Population Structure and Inheritance of Coat Colour in Kermode Bears (*Ursus americanus kermodei*) on the Northwest Coast of British Columbia

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Executive Summary

This project is an approved FRBC wildlife inventory project (Activity 10487) regarding Genetics and Distribution of Kermode Bears in Northwestern BC. The Kermode bear is a white-phase of the black bear (*Ursus americanus*), and it occurs at varying frequencies, of up to 10%, on the islands and coastal mainland areas of the North Coast and Mid-Coast Forest Districts. This project gathers and analyzes information about (a) the relative frequencies of Kermodism across this region, (b) genetic variation within these populations, genetic differences of these populations, and indirectly inferred rates of migration among populations, and (c) the genetic mechanism behind white-phase coat colouration.

The relative frequencies of Kermodism, determined from genetically distinct hair samples, averaged 8% in our sample (18 of 216) and ranged from 1% (1 of 80) on the mainland to 50% (8 of 16) on Gribbell Island. At the genetic marker loci we surveyed (microsatellites), genetic variation (expected heterozygosity H_e) was 4% lower on island populations, and interestingly, Gribbell Island showed the least variability (H=0.664), although this reduction is small (average $H_e = 0.693$ on island populations). Genetic differentiation (the proportion of genetic variation distributed among populations) was moderate (10%) among populations, irrespective of whether island-island, island-mainland, or mainland-mainland comparisons were made. This level of differentiation is consistent with ca. 3 migrants per generation among populations. However, island-island and island-mainland differentiation was three times more variable than mainland-mainland differentiation. This is likely due to variation of migration rate and population size among islands, and reflects the complex topography of the island area.

Coat color inheritance was indirectly inferred to be single-gene recessive. Interestingly, the data strongly suggest the presence of assortative mating, but this could be an artifact of local population structure. The frequency of the Kermode coat color gene showed greater differentiation among populations than that expected from the microsatellite differentiation. The causes of this are speculative but assortative mating, if present, may have magnified the rate of drift for this gene, resulting in the observed excess differentiation for the coat color gene. Attempts to directly identify the DNA sequence changes responsible for Kermodism have not yet been successful.

Our study finds some genetic differentiation of island populations where Kermodism is present, but this genetic differentiation is no more than that found among exclusively black bear

populations in our sample, and in fact, are less than that found for other vertebrate island populations. While one might argue that any degree of genetic divergences indicates "uniqueness" at this subspecies level, the actual boundaries of uniqueness are blurry, and conclusive statements are bound to be subjective. Finally, while information about population differentiation and mode of inheritance might enable us to predict the fate of Kermodism under various forest practices, there is not sufficient information about the quantitative impacts of such practices upon migration and population sizes for us to make predictions. Thus, our findings by themselves do not have implications for bear management practices.

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Introduction

The major objectives of this study were:

1) To obtain microsatellite profiles from the hair samples collected by the field team in September, 1999, integrate these results with the 1997/98 and 1998/99 microsatellite results, and determine the number of individual bears, both black and white, sampled in each of the localities for the three years of this study.

2) Using the frequencies of microsatellite profiles among populations, to make inferences about effective population sizes and migration among localities. We will test the hypothesis that coastal populations which contain Kermode bears are genetically isolated and consequently exhibit less genetic variation. This reduction of variation is commonly caused by reduced population sizes over historical time. We can also infer, from the frequency distribution of alleles, whether this reduction in variation is due to an ancestral bottleneck, or to continued drift in a small population of constant size.

3) To attempt to determine the mode of inheritance of the white coat colour. We are taking two approaches. First, a model of coat color inheritance will be fitted to the observed distribution of coat-color sharing among relatives of differing relationship. The model is a function of the mode of inheritance, and the likelihood of dominant vs. recessive inheritance will be determined. Second, we are searching for the DNA mutation underlying the coat color difference, and are sequencing putative coat-color genes, found by analogy with mouse mutations.

4) To make some projections regarding the effects of changes of gene flow upon the frequency of white coat colour, changes of which might result from various proposed land uses.

In additional to ourselves at the University of British Columbia (UBC) credit for this project goes to the field team headed each year by Helen Davis (R.P.Bio, Artemis Wildlife Consultants), the bear advisory group meeting attendants (Dr. John Barker, UBC; David Byng, Western Forest Products; Tony Hamilton, Ministry of Environment, Lands and Parks (MoELP); Don Reid, MoELP; Dr. Fred Bunnell, UBC; James McCormick, UBC; Dr. Alton Harestad,

Simon Fraser University; and to Kelly Stalker (Abacus Wildlife Consultants), Jason Maydan (UBC), and Dr. Craig Newton (BC Research) for laboratory assistance.

Study Area

The study area encompasses much of the geographic range of *Ursus americanus kermodei,* which inhabits the coastal mainland from the Burke Channel north to approximately the Nass River and inland to Terrace, and the adjacent islands including Princess Royal Island, as well as Gribbell, Hawkesbury, Roderick, Pooley and Yeo Islands (Figure 1). The sampling area occurs primarily in the Coastal Western Hemlock biogeoclimatic zone of British Columbia.

