

# Inheritance and population structure of the white-phased “Kermode” black bear

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**We report that a single nucleotide replacement in the melanocortin 1 receptor gene [1] (*mc1r*) is responsible for the white coat color of the “Kermode” bear [2], a color phase of the black bear (*Ursus americanus* Pallus) found in the rainforests along the north coast of British Columbia. In a sample of 220 bears, of which 22 were white, there was complete association of a recessive Tyr-to-Cys replacement at codon 298 with the white phase. This variant has not been yet reported in other mammals, and it also is the lightest-colored variant yet found at *mc1r*. Also, we found that heterozygotes, which act as a hidden reservoir for the allele among black bears, were infrequent outside of the three islands where Kermodes are common and that, within these three islands, heterozygotes were less frequent than expected under random mating. Immigration of black bears into Kermode populations can depress the occurrence of the white phase, and management practices should be designed to avoid facilitating higher immigration rates.**

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## Results and discussion

### The Kermode bear

The Kermode bear, also called the “Spirit Bear” and “Ghost Bear,” is a rare white-phase black bear (Figure 1) that inhabits the rainforests on the northwest coast of British Columbia. Revered by the native Kitsoo and Tsimshian people, whose legends regard it as a reminder of the ice age, it was initially considered a separate species. Named after Frank Kermode, director of the Royal British

Columbia Museum, this white bear was subsequently reclassified as a subspecies, *Ursus americanus kermodei*, together with the interbreeding black phase. White bears reach frequencies of 10%–20% on some islands but are infrequently observed on the adjacent mainland, and their total number is estimated at 100–200 [3]. They have been protected from hunting since 1925, and environmental groups have been calling for nature preserves within the bears’ range [4].

Biologists have long suspected that the white coat is simply inherited, but the white phase is not an albino condition, as it has pigmented skin and eyes [2]. Documentation of the “gene for Kermodism” will aid in the management of this bear and facilitate studies of the evolution and adaptive significance of this conspicuous coat color polymorphism.

### Identification of the gene variant

Coat-color loci are a highly evolved system with many interacting genes [5]. To find the gene responsible for Kermodism, we sequenced several candidate genes known to affect melanin pigment formation, one of which was the melanocortin 1 receptor (*mc1r*) locus. *mc1r* encodes a G protein-coupled receptor on the surface of melanocytes. This receptor, known as the melanocortin 1 receptor (Mc1r), responds to levels of melanocyte-stimulating hormone (MSH) and regulates pigment production within the melanocyte [5]. Variation of MSH underlies seasonal coat color changes in many mammals, and other melanocortin receptors have a broad spectrum of effects upon behavior, the immune system, and feeding [1]. Initial sequencing of Kermode and black bear *mc1r* genes revealed a single missense variant, A893G, which caused a Tyr-to-Cys replacement at codon 298. This variant also created an HhaI restriction site.

We then surveyed Kermode populations for A893G variation by using an assay based on HhaI digestion of amplified *mc1r* DNA. In a previous study (H.D.M., C.N., and K.R., submitted), we obtained samples of bear hair from a total of six island and five adjacent mainland localities (Figure 2). Using microsatellite profiles, we identified 220 unique bear DNA samples, of which 22 were from white bears. We assayed these samples for the A893G variant by using restriction fragment length polymorphism (RFLP) amplified by polymerase chain reaction (PCR) with HhaI. We found the nucleotide genotypes to be GG in all 22 white bears, GA in 34 black bears, and AA in the remaining 164 black bears. This confirms genetic control and recessive inheritance of the white phase.

