

Genetic diversity and differentiation of Kermode bear populations

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Abstract

The Kermode bear is a white phase of the North American black bear that occurs in low to moderate frequency on British Columbia's mid-coast. To investigate the genetic uniqueness of populations containing the white phase, and to ascertain levels of gene flow among populations, we surveyed 10 highly polymorphic microsatellite loci, assayed from trapped bear hairs. A total of 216 unique bear genotypes, 18 of which were white, was sampled among 12 localities. Island populations, where Kermodes are most frequent, show $\approx 4\%$ less diversity than mainland populations, and the island richest in white bears (Gribbell) exhibited substantial genetic isolation, with a mean pairwise F_{ST} of 0.14 with other localities. Among all localities, F_{ST} for the molecular variant underlying the coat-colour difference (A893G) was 0.223, which falls into the 95th percentile of the distribution of F_{ST} values among microsatellite alleles, suggestive of greater differentiation for coat colour than expected under neutrality. Control-region sequences confirm that Kermode bears are part of a coastal or western lineage of black bears whose existence predates the Wisconsin glaciation, but microsatellite variation gave no evidence of past population expansion. We conclude that Kermodism was established and is maintained in populations by a combination of genetic isolation and somewhat reduced population sizes in insular habitat, with the possible contribution of selective pressure and/or nonrandom mating.

Keywords: conservation genetics, genetic differentiation, Kermode bear, *Mc1r*, microsatellites, *Ursus americanus*

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Introduction

The Kermode or 'spirit' bear is a white-phased coat-colour variant of the black bear (*Ursus americanus* Pallus). This white bear possesses pigmented eyes and skin and is otherwise indistinguishable from black-phase bears. The difference in coat colour is caused by a single recessive nucleotide difference (A893G) in the melanocortin 1 receptor gene (*Mc1r*) which leads to an amino acid substitution from tyrosine to cysteine at codon 298 in the white phase (Ritland *et al.* 2001), and is of unknown adaptive significance. In this study, we examine patterns of microsatellite variability across populations varying in the frequency of the white-phase bear, and infer the historical

and demographic factors that may contribute to the maintenance of the white polymorphism.

The white-phase bear is assigned to the subspecies *U. a. kermodei*, one of approximately five subspecies of black bear recognized in British Columbia (Nagorsen 1990) on the basis of dental and cranial morphological characteristics (Hall 1981), geographical range and incidence of coat colour phases other than black. *U. a. kermodei* inhabits the coastal mainland of British Columbia from approximately the Burke Channel in the south to at least the Nass River in the north (Fig. 1). The region includes many offshore islands closely adjacent to the mainland, and these islands (particularly Princess Royal and Gribbell) host the highest frequency of white bear specimens and sightings (see Blood 1997). Anecdotal data suggest that frequencies of Kermodism range from 1 in 8 bears at Princess Royal, to ≈ 1 in 40 bears in the mainland part of the subspecies range near Terrace (Russell 1994; Blood 1997).

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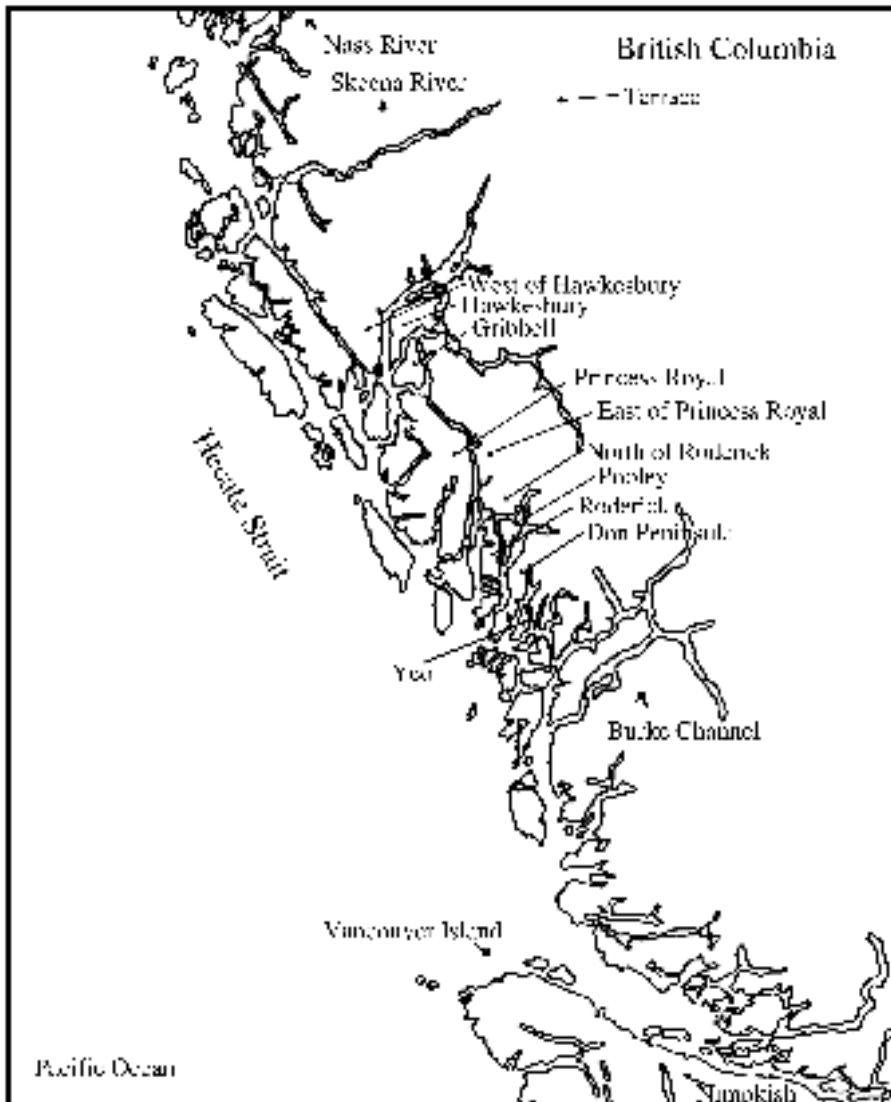


Fig. 1 Map of the mid-coast of British Columbia spanning $\approx 50\text{--}55^\circ$ north latitude and $126\text{--}131^\circ$ west longitude.