Methods

Sampling localities, DNA extraction from hairs, and microsatellite assays

The sampling localities were chosen such that each of the major Kermode-rich islands was sampled in addition to the mainland closely adjacent to each island where possible. Additional mainland localities and Hawkesbury Island (where Kermodes have not been sighted) were included for comparative purposes. Details of the hair trapping procedures and localities are described in the report from the field team (Davis 1999).

DNA was extracted from 560 hair samples from the 1999 collection. Samples were excluded which appeared very poor in quality and looked unlikely to produce a microsatellite profile, or which were deemed most likely to belong to another species (wolf or grizzly bear). Up to 10 roots, whole hairs, or pieces of hair were included in each DNA extraction and DNA was extracted by the Qiagen spin column method as per the manufacturer's instructions (Qiagen Inc., 1999). An additional 100 hair samples from the 1997 and 1998 hair collections were also extracted using Qiagen spin columns, to verify or improve the microsatellite profiles of some of the individual bears observed during those two years or to increase sample sizes. DNA samples from all three years are now being stored at -80°C at UBC, and may be useful for future genetic studies. The remaining hair samples are in storage at -20°C. Finally, DNA was extracted from 22 additional hair samples from bears from Nimpkish (*U. a. vancouveri*), which were provided by A. N. Hamilton and should serve as a useful comparison from outside the range of *U. a. kermodei.*

Microsatellite profiles were obtained by first amplifying the appropriate loci from the DNA sample via the polymerase chain reaction (PCR) using dye-tagged primers specific for each locus. Then a fluorescence-based DNA analysis system was employed to electrophoretically separate and detect the dye-tagged DNA amplification products. A ten-locus microsatellite profile for a single sample consists of 20 alleles identified by size in DNA base pairs or number of microsatellite repeats. The loci used here were isolated from a genomic library specifically for bears and described by Paetkau and Strobeck (1994), Paetkau et al. (1995), and Taberlet et al. (1997). First, eight loci were amplified and scored for each hair sample. These are the same eight loci (G10L, G10B, G10X, G10C, G1A, G1D, G10P, and G10M) that were scored in the two previous years of hair collection. Then the profiles from each hair sample were sorted and grouped into individuals (the probability of two different bears possessing the same 8-locus profile is less than 1 in 10^7; Paetkau et al. 1995). In the past year, we assayed two additional loci (G10H and G10U) for all 1999 samples, as well as for all 1998 and 1997 samples (only unique genotypes, identified by the other 8 loci, were assayed). The sex of a subset of the individual bears was also determined by co-amplifying a Y-chromosome locus and a mitochondrial DNA locus (Taberlet et al. 1993). Genetic profiles from all three years of hair collection were then integrated into one data set.

Genetic statistics of population structure

The following genetic statistics were then calculated. As a measure of within-population genetic diversity, average expected heterozygosity was calculated for each locality, as described by Nei (1987). To determine whether reductions in heterozygosity (if observed) are due to ancestral bottlenecks, the method and program of Cornuet and Luikart (1996) were employed. The method is based on the following premise. Populations that have experienced a recent reduction in effective population size exhibit a correlated reduction in number of alleles (k) and expected heterozygosity (H_e) at polymorphic loci, but the allele numbers is reduced faster than the expected heterozygosity. Thus, in a recently bottlenecked population, the observed H_e is higher than the expected equilibrium H_e. The program of Cornuet and Luikart (1996) computes for each sample and for each locus the distribution of H_e expected from the observed k given the sample size (number of genes, n) under the assumption of mutation-drift equilibrium. This distribution is obtained through simulating the coalescent process of n genes under three possible

mutation models, the IAM (infinite-alleles model), the TPM (two-phase model), and the SMM (stepwise-mutation model). Observed H_e is then compared with expected H_e to establish whether there is a heterozygosity excess or deficit at each locus. There is an approximately equal probability of each at a given locus, and a Wilcoxon signed-rank test is used to determine whether a sample exhibits a significant number of loci with a heterozygosity excess, indicative of a recent bottleneck.

To determine the partitioning of genetic variability within and between populations, and the extent of inbreeding within populations, we computed Wright's *F*-statistics via the method of Weir and Cockerham (1984). The use of *F*-statistics to determine population structure corresponds to a hierarchical analysis of variance. At the simplest, this approach partitions the correlation of a pair of alleles into two components: (1) between-alleles within-individuals within-populations (termed F_{IS}) and (2) between-alleles among-individuals within-populations (termed F_{ST}). The first, F_{IS}, measures the extent of inbreeding due to mating between relatives, while the second, F_{ST}, measures the extent of inbreeding due to genetic drift within populations. When F_{ST} is computed using just two populations, F_{ST} is a "genetic distance" measure, which is proportional to the number of generations of genetic drift. For multiple populations, F_{ST} measures the extent of population differentiation. The estimation of *F* statistics relies upon measures of heterozygosity taken within each locality first, and then averaged, with appropriate weightings for sample sizes and numbers of alleles (Weir and Cockerham 1984).

