Microsatellite population structure of Newfoundland black bears
(Ursus americanus hamiltoni)

H. Dawn Marshall, Edward S. Yaskowiak, Casidhe Dyke, and Elizabeth A. Perry

Abstract: We investigated population structure of black bears (Ursus americanus hamiltoni Cameron, 1957) from insular Newfoundland using the microsatellite profiles of 12 loci from three broadly distributed areas (Northern, Baie Verte, and Bonavista peninsulas). Our goals were to revisit earlier findings of low heterozygosity in Newfoundland and increase knowledge of intraspecific variability in black bears, and make inferences about postglacial colonization and contemporary movements of island black bears. Ninety-three individuals (42 males) were identified among 543 hair samples: 21 from Bonavista, 25 from Northern Peninsula, and 47 from Baie Verte. Genetic diversity is relatively low ($H_e = 0.42$) and decreases from northwest to southeast. Small but significant subpopulation differentiation revealed by $F$ statistics is greatest between Northern and Baie Verte peninsulas; it is lower and comparable in the remaining pairwise comparisons. We hypothesize that postglacial colonization proceeded from the Northern Peninsula southeastward. Bears migrated from the Northern Peninsula to Baie Verte at some more distant time in the past, then diverged by genetic drift. More recently, migration occurred from these two populations to Bonavista, characterized by positive $F_{IS}$ indicative of admixture. Tests of biased dispersal and posterior probability of correct assignment to locality reveal contemporary movements of both males and females with historical dispersal attributable to males.

Résumé : Nous étudions la structure des populations d’ours noirs (Ursus americanus hamiltoni Cameron, 1957) de l’île de Terre-Neuve à l’aide des profils microsatellites à 12 loci dans trois régions à large répartition (les péninsules Nord, de la Baie Verte et de Bonavista). Nos objectifs sont de revoir les découvertes antérieures d’une faible hétérozygotie à Terre-Neuve, de préciser la variabilité intraspécifique chez les ours noirs et de formuler des hypothèses sur la colonisation post-glaciaire et les déplacements actuels des ours noirs de l’île. Nous avons identifié 93 individus (42 mâles) dans 543 échantillons de poils, 21 de Bonavista, 25 de la péninsule Nord et 47 de la Baie Verte. La diversité génétique est relativement faible ($H_e = 0.42$) et elle décroît du nord-ouest vers le sud-est. La statistique $F$ révèle l’existence d’une différenciation petite mais significative entre les populations des péninsules Nord et de la Baie Verte ; elle est plus faible mais comparable dans les autres comparaisons appariées. Nous émettons l’hypothèse selon laquelle la colonisation postglaciaire a procédé de la péninsule Nord vers le sud-est. Les ours ont migré de la péninsule Nord vers la Baie Verte à un moment donné dans un passé éloigné, puis ont divergé par dérive génétique. Une migration plus récente s’est produite à partir de ces deux populations vers Bonavista, une population caractérisée par un $F_{IS}$ positif indicateur de mélange. Des tests de dispersion différentielle et de probabilité à posteriori d’assignation correcte aux différents sites montrent qu’il y a des déplacements actuels à la fois des mâles et des femelles, alors que la dispersion du passé est attributable aux mâles.

[Traduit par la Rédaction]

Introduction

The mammalian fauna of the island of Newfoundland, Canada, is striking both in terms of number of indigenous terrestrial species (14) and the disproportionate number of those species that are members of the order Carnivora (7). The unique biogeography of the island owes itself to its particular features of climate, soil conditions, and geology. Notably, the island was most likely covered in glacial ice until only 7000 years ago (South 1983), yet the present-day Newfoundland representative of many species is sufficiently distinct from its continental counterpart to be considered separate subspecies. Although there is little information available on the black bears of Newfoundland, except for a few molecular studies that have included some Newfoundland samples of black bears (Paetkau and Strobeck 1994, 1996), they are currently recognized as a distinct subspecies (Ursus americanus hamiltoni Cameron, 1957; Cameron 1956), based...
on differences in skull size and shape (Cameron 1956). Black bears in Newfoundland are generally larger (females average 101 kg (37% larger) and males average 179 kg (55% larger)), than black bears in New Brunswick, Ontario, and Quebec, Canada, and Maine and Alaska, USA (Mahoney et al. 2001). Mahoney et al. (2001) suggested that this difference in body size likely reflects a response to environmental selective pressures which affects the bears’ ability to exploit seasonal availability of protein, but may be attributed to genetic divergence of the Newfoundland population.

Genetic diversity and phylogeographic structure of black bears have been studied across much of their distribution, in part to assess the pattern of genetic structuring in a large, widespread North American carnivore species (Cronin et al. 1991; Wooding and Ward 1997). Both allozyme (Wathen et al. 1985) and mitochondrial DNA (mtDNA) RFLP (Cronin et al. 1991) studies have indicated that black bears have relatively low genetic variation, although more than has been found in North American populations of either polar bears (Ursus maritimus Phipps, 1774) or brown bears (Ursus arctos L., 1758) (Cronin et al. 1991). Despite this, highly divergent (~5%) mtDNA haplotypes were identified by Cronin et al. (1991) in populations in Montana and Oregon, and by Wooding and Ward (1997) in eastern British Columbia and western Alberta, Canada, and Montana, USA. Wooding and Ward (1997) suggested that this pattern of haplotype divergence and distribution is due to long-term isolation of bear lineages in separate refugia during the time of Pleistocene forest fragmentation, followed by recent regional admixture along a zone of contact associated with the Continental Divide.

In contrast, much more similar haplotypes are distributed across the rest of the range east of the Continental Divide (Wooding and Ward 1997; Van Den Bussche et al. 2009). For example, Van Den Bussche et al. (2009) surveyed mtDNA data from black bears in the central part of the species distribution (Arkansas, Oklahoma, and Louisiana, USA, and Manitoba, Canada) and reported very low levels of nucleotide diversity (mean $\pi = 0.003$) among nine localities. This lack of deep phylogenetic structuring with respect to geographic distribution east of the Continental Divide is consistent with considerable gene flow throughout the history of the species (Avise et al. 1987; Cronin et al. 1991), such as may result from rapid distribution of haplotypes across the range during postglacial expansion. Additionally, although home-range size and dispersal characteristics of black bears vary with population density and habitat quality, among other factors (Wathen et al. 1985; Rogers 1987), the high dispersal capacity of this species (e.g., one-way movement distance of up to 214 km were reported by Hellgren et al. 2005) may contribute to the spread of mtDNA haplotypes among areas. There is, however, as shown by Paetkau and Strobeck (1996) and Wooding and Ward (1997), a small level of phylogenetic differentiation between the western and eastern parts of this more restricted range. This may be indicative of currently restricted gene flow and developing population structure on a broad geographic scale.

When mtDNA genetic diversity of black bears across Canada was compared with that of Newfoundland black bears, there was no evidence of differentiation between mainland Canada and the island of Newfoundland (Paetkau and Strobeck 1996). Furthermore, the results of a preliminary microsatellite analysis of four loci showed quite reduced genetic variation in Newfoundland black bears compared with mainland bears (Paetkau and Strobeck 1994). Paetkau and Strobeck (1996) implicated a recent (<7000 years ago) postglacial founder event rather than long-term small effective population size maintained throughout the last glaciation as causative of this reduced genetic diversity, because mtDNA haplotypes of Newfoundland black bears are closely related to other eastern haplotypes.

Although Newfoundland black bears have been considered in the context of genetic diversity and phylogeography in previous studies (Paetkau and Strobeck 1994, 1996; Wooding and Ward 1997), they have been represented by only a single locality from the eastern part of the island. In this study, we seek to document genetic diversity of bears from three geographically widespread localities to confirm and expand upon the earlier findings of reduced genetic diversity; to investigate long-term, ongoing, and sex-biased patterns of gene flow among island black bears; and to make inferences about the postglacial colonization history of the population of Newfoundland bears. We use genotypic data from 12 nuclear microsatellite loci in combination with genetic determination of gender from noninvasively collected bear hair samples.

**Materials and methods**

**Hair trapping**

Hair snagging sites were located in three areas on the Island of Newfoundland (Fig. 1): the Northern Peninsula (NP), the Baie Verte Peninsula (BVP), and the Bonavista Peninsula (BP). A systematic grid (75 km$^2$) was overlaid in each of these locations and hair traps were constructed as close to the centre of each individual cell as possible. Fifty-one hair traps were established on the Northern Peninsula, 49 on the Baie Verte Peninsula, and 46 on the Bonavista Peninsula. Traps were triangular in shape and consisted of a single line of barbed wire around the perimeter. Trees present at the site were used as the attachment points at each corner and each side was ~3 m in length. Traps were baited using approximately 2.5 kg of moose meat and vanilla extract was spread around the trap. Sampling was done during the months of June and July. Traps were checked once every 10 days and a total of four checks were performed. Hair samples present on barbs were removed and placed into labelled envelopes. Separate envelopes were used for samples collected on individual barbs. After collection, a hand-held torch was used to ensure the removal of any remaining hair. After the 40-day sampling period, traps were removed and baiting ceased.

**DNA analysis**

A total of 543 samples were included in this study. DNA was extracted from approximately 10–20 roots of hair using Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, Ontario, Canada) and following the manufacturer’s protocol. DNA was resuspended in 150 µL of elution buffer. The samples were screened at 12 microsatellite loci: CXX20, CXX110, G1A, G1D, G10B, G10C, G10H, G10I, G10L, G10M, G10P, and G10X (Onorato et al. 2004; Paetkau and Strobeck 1994, 1998; Paetkau et al. 1995; 1998, 1999; Taberlet et al. 1997). Polymerase chain reactions (PCR) were run in a total volume of 10 µL and reactions contained 1X PCR.
Fig. 1. Map of Newfoundland, Canada, showing sampling locations: (1) Northern Peninsula, (2) Baie Verte Peninsula, (3) Bonavista Peninsula, and (4) Terra Nova National Park (sampling locality in previous studies of black bears, *Ursus americanus hamiltoni*).

Master Mix (Promega Corp., Madison, Wisconsin, USA), 2 mmol/L MgCl2, 0.04 µg/µL BSA, 1 µmol/L of each primer (one primer in each pair was fluorescently labelled; Applied Biosystems Inc., Foster City, California, USA), and 2.5 µL of DNA template (5–20 ng). All PCRs included a negative control. Thermal cycling was performed in a GeneAmp 9700 (Applied Biosystems Inc.) under the following conditions: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 4 min. The microsatellite PCR products were pooled and run against an internal standard (LIZ500; Applied Biosystems Inc.) on an Applied Biosystems 3730 DNA Analyzer using GeneScan software (Applied Biosystems Inc.). Allele sizes were read by two readers.

Gender determination of hair donors was achieved by simultaneously amplifying a 130 bp region of the Y-chromosome *SRY* gene (*SRY29F* and *SRY121R*; Taberlet et al. 1997) and ~1 kb of the zinc finger X-chromosome gene (*ZF*; Shaw et al. 2003). PCRs were run in a total volume of 10 µL and contained 1× PCR Master Mix (Promega Corp.), 2 mmol/L MgCl2, 0.04 µg/µL BSA, 0.5 µmol/L of each primer and 2.5 µL of DNA template (5–15 ng). Cycling conditions were 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min, and a final extension at 72 °C for 7 min. Samples with only the upper X-specific band were identified as females. All PCRs included a negative control.

Samples that produced complete (scored at all 12 loci) genotypes were further investigated with GENECPAP version 1.3, a Microsoft Excel macro that compares each individual multilocus genotype with all other genotypes within the data set to determine matching genotypes (Wilberg and Dreher 2004).

**Data analysis**

Per-locus measures of variability and fit to Hardy–Weinberg equilibrium (number of alleles, observed and expected heterozygosity, and *F*<sub>IT</sub>) for the entire sample were calculated using ARLEQUIN version 3.5.1.2 (Excoffier and Lischer 2010). *F*<sub>IT</sub> was estimated via the algorithm of Weir and Cockerham (1984); significance was tested with 1000 permutations. To assess the utility of each locus to detect population structure, per-locus *F*<sub>ST</sub> measures were calculated with individuals allocated to each of the three subpopulations using ARLEQUIN, via the algorithm of Weir and Cockerham (1984). Linkage disequilibrium among all pairs of loci was evaluated with the exact test implemented in ARLEQUIN, with the default Markov chain and dememorization parameters.

Per-locality measures of variability (observed and expected heterozygosity) and estimates of *F*<sub>IS</sub> (Weir and Cockerham 1984) were made in ARLEQUIN, with significance testing by 1000 permutations. Allelic richness (*k*), a measure of the number of alleles in each *s* independent of sample size, was estimated in FSTAT version 2.93 (Goudet 1995).

Estimates of *F*<sub>ST</sub> and *R*<sub>ST</sub> among all population pairs were made in ARLEQUIN. The significance of each estimate was evaluated using 1000 randomizations. Assessment of population structure was also conducted with the Bayesian methodology implemented in STRUCTURE version 2.3.3 (Pritchard et al. 2000). The no-admixture model with correlated allele frequencies and sampling location as prior information was used to perform 10 independent runs for each of *K* = 1–5, with 500 000 Markov chain Monte Carlo repetitions following 100 000 burn-in iterations. The most probable number of subpopulations was taken as the highest mean log-probability of the data given *K*.

To investigate differences in movement patterns between males and females, FSTAT was used to calculate a variety of descriptive statistics (*F*<sub>ST</sub>, *F*<sub>IS</sub>, observed heterozygosity, gene diversity (*H*<sub>G</sub>; sensu Nei 1987), relatedness [*2F*<sub>ST</sub>/(1 + *F*<sub>ST</sub>)], and mean and variance of assignment index (Favre et al. 1997)). To determine whether the larger value of the statistic was significantly larger than the smaller value, 10 000 randomizations were performed. These tests and their applications are described in Goudet et al. (2002). Recent movement patterns were also investigated by implementing the USEPOPINFO model in STRUCTURE, for 10 independent runs of *K* = 3, with 500 000 Markov chain Monte Carlo repetitions following 100 000 burn-in iterations.

**Results**

Five hundred and forty-three samples (125 BP, 123 NP, and 295 BVP) were processed. Four-hundred and forty-four (82%) of these samples produced complete genotypes (113 BP (90%), 108 NP (88%), and 223 BVP (76%)). The data were screened with Micro-Checker (Van Oosterhout et al. 2004) and there was no evidence of large allelic dropout,
null alleles, or errors owing to stutter. When any genotypes that were found only once in all of the samples, and therefore containing possible genotyping errors, were removed from the data, a total of 93 individuals (distinct genotypes) were identified (21 from BP, 25 from NP, and 47 from BVP).

According to the per-locus measures of variability, fit to Hardy–Weinberg equilibrium, and population structure presented in Table 1, the number of alleles per locus ranges from 2 (G10L) to 6 (CXX20), with a mean of 3.9, and expected heterozygosities are on the order of 0.073 (G10H) to 0.645 (G10M), with a mean of 0.422. Observed heterozygosities correlate well with expected heterozygosities; $F_{ST}$ is significantly greater than zero for only one locus (G10M; $P = 0.029$); no locus showed significant departure from Hardy–Weinberg expectations at $P = 0.05$ after correction for multiple comparisons (adjusted $\alpha = 0.004$). $F_{ST}$ is significantly greater than zero at $P = 0.05$ for five loci (CXX110, G10X, CXX20, G10M, G10C) and for the latter two even after correction for multiple comparisons (adjusted $\alpha = 0.004$). Across all loci, $F_{ST}$ is significantly positive (0.033; $P = 0$), suggesting some level of genetic differentiation among the three subpopulations. No pair of loci demonstrated significant linkage disequilibrium at $P = 0.05$ after correction for multiple comparisons (adjusted $\alpha = 0.0008$). In summary, these measures indicate that the loci selected for this study are moderately variable, unlikely, in Hardy–Weinberg equilibrium, show no evidence of locus-specific nonrandom mating or null alleles, and demonstrate the ability to detect subpopulation differentiation.

When individuals are allocated to their subpopulation of origin (Table 2), expected heterozygosities are highest in the NP sample (0.441) and lowest in the BP sample (0.383). Again, observed heterozygosities do not differ significantly from expected heterozygosities; $F_{IS}$ is slightly positive in the Northern Peninsula (NP), with a mean of 0.422. Observed heterozygosities do not differ significantly from expected heterozygosities; $F_{ST}$ is significantly positive ($P \leq 0.01$) among all pairs of subpopulations, with the highest $F_{ST}$ (0.041) attributable to the NP–BVP comparison and the lowest to the NP–BP comparison (0.023). The level of differentiation between the BP and NP is approximately intermediate (0.029). The pattern of differentiation revealed by $R_{ST}$ (Table 3), which takes mean squared difference in allele size into account, is similar to that observed with $F_{ST}$ but the magnitude of the values is lower, and only the NP–BP comparison approaches significance ($P = 0.045$). Furthermore, while the NP–BP comparison maintains the greatest magnitude of differentiation ($R_{ST} = 0.017$), the BP and NP samples are the least differentiated ($R_{ST} = -0.010$).

The STRUCTURE analysis identified three as the most likely number of genetic clusters ($K$) among the 93 individuals (mean $\ln P(X/K) = -1708$ for $K = 3$; mean $\ln P(X/K) = -1711$ for $K = 1$; mean $\ln P(X/K) \leq -1722$ for all other $K$). Although $K = 3$ does not differ too greatly from $K = 1$ in terms of likelihood, $K = 3$ is consistent with the biological expectation and with the small significant levels of population differentiation suggested by $F_{ST}$ and $R_{ST}$. The latter observation also validates the use of sampling location as prior information in the STRUCTURE model. Each locality attributes its ancestry, on average, differently to each of the three clusters; NP owes 54% of its ancestry to one cluster, BVP owes 43% to one cluster, and BP owes 53% to one cluster. One example (for the run with the highest likelihood) of a triangle plot showing individual membership in each cluster is given in Fig. 2.

Figure 3 presents one example of a bar plot showing posterior probabilities of membership of individual bears to their assigned population, derived using the USEPOPINFO model in STRUCTURE, and details 16 (8 male) individual bears whose posterior probabilities of being correctly assigned to their given population are <0.5. These include one female in NP, five males and six females in BVP, and three males and one female in BP. Rather than literally interpreting these individuals as recent immigrants or dispersers, we suggest that this set of 16 individuals represents the characteristics of the class of individuals experiencing ongoing movement. This is due to the low levels of subpopulation differentiation that we observe, which make it difficult to differentiate between mis-
Table 3. Pairwise differentiation ($R_{ST}$ above the diagonal and $F_{ST}$ below the diagonal) of three subpopulations of black bears (Ursus americanus hamiltoni).

<table>
<thead>
<tr>
<th></th>
<th>Northern Peninsula (NP)</th>
<th>Baie Verte Peninsula (NVP)</th>
<th>Bonavista Peninsula (BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Peninsula (NP)</td>
<td>—</td>
<td>0.017, $P = 0.045$</td>
<td>0.006, $P = 0.288$</td>
</tr>
<tr>
<td>Baie Verte Peninsula (BVP)</td>
<td>0.041, $P = 0$</td>
<td>—</td>
<td>-0.010, $P = 0.847$</td>
</tr>
<tr>
<td>Bonavista Peninsula (BP)</td>
<td>0.023, $P = 0.010$</td>
<td>0.029, $P = 0$</td>
<td>—</td>
</tr>
</tbody>
</table>

Fig. 2. Triangle plot of individual membership of black bears (Ursus americanus hamiltoni) in each of three genetic clusters for three Newfoundland localities ($\ln P(X/K) = -1703$). NP, Northern Peninsula; BVP, Baie Verte Peninsula; BP, Bonavista Peninsula.

Discussion

We investigated population structure and demography of black bears from insular Newfoundland by analysing 12 microsatellite loci from three broadly distributed sampling localities (the Northern, Baie Verte, and Bonavista peninsulas). Our goals were to re-examine earlier findings of low heterozygosity found in 23 bears from one Newfoundland location (Terra Nova National Park; Paetkau and Strobeck 1994) and increase knowledge of intraspecific variability in North American black bears, to make inferences about postglacial colonization history of black bears on the island, and to gain insight into patterns of contemporary movements of black bears.

We found that overall heterozygosity is low in populations of black bears from insular Newfoundland, consistent with Paetkau and Strobeck (1994). We report a mean expected heterozygosity of 0.42, compared with 0.36 at four loci reported by Paetkau and Strobeck (1994) in their sample of 23 bears from Terra Nova National Park versus ~0.80 from other regions in Canada. Notably, the lowest expected heterozygosity in our study was 0.38, found in the easternmost Bonavista Peninsula. Other measures of diversity (number of alleles per locus, allelic richness, and $\theta$) were corresponding low in our study. Low heterozygosity at neutral loci is consistent with low long-term effective population size and (or) a recent colonization founder event, as suggested by Paetkau and Strobeck (1994). This is supported by the fact that the island of Newfoundland was largely covered in glacial ice until the end of the Wisconsinan glaciation, ~7000 years ago (South 1983).

The island of Newfoundland is home to only 14 extant indigenous terrestrial mammal species, a number of which are rodents, bats, and lagomorphs. This fauna is also unbalanced with a predominance of larger species (South 1983), represented by the orders Artiodactyla (woodland caribou, Rangifer tarandus caribou (Gmelin, 1788)) and Carnivora, which includes the Canada lynx (Lynx canadensis Kerr, 1792) and red fox (Vulpes vulpes (L., 1758)) in addition to the black bear. As insular Newfoundland was largely glaciated until ~7000 years ago, the present-day distribution and population size of each of these mammals represent a relatively recent history of colonization and population growth. The depauperate and disproportionate mammalian fauna in combination with the glaciation history and the isolation of the island postglacially renders the mammalian colonization history of the island of particular interest. In terms of the large mammals, such investigations have been undertaken from a genetic perspective only in the caribou (Willkerson 2010) and to some extent in the black bear (Cronin et al. 1991; Paetkau and Strobeck 1994, 1996; Wooding and Ward 1997). Based on mtDNA sequences, Wilkerson (2010) suggested that the most likely postglacial route for the recolonization of New-
Bonavista Peninsula.

The hypothesis for the path of colonization of Newfoundland is consistent with not having been isolated in a separate refugium, as the high levels of mtDNA divergence within black bears and the paraphyly of brown bears, it is important to accommodate intraspecific variation in phylogenetic analysis of black bears.

Several studies on black bears have included a number of individuals from Terra Nova National Park on the northeast coast of Newfoundland (Cronin et al. 1991; Paetkau and Strobeck 1996). Phylogenetic analysis of the two mitochondrial haplotypes represented among these individuals shows that they are most closely related to haplotypes from Fundy National Park in New Brunswick and La Mauricie National Park in Quebec (Paetkau and Strobeck 1996; Wooding and Ward 1997), and that eastern lineages of the North American black bear in general are phylogenetically distinct from western ones. Paetkau and Strobeck (1996) made the case that the close relationship between Newfoundland and eastern lineages relative to western ones suggests that Newfoundland lineages of black bear belong to the same refugial source as other eastern lineages, that is the southern refugium. Furthermore, the low diversity of the Newfoundland sample is consistent with not having been isolated in a separate refugium, such as the Grand Banks, for the duration of the Wisconsinan glacial period. Therefore, they conclude that the oldest and hence expected to have the greatest diversity at neutral loci. A subsequent spread of bears in a southeasterly fashion would result in progressively decreasing levels of diversity across the island. The alternative of a recolonization from a refugial source on the Grand Banks, for example, would most likely have resulted in a colonization route through the Avalon Peninsula or southeastern part of the island northward, and would have the reverse pattern of diversity. In addition to the pattern of diversity, the paucity of bears on the Avalon Peninsula provides evidence against this hypothesis.

We found that diversity decreases from northwest to southeast, and this trend is even more apparent when the diversity measure from Terra Nova National Park is included. This is consistent with colonization of Newfoundland via Labrador, as the earliest established population would be the oldest and consistent with colonization of Newfoundland via Labrador, as the earliest established population would be the oldest and hence expected to have the greatest diversity at neutral loci. A subsequent spread of bears in a southeasterly fashion would result in progressively decreasing levels of diversity across the island. The alternative of a recolonization from a refugial source on the Grand Banks, for example, would most likely have resulted in a colonization route through the Avalon Peninsula or southeastern part of the island northward, and would have the reverse pattern of diversity. In addition to the pattern of diversity, the paucity of bears on the Avalon Peninsula provides evidence against this hypothesis.

We note that the relationship among haplotypes reported by Paetkau and Strobeck (1996) and Wooding and Ward (1997), in which the Newfoundland haplotypes are paraphyletic and one is basal to a clade containing the Fundy and La Maurice haplotypes, is intriguing. Under a hypothesis in which bears colonized eastern North America from a southern refugium through Quebec and Labrador and then into Newfoundland, all Newfoundland haplotypes should be monophyletic and derivative. The presence of paraphyly and a haplotype ancestral to the other eastern lineages may indicate a more complex pattern of colonization, possibly with an alternative refugial source. However, bootstrap support was not reported for the node placing one Newfoundland haplotype together with the Quebec and New Brunswick lineages internal to the other Newfoundland haplotype, and is presumably low. In an endeavour to fully resolve intraspecific phylogenetic relationships and address this issue more thoroughly, we are currently sequencing mtDNA genomes of a more geographically extensive sample of a population of black bears. We concur with Cronin et al. (1991) who suggested that because of the high levels of mtDNA divergence within black bears and the paraphyly of brown bears, it is important to accommodate intraspecific variation in phylogenetic analysis of black bears.

Table 4. Population parameters partitioned by sex as a measure of biased dispersal of black bears (Ursus americanus hamiltoni).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean assignment index</td>
<td>0.338</td>
<td>-0.278</td>
<td>0.149</td>
</tr>
<tr>
<td>Variance of the index</td>
<td>7.91</td>
<td>6.691</td>
<td>0.321</td>
</tr>
<tr>
<td>$F_{ST}$</td>
<td>0.014</td>
<td>0.047</td>
<td>0.067</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.037</td>
<td>-0.046</td>
<td>0.040</td>
</tr>
<tr>
<td>Relatedness</td>
<td>0.026</td>
<td>0.094</td>
<td>0.055</td>
</tr>
<tr>
<td>$H_0$</td>
<td>0.383</td>
<td>0.443</td>
<td>0.111</td>
</tr>
<tr>
<td>$H_S$</td>
<td>0.398</td>
<td>0.424</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Note: $H_S$ is gene diversity. The one-tailed P value in each case is for the one-sided test that the larger value is larger than the smaller value. In the case of the mean assignment index, a Student’s t test was performed. Boldface type indicates the value associated with the dispersal.
The pattern of subpopulation differentiation revealed by F statistics is consistent with a scenario of colonization from the Northern Peninsula southeastward. F$_{ST}$ and R$_{ST}$ reveal a similar pattern of differentiation, with the greatest level of genetic differentiation between the Northern and Baie Verte peninsulas, and lower and comparable levels between samples from the Northern and Bonavista peninsulas and from the Baie Verte and Bonavista peninsulas. F$_{ST}$ values are higher and more strongly significant than R$_{ST}$ values, suggesting that drift rather than mutation is the predominant factor contributing to differentiation. In this situation, F$_{ST}$ is recommended as the metric of differentiation because of its lower variance (Hardy et al. 2003). Accordingly, we hypothesize that bears migrated from the Northern Peninsula in a southeasterly fashion to the Baie Verte Peninsula at some more distant time in the past, after which these two populations diverged by genetic drift. More recently, migrants from each of these populations made their way to the Bonavista Peninsula, where according to F$_{ST}$, this population diverged from its two source populations by drift.

The STRUCTURE result is consistent with the presence of three clusters in the sample, suggesting that indeed the three geographical sampling locations are significantly if not three clusters in the sample, suggesting that indeed the three geographical sampling locations are significantly if not distinct from its two source populations by drift. The STRUCTURE result is consistent with the presence of three clusters in the sample, suggesting that indeed the three geographical sampling locations are significantly if not three clusters in the sample, suggesting that indeed the three geographical sampling locations are significantly if not distinct from its two source populations by drift. In other words, the subpopulation from the Northern Peninsula is isolated from the two more easterly localities, and supports a more recently evolved differentiation between the latter two localities relative to the former. Support for this hypothesis also comes from the positive F$_{IS}$ signature of this population, which is consistent with admixture. The posterior probability of assignment results assign the ancestry of 4%, 23%, and 19% of individuals within each of Northern, Baie Verte, and Bonavista peninsulas, respectively, to one or both of the other populations (Fig. 3). These results can be used to make inferences about contemporary patterns of gene flow and suggest that the historical pattern indicated by F$_{ST}$ is ongoing. In other words, the subpopulation from the Northern Peninsula is isolated from the two more easterly localities, which show clear evidence of mixture. The misassigned individuals (indicated by stars in Fig. 3) in the Baie Verte Peninsula are primarily from the Bonavista Peninsula, whereas the misassigned individuals in the Bonavista Peninsula come from both of the other two localities, again consistent with admixture in this locality.

Although little is known about the ecology and movements of Newfoundland black bears, there are few geographic barriers like habitat fragmentation to limit movement between the areas included in this study, as have been found in other studies (e.g., Dixon et al. 2007; Hellgren et al. 2005). Even those that do so (Payne 1975). The lack of barriers to bear movement would suggest that there should be continuous gene flow among study sites, as we find between bears in the most easterly study area (Bonavista Peninsula) and the two other sites. In contrast, we find clear genetic differentiation between our two most westerly study areas, Northern Peninsula and Baie Verte Peninsula. These results suggest that the Northern Peninsula may be at the capacity for bears and there is movement between the easterly localities where bears are less densely distributed. As there are no data available on bear densities, we are not able to test this hypothesis.

The results of the biased dispersal analysis, in which differentiation statistics were compared between sexes (Table 4), make it clear that males were, at least historically, the predominant dispersing sex, as is observed in many mammals (see Chepko-Sade and Halpin 1987). However, the posterior probability of misassignment results from STRUCTURE (Fig. 3) indicated that half of the class of individuals experiencing ongoing movement are female.

Movement of both male and female black bears has been reported in a number of other studies. Reported ranges of bear movements vary dramatically depending on geographic barriers and human activity affecting each population (Hellgren et al. 2005). Subadult females can be recruited into their natal areas (Alt 1978; Reynolds and Beecham 1980; Rogers 1987); however, high bear density (Wathen et al. 1985), less productive ecosystems (Mowat et al. 2005), and increased human activity (Clark et al. 2002) have all implicated as pressures that may lead to movement. As little is known about the ecology of black bears in Newfoundland, including densities and home-range sizes, it is difficult to know which factors are driving movement in this island population.

This is the first island-wide intensive effort to study the genetics of black bears in Newfoundland, and one of the few genetic studies of any terrestrial Newfoundland mammal. We find levels of genetic variation are low, as previously reported, but sufficient to detect population structure. We note that the levels of genetic variation are slightly higher in more northwesterly localities, and that the location studied by previous authors (Terra Nova National Park) is adjacent to our least diverse locality (Bonavista Peninsula). We find a pattern of diversity that is consistent with inferred routes of colonization history of other mammals on the island. We find a historical pattern of classic mammalian dispersal and report the intriguing observation that females and males are equally likely to be moving.

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**References**


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**Note:** The text contains references to figures and tables that are not visible in the image, and some technical terms related to genetics and population biology. The natural text representation maintains the flow and coherence of the original content, focusing on the main points and arguments presented in the document.