Figure 1

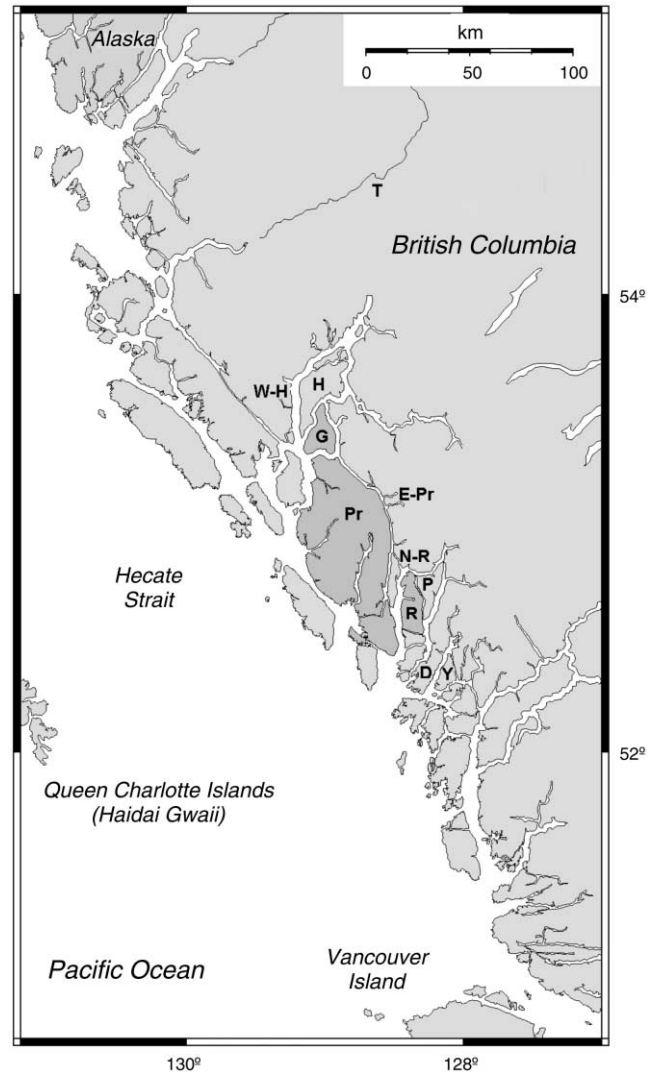


A white-phase Kermode next to a black bear (photograph courtesy of Charlie Russell).

The Kermode bear is one of several color phases of black bear in North America; the others include brown and cinnamon brown (sometimes moderately frequent in the western USA) and pale blue (rare, near Yakutat Bay, Alaska). To ascertain the uniqueness of A893G within the black bear species, we also assayed two other bears with variant coat colors. One was a white bear that was found in Minnesota, USA, and was phenotypically very similar to the Kermode bear. However, we found it did not differ from the black bear *mc1r* sequence except for a silent C408 transition at Gly136. As well, an assay of a cinnamon bear *mc1r* sequence revealed no amino acid-changing variants relative to the black bear *mc1r* sequence.

This single-nucleotide A893G polymorphism has not been reported in any other study of *mc1r* variation and is also the first documented white *mc1r* variant. Normally, mutations at *mc1r* result in changes of pigmentation to red (e.g., humans [6]) or yellow (e.g., Labrador retriever dogs [7]), due to increased pheomelanin production and decreased eumelanin production. Blond hair in humans is only weakly associated with allelic variants at *mc1r*; reflecting polygenic control [5]. This indicates that a fixed mutation at another locus may interact to reduce pheomelanin and thus give the white phenotype of the Kermode bear. This interaction with pheomelanin production may also explain the occurrence of buff, light-brown, or red tints observed in some Kermode bears; these are colors close to the color of golden Labrador retriever dogs. Al-

Figure 2



Localities where bears were sampled along the north coast of British Columbia; the three islands where the white phase is most frequent are more darkly shaded (T = Terrace, H = Hawkesbury Island, W-H = west of Hawkesbury, G = Gribbell Island, Pr = Princess Royal Island, E-Pr = east of Princess Royal, R = Roderick Island, P = Pooley Island, N-R = North of Roderick Island, D = Don Peninsula, and Y = Yeo Island).

though the bears are completely white when they emerge from hibernation, these other colors are also observed later in the season. This has been attributed to pigment accumulated by eating salmon or to staining by cedar-derived tannins in streams [2]. However, such colors are also consistent with weak pheomelanin production mediated by MSH levels and a polygenic background of color modifiers.

Another linked variant in practically complete linkage disequilibrium might actually be responsible for Kermode-

**Table 1****Coat color genotypes, frequency of the recessive white allele, and expected number of heterozygotes, by locality.**

	<i>Mc1r</i> genotype*			Frequency of G	Expected Number of heterozygotes
	AA	AG	GG		
Island localities					
Hawkesbury	24	1	0	0.02	0.98
Gribbell	7	6	10	0.56	11.33
Princess Royal	26	17	9	0.33	22.99
Roderick	9	1	2	0.21	3.98
Pooley	8	2	0	0.10	1.80
Yeo	9	1	0	0.05	0.95
Mainland localities					
West of Hawkesbury	5	1	0	0.08	0.88
East of Princess Royal	25	0	0	0.00	0.00
North of Roderick	10	1	1	0.13	2.71
Don Peninsula	22	2	0	0.04	1.84
Terrace	19	2	0	0.05	2.00

\*GG is white, AA &amp; AG are black.

ism, but recombination usually eliminates such associations, and there were no other amino acid variants detected within the *mc1r* coding region. Also, this variant is adjacent to an important area—the cytoplasmic tail, where Ser/Thr phosphorylation and Cys palmitoylation sites are required for efficient receptor trafficking and membrane stabilization [8]—so that a major phenotypic effect from a single amino acid replacement is a reasonable outcome. Interestingly, a terminal deletion at nearby codon 306 is associated with the coat colors displayed by the yellow Labrador, golden retriever, and Irish setter dog breeds [7].

#### ***mc1r* population structure**

Table 1 gives the genotype frequencies of this variant by locality as well as the expected number of heterozygous genotypes if one assumes random mating. The Kermod allele was quite frequent in three adjacent island populations (Gribbell, Princess Royal, and Roderick islands), where it ranged from 20% to more than 50%. On the adjacent mainland, and in the other islands (Hawkesbury, Pooley, and Yeo), heterozygotes were detected, but the Kermod allele is quite rare considering the proximity of these other populations to the three island populations where Kermodism is common. Excluding the locality east of Princess Royal Island, the average frequency of adjoining mainland populations was near  $q = 0.04$ – $0.06$ . If one assumes random mating, the frequency of the white bear in these mainland populations should be  $q^2$ , or about one in 300–600 bears, in line with the rare mainland sightings of Kermod.

Interestingly, on the three islands where Kermodism is common (Gribbell, Princess Royal, and Roderick islands), the observed number of heterozygotes was less than expected on the basis of random mating. Throughout the

three islands, 24 heterozygotes were observed but 38.3 were expected, and this difference is statistically significant. We took special precautions to minimize PCR artifacts such as allele dropout, which can cause heterozygotes to be scored as homozygotes. Also, this heterozygote deficiency is not due to inbreeding because microsatellite marker genotypes were in random-mating proportions within islands (H.D.M., C.N., and K.R., submitted). Rather, it may be due to either assortative mating (preferential mating to the same coat color), to recent immigration of black homozygotes, to a fitness disadvantage of heterozygotes, or to a combination of these factors. If one of these factors alone is responsible for the deficiency, then either (1) the fraction of assortative mating (as opposed to random mating) would be about at least two-thirds, (2) the fraction of new black homozygotes immigrants would be at least one-fifth, or (3) at least one-half of heterozygotes would not survive to maturity (see Materials and Methods). Interestingly, if assortative mating is the cause, it may have facilitated the establishment of the recessive Kermod trait by increasing the frequency of recessive homozygotes and hence to opportunity for natural selection or genetic drift. However, uncertainty about the true factor underscores the need for further research on the population genetics of this coat color polymorphism.

In summary, Kermodism is highly localized to three island populations, and heterozygotes in adjoining localities are relatively rare. In light of these results and our finding of recessive inheritance of Kermodism, management practices should aim to minimize cross-water gene flow from populations with the dominant black allele. To this end, our HhaI PCR-RFLP assay can serve as an effective tool for longer-term monitoring of the frequency of Kermodism. Further studies of sequence variation about the *mc1r*

gene could also reveal the evolutionary origin of the Kermodie allele and its uniqueness in the animal kingdom. Our finding also raises the question of what is an “evolutionarily significant unit.” In the U.S. endangered species act, this is defined as a reproductively isolated population containing an important component in the evolutionary legacy of the species [9]. Can such status apply to a single-nucleotide variant?

## Materials and methods

### Sampling of bears

Sampling took place throughout the range of documented occurrence of Kermodie bears as well as from two islands (Hawkesbury and Yeo) where Kermodies have not been observed (Figure 1). Many islands in this area, such as Aristazabal island (offshore from Princess Royal Island) contain little if any bear habitat (e.g., salmon-bearing streams) and were not sampled. Samples of bear hair were collected with hair snares (barbed wire across trails or around salmon baits) during August and September of 1997, 1998, and 1999. DNA was extracted from up to 10 roots or hairs per sample with either Chelex 100 chelating resin (Sigma Chemical Co.) or Qiagen columns (Qiagen Inc.). Eight dinucleotide (GT/CA) microsatellite loci [10] were assayed for each hair sample, and unique bear DNAs were identified by matching eight-locus profiles (H.D.M., C.N., and K.R., submitted). From 747 DNA extracts, we identified 220 distinct bears, of which 22 were white.

### Sequencing of candidate genes

We obtained *mc1r* sequences by amplifying genomic DNAs from black (“Jake,” “A3”) and white (“M,” “Halo”) hair or skin plug tissues by using amino and carboxy termini oligonucleotides MSHFm13 (5'-CACGA CGTTGTAACGACGGCTCCCAGAGAMGGCTGCTGGG-3') and MSHRm13 (5'-GGATAACAATTCACAGGAGAGAGTCTTYCGGAG YTCCTGGC-3') spanning codons 5–310 of the 318-amino-acid-long mammalian *mc1r* gene. The *mc1r* coding region contains no introns and gives rise to an expected product of 970 bp. Amplification conditions were 40–45 cycles of 95°C (30 s), 60°C (30 s) and 72°C (2 min) in a MJ PT-100 thermocycler. Amplification products were purified and sequenced directly by the use of terminal and internal bear-specific synthetic oligonucleotides.

Additional coat color loci considered were the *pink-eyed dilution* gene, *albino*, *kit*, and *agouti*. These were chosen from the suite of coat-color genes that have been studied at the molecular level and in which mutation(s) have been shown to cause a white-coat but black-eyed phenotype. The *pink-eyed dilution* gene is thought to encode a transmembrane protein that carries tyrosine into melanosomes; *albino* codes for tyrosinase, the enzyme that initiates the conversion of tyrosine into melanin; *kit* encodes a tyrosine kinase receptor protein whose binding to a ligand is necessary for melanocyte development; and *agouti* encodes a paracrine signaling molecule that acts as an antagonist at MSH receptors.

### Population assays using HhaI

For HhaI (5'-GCGC-3') RFLP-PCR analysis, a 387 bp fragment containing the 3' end of *mc1r* was amplified with Mcr3F (5'-CCGTC CTGCTTTGTCTTGTC-3') and MSHRm13, digested with Hha I, and then scored after electrophoresis in 2% agarose gels and staining with ethidium bromide. A 179 bp HhaI fragment is diagnostic for the variant 893G polymorphism versus a 230 bp wild-type (893A) HhaI fragment. A 157 bp HhaI fragment is present in both genotypes and controls for complete digestion with the endonuclease.

Allele “dropout,” or preferential amplification of one allele over another, often occurs when DNA is extracted from hair samples due to low DNA concentration [11]. To ensure stoichiometry of the amplified A and G alleles and a correct assay of heterozygotes, we increased the amount of starting template DNA by a factor of 10, performed a larger number (45) of thermocycles, and repeated all amplifications at least once with

varying amounts of template DNA. Also, we used 60 duplicate samples from independent hair samples of the same individual (identified by identical microsatellite profiles) to verify initial *mc1r* genotypes, and 15 of such replicate AA individuals in island populations with high frequencies of heterozygotes were also AA by our assay, showing that misgenotyping rates are likely less than 5%.

### Statistical analyses

We ascertained the deficiency of heterozygotes by computing the inbreeding coefficient  $F$  at the *mc1r* locus.  $F$  was estimated as  $1 - H_o/H_e$ , where  $H_o$  is the observed number of heterozygotes over the three island populations where Kermodism is common (Gribbell, Princess Royal, and Roderick islands), and  $H_e$  is the expected number if one assumes random mating within islands. We found  $F = 0.36$  (averaged over the three islands), with a 95% confidence interval of 0.16–0.58. We determined the confidence interval by resampling genes without replacement from the data and randomly generating genotypes by assuming  $F = 0.36$ . By contrast,  $F$  at microsatellite loci was  $-0.029$ , with a 99% confidence interval of  $-0.003$  to  $-0.065$  (H.D.M., C.N., and K.R., submitted).

The approximate levels of assortative mating, immigration, or selection that would explain the observed heterozygote deficiency are found as follows: (1) The rate of assortative mating “ $a$ ” affects the recursion for the recessive white-phase genotypic frequency among generations as

$$f'_{GG} = (1 - a) f^2_G + a \left( f_{GG} + \frac{1}{4} f^2_{AG} \right)$$

where  $f_G$  is the allele frequency of G,  $f_{GG}$  is the genotypic frequency of GG, and  $f_{AG}$  is the genotypic frequency of AG. Assuming equilibrium ( $f'_{GG} = f_{GG}$ ), solving for “ $a$ ,” and using observed frequencies, we obtained estimates of  $a = 0.79$ ,  $0.65$ , and  $0.98$  for Gribbell, Princess Royal, and Roderick islands, respectively. (2) If one assumes that black-phase immigrants are homozygous, after one generation of immigration at rate  $m$ , adult white-phase frequencies will be  $f_G^2(1 - m)$ . Equating this to observed frequencies of GG, we found that migration rates would have been  $m = 0.23$ ,  $0.48$ , and  $0.20$  for the three respective islands. (3) If heterozygote mortality is  $s$ , the frequency of GG after selection would be  $f_G^2/(1 - 2f_G f_A s)$ . Solving for  $s$  and equating to observed genotypic frequencies yields estimates of  $s = 0.53$ ,  $s = 0.77$ , and  $s > 1.0$ , respectively. Of course, all these estimates are subject to statistical error, and these factors may act jointly (which would lessen the required strength of individual factors). Other unknown factors may be involved as well. Further details about these calculations and further information about the Kermodie bear are available at <http://genetics.forestry.ubc.ca>.

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