From F_{ST}, one can estimate the number of migrants being exchanged between localities, termed *Nm*, using the equilibrium relationship $F_{ST} = 1/(4Nm + 1)$. This holds if each sample was randomly collected from a randomly mating population, if no selection is occurring on the genetic markers, and if mutation is of negligible effect in the short-term genetic divergence of populations. Most importantly, populations are assumed to be at equilibrium between migration and drift, a process that can take hundreds or even thousands of generations.

Highly variable markers such as microsatellites provide the unique opportunity to compare these "indirect" estimates of migration (*Nm*), which assume equilibrium of processes acting over long time intervals, with "direct" identification of recent immigration based upon assignment tests. We used one such test proposed by Rannala and Mountain (1997), which assigns individual bears as derived from specific localities. This test determines the likelihood that a bear is not a migrant, versus the likelihood that a bear is an immigrant (including the

possibility that it is the progeny of an immigrant parent). A significantly greater likelihood of the latter indicates immigration. The test uses the allele frequency distributions in each locality to calculate genotype probabilities, and then determines the relative probabilities that each individual genotype in each locality was derived from nonimmigrant parents in its own locality versus each of the other localities.

Testing models of coat-color inheritance using inferred pedigrees

An innovative analysis was conducted wherein a model of coat color inheritance is fitted to the observed distribution of coat-color sharing among relatives of differing relationship. Given some small number of possible relationships, the general likelihood model for inferring inheritance using markers is the mixture of probabilities, summed over k types of possible relationships,

$$\sum_k \Pr(M_i|R_k)\Pr(S_i|R_k)\Pr(R_k).$$

In this expression, $\Pr(M_i|R_k)$ is probability of the genetic markers M_i observed for pair i, conditioned their relationship k (full sibs, half sibs, etc.), $\Pr(S_i|R_k)$ indexes the probabilities that two phenotypes are either (a) both white, (b) both black, or (c) one black and one white, and $\Pr(R_k)$ is the prior probability of relationship k. The total likelihood is the product over all pairs i, and the $\Pr(r_k)$ are estimated using the information from all pairs. $\Pr(S_i|R_k)$ depends on the mode of inheritance, e.g., single-gene recessive vs. single-gene dominant (formula will be given in the journal paper that will arise from this work). In our analysis, we assumed a mixture of full-sibs, parent-offspring, half-sibs, first-cousins, and unrelated.

In addition, the probabilities of coat-color sharing among relatives can depend on the mating system. In initial data analyses, excesses of white-white pairs, and black-black pairs, were found among full-sibs, indicating positive assortative mating. Thus, an estimation program was written that included the modification by assortative mating upon the sharing of coat colors between full-sibs. Sharing of coat colors between other classes of relatives would be little affected by assortative mating, compared to full sibs, so these probabilities were assumed to be a function of random mating. The specific formulae are available from KR, and will appear in the paper about inferred coat color inheritance.

Under two models of inheritance, and under two types of mating (random and assortative, where the assortative mating parameter was set to 0.5), likelihoods of the data were computed. In addition, the likelihood of the data with no genetic determination of coat color was found. Under recessive inheritance, the entire parameter of assortative mating was varied from zero to its maximum value (near 1). The maximum possible value is not one because assortative mating involving dominant and recessive phenotypes places restrictions on the allowable values of this parameter.

The bootstrap method (Efron 1982) was used to determine statistical significance. In this procedure, individual bears were resampled, with replacement, to form replicate datasets. In the analysis of replicate datasets, comparisons between identical bears were omitted. This method assumes that pairs are independent, which is approximately true as it is unlikely that groups of three or more full-sibs will be sampled in our study. Bootstrap datasets in which no inheritance was the most likely were omitted as these were situations in which almost no relatives were sampled by chance (these occurred ca. 10% of the time), resulting in little power to discriminate among alternative inheritance models. This probably does not bias conclusions about inheritance, as we compared only among alternative models of inheritance, but before publication this assumption needs verification.

Molecular work with putative coat colour genes

With respect to determining the molecular cause of the white coat in Kermodes, we have continued to assay the DNA sequence of the pink-eyed dilution gene in Kermode bears and in non-*kermodei* black bears. For this, primers were designed to target portions of the pink-eyed dilution gene in bears by comparing the published sequences for this gene from mice and humans. The coding portion of the pink-eyed dilution gene is about 2500 base pairs (bp) long but it is divided into 24 exons separated by highly variable non-coding sequences called introns, so exons or groups of exons must be separately amplified via PCR and subsequently sequenced using the same fluorescence based DNA analysis system described above. We have also designed primers for four other coat-colour loci (albino, kit, agouti, and melanocyte stimulating hormone receptor (MSH-R)) identified in the literature as capable of producing a white pelage/pigmented eye phenotype. An alternative approach, amplification of only the coding portion of these genes by a procedure called reverse transcriptase PCR (RT-PCR) is being tested

on these latter four loci. This involves the isolation of RNA from a tissue where the genes are being expressed (we used earplugs from white and black bears) and performing the PCR on the mRNA of each gene, rather than directly on the DNA. Some of the sequencing and RT-PCR work was contracted to Dr. Craig Newton at BC Research.

Results

Hair collections and microsatellite assays

The hair amplification success and microsatellite profile results from the third year of sampling were integrated with the previous two years' results (Table 1). This includes number of samples extracted per year, number of samples producing scorable microsatellite results, number of individual bears identified each year, and numbers of among-creek and among-year replicates identified. Among-creek replicates stem from the fact that in each coastal study locality between two and nine creek valleys were sampled. Of 1706 hair extractions 751 produced scorable microsatellite profiles, with the highest success (63%) in the final year. In total 197 individual bears were identified; 179 black bears and 18 white bears. The highest proportion of white bears was observed on Gribbell Island, in agreement with historical anecdotal evidence (Blood 1997). One white bear was identified on the mainland (Bolin Bay).

Only eight replicate samples were identified among years, and nine among creeks. (Discrepancies in the sum of the three years relative to the total sample in Table 1 are due to these among-year replicates). However, each locality was not sampled every year, nor were the same creeks necessarily sampled in subsequent years in the same locality. Therefore the among-creek and among-locality data are not standardized and should be viewed anecdotally. One replicate was also discovered in two different localities (Princess Royal Island near Laredo Inlet, and on the mainland east of Princess Royal Island) within the same year (1998). Of the additional 22 extractions performed on the Nimpkish samples, 19 proved successful (19 additional bears or 216 in total). The sex was determined for 129 bears: 77 were male and 52 female.

The success of the microsatellite analysis of each hair sample was also examined in relation to the number of roots used in the extraction (Figure 2), and whether the hair was collected by a snare or by investigation (Figure 3). "Snared" samples are ones that were collected by the use of a barbed wire hair trap (see Davis 1999) while "investigative" samples

were collected either from the ground, vegetation, or from mark trees while investigating a creek (Davis 1999). The proportion of successes steadily increased with number of roots; at five roots the chance of success outweighed the chance of failure. The chance of success for snared samples was almost 60% while that for investigative samples was less than 15%. An EXCEL spreadsheet which contains collection and extraction information on each of the 1728 hair samples from which DNA was extracted is included with this report. This contains dates of collection and extraction, type of DNA extraction, number of roots, year of collection, creek and locality, whether collected by snare or investigation, success of microsatellite analysis, number of replicates of each sample, and a list of the replicates. This will aid in future genetic studies of the hair and DNA collections from this study.

Genetic diversity, and inferences from diversity patterns

Average expected heterozygosity for each locality is presented in Table 2. Heterozygosity within all localities is reasonably high (as would be expected for highly variable microsatellite markers), ranging from 0.621 in the Nimpkish sample to 0.793 in Terrace. Generally, the coastal localities (island and mainland) exhibit relatively lower diversity than the inland sample (Terrace), both in this study and relative to samples from Banff National Park in Alberta and LaMauricie National Park in Quebec (0.801 and 0.783 respectively, Paetkau and Strobeck 1994). The low Nimpkish result may reflect a non-random sample consisting of related bears, and are likely relatives of each other (A. N. Hamilton, personal communication).

Expected heterozygosity (H) is a function of both longer-term effective population size (N) and the per-generation mutation rate (u). Under an infinite-alleles model of microsatellite locus evolution, where mutations occur to any other possible allele length, $H=4Nu$, while under a stepwise model of evolution, where mutations occur only to alleles of neighboring length, H estimates the square root of $4Nu$. It is generally difficult to use observed data to distinguish between these models of evolution, but at least under either model, H provides an indicator of relative effective population sizes in the different localities, as the mutation rate should be consistent among localities (mutation rate is generally accepted to be a constant quantity, although generation time, and elevation [UV light intensity] have been shown to affect mutation rates; in our study, populations do not differ for these factors).

The results of the Bottleneck simulations were somewhat equivocal. Under the IAM all but four localities (Gribbell, Don Peninsula, Mainland West of Hawkesbury, and Mainland East of Princess Royal Island) exhibited significant ($P < 0.05$) heterozygosity excesses (evidence for recent bottlenecks), while under the SMM both Gribbell and the Mainland East of Princess Royal showed significant ($P < 0.05$) reductions in heterozygosity relative to number of alleles (evidence for recent population growth). Most researchers (see Estoup and Cornuet 1999) now agree that microsatellites evolve according to neither the IAM nor the SMM in a strict fashion, but rather something in between (such as the TPM). Under the TPM three localities (Hawkesbury, Yeo, and Nimpkish) exhibited a significant heterozygosity excess ($P < 0.05$). In any event, the locality with the lowest reported expected heterozygosity (Gribbell) does not appear to have been bottlenecked in the recent past.

Genetic differentiation, and inferences from differentiation patterns

To examine genetic differentiation among localities, *F*-statistics were calculated in two ways. First, we found pairwise estimates of F_{ST} and *Nm* between all pairs of localities (Table 3). Second, we found F_{ST} and *Nm* between various groups of localities (Table 4), where populations within a group were pooled. This allows a more general picture of where barriers to migration may exist. Several trends are apparent from the results of the F_{ST} analyses. First, the pairwise estimates indicate that reasonably high levels of differentiation exist among many of localities surveyed in this study. Interesting, in Table 3 average pairwise F_{ST} values were nearly the same between mainland populations (average $F_{ST} = 0.099$), between island populations (average $F_{ST} = 0.106$) and between mainland and island populations (average $F_{ST} = 0.097$). Even more interestingly, the variance of pairwise F_{ST} was about three times greater in the latter two catagories of comparisions, indicating that island populations differ for effective population sizes, while mainland populations are more constant in size.

These pairwise F_{ST} values give *Nm* estimates usually between 0 and 2. These values are low enough to allow population differentiation to occur by random genetic drift in each locality (Hartl and Clark 1989). In particular, both Gribbell Island and the Nimpkish locality are well differentiated from the other localities with *Nm* of approximately 1 in many comparisons. (The Nimpkish result may be slightly misleading if this sample consists of highly related individuals.)

The first and last entries in Table 4 quantify these results; F_{ST} among all *U. a. kermodei* localities and between *U. a. kermodei* and *U. a. vancouveri* (Nimpkish) are fairly high.

Among groups of localities, high levels of differentiation (F_{ST} = 0.09-0.10) occur among *U. a. kermodei* groups, among island groups, and among mainland groups (Table 4). However, as a group, island localities did not differ from mainland localities, nor did white bear populations differ from black bear populations (Table 4). This is not surprising, as the mainland topography we sampled is quite "island" like, with few opportunities for migration among watersheds of the mainland. Examination of the pairwise matrix suggests that Hawkesbury Island in addition to Gribbell Island contributes to the overall among-islands differentiation, while the sample from the mainland West of Hawkesbury Island is the most genetically distinct of the mainland localities.

We also compared the relationship between F_{ST} and shortest water distance separating two sampled localities, where we excluded comparisons between localities on the same island, or both on mainland. There is a suggestion that pairwise F_{ST} increase with shortest water distance between localities (Figure 4). However, the proportion of variance explained was only r^2 = 0.104 and the regression coefficient was non-significant (p =0.30).

The "direct" test of immigration of Rannala and Mountain (1997) identified 21 individuals as recent immigrants (Table 5). The significance level of each comparison presented was $P < 0.01$, so that the issue of multiple comparison must be considered (when many pairwise comparisons between populations are performed for each of a large number of individuals, some individuals may appear to immigrants purely by chance). Nonetheless, we can comment on the patterns apparent from this analysis. First, the recent migrants are largely black males. There were 11 male migrants identified, three females, and seven of unknown sex. Two of the three females migrated between mainland localities, and the third between a mainland and an island locality. Two white migrants were detected, one from Roderick Island to Gribbell Island, and the other from Gribbell Island to Princess Royal Island. Overall, nine of the migration events occurred between islands, six between mainland localities, and six between an island and a mainland locality. Of the 21 migrants, most are either mainland-to-mainland migrants, or are migrants between neighboring localities. The results of this analysis are therefore generally consistent with the Nm/F_{ST} results, although they cannot be directly compared without correction for sample size.

F_{IS}, an indicator of inbreeding, was also estimated for each locality. It was not significantly different from zero in any locality. Besides indicating a lack of consanguineous matings (mating between relatives), this finding of zero F_{IS}, also indicates that "null" alleles (alleles that do not yield a PCR product) are of undetectable and negligible frequency in our samples. Null alleles, if present, cause a spurious increase in homozygosity, as an individual heterozygous for a null allele and a normal allele looks like a homozygote, while being actually heterozygous.

Coat color inheritance inferred from pedigrees, and assortative mating

The likelihoods under alternative models of inheritance (dominant vs. recessive) and for alternative mating systems (random mating, assortative mating) are given in Table 6, part A. The significance of their differences are given in Table 6, part B, in terms of the number of bootstraps in which the difference occurred. It should be noted that these are log-likelihoods, and they are always negative. A "less-negative" likelihood is more likely and more acceptable.

The most likely mode of inheritance was recessive with assortative mating, with a likelihood of -2601.47, which was ca. 100 times more likely than the next most likely, dominant with no assortative mating (Table 6A). This difference was statistically significant, as 97 of 100 bootstraps maintained this difference over two alternative models: vs. recessive/no assortative mating (more likely in 97 of 100 bootstraps, Table 6B), and vs. dominance/no assortative mating (more likely in 96 of 100 bootstraps). Interestingly, if mating was assumed to be random, dominant inheritance was slightly more likely than recessive, but this was not statistically significant (more likely in 58 of 100 bootstraps). If assortative mating was assumed, dominance was almost as likely as recessiveness (more likely in 48 of 100 bootstraps).

Unfortunately, we cannot test the possibility that variation at two genes, either linked or unlinked, act together in an epistatic manner to determine coat colour, as we cannot estimate their frequencies. For example, white coat colour might be due to two loci, both with two recessive alleles, while black occurs otherwise. However, there is just one degree of freedom in the data (frequency of white) and a two-locus model demands two degrees of freedom, corresponding to frequencies at the two loci. It is conceivable that there may be an exception to this rule when pairwise relationship is considered, but the statistical power to accept two-locus inheritance would probably be minuscule with our sample size.

DNA sequencing for the coat color gene

Further progress in identifying the mutation responsible for coat colour consists of the following. Current sequences that have been scored are given in Appendix 1. First, exons 6 to 24 (of 24 in total) of the Kermode pink-eyed dilution gene have now been almost entirely sequenced (1880 bp of DNA sequence). This constitutes the latter three-quarters of the gene, which is the most conserved portion in human-mouse comparisons and where most if not all of the mutations associated with p-gene albinism have been identified. The Kermode gene is 89% similar to the human gene, and contains no deletions or stop codons, which would disrupt its function. It does contain amino-acid changing substitutions relative to the human and mouse genes, but we need to obtain the comparable sequence of the black bear to know if any of these is associated with Kermodism. Of the portion of the gene already sequenced in a black-phase bear (and reported previously by us) no amino-acid substitutions were apparent between white and black bears.

Large portions of the remaining black bear sequence have been amplified via PCR, but still need to be sequenced. These include exons 10-13 and 15-21. To sequence the remainder of the black bear pink-eyed dilution gene, it will probably be necessary to make more mRNA from the black bear, using a better tissue sample. The 5' end of the gene (exons 1-5) is proving difficult to obtain in both black and white bears, due to its high variability. This variability suggests however that mutations within this portion of the gene are less likely to disrupt its function.

Regarding the other coat colour genes being examined, the RT-PCR analysis indicates that both the albino and kit genes are being expressed in Kermode and black bears. Therefore mutations leading to lack of gene expression of these two genes are not likely the cause of Kermodism. The RT-PCR products obtained are probably sequencable, so missense or nonsense mutations that would prevent protein production or interrupt protein function may still be detectable. The RT-PCR and sequencing work needs to be completed with these genes and with the agouti and MSH-R genes; these latter two did not produce RT-PCR products in initial attempts.

We have also had some success sequencing a portion of the kit gene using the direct PCR approach. The kit gene encodes a tyrosine kinase receptor protein necessary for the proper

development of hair-bulb (but not eye) melanocytes. It is responsible for the dominant white black-eyed phenotype observed in pigs, as well as for piebaldism in humans. The portion of the gene we were able to sequence was exon 18 and the adjacent intron; this is the region in which the dominant white mutation occurs in pigs. Our sequencing revealed no mutations between white and black bears so we can rule out this particular possibility as the cause of Kermodism. This is also consistent with the finding that recessive inheritance is more likely than dominant for the white coat in bears. Nonetheless, because kit and certain other pigmentation mutations resulted in either melanocyte absence or altered melanocyte morphology it may prove informative to have some Kermode hairs examined using electron microscopy in the future.

Discussion

Sampling

On the sampling side, in the third year, we succeeded in increasing the range of sampling to somewhat include neighboring subspecies of black bear. This is enabling a more accurate picture of the distinctness of the Kermode island populations. In addition, if we eventually locate the DNA change responsible for the coat-color difference, this will provide a better opportunity to determine the extent of Kermodism outside of the Kermode range using the heterozygote assay (assuming recessive inheritance). Additionally, we might be able to assess the distinctiveness of the *kermodei* subspecies relative to the other coastal subspecies of black bear, and the historical factors (such as different glacial refugia and/or population bottlenecks) involved in shaping the current distribution and genetic variation of present-day populations.

Comparisons to similar studies

Using our microsatellite data we can compare coastal black bear populations with those in other evolutionary and genetic studies of black bears throughout the North American range. Previous work in this area is represented by three studies. Wooding and Ward (1997) described the phylogeographic structure (the topological relationships of genetic lineages with respect to geography) among black bears from across North America, using mitochondrial control-region sequences. Paetkau and Strobeck (1994) assessed the microsatellite variability within three populations. They reported levels of heterozygosity in two inland localities which were similar

to the level we observed in Terrace. This enabled us to conclude that coastal localities exhibit somewhat reduced heterozygosities relative to inland localities, most likely reflecting reduced long-term effective population size due to inhabiting islands. Byun et al. (1997) examined the phylogeography of coastal black bears using mitochondrial cytochrome *b* sequences (but found relatively little variation.)

Taken together, the two mitochondrial DNA studies point to the existence of three distinct evolutionary lineages of black bear, eastern and western lineages separated by the Rockies, and a coastal lineage in which both *U. a. kermodei* and *U. a. vancouveri* occur. Our preliminary comparison of the Kermode and Nimpkish samples points to the possibility of identifying subdivision within the coastal lineage, perhaps along subspecies lines. However, the Nimpkish sample poorly represents *U. a. vancouveri* as it is a sample of a small inbred population. Larger sample sizes of this subspecies would be helpful.

Origin and maintenance of Kermode bears

A plausible explanation for the origin and maintenance of the white pelage polymorphism within *U. a. kermodei* can be inferred from the patterns of diversity, gene flow and white-phase frequency. Since Gribbell Island has the highest proportion of white bears, and has the lowest genetic diversity, it is likely that the mutation may have become established at appreciable frequency on this island (or an adjacent, perhaps Princess Royal) from pure genetic drift. The lower heterozygosity (*H*) on Gribbell is evidence for a higher rate of drift on this island (in fact, Gribbell had the lowest *H* of all islands). It is unlikely that the original mutation occurred on this island, since mutation is rare in evolution, and similar coat-color variants suggestive of the same mutation occur elsewhere in the black bear range, for example Minnesota. The mutation may also involve several genetic changes, which would make recent origin even less likely (molecular knowledge of the mutation would be useful here). Most likely, high genetic drift accentuated a chance increase in the frequency of an initially rare allele for the white-phase.

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 neighboring localities. Princess Royal Island was probably a major player in this migrant exchange, as its population is most similar to Gribbell, and it exhibits the second highest proportion of white bears. In fact, the assignment test for immigrant ancestry (Table 5) identified two recent

migrants between Princess Royal and Gribbell Islands, so this process may be ongoing. Only a small portion of the relatively substantial genetic differentiation among localities can be attributed to shortest water distance between them, suggesting the involvement of several or perhaps many other factors affecting the motivation for bears to emigrate. This topic remains to be addressed in future work.

It must also be noted that inferences about migration rates from F_{ST} rely upon the assumption that populations are in equilibrium between gene flow and genetic drift. Therefore if changes in population size or migration rate occur as the result of future forestry practices, F_{ST} measurements taken in subsequent generations will not reflect then-ongoing migration rates. The observed departure from random mating in the direction of assortative mating should not unduly affect the estimates of migration from F_{ST} because assortative mating only affects the loci involved in determining the trait involved in the assortative mating (Hartl and Clark 1989).

Inheritance

We found the most likely mode of inheritance of the white pelage to be recessive, and interestingly, there was strong evidence of assortative mating. While recessive inheritance has always been assumed for Kermodism, there are examples of dominance of white over black coat color in mice. The significance of recessive inheritance is that any mixing of populations will result in a much faster decline of white-phase bears than if white was dominant over black. This is the first time the mating system of an animal has been detected as "assortative" with the aid of genetic markers. However, the reliability of inferences suffer from a relatively small sample size of 77 bears in the polymorphic populations that were analyzed, as well the problem of defining independent units of observation for the purposes of significance tests.

Knowledge of the DNA sequence changes responsible for the Kermode white-phase will enable us to greatly refine this scenario. In evolutionary biology, many studies have inferred the evolutionary history of major polymorphisms, such as the alcohol dehydrogenase isozyme polymorphism in Drosophila, based upon DNA sequence variation surrounding the mutation responsible for the polymorphism. From the patterns of nucleotide variation among many sequences, one can infer the ages of alleles, the pattern of natural selection acting on the alleles, and the geographic origins of alleles (albeit, in an ideal world). This is one of the reasons why the hunt for the DNA change responsible for Kermodism is so important. In addition, we could

develop assays to determine whether black bears are heterozygous (contain one copy) for the Kermode gene, and hence more accurately determine the expected frequency of Kermodism.

Are Kermodes genetically unique?

Our study finds some genetic differentiation of island populations where Kermodism is present, but these levels are typical for vertebrate island populations. One method of ascertaining genetic "uniqueness" is to see how accurately individuals can be assigned to their population of origin. For example, among populations of foxes on California's Channel Islands, all but one of 183 foxes could be correctly assigned to its island of origin by reconstructing the phylogenetic relationships among foxes using 19-locus microsatellite profiles (Goldstein et al. 1999). A comparable analysis with our data does groups most (but not all) Gribbell, Hawkesbury and Nimpkish bears into their own separate clades (or phylogenetic groupings), but bears from all other localities are admixed phylogenetically. Of course the fox study clearly differs from ours in terms geographic scale (the closest islands are 4 km apart) and number of loci examined, and pairwise levels of differentiation were not reported, so the two studies are not directly comparable.

With respect to other bear populations, Paetkau et al. (1999) reported F_{ST} values on a scale of 0.002 to 0.108 among polar bear localities. 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 travel over very large distances (on the order of 50,000 km^2 in a year; Domico and Newman 1988) and maintain a circumpolar, connected distribution. Clearly, the island habitat of coastal black bears is most likely restricting movement of the bears relative to what they may experience in a large, continuous mainland habitat.

There is considerable activity by conservation biology that is attempting to identify populations unique enough to warrant preservation attempts. Conservation geneticists define an evolutionarily significant unit (ESU) as the smallest taxonomic unit with an independent evolutionary trajectory. Rationales for ESU protection include avoidance of outbreeding depression and behavioral isolation, maintenance of genetic variability, and preservation of "natural" or historical patterns of variation.

One might argue that any significant genetic divergence indicates "uniqueness". However, genetic uniqueness is a complex, and often fuzzy, quantity, and numerous debates

have occured in the United States over the genetic uniqueness of various populations of organisms including salmon and mammals. At one extreme, every population could be claimed unique, while at the other extreme, only reproductively isolated populations with unique life history, behavioral and morphology characteristics would be deemed unique. The societal decision about uniqueness is often the outcome of perceptions and values, and not any objective biological reality. At least, in population genetics, values of $Nm < 1$ (less 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.

Interestingly, in the U.S., The Endangered Species Act (ESA) places higher priority on vertebrates, such as bears, than on other species. Vertebrates are given first class status, invertebrates second class, and plants third class. The rationale for this is that such "indicator vertebrates" serve as umbrellas to both described and undescribed species. Perhaps this is why the "Great Bear Rainforest" is called so by environmental activists. However, in actuality, focus on a single major vertebrate species leaves several gaps in their protection of co-existing species.

However, its occurrence is not that surprising, as local populations at the edge of a species distribution are usually genetically more distinct than a geographically isolated subspecies near the middle. To really ascertain the uniqueness of Kermodism, we need more information about the nature of the DNA changes underlying Kermodism, and the factors responsible for its relatively high frequencies at locations along the North Coast of B.C. If we could identify the mutation responsible for Kermodism, we could then assay sequence variation about this mutation to estimate the age of the mutation, and whether it occurred more than one time, as mentioned. Perhaps, through comparisons with identical mouse and/or human mutations, possible behavioral and/or life history differences between Kermodes and black bears might be ruled out, although the scientific credibility of such inferences would probably be very dubious.

Future work

There are the following possibilities for future work. (1) Further work to find the molecular basis for the coat color polymorphism, which if elucidated, can lead to further studies of Kermode allele frequency on the islands and adjacent mainland, and the evolutionary history

of this polymorphism (2) Integration of our data with other studies of bears (black and grizzly) in British Columbia. Alleles should be matched across studies. Common objectives and methodologies should be identified and shared. Easier said than done. (3) A landscape-level study of bear populations to identify the impacts of forest practices in terms of movements and changes of population size, as inferred from DNA markers. The amount of sampling and genetic assays would be larger and consequentially more expensive. This is more generally concerned with black bears and not the Kermode bear. There is a graduate student possibly starting Fall 2000 interested in such work.

In addition to the final report, we intend to write several papers from this work:

(1) Biogeography and evolution of *U. a. kermodei* populations and the role of factors such as long-term effective population size, inbreeding, and historical and current patterns in effecting genetic differentiation among populations (Evolution),

(2) Fine-scale and sex-specific patterns of migration and genetic differentiation and the roles of relatedness structure and female philopatry (Molecular Ecology).

(3) Inference of coat-color and assortative mating based upon pedigree analysis of coat color variation (Heredity).

(4) An article of interest for laypeople that summarizes our research findings and their implications for conservation (American Scientist). Included in this will be calculations of the effect of migration upon the frequency of Kermodism, assuming recessive inheritance and assortative mating.

(5) The molecular findings from our coat colour work (Nucleic Acids Research).

In addition, Ritland is developing an estimator of population size based upon the distribution of pairwise relationships (this is analogous to the hair "recapture" method used for grizzly bear population censuses, but it differs in using the frequency of relatives, and not the frequency of identical samples); it will use the Kermode data as an example dataset.

Critique of Inventory Protocols

The success rate of obtaining microsatellite profiles from hair samples varied substantially from year to year and also among localities. There were at least three factors responsible for this variation: type of sampling (snare versus investigation), number of roots

included in the DNA extraction, and type of DNA extraction (Chelex or Qiagen). Because each of these factors varied in any comparison it is difficult to quantitatively investigate the influence of each.

Nonetheless we are able to comment qualitatively on each. First, the type of sampling is of utmost importance. Snared samples are substantially more likely to produce usable results. This may in part be due to the fact that snared samples in general consist of many more hairs and therefore provide many more roots per DNA extraction. Additionally the history of investigatively collected samples is uncertain - they may be older or may have been shed rather than pulled from the bear (shed hairs are usually in a different cell cycle phase in which less DNA may be available). Despite these observations, the investigatively collected samples were a useful addition to this study because when they worked they were usually from individual bears whereas the snared samples are more often replicates of each other.

The number of roots included in the extraction also greatly affects success. Clearly, the more the better, with five roots being the minimum necessary for the sample to have a greater probability of success. This affected the variation of our success rates in two ways. First, because the initial year of sampling was primarily investigations in this year we were unable to put five or more roots in most extractions. Second, following then-established protocols, in the first two years of the study we were including a maximum of six roots per extraction, whereas in the third year 10 roots were used when available.

Finally, the use of Qiagen columns as opposed to Chelex resin improved the DNA extractions at least to the extent that the extractions performed with Qiagen will be stabler over a longer period than those performed with Chelex. Chelex-extracted DNA is thought to degrade within a year of extraction, and indeed when analyzing the last two loci on the first year of DNA extractions in the third year of this study many samples were difficult to score and had to be repeated or simply scored as missing. The Qiagen approach may also have improved the success rate of the hair samples, but it is difficult to isolate this effect because we combined the use of Qiagen with the inclusion of 10 roots where possible and both factors may have improved the observed success rate. In other bear studies in British Columbia the use of Qiagen has been shown to improve success rates of obtaining microsatellite profiles (A. N. Hamilton, personal communication).

Management Recommendations

Predictions about changes of coat-color frequency depend mainly upon how forestry practices affect bear migration. This study does not provide such information. To obtain such information, we need a contrast between logged vs. undisturbed regions, as well as between different silvicultural practices. The landscape-level approach mentioned above may allow such inferences, or previous work involving tagging experiments may give us an idea of the impacts upon migration.

Conclusions

The highlights of our genetic survey to determine population structure and coat colour inheritance in Kermode bears are as follows.

1) Using 10-locus microsatellite profiles we were able to examine 197 bears distributed throughout the *U. a. kermodei* