 20%; Mb = 70%; GR = 5%	220	12	high	high	high	high
	Hypothesis 5b: Ma = 20%; Mb = 70%; GR = 10%	220	12	high	high	high	high
	Hypothesis 6a: Ma = 40%; Mb = 70%; GR = 5%	220	12	high	high	high	high
	Hypothesis 6b: Ma = 40%; Mb = 70%; GR = 10%	220	12	high	high	high	high
Recently reduced migration							
Scenario 4	0 migrant per generation	220	40	low	low	low	low
	0.1 migrant per generation	220	40	low	low	low	low

0.2 migrant per generation	220	40	low	low	low	low
0.5 migrant per generation	220	40	**yes**	**yes**	**yes**	**yes**
1 migrant per generation	220	40	high	yes	yes	yes
2 migrant per generation	220	40	high	high	high	high
10 migrant per generation	220	40	high	high	high	high
Recently reduced migration + demographic bottleneck						
0 migrant per generation						
Hypothesis 1a	220	40	low	low	low	low
Hypothesis 1b	220	40	low	low	low	low
Hypothesis 2a	220	40	low	low	low	low
Hypothesis 2b	220	40	low	low	low	low
Hypothesis 3a	220	40	low	low	low	low
Hypothesis 3b	220	40	low	low	low	low
Hypothesis 4a	220	40	low	low	low	low
Hypothesis 4b	220	40	low	low	low	low
Scenario 5 Hypothesis 5a	220	40	low	low	low	low
Hypothesis 5b	220	40	low	low	low	low
Hypothesis 6a	220	40	low	low	low	low
Hypothesis 6b	220	40	low	low	low	low
0.1 migrant per generation						
Hypothesis 1a	220	40	low	low	low	low
Hypothesis 1b	220	40	low	low	low	low
Hypothesis 2a	220	40	low	low	low	low
Hypothesis 2b	220	40	low	low	low	low
Hypothesis 3a	220	40	low	low	low	low

Hypothesis 3b	220	40	low	low	low	low
Hypothesis 4a	220	40	low	low	low	low
Hypothesis 4b	220	40	low	low	low	low
Hypothesis 5a	220	40	low	low	low	low
Hypothesis 5b	220	40	low	low	low	low
Hypothesis 6a	220	40	low	low	low	low
Hypothesis 6b	220	40	low	low	low	low
0.2 migrant per generation						
Hypothesis 1a	220	40	low	low	low	low
Hypothesis 1b	220	40	low	low	low	low
Hypothesis 2a	220	40	low	low	low	low
Hypothesis 2b	220	40	low	low	low	low
Hypothesis 3a	220	40	low	low	low	low
Hypothesis 3b	220	40	low	low	low	low
Hypothesis 4a	220	40	low	low	low	low
Hypothesis 4b	220	40	low	low	low	low
Hypothesis 5a	220	40	low	low	low	low
Hypothesis 5b	220	40	low	low	low	low
Hypothesis 6a	220	40	low	low	low	low
Hypothesis 6b	220	40	low	low	low	low
0.5 migrant per generation						
Hypothesis 1a	220	40	low	low	low	low
Hypothesis 1b	220	40	low	low	low	low
Hypothesis 2a	220	40	low	low	low	low
Hypothesis 2b	220	40	low	yes	low	low

Hypothesis 3a	220	40	low	low	low	low
Hypothesis 3b	220	40	low	low	low	low
Hypothesis 4a	220	40	low	yes	yes	low
Hypothesis 4b	220	40	low	yes	yes	low
Hypothesis 5a	220	40	low	yes	low	low
Hypothesis 5b	220	40	low	yes	low	low
Hypothesis 6a	220	40	low	yes	low	low
Hypothesis 6b	220	40	low	yes	low	low
1 migrant per generation						
Hypothesis 1a	220	40	low	yes	yes	yes
Hypothesis 1b	220	40	low	yes	yes	yes
Hypothesis 2a	220	40	low	yes	yes	yes
Hypothesis 2b	220	40	low	yes	yes	yes
Hypothesis 3a	220	40	low	low	yes	yes
Hypothesis 3b	220	40	low	yes	yes	yes
Hypothesis 4a	220	40	**yes**	**yes**	**yes**	**yes**
Hypothesis 4b	220	40	**yes**	**yes**	**yes**	**yes**
Hypothesis 5a	220	40	**yes**	**yes**	**yes**	**yes**
Hypothesis 5b	220	40	**yes**	**yes**	**yes**	**yes**
Hypothesis 6a	220	40	**yes**	**yes**	**yes**	**yes**
Hypothesis 6b	220	40	**yes**	**yes**	**yes**	**yes**
2 migrants per generation						
Hypothesis 1a	220	40	low	yes	yes	yes
Hypothesis 1b	220	40	**yes**	**yes**	**yes**	**yes**
Hypothesis 2a	220	40	low	yes	yes	yes