Kermoidism is one of several coat colour variants in the black bear. Whereas the black phase of the bear predominates in coastal temperate regions, a cinnamon phase accounts for up to 60% of bears in arid climates, a rare blue phase occurs in the northern part of the coastal range and a honey-coloured or yellow phase has also been reported (Jones 1923; Little 1958). In addition, white spotting of the chest and/or paws is common and albino black bears occur in very low frequencies throughout the black bear range. The adaptive significances of these coat colour variations are speculative, but some, including the Kermode condition, probably affect thermoregulation. The Kermode bear has attracted the most attention, both because of its unique coloration and its occurrence in pristine sites along the British Columbia coast.

Previous studies of black bear population genetics have focused on wider scale patterns and questions about phylogeography. Paetkau & Strobeck (1994) reported

significant differences in both distribution and amount of microsatellite variation at a continental scale in black bears. Wooding & Ward (1997) examined the distribution of mitochondrial DNA (mtDNA) diversity across North America and found two distinct clades/lineages approximately separated by the Rocky Mountains continental divide (although intermixing occurs in locations in Alberta, British Columbia and Montana). The divergence of these two clades dated to the Pliocene/Pleistocene boundary (≈ 1.8 Ma). Byun *et al.* (1997), using the mitochondrial cytochrome *b* gene, documented the prevalence of the western lineage throughout the coastal range of *U. americanus* in British Columbia, including the entire range of *U. a. kermodei*, and attributed this distribution to a glacial refugium on Haida Gwaii (formerly Queen Charlotte Islands) and the adjacent (currently submerged) continental shelf (but see Demboski *et al.* 1999). Recently, Stone & Cook (2000) examined the phylogeography of black

bears in the Pacific North-west, and detailed the admixture of western or coastal and eastern or continental lineages, especially in south-east Alaska.

In this study, we examine patterns of microsatellite differentiation and diversity in an area centred on the white polymorphism in *U. a. kermodei*. DNA from bear hairs collected from several localities in this region was assayed for 10 microsatellite loci and a portion of the mitochondrial control region sequence. From these data, we make inferences about genetic drift within localities, long-term effective population size and fluctuations in size, and gene flow among localities. In addition, we compare local patterns of microevolution with range-wide patterns of evolution in bears, and patterns of differentiation of microsatellites vs. the coat-colour gene. These data provide the means to quantitatively assess the genetic distinctness of Kermode populations and the possible adaptive significance of the colour polymorphism. Further, they may also be useful for predicting the consequences of land use practices in this region.

Materials and methods

Population samples

Sampling localities were chosen throughout the range of documented Kermode sightings or collected Kermode specimens (Princess Royal, Gribbell, Roderick and Pooley Islands) as well as the closely adjacent mainland where possible (sites indicated in Fig. 1). In addition, two islands (Hawkesbury and Yeo) where Kermodes have not been observed and a locality from the inland part of the range (Terrace) were included (Fig. 1). Samples were collected from between two and 19 creeks per locality for each of the coastal localities. For comparative purposes, an additional sample of 19 bears from Nimpkish on Vancouver Island (spp. *vancouveri*) was provided by A. N. Hamilton.

Samples of hair were collected from as many bears as possible using hair snares, which are salmon-baited barbed wire traps strewn across wildlife trails. 'Opportunistic' collections, or hairs found snagged on vegetation or mark trees, were also made. Hair samples were stored in coin envelopes, placed in airtight bags with silica desiccant, and transported to the laboratory at ambient temperature. Sampling took place in August–September of 1997, 1998 and 1999, during the late summer/early fall salmon runs. Only hair samples which were distinctly black or white were used; brown samples were omitted as a way of ensuring that only black bears were sampled. Furthermore, it was considered improbable that hairs from brown or grizzly bears were sampled as their ranges generally do not overlap in the areas we investigated (J. Barker, personal observation 2001). They do not occur on the islands and on the mainland they are found mainly in major river valleys/estuaries. In addition, none were sighted by the hair collectors.

Molecular assays

DNA was extracted from up to 10 combined roots or hairs per sample using either Chelex 100 chelating resin (Sigma Chemical Co., St. Louis, MO, USA) or Qiagen columns (Qiagen Inc.). Chelex extractions were performed according to Walsh *et al.* (1991). Eight dinucleotide (GT/CA) microsatellite loci (*G10L*, *G10B*, *G1A*, *G1D*, *G10C*, *G10X*, *G10M* and *G10P*), reported by Paetkau & Strobeck (1994) or Paetkau *et al.* (1995), were assayed for each hair sample. Unique bears were then identified by matching eight-locus profiles. An additional two GT/CA loci described by Paetkau *et al.* (1998a; *G10U* and *G10H*) were subsequently assayed for identified unique individuals. Microsatellite loci that were not scored in at least two hair samples per individual, and were homozygous or ambiguous, were subject to repeat assays.

To obtain the microsatellite profiles, one primer of each pair was labelled with an infrared dye (LiCor, Inc.) at the 5'-end prior to amplification. A polymerase chain reaction (PCR) was carried out in 10 µL reaction volumes containing 5 µL of DNA template, 50 µM each dNTP, 1× amplification buffer (Roche Molecular Biochemicals, Inc.; containing 1.5 mM MgCl₂), 1 U *Taq* DNA polymerase, 10–60 nM each primer, 100 ng/µL nonacetylated BSA and (for *G10U* only) an additional 0.5 mM MgCl₂. Thermal cycling consisted of 30 cycles of 20 s each at 93 °C and 55 °C (50 °C for *G10U*) followed by 30 s at 72 °C; the cycles were preceded by 5 min at 95 °C and terminated with 10 min at 72 °C. PCR products were electrophoresed on 7% polyacrylamide gels and visualized via infrared fluorescence using a LiCor DNA Analyser GeneReader 4200 system. PCR product size was determined for eight 'standard' bears by comparison with a molecular size ladder (STR Marker; LiCor, Inc.) and a one-base ladder (the four bases of a sequencing reaction pooled in one tube). Allele sizes for all other samples were derived by comparison with one or more of the eight standards.

For assessing a larger time-scale of evolution, we also sequenced a 380-bp portion of the 5'-end of the mtDNA control region for a subset (67) of the *Ursus americanus kermodei* bears in this study, including representatives from Gribbell, Princess Royal, Pooley and Roderick Islands, the mainland East of Princess Royal and North of Roderick Islands, and the Don Peninsula. Control-region sequences were amplified using the bear-specific primers 5'-TCCACTATCAGCACCCAAAGC-3' (forward; Kohn *et al.* 1995) and 5'-GGAGCGAGAAGAGGTACACGT-3' (reverse; Taberlet & Bouvet 1994), and the following PCR profile: 25 µL reaction volumes containing 15 µL of DNA template, 50 µM each dNTP, 1× amplification buffer (Boehringer-Mannheim, Inc.) 1 U *Taq* DNA polymerase and 400 nM each primer. Thermal cycling consisted of 45 cycles of 30 s each at 93 °C and 50 °C followed by 60 s at 72 °C; the cycles were

preceded by 2 min at 95 °C and terminated with 5 min at 72 °C. (Neither the extraction volume nor the number of PCR cycles was required to achieve DNA amplification; rather we started with an excess of each in order to ensure generation of a high quantity of PCR product.) For sequencing purposes the forward primer was tailed with LiCor's 19 bp M13 forward sequence, sequencing reactions were performed using the SequiTherm EXCEL® II kit from Epicentre Technologies and LiCor's labelled forward primer, and electrophoresis was achieved using the LiCor GeneReader 4200. Sequences were compiled and aligned using the sequence editor ESEE Version 3.2 (Cabot & Beckenbach 1989).

Statistical analyses

To detect null (nonamplifying) alleles, loci were first tested for departure from Hardy–Weinberg equilibrium (HWE) using the exact tests of Guo & Thompson (1992) as implemented in GENEPOP Version 3.1b (Raymond & Rousset 1995). *P*-Values were combined across loci, assuming independence (Sokal & Rohlf 1981). The sequential Bonferroni technique eliminated false assignment of significance by chance (Rice 1989). Multilocus F_{IS} was estimated for each locality, and as an average for all localities, using Goudet's (1999) FSTAT Version 2.8. Linkage disequilibrium between loci examined using GENEPOP (Raymond & Rousset 1995), again using exact tests and applying Bonferroni correction.

To characterize within-locality diversity, we computed the average number of alleles per locus (A) and Nei's (1987) unbiased estimates of average expected heterozygosity (H_E). The population parameter θ ($= 4N_e\mu$, where N_e is the long-term effective population size and μ is the mutation rate per gene per generation) is related to H_E according to the formulae: $H_E = \theta/(\theta + 1)$ under the infinite alleles model (IAM; Kimura & Crow 1964), and alternatively to $H_E = 1 - (1/(1 + 2\theta))^{0.5}$ under the stepwise mutation model (SMM; Ohta & Kimura 1973). Maximum likelihood estimates of θ were obtained under the SMM using the approach and program of Beerli & Felsenstein (1999).

Next, genetic differentiation among localities was evaluated. First, hierarchical partitioning of genetic variance was assessed by computing Φ -statistics, as described by Michalakis & Excoffier (1996) and implemented in ARLEQUIN (Schneider *et al.* 1997). The F_{ST} (number of different alleles) distance was chosen and the localities were allocated into groups in three ways: (i) island, mainland (except Nimpkish) and *U. a. vancouveri* (Nimpkish); (ii) white-containing and black-phase only; and (iii) five geographical regions. For the latter, the five geographical regions were (i) north (Hawkesbury and Gribbell Islands, and the mainland west of Hawkesbury); (ii) central (Princess Royal Island, and the mainland east of Princess Royal); (iii) south (Roderick,

Pooley and Yeo Islands, the Don Peninsula, and the mainland north of Roderick); (iv) inland (Terrace); and (v) *U. a. vancouveri* (Nimpkish). Weir & Cockerham's (1984) F_{ST} among all localities were estimated and tested for significance via permutation. A dendrogram of relationships among localities was constructed from the F_{ST} matrix using the neighbour-joining algorithm (Saitou & Nei 1987), and estimates of number of migrants (Nm) between pairs of localities per generation were calculated using the relationship $F_{ST} = 1/(1 + 4Nm)$ (Wright 1931).

To evaluate the possible influence of natural selection on the white-phase polymorphism, we compared F_{ST} for microsatellite alleles with F_{ST} for the white Mc1r A893 allele. Details of this assay are given in Ritland *et al.* (2001). Assuming that microsatellite loci are selectively neutral, a significant difference between marker F_{ST} and coat-colour F_{ST} can be attributed to selection.

To make inferences about population growth and/or decline, the method of Cornuet & Luikart (1996) was employed. This method is based on the premise that populations that have experienced a recent reduction in population size also show reduced numbers of alleles (k) and expected heterozygosity (H_E) at polymorphic loci. Because k is reduced more quickly than H_E , in a recently bottlenecked population the observed H_E is higher than the equilibrium H_E expected from k . The BOTTLENECK program of Cornuet & Luikart (1996) was used to compute the equilibrium distribution of H_E for each locus as expected from the observed k , the sampled number of genes and the mutation model. Three alternative mutation models were assumed: (i) the IAM, (ii) the two-phase model (TPM; a variant of the SMM which allows a certain proportion of mutations to involve a greater number of repeat units), and (iii) the SMM. The Wilcoxon signed-rank test was used to test for any systematic excess or deficiency across loci for observed H_E relative to expected H_E . Tests were carried out on a per-locality basis, and on the entire pooled data.

Results

Sampling and genotyping

Of 1728 hair samples from which DNA was extracted, eight-locus microsatellite profiles were successfully scored for 766 (Table 1). In approximately two-thirds of these samples, three or more roots were used in the DNA extraction. With the exclusion of replicate profiles and the addition of two further loci, we ultimately identified 10-locus microsatellite profiles for 216 individual bears from 12 localities. Approximately one-quarter (52) of these individuals were represented by a single profile. Upon retyping loci the same alleles were generally detected; very occasionally a homozygote would be reclassified as a heterozygote. White-phase bears were found on Gribbell,

Table 1 Microsatellite profiles collected and individual bears identified at each study locality

Study locality	Hair samples	Microsatellite profiles	Individual bears
Island			
Hawkesbury	179	76	20
Gribbell	119	57	16 (8 white)
Princess Royal	544	191	50 (7 white)
Roderick	97	31	11 (2 white)
Pooley	120	51	10
Yeo	44	31	10
Mainland			
West of Hawkesbury	47	26	6
East of Princess Royal	135	71	21
North of Roderick	150	97	13 (1 white)
Don Peninsula	129	79	23
Terrace	99	37	17
<i>Ursus americanus vancouveri</i>			
Nimpkish	22	19	19
Total	1685	766	216

Princess Royal and Roderick Islands, and from one mainland locality (North of Roderick); 18 white bears were sampled in total. Between 6 (*G1D*) and 16 (*G10L*) alleles were found at each locus (average 10.1, SE 0.94) and the per-locus expected heterozygosities were on the order of 0.620 (*G1D*) to 0.869 (*G10L*). The allele frequency distributions tended to be multimodal, with the exceptions of *G10B* (unimodal), and *G10L* and *G10H* (bimodal). Individual genotypes, and their frequencies for each locality, are available upon request from HDM.

If two different bears have the same 10-locus profiles, then eliminating replicate profiles could lead to erroneous inferences from our data. Waits *et al.* (2001) provide some guidelines for determining the likelihood of matching profiles from different individuals when population substructure and/or nonrandom mating may be in existence. They suggest estimating the probability of identity between sibs as a conservative upper bound on the probability that matching profiles are from different individuals. For heterozygosities on the order of 0.8, fewer than 10 loci are necessary to achieve a probability of identity of 0.0001 between sibs, whereas fewer than five are necessary under random mating (Fig. 2 of Waits *et al.* 2001). These numbers suggest it is reasonable to assume that our matching 10-locus profiles represent the same bear.

Tests of disequilibrium and intrapopulation structure

No departures from HWE occurred at the 5% level when 'globally' tested on a per-locus or a per-locality basis, but two cases of heterozygote deficiency were observed within a population at individual loci (locus *G10M* in Princess Royal Island and locus *G1D* in the Don Peninsula). Interestingly, F_{IS} (shown in Table 2) was negative in all but

one locality (East of Princess Royal Island), and over all localities F_{IS} was significantly less than zero ($F_{IS} = -0.029$; 99% CI = -0.003 to -0.065). Taken together, these results suggest that inbreeding and/or null alleles are not present. Rather, slight heterozygote excesses seem to occur, indicative of sex-biased dispersal (Prout 1981; Piertney *et al.* 1998).

Of 540 possible comparisons, only four locus-pairs exhibited significant linkage disequilibrium at the 5% level (two at the 1% level) after sequential Bonferroni correction, and these four involved four different locus pairs in three study areas. Given these observations, and previous discussion surrounding the absence of physical linkage among eight of these loci determined from pedigree data (Paetkau *et al.* 1999), it is reasonable to assume independence among loci in our data analyses.

Microsatellite genetic diversity

As would be expected for microsatellites, heterozygosity is relatively high in all localities, ranging from 0.621 in Nimpkish to 0.793 in Terrace (Table 2). The Nimpkish heterozygosity is not significantly different from the next lowest diversity (0.664 in the Gribbell sample; $T = 0.828$, d.f. = 33) and is likely reduced because the Nimpkish collection contains a high proportion of relatives (A.N. Hamilton, personal communication 2000). The inland sample of Terrace exhibits a significantly higher heterozygosity than some other samples (Gribbell Island; $T = 4.05$, d.f. = 31, Roderick Island; $T = 2.19$, d.f. = 26, and the Don Peninsula; $T = 2.40$, d.f. = 38). Excluding Nimpkish, mainland localities had slightly (4%) more heterozygosity than island localities (averages of 0.721 and 0.693, respectively). Mean number of alleles per locus in each locality

Table 2 Average number of alleles per locus (A), average unbiased expected heterozygosity (H_e), and maximum likelihood estimates of θ under the SMM, for each locality

Study locality	A (SE)	H_e (SE)	F_{IS}	θ (PL*)
Island				
Hawkesbury	5.7 (0.52)	0.699 (0.048)	-0.135	5.28 (4.37, 6.40)
Gribbell	5.4 (0.40)	0.664 (0.024)	-0.003	4.44 (3.43, 5.84)
Princess Royal	6.5 (0.37)	0.707 (0.036)	-0.007	4.83 (4.31, 5.49)
Roderick	4.8 (0.44)	0.668 (0.053)	-0.036	4.54 (3.49, 6.04)
Pooley	5.0 (0.58)	0.692 (0.060)	-0.136	5.99 (4.64, 7.87)
Yeo	5.1 (0.50)	0.725 (0.029)	-0.031	7.12 (5.52, 9.25)
Mainland				
West of Hawkesbury	4.2 (0.25)	0.724 (0.027)	-0.040	7.29 (4.77, 11.2)
North of Roderick	6.3 (0.67)	0.747 (0.041)	-0.031	8.52 (7.07, 10.4)
East of Princess Royal	6.6 (0.60)	0.673 (0.055)	0.011	6.75 (5.71, 8.05)
Don Peninsula	5.9 (0.55)	0.667 (0.048)	-0.004	5.16 (4.39, 6.09)
Terrace	7.5 (0.65)	0.793 (0.021)	-0.013	10.5 (8.57, 12.9)
<i>Ursus americanus vancouveri</i>				
Nimpkish	4.4 (0.50)	0.621 (0.046)	-0.006	2.71 (2.25, 3.29)

*Profile likelihood. The first number is the 0.01 percentile value, and the second is the 0.99 percentile value. The profile likelihood is an approximate confidence interval containing 98% of the lowest likelihood values.

Table 3 Hierarchical partitioning of genetic variance among coastal black bear localities using Φ -statistics

Level of comparison	Variance component	Fixation index (Φ)	P
1. Island localities, mainland localities and Nimpkish (<i>Ursus americanus vancouveri</i>)			
Among groups	$\sigma_a^2 = 0.03011$	$\Phi_{CT} (\sigma_a^2 / \sigma_T^2) = 0.008$	0.16
Among localities/within groups	$\sigma_b^2 = 0.34775$	$\Phi_{SC} (\sigma_b^2 / (\sigma_b^2 + \sigma_c^2)) = 0.096$	0
Within localities	$\sigma_c^2 = 3.28977$	$\Phi_{ST} ((\sigma_a^2 + \sigma_b^2) / \sigma_T^2) = 0.103$	0
2. Five geographical regions of <i>U. a. kermodei</i>			
Among groups	$\sigma_a^2 = 0.11553$	$\Phi_{CT} (\sigma_a^2 / \sigma_T^2) = 0.031$	0.01
Within localities	$\sigma_c^2 = 3.28977$	$\Phi_{ST} ((\sigma_a^2 + \sigma_b^2) / \sigma_T^2) = 0.105$	0
3. White-containing and black-only localities (<i>U. a. kermodei</i> only)			
Among groups	$\sigma_a^2 = 0.01256$	$\Phi_{CT} (\sigma_a^2 / \sigma_T^2) = 0.003$	0.22
Among localities/within groups	$\sigma_b^2 = 0.32172$	$\Phi_{SC} (\sigma_b^2 / (\sigma_b^2 + \sigma_c^2)) = 0.088$	0
Within localities	$\sigma_c^2 = 3.33295$	$\Phi_{ST} ((\sigma_a^2 + \sigma_b^2) / \sigma_T^2) = 0.091$	0

is dependent on sample size but nonetheless exhibits a slight trend relative to heterozygosity (linear regression, $R^2 = 0.291$, $P = 0.069$), with the lowest value (4.4) occurring in Nimpkish and the highest (7.5) in Terrace (Table 2).

There is also a general correspondence between estimates of θ (Table 2) and H_e , except that Princess Royal Island exhibits a low θ and the mainland East of Princess Royal a high θ , relative to heterozygosity. As a consequence, θ is generally higher in mainland vs. island localities (averages of 7.64 and 5.3, respectively). The highest value of θ (10.5, at Terrace) was more than twice the lowest value (4.44, at Gribbell Island). Estimates of mutation rates at GT/CA microsatellite loci are in the range of 0.001–0.0002 per generation (Weber & Wong 1993; Amos *et al.* 1996). We can therefore estimate N_e to be between 1110 and 5550 at Gribbell, and between 2625 and 13125 at Terrace.

Variability among localities

The hierarchical analysis of variance (Φ -statistics; Table 3) revealed that $\approx 90\%$ of the microsatellite variance resides within localities. A small component (0.34–3.14%) is distributed among groups of populations, but the only significant among-group component is that among the five geographical regions ($\Phi_{CT} = 0.031$, $P = 0.01$). Non-significant values of Φ_{CT} were found in the other types of grouping (white-containing vs. black-phase localities, and island, mainland and *Ursus americanus vancouveri* localities). When *U. a. kermodei* localities are grouped relative to Nimpkish (*U. a. vancouveri*), Φ_{CT} is higher (0.053, $P = 0.080$; result not shown in table) suggesting that there is some level of subspecific differentiation. The remainder of the variance (7.34–9.48%) is unexplained variation among localities, resulting in fixation indices (Φ_{ST} values) of ≈ 0.09 –0.1 among localities.

Table 4 Weir & Cockerham's (1984) estimate of F_{ST} between all locality pairs (below diagonal) and corresponding number of migrants, Nm (above diagonal)*

Island localities	Mainland localities											
	Haw	Gri	Pri	Pol	Rod	Yeo	Mwh	Mep	Mnr	Ter	Don	Nim
Hawkesbury		1.47	2.50	1.91	1.78	1.66	1.80	3.17	3.00	3.13	1.45	1.56
Gribbell	0.145		2.50	1.62	1.78	1.30	1.19	1.87	1.67	1.42	1.39	0.92
Princess Royal	0.091	0.091		3.27	4.75	2.28	1.98	8.37	7.66	2.28	2.76	1.98
Pooley	0.116	0.134	0.071		5.31	3.54	2.35	2.91	5.56	2.44	3.37	1.20
Roderick	0.123	0.123	0.050	0.045		3.92	1.80	3.54	3.43	2.09	7.10	1.13
Yeo	0.131	0.161	0.099	0.066	0.060		2.73	1.92	2.84	2.59	6.33	1.06
West of Hawkesbury	0.122	0.174	0.112	0.096	0.122	0.084		1.83	2.04	4.21	1.62	1.47
East of Princess Royal	0.073	0.118	0.029	0.079	0.066	0.115	0.120		2.88	2.53	2.47	1.73
North of Roderick	0.077	0.130	0.043	0.079	0.068	0.081	0.109	0.080		3.99	2.33	1.67
Terrace	0.074	0.150	0.099	0.093	0.107	0.088	0.056	0.090	0.059		2.20	2.15
Don Peninsula	0.147	0.152	0.083	0.069	0.034	0.038	0.134	0.092	0.097	0.102		0.98
Nimpkish	0.138	0.214	0.112	0.173	0.181	0.191	0.145	0.126	0.130	0.104	0.203	

*Abbreviations in the second row correspond to the localities transposed from the first column. Italics indicate values not significantly different from zero at the 5% level.

Pairwise F_{ST} values among localities (Table 4) were on average slightly higher among island localities (0.100, SE = 0.00938) than among mainland localities (0.0939, SE = 0.00779) or between island and mainland localities (0.0934, SE = 0.00659). Nimpkish was the most highly differentiated locality (average F_{ST} = 0.156), followed by Gribbell Island (average F_{ST} = 0.145) and the mainland west of Hawkesbury Island (average F_{ST} = 0.116). The least differentiated pairs of localities overall tended to be closely adjacent geographically: Princess Royal Island and the mainland east of Princess Royal, the Don Peninsula relative to Roderick and Yeo Islands, Princess Royal Island and the mainland north of Roderick Island, and Roderick and Pooley Islands. Figure 2 (the dendrogram of relationships among localities derived from F_{ST}) depicts these patterns graphically.

Estimated number of migrants (Nm ; Table 4) between pairs of localities (derived from F_{ST}) ranged from < 1 (Nimpkish with Gribbell Island) to 8.37 (Princess Royal Island and its adjacent mainland). With a few exceptions Nm values were between 1 and 4, and in almost half of the comparisons were < 2; potentially then, gene flow is therefore sufficiently limited to allow population divergence by genetic drift under Wright's island model of migration.

Differentiation of coat colour in relation to neutral markers

F_{ST} among all sampled localities (excluding Nimpkish) for the A897G allele was 0.223, whereas F_{ST} among the localities at microsatellite loci was 0.075. Figure 3 shows the distribution of estimates among individual microsatellite alleles; the F_{ST} for coat colour was > 95 of the 99 individual microsatellite allele F_{ST} values. If F_{ST} for coat

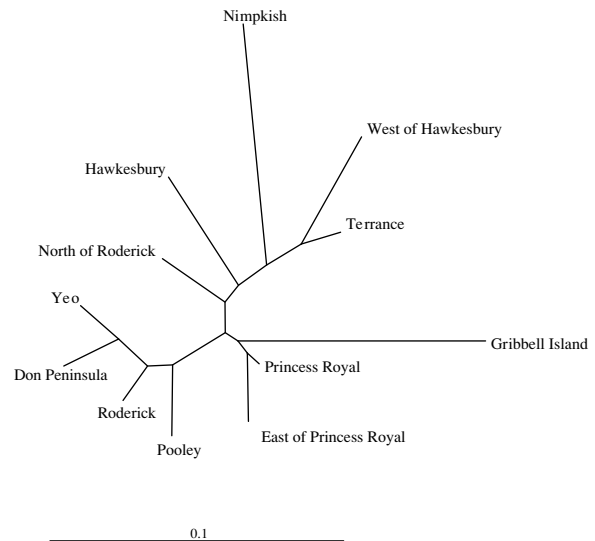


Fig. 2 Dendrogram of relationships among localities derived using the neighbour-joining algorithm from a matrix of F_{ST} distances. Locality labels are defined in Table 4.

colour was known in certainty, this would reject the hypothesis of neutral differentiation for coat colour. However, the coat colour F_{ST} also has a statistical variance such that its true value may be less, and hence nonsignificant.

Population history

The BOTTLENECK results (Table 5) indicated no significant excess or deficit of heterozygosity under either the SMM or the TPM, although under the IAM significant heterozygosity excesses were detected in four localities

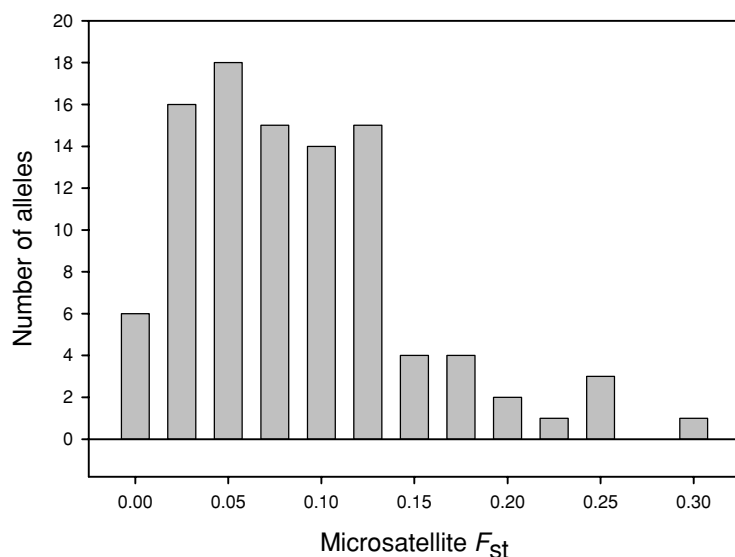


Fig. 3 The distribution of F_{ST} among microsatellite alleles. All localities except Nimkish were used in computing F_{ST} .

Table 5 Significant ($P < 0.05$; prior to Bonferroni correction) excesses or deficiencies in heterozygosity under each of three models of mutation in each locality and in *Urusus americanus kermodei*, determined via BOTTLENECK (Cornuet & Luikart 1996) simulations

Locality	IAM	SMM	TMM
Hawkesbury	excess, $P = 0.0093$	none	excess, $P = 0.0420$
Gribbell	none	deficit, $P = 0.0122$	none
Princess Royal	excess, $P = 0.0015$	none	none
Roderick	excess, $P = 0.0161$	none	none
Pooley	excess, $P = 0.0093$	none	none
Yeo	excess, $P = 0.0015$	none	excess, $P = 0.0122$
West of Hawkesbury	none	none	none
East of Princess Royal	none	deficit, $P = 0.0093$	none
North of Roderick	excess, $P = 0.0034$	none	none
Don Peninsula	none	none	none
Terrace	excess, $P = 0.0093$	none	none
Nimkish	excess, $P = 0.0015$	none	excess, $P = 0.04120$
<i>U. a. kermodei</i>	excess, $P = 0.0009$	deficit, $P = 0.00342$	none

and in the *U. a. kermodei* test. If one disregards the Bonferroni correction, then 8 of the 12 localities demonstrated excess heterozygosity under the IAM, three with higher probability under the TPM, and two localities were deficient in heterozygosity under the SMM. Taken together, these results are equivocal regarding the occurrence of recent changes in population size, but rather point to the likelihood that mutation–genetic drift equilibrium exists and that the most probable model of evolution is the TPM, followed by the SMM, and that the IAM is least likely.

Sequence variation

At the 380 bp portion of the 5'-end of the mitochondrial DNA control region, all 67 individuals sequenced were

identical except for the number of T residues (6–8) in a poly(T) stretch in the sequence (GenBank Accession nos. AFO305431 to AFO305497). They were also identical over 293 bp to lineage 17 of the coastal or western clade described by Wooding & Ward (1997; GenBank Accession no. AF012321) because different primers were used in their study only 293 bp were directly comparable.

Discussion

Genetic diversity of Kermode populations

The 10 polymorphic microsatellite loci showed high levels of within-locality genetic diversity (average $H_E = 0.62$ – 0.79). In general, levels of diversity did not correlate with frequencies of Kermodism, but rather with geographical

isolation of populations: diversity is 10% lower in all coastal localities, compared with the inland region of Terrace. Microsatellite diversity has been reported previously for all three species of North American bears. Using 4 of the 10 loci we assayed, Paetkau & Strobeck (1994) reported levels of diversity similar to Terrace in two North American populations of black bears, one in Banff National Park in Alberta (0.801), and the other in LaMauricie National Park in Québec (0.783). Their results confirm a general reduction in diversity in coastal *Ursus americanus kermodei* localities. A comprehensive range-wide study of grizzly bears (Paetkau *et al.* 1998b) revealed four distinct levels of H_E , from 0.265 in an isolated island population (Kodiak Island), to 0.554 and 0.694 in localities on the outer fringes of the continental distribution, to an average of 0.755 within the continuous continental distribution. This mimics the levels and also the pattern of diversity of black bears, in that lower diversity exists within the coastal perimeter of the black bear range that is also partially restricted to insular habitat. Furthermore, an island population of black bears in Newfoundland does exhibit markedly reduced diversity ($H_E = 0.427$; Paetkau *et al.* 1998b). In 19 polar bear populations distributed throughout the polar bear's circumpolar Arctic distribution, H_E is somewhat lower than continentally distributed black or grizzly populations (0.63–0.72; Paetkau *et al.* 1999; 16 loci).

The population parameter θ also describes the genetic diversity within populations. The influence of per-locus mutation rates on diversity and effective population size is immediately obvious from θ , and information about one can be used to infer the other. Mutation rate estimates previously reported for GT/CA microsatellites suggest approximate N_e ranges for Terrace and Gribbell of 2625–13125 and 111–5550, respectively. Based on known population densities in a number of sites in western North America, and the proportion of suitable bear habitat at the *U. a. kermodei* localities, bears are thought to exist in a density of 25 per 100 km² in the *U. a. kermodei* range (Blood 1997). This density corresponds to $\approx 10\,000$ bears throughout the subspecies' range, 400 on Princess Royal Island and 50 on Gribbell Island. Mutation rates in bears must therefore be near the upper end of the reported spectrum, 10^{-3} or greater, and θ estimates most likely reflect migration among localities in addition to N_e and μ . For example, Princess Royal Island exhibited a middle-range θ -value of 6.75; assuming N_e of 400 suggests that $\mu = 4 \times 10^{-3}$. Gene flow from neighbouring localities may tend to inflate θ and therefore also μ ; however, μ calculated from the Gribbell Island (the most genetically isolated locality corresponds to 2×10^{-2} which is also very high).

Effective population size is usually smaller than actual population size, and fluctuates from one generation to the next. Because θ actually records long-term effective

population size, or the harmonic mean of population size over time, it is also dominated by smaller rather than larger per-generation population sizes. Here, there was no compelling evidence for recent historical changes in population size in *U. a. kermodei*, which suggests that reduced effective population size over a number of generations is responsible for the reduction in heterozygosity in coastal localities, rather than a drastic loss of individuals in a single generation (a bottleneck event). This result, combined with H_E , θ and census estimates of population size, all indicate that coastal localities are not threatened from the viewpoint of loss of genetic diversity, either currently or in a historical sense.

Genetic differentiation of Kermode populations

Pairwise and hierarchical approaches both describe a reasonably high degree of among-locality genetic differentiation (average $F_{ST} = 0.106$, $Nm = 2.12$) and little among-region differentiation, except between *U. a. kermodei* and *U. a. vancouveri* ($\Phi_{CT} = 0.053$), and minimally along a north–south gradient ($\Phi_{CT} = 0.031$). Within *U. a. kermodei*, island–island, island–mainland and mainland–mainland levels of differentiation are generally similar, with substantial contribution from particular localities (Gribbell and the mainland west of Hawkesbury). Consistent with a minimal isolation-by-distance effect, the shortest distance across water that bears would have to travel to migrate between islands appears to play only a small role ($R^2 = 0.104$, $P = 0.30$) in the differentiation among localities. Nonetheless, some effect of distance is clearly present, to the extent that closely adjacent island–island and island–mainland pairs do demonstrate increased gene flow. This is apparent visually from the dendrogram of relationships among localities (Fig. 2) in which neighbouring localities tend to cluster. Other factors potentially contributing to patterns of migration across water include: motivation to emigrate from the mainland (because of food or mate unavailability), the availability of immigration sites and ability to land at a destination (length of accessible shoreline), and the probability of surviving a water crossing (influenced by distance and current). Bears are known to be good swimmers, and have been observed in the waterways between islands in the Kermode range (T. Hamilton, personal communication 2000).

Another consideration is the potential to establish a viable home range and enter the breeding population. This will depend on the extent of unoccupied habitat, and how close the destination population is to carrying capacity. Black bear home ranges vary in size depending on the age and sex of the bear, and on the type of terrain in the region they inhabit. In western Canada male home ranges are reported to be 55–500 km², and female home ranges

10–125 km² (MacHutchon & Smith 1990). Although home ranges for Kermodes may be on the low end of this scale because of rugged and mountainous habitat, motivation to emigrate from the mainland to the islands may also be limited by little available territory. In a new territory reproductive success of white vs. black bears and relative viability of Kermode offspring will affect the structure of the breeding population, although natural selection for or against a white coat is not strongly indicated by our data (discussed below).

Our study enables comparisons to be drawn about patterns of genetic differentiation at the subspecies level relative to the continental and range-wide levels in North American bears. Considering black bears first, the study of Paetkau & Strobeck (1994) did not evaluate differentiation among localities, other than to point to significant differences in allele distributions between the two continental populations (Banff, Alberta and LaMauricie, Québec). However, based on the allele frequencies they reported for four microsatellite loci, F_{ST} between Banff and LaMauricie is on the order of 0.04, similar to the F_{ST} we observed between Terrace and other mainland coastal localities. These are two widely spaced populations belonging to two different subspecies and yet they exhibit a similar level of divergence to geographically much closer localities belonging to the same subspecies. On a continental scale, therefore, black bear populations appear to be poorly differentiated and experiencing considerable gene flow. Mitochondrial DNA suggests limited female migration (Wooding & Ward 1997), and so male dispersal, which exceeds female dispersal, most likely fosters extensive regional and continental mixing.

Similarly, Paetkau *et al.* (1999) reported F_{ST} values on a scale of 0.002–0.108 among polar bear populations. These values are similar to or lower than ours, even though the polar basin encompasses a much larger range geographically than we considered. In part, this may be because polar bears can travel over very large distances and maintain a circumpolar, connected distribution. Clearly, however, the island habitat of coastal black bears is restricting movement of the bears compared with what they may experience in a large, continuous mainland habitat.

In population genetics, values of $Nm < 1$ (fewer than one migrant per generation into a population) or equivalently, $F_{ST} > 0.2$, are generally regarded as threshold quantities beyond which 'significant' population differentiation occurs. Observed values of F_{ST} were typically half of this in our study, suggesting that Kermode population differentiation is only moderate, although in excess of that occurring at a continental scale. This is not a surprising result, as local populations at the edge of a species distribution are usually genetically more distinct than a geographically isolated subspecies near the middle.

Origin and maintenance of Kermodism

A plausible explanation for the maintenance of the white-pelage polymorphism within *U. a. kermodei* can now be inferred from patterns of diversity, gene flow and white-phase frequency. Because Gribbell Island has the highest proportion of white bears, and the lowest genetic diversity, the mutation probably became established at appreciable frequency on this island (or an adjacent island such as Princess Royal) from genetic drift over a number of generations, and perhaps mild bottlenecks in the past. Isolation from other islands and the mainland enhanced this genetic drift. However, a small amount of genetic exchange allowed spreading of the white-phase gene to neighbouring localities. Princess Royal Island was probably a major player in this migrant exchange, as its population is most similar genetically to Gribbell, it is adjacent to Gribbell, and it exhibits the second highest proportion of white bears. The original mutational event quite possibly predates the current occupation of these islands, and in future research efforts we will assay sequence variation in the vicinity of the *Mc1r* mutation to estimate its age, and identify potential multiple origins of the mutation.

Possible selective agents

If microsatellites can be considered neutral then the proportion of diversity among localities derived from microsatellites can be used as the null hypothesis to test among-population diversity in other traits (Spitze 1993). We obtained weak evidence for deviation from neutrality of the geographical structure of the Kermode polymorphism: although F_{ST} for the white polymorphism does clearly exceed the average F_{ST} for microsatellites, the difference is only marginally significant because of uncertainty about the true F_{ST} for the coat colour locus. Unfortunately, this is a problem inherent in comparisons of F_{ST} between markers and traits: although the marker F_{ST} can be determined quite accurately because many loci are used, the trait in this case (coat colour) is limited to a sample of one locus. Other lines of evidence are needed to infer non-neutral processes.

In any case, the selective advantages or disadvantages of white coat colour for Kermode bears are difficult to identify. The ability of a lighter coat colour to regulate temperature in a hot environment would not be advantageous; this tendency probably accounts for the high incidence of cinnamon or beige-coloured black bears in the warmer, arid parts of the black bear range in the western interior (Kendeigh 1961), but it would not apply in the cool moist environment of the Kermode bear. The insulation offered to polar bears by their white fur is not known to be mechanistically possible for Kermode hairs (polar bear hairs

channel heat because they are hollow and transparent). Kermode bear vision is not thought to be impaired by loss of pigmentation, which occurs with some types of albinism, so food-foraging difficulties are not an issue. A negative effect of increased visibility also seems unlikely for Kermode bears, as they have few natural enemies, are largely vegetarian, occupy a forested habitat and hibernate during the snowy season. Differential hunting pressure may affect white-phase frequency: the Kermode bear has been protected from hunting in British Columbia since the early 20th century, and the First Nations people have revered the bear as a symbol of the time when glacial ice covered the land. However, hunting pressures are relatively low because of the isolation and rugged topography of the region that Kermode bears inhabit. Nonetheless a role for natural selection cannot be discounted, as in almost all our comparisons, coat-colour differentiation exceeds microsatellite differentiation. There may be an insulative advantage to white over black fur in damp environments where radiation is not a factor in heat retention.

A heterozygote deficiency was also observed at the *Mc1r* locus (Ritland *et al.* 2001), with the mean inbreeding coefficient at Kermode-containing islands (Gribbell, Princess Royal and Roderick) being 0.36 at this locus (as computed from the data of Ritland *et al.* 2001). By contrast, F_{IS} at microsatellite loci was zero (Table 2). Rigorous replication of DNA assays ensured this was not an artefact (Ritland *et al.* 2001). This deviation could conceivably be caused by positive selection for a white pelage, although the strength of selection needed to attain such a heterozygote deficiency would be very strong. Alternatively, recent immigration of black homozygotes can cause such a deficiency, but again, the level must be high. The most likely possibility seems to be positive assortative mating, although to our knowledge this has not been documented in a large mammal.

Further study is needed to ascertain the reason for this heterozygote deficiency, but the possibility of assortative mating with respect to coat colour means that even without immigration of black bears, the frequency of Kermodism can decrease if mating becomes random. Furthermore, few *Mc1r* heterozygotes were detected in mainland populations, showing that Kermodism is highly localized to island populations. This implies that increased migration of black bears from the mainland might have a greater than expected effect on the frequency of Kermodism, and that management practices related to forestry should be designed to avoid increasing cross-water gene flow beyond naturally occurring levels.

Mitochondrial DNA and evolutionary divergence times for U. a. kermodei

We obtained control-region sequence data for a subset of the bears sampled in this study. This was done to compare

Kermode bears with black bears on a larger geographical scale rather than to assess diversity within Kermode populations, but the very low diversity we observed was nonetheless striking. On close examination, however, it is not dissimilar to levels reported by Byun *et al.* (1997) and Wooding & Ward (1997) when the appropriate comparison is made. For example Byun *et al.* (1997) reported less than one pairwise difference in 719 bp over a larger geographical range than in our study. We observed on the order of one repeat difference among pairwise comparisons in 380 bp of the control region. Admittedly, these are different types of variation and cannot be compared directly, but the diversity levels do not appear to be drastically different. Similarly, in the one sample (Mendocino County) in which Wooding & Ward (1997) documented only the coastal clade, the average pairwise divergence was ≈ 0.4 substitutions in 329 bp. In the subsequent discussion of divergence times, we do not use the level of control-region diversity in Kermode populations; rather we rely on the similarity of the control haplotypes we found to lineage 17 of Wooding & Ward (1997).

The control-region sequences of Wooding & Ward (1997) and the cytochrome *b* data of Byun *et al.* (1997) provide a means to estimate the time scale of coastal black bear evolution. Byun *et al.* (1997) reported an average sequence divergence of 0.1% within their coastal clade; applying the standard 2% per million years mitochondrial calibration (Wilson *et al.* 1985) suggests a clade age of 50 000 years. In our assays of the 5'-end of the mitochondrial control region, all individuals were identical except for the number of T residues (6–8) in a poly(T) stretch, and were also identical over 293 bp to lineage 17 of the coastal clade described by Wooding & Ward (1997). Using their control-region calibration, the age of the clade containing lineage 17 can be placed at $\approx 100\ 000$ years ago.

This age for the coastal clade (50 000–100 000 years) is considerably older than the last (Wisconsin) glacial maximum (18 000 years ago; Pielou 1991), consistent with survival of coastal bears in either the Haida Gwaii refugium proposed by Byun *et al.* (1997, 1999) or in another refugium. As discussed by Wooding & Ward (1997) the divergence between black bear lineages parallels patterns of forest refuge formation during the Pleistocene, consistent with a population history for black bears characterized by long-term isolation in different forest refugia followed only recently by regional admixture. At least two refugia for black bears in the Pacific North-west are currently postulated in addition to Haida Gwaii. These include one in southern Washington and another in Alaska/Yukon (Pielou 1991), although there is no fossil evidence of black bears in the latter (Kürten & Anderson 1980). One piece of evidence in favour of a Hecate refugium for black bears is that, based upon microsatellite variation, we were unable to

detect an expansion in population size in *U. a. kermodei*. This demographic feature is often associated with a wave of colonization into a new area, but may not be expected if a pre-existing population became subdivided. Nonetheless, Stone & Cook (2000) point out that although the existence of a Hecate refugium is well supported, the extensive range of the coastal lineage in conjunction with a lack of fossil evidence for black bears makes the existence of black bears in the Haida Gwaii refugium speculative.

Conclusions

The white-phase pelage of the North American black bear is one of the most striking examples of a conspicuous morphological polymorphism occurring in nature. By examining the relative effects of genetic drift and migration at neutral microsatellite loci, we attempted, in conjunction with observations of the gene frequencies of the *Mcl1r* coat-colour gene, to gauge the relative roles of genetic drift and natural selection in the maintenance of this polymorphism. Kermode populations represent a component of the coastal lineage of black bears whose current distribution may describe part of a glacial refugium. The presence of the white-phase bear in appreciable frequencies in this region can be attributed to restricted population size and isolation in insular habitat relative to mainland populations, in combination with a fragile population structure and possible selection pressure on the coat-colour locus associated with the white phase. Future management decisions will need to take into account the implications of habitat disturbances which would increase immigration from neighbouring mainland populations, and potentially those that would affect mating opportunities for white bears.

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