Hypothesis 2b	220	40	yes	high	yes	yes
Hypothesis 3a	220	40	low	yes	yes	yes
Hypothesis 3b	220	40	yes	high	yes	yes
Hypothesis 4a	220	40	yes	high	high	high
Hypothesis 4b	220	40	yes	high	high	high
Hypothesis 5a	220	40	yes	high	high	high
Hypothesis 5b	220	40	yes	high	high	high
Hypothesis 6a	220	40	yes	high	high	high
Hypothesis 6b	220	40	yes	high	high	high
10 migrants per generation						
Hypothesis 1a	220	40	high	high	high	high
Hypothesis 1b	220	40	high	high	high	high
Hypothesis 2a	220	40	high	high	high	high
Hypothesis 2b	220	40	high	high	high	high
Hypothesis 3a	220	40	high	high	high	high
Hypothesis 3b	220	40	high	high	high	high
Hypothesis 4a	220	40	high	high	high	high
Hypothesis 4b	220	40	high	high	high	high
Hypothesis 5a	220	40	high	high	high	high
Hypothesis 5b	220	40	high	high	high	high
Hypothesis 6a	220	40	high	high	high	high
Hypothesis 6b	220	40	high	high	high	high

CHAPTER 5

GENERAL DISCUSSION

My thesis shows clear evidence of both historical and contemporary genetic structuring patterns among black bears in Ontario, and provides insights into natural and anthropogenic influences on local and regional levels of genetic diversity. Phylogeographic analyses of mtDNA identified historical postglacial lineages among Ontario populations, and added genetic data from the mid-eastern part of the black bear range, which was lacking from previously published studies. These phylogeographic analyses were complemented by microsatellite genotyping and individual assignment tests that revealed contemporary, large-scale, gene flow patterns across the province. Genetic substructure at smaller spatial scales potentially linked to anthropogenic activities was also observed. The suggested influence of human activities on small-scale genetic differentiation was confirmed through the investigation of reduced genetic variability in an isolated black bear population located in the southeastern portion of the province. For this population, our genetic results added information to previous demographic data, and gave further support to conservation concerns raised following population viability analyses (Howe et al. 2007).

Historical and contemporary gene flow patterns of Ontario black bears

Several North American species are divided into 2 or more historical phylogeographic clades as a result of isolation in glacial refugia respectively located on the east and west sides of the continent during the Last Glacial Maximum (Arbogast 1999; Demboski et al. 1999; Aubry et al. 2009). In this study, all black bear haplotypes identified in Ontario through mtDNA analyses (n = 36) were added to haplotypes previously identified across North America (Wooding and Ward 1997; Paetkau and Strobeck 1996; Onorato et al. 2004; Robinson et al. 2007; Van Den Bussche et al. 2009). These data allowed me to address the lack of information for the mid-eastern part of the American black bear's range. Results confirmed that historically, Ontario black bears belonged to the widespread continental phylogeographic clade (Pelletier et al. 2011). Further, the mtDNA data from Ontario showed that this large continental clade was divided into 2 subclades respectively located on the western and eastern side of the Great Lakes. This division had previously been observed by Wooding and Ward (1997), but had been deemed spurious, as it was thought to result from the lack of data that the present study was able to fill. Here, we showed that black bears from northwest Ontario, located on the western side of this lake, are more related to the western subclade (e.g., Manitoba, Montana, California), whereas individuals from central and southeast Ontario, located on the eastern side of this lake, are more related to the eastern subclade (e.g., Québec, Louisiana). These results suggest that the subdivision of the continental phylogeographic clade could be due to recolonization routes situated on either side of the Great Lakes following the last glaciation, and that the region north of the Great Lakes, around Lake Nipigon, represents the contact zone (Pelletier et al. 2011). As a glacial lake,

Lake Nipigon likely acted as a barrier to movement for terrestrial species until after its deglaciation, 10,000 years ago (Dyke 2004). As such, the secondary contact between American black bear continental subclades must have occurred after that time. Secondary contact of phylogeographic lineages have also been observed in several terrestrial species in the same area (e.g., garter snake – Rye 2000; woodland caribou – Klütsch et al. 2012), suggesting that such genetic signals could also be observed in other species whose ranges include the Great Lakes area. For this reason, studying gene flow patterns in this region could give insights into postglacial recolonization dynamics of multiple species, and could help identify contemporary processes that maintain historical lineages. In Ontario black bears, for example, male-biased dispersal, detected through stronger levels of genetic differentiation in mitochondrial analyses ($0.09 < F_{ST} < 0.44$) relative to microsatellite analyses (Mills 2005), and through lower differentiation among males than females, could be maintaining the subclade division.

In addition to the historical genetic differentiation detected at a small spatial scale through phylogeographic analyses, individual assignment tests based on microsatellite profiles revealed the existence of 3 contemporary genetic clusters in Ontario black bears (Pelletier et al. 2012). Two large clusters corresponded to individuals from the northwest and southeast regions of the province, whereas the third, much more restricted geographically, corresponded to individuals from the BP. Although most sampling sites were separated by shorter distances than male black bears can disperse (Rogers 1987; Lee and Vaughan 2003), isolation by distance was significant across the province ($r = 0.552$, $P = 0.001$), and genetic variation between the Northwest and Southeast clusters was clinal. This gradient in genetic variation illustrated the difficulties encountered when

defining population boundaries from genetic data in wide-ranging species that have large home ranges, as when genetic variation is clinal, clearly delineating populations might not be possible. Still, the general congruence between the contemporary structuring patterns and the phylogeographic lineages shows that if management decisions were to be made based on defined groups rather than at the Wildlife Management Unit level, these cluster delineations could be used.

Genetic diversity was high in both of the large clusters (Ho = 0.76 and 0.77 for Northwest and Southeast, respectively), and moderate genetic differentiation was found between them (F_{ST} = 0.01). In comparison, BP black bears showed stronger genetic isolation with each of those clusters (F_{ST} = 0.12 and 0.13, respectively), and a lower level of diversity (Ho = 0.55), consistent with what can be observed in other geographically isolated black bear populations across the continent (Paetkau and Strobeck 1994; Warrillow et al. 2001; Triant et al. 2004; Onorato et al. 2007; Robinson et al. 2007). Each of the 3 main clusters contained substructuring, although the level of differentiation between the Southeast subclusters (F_{ST} = 0.007, P < 0.0001) was lower than the one observed between the Northwest subclusters (F_{ST} = 0.01, P < 0.0001), despite the higher anthropogenic pressures that exist in the south of the province (Statistics Canada 2002). This suggests that gene flow has been high enough to lessen the effects of habitat fragmentation in areas located at the periphery of the current Ontario distribution, where pressures on the landscape are higher. Similarly, due to their geographic overlap, the presence of 2 genetic subclusters on the BP is unlikely to be the consequence of contrasting levels of human influences, but rather, could be explained by the existence of family groups resulting from females mate selection decisions following the demographic

bottleneck. The genetic divergence of BP black bears from the core SE cluster, despite a close geographical proximity, gives further support to the concerns raised by Howe et al. (2007) regarding their persistence. As this population is small and under high levels of anthropogenic pressure, there could be a need for future conservation actions, which needed to be assessed by first identifying the reasons for their high level of differentiation with the rest of the Ontario individuals.

Results from Chapters 2 to 4 show that in Ontario, both historical and contemporary processes have led to the current patterns of genetic diversity and differentiation in American black bears. Although the overall contemporary genetic diversity across the province is high, the existing genetic clusters, whose boundaries mostly correspond to the historical subclades, could experience further differentiation if long-distance gene flow was highly impeded (see simulations of scenario 4: recently reduced migration – one-migrant rule; Chapter 4). This integrative understanding of black bear gene flow patterns, and the delineation of historical and contemporary genetic boundaries, provide important contributions to inform potential management plans that would seek to maintain large-scale genetic diversity and connectivity for this species in the long-term.

Potential population trajectories of Ontario black bears

Although BP black bears do not have unique haplotypes nor alleles that could make this population an evolutionary significant unit as defined by Moritz (1994), they could be considered a Management Unit based on their substantial divergence with the larger genetic clusters (Palsbøll et al. 2007), which indicates reduced gene flow between

BP and the rest of the Ontario. BP black bears also display lower haplotypic and nuclear genetic diversity levels compared to the other clusters (Pelletier et al. 2011 and 2012), and habitat connectivity with the neighboring core population is highly reduced (Howe et al. 2007). These characteristics make the BP population a useful biological model to determine the factors that could lead to a similar situation in other wide-ranging species that can disperse over long distances, and for which populations are still geographically connected and genetically diverse. For this reason, I used a modeling approach to identify which contemporary or historical processes could have led to the reduced diversity in BP black bears. Using forward simulations greatly enhanced the existing genetic data through their incorporation into a hypothesis-testing framework. I tested for: 1) historical genetic drift following the colonization of the BP by black bears; 2) a recent and sudden decrease in population size resulting from forest fires that destroyed a portion of black bear habitat in the early 1900s as a consequence of agricultural activities (Suffling et al. 1995); 3) a highly reduced level of migration between BP and SE individuals as a consequence of anthropogenic influences following European settlement; and 4) a combination of a recent population crash and highly reduced migration. Comparisons of genetic diversity measures between the simulated populations and the empirical data suggested that the low diversity of BP black bears and their differentiation with SE individuals could be a consequence of sequential recent demographic bottlenecks combined with reduced migration. Despite this, the BP population could retain 80% of its current diversity over the next 100 years, even under complete isolation, and under these circumstances, translocation efforts could increase genetic diversity only on the short-term.

Several populations of large carnivores with low genetic diversity have been shown to persist under geographic isolation (Paetkau et al. 1998a; Paetkau et al. 1998b; Walker et al. 2001). In Ontario, BP black bears represent a unique situation, as it is the only population that is both geographically and genetically isolated for which data are available (Howe et al. 2007; Pelletier et al. 2011 and 2012). My thesis shows that although landscape management allowing a continuous intake of migrants from the mainland would be an effective method to enhance genetic diversity of BP black bears in the long-term, economic interests associated with private land-use would likely prevent actions that would aim to restore habitat connectivity between BP and SE. Because of this, translocations of individuals from SE would be a reasonable compromise to increase diversity in the short-term. Such a strategy would give time to design a more long-term plan before BP black bears become more genetically isolated.

Using simulations to understand the factors and length of time required to establish population structuring and diversity patterns similar to the ones currently observed on the BP emphasized concerns raised in previous demographic analyses regarding the persistence of this population (Howe et al. 2007). This approach enabled me test alternative demographic hypotheses, and also to generate predictive scenarios regarding the future of BP black bears. The results presented here suggest that although most wide-ranging mammals located in the northern portion of the continent might not show elements warranting conservation concerns, the high latent extinction risks that exist in this region due to future human expansion (Cardillo et al. 2006) should raise awareness as to how to implement efficient management actions to ensure their long-term viability. Thus, before further fragmentation occurs, it would be advisable to start

implementing large-scale landscape management plans that aim to maintain large networks of habitat allowing for population connectivity to be retained.

Future directions

Predicting movement and genetic differentiation across a changing landscape

As human expansion continues, North American landscapes will likely experience radical changes (Cardillo et al. 2006). Populations living in previously undisturbed regions will become more vulnerable to geographic isolation, stochastic events (Lande 1993), and genetic drift (Keyghobadi 2007), and as such, will have a higher probability of extinction (Frankham 2005). For this reason, efforts should be conducted to implement large-scale landscape management plans that would prevent local extirpation of currently healthy mammalian populations. To do so, the relationships between critical landscape variables that promote habitat connectivity, and thus gene flow and genetic diversity, have to be properly identified. Taking a landscape genetic approach would allow this, as this method enables defining the links that exist between gene flow patterns that reflect animal movements, and specific landscape features (Manel et al. 2003). The fact that landscape configuration influences animal movements (McRae and Shah 2009) can be used to determine where animals are most likely to be found based on habitat selection data. From these hypothesized movement patterns, it is possible to identify areas where reduced gene flow and higher differentiation between populations may arise as a result of fragmentation. Thus, combining ecological projections with genetic data would allow the identification of landscape features that need to be conserved to decrease mortality risks, and maintain genetic diversity and connectivity between populations that could become

genetically differentiated. To identify which populations of Ontario black bears are more likely to become geographically and genetically fragmented, data should be obtained regarding: 1) their current and expected use of food resources in case of local changes in mast species availability, 2) the amount and types of roads and developed areas they encounter, and 3) their local and regional scale genetic structuring patterns, and their potential correlations with landscape variables.

Isolation by distance is significant in Ontario black bears (Pelletier et al. 2012), although natural landscape variables likely play an important role on genetic structure on more local scales (Short Bull et al. 2011), as shown for several populations in North America (Paetkau and Strobeck 1994; Cushman et al. 2006; Peacock et al. 2007). As weak trends of genetic structure have been detected within the large Ontario clusters, using landscape resistance surfaces (McRae and Shah 2009) may help detect more local patterns if fine scale habitat data were available. However, landscape features need to be highly variable for an effect on gene flow to be detected (Short Bull et al. 2011), and thus, studying structuring patterns at several spatial scales, and across several study sites, would be necessary.

Although generalists (Schoen 1990), black bears are highly dependent on seasonal food resources. Easily digestible green vegetation and fruits provide soft mast, critical to quickly obtain the necessary calories following den emergence (Inman and Pelton 2002), a diet which can be supplemented with insects for higher protein intake (Rogers 1987). Acorns and nuts provide hard mast, and are essential to survive the denning season and recover from the subsequent energy losses (Jonkel and Cowan 1971; Rogers 1987). Food failures, which currently occur every 3 to 5 years (Howe et al. 2010), have been shown to

be correlated to higher levels of black bear mortality (e.g., road kills, illegal kills, nuisance control [Ryan et al. 2007], hunting [Noyce and Garshelis 1997; Rogers 1976]). This increased mortality has been associated with bears travelling longer distances to forage, as well as a higher probability of using unnatural food sources (Pelton 1989; Rogers 1976). Food scarcity is also linked to decreased reproductive success, as females that do not gain enough weight prior to denning usually fail to produce a litter, or produce cubs that are smaller than average and less likely to survive (Rogers 1976; Garshelis and Pelton 1981; Obbard and Howe 2008), which negatively affects population growth rate.

Beech bark disease, which increases the mortality of beech trees, could reach the northern core of the beech distribution by 2040 if its spread was linked to climate conditions (Noble 2010). This could have a negative effect on American black bears access to resources in the mid-portion of Ontario, since beech is sometimes the only hard mast producing species in localized forested areas of this region (Noble 2010). In such a case, it might be possible to observe an increase in distances travelled by bears that inhabit areas mostly forested by beech, as well as an increase in home-range size that would allow them to gain access to a higher diversity of hard-mast producing species to prepare for the winter (Inman and Pelton 2002). It might also be possible to detect an increase in human-induced mortality (Pelton 1989; Rogers 1976), and a reduction in reproductive success. Finally, as black bears in Ontario are likely close to carrying capacity, a higher number of overlapping home-ranges might be observed.

In addition to changes in resource availability and natural landscape features that may influence genetic structure in wide-ranging mammals, the effects of anthropogenic structures such as roads and settlements should also be taken into account, as they can result in habitat loss and fragmentation. In black bears, population dynamics and genetic structuring patterns can be influenced by road types (Coster and Kovach 2012), road density (Mills 2005), and vehicular traffic (Hostetler et al. 2009). In Ontario, it has been shown that even moderate levels of road density can lead to contemporary genetic differentiation (road density < 0.5 km/km^2; Mills 2005), and thus, the expected increase in anthropogenic pressures in the province, in addition to increasing mortality (Forman and Alexander 1998; Saunders et al. 2002), could decrease connectivity, lower gene flow, and lead to higher genetic structure between sites that currently share migrants. Thus, projections of climate variables and land-use patterns would be useful to estimate the potential landscape modifications that will occur in Ontario, and predict the subsequent changes in bear movement and local genetic structuring patterns across the province.

Species that have similar ecological requirements and comparable life history traits may be similarly affected by natural and anthropogenic landscape variables. As such, the landscape genetics approach presented above could be used to conduct studies that aim to identify the effects of habitat fragmentation on gene flow patterns of multiple species. This would inform integrative and large-scale landscape management actions to conserve habitat features that are important for several taxa, which would allow for such actions to have a broader ecological impact. A multi-species framework would also likely help other organisms than large mammals due the potential umbrella effects of habitat conservation actions.

Despite the benefits of the landscape genetics framework, the weak isolation by distance detected in Ontario black bears (Pelletier et al. 2012) could undermine our ability to correlate local genetic structuring patterns to specific landscape features. For this reason, it might be advantageous to look at genes under selection, as we could detect genetic changes illustrating adaptive responses to specific environmental conditions (Hoffmann and Willi 2008). An adaptive landscape genetics approach could help determine if functional alleles are geographically segregated (Schoville et al. 2012), and thus, might be used to detect patterns of local adaptation. Such information would be useful to predict how populations would respond to environmental changes, and thus could help define preventative management strategies that would aim to conserve evolutionary potential in species.

Genetic assessment of the role of protected areas

Source-sink dynamics patterns have been observed in several carnivore species (Mace and Waller 1998; Novaro et al. 2005; Robinson et al. 2008), and it has been shown that open populations that are being hunted are able to keep stable densities as a result of compensatory immigration from adjacent areas (Robinson et al. 2008). Migration of animals from unhunted to hunted areas might be essential for hunters to obtain harvest material (Slough and Mowat 1996), and such movement patterns may also play a role in the presence of fine scale genetic structure, which can be identified through the estimation of net gene movement and local differences in allelic diversity (Andreasen et al. 2012).

In Ontario, where black bears are hunted for sport, several protected areas (Chapleau Crown Game Preserve, Bruce Peninsula National Park, Algonquin Park) are surrounded by regions where hunting is allowed. As such, this region provides a good study system to assess what role protected areas may have in shaping black bear fine scale genetic variation, and in providing a source for harvest material.

Estimating temporal genetic turnover

As large scale landscape changes are expected in North America as a consequence of human expansion and climate change, it might be expected that animals show responses to these modifications over time. These responses may be detected by comparing the situation at similar sites at various timepoints. Contemporary temporal variations in genetic structure and diversity have been observed in several vertebrates as a result of seasonal immigration (Norén et al. 2011), habitat fragmentation (Delaney et al. 2010), or genetic drift due to small effective population size (Ortego et al. 2011). To provide a theoretical baseline regarding the stability of structuring patterns expected in connected or contiguous natural populations, the level of genetic turnover should first be evaluated under undisturbed conditions. Following this, the information regarding temporal genetic variation could be combined to habitat selection and genetic differentiation data to help improve the predictive power of landscape genetic models, and better determine the probability of persistence of wild populations.

The sampling design conducted in Ontario to obtain population estimates of black bears allows for this type of study to be carried out, as 18 Wildlife Management Units were sampled at least 4 years apart, allowing enough time for a litter produced the first

sampling year to have reached the subadult stage, and in the case of males, to have already dispersed from their natal site. The fact that these repeated sampling events encompass WMUs located in each genetic cluster previously identified in Ontario (Pelletier et al. 2012) can give information as to the amount of short-term genetic variation that occurs in each region. Thus it would be possible to determine if the same individuals are found at specific sites at different timepoints, and if genetic diversity and clustering patterns are constant or vary over short timescales. This could then be used to compare the genetic turnover from undisturbed to disturbed conditions, and could be integrated to landscape genetic analyses to obtain a reliable assessment of the predicted effects of habitat fragmentation on changes in genetic structure and diversity.

Conclusion

In my thesis, I identified the historical and contemporary processes influencing American black bears genetic structure and diversity across a vast landscape that shows gradations in anthropogenic pressures. I also used a predictive approach to assess the future of a geographically isolated population with low genetic diversity. This research provides an integrative understanding of gene flow patterns in a widely distributed large mammalian carnivore, and gives insights into the factors that may lead to differentiation in other species with similar life history traits. The data presented here can be included into predictive models to inform preventive management actions that could not only benefit American black bears, but a multiple array of species whose habitat may become more fragmented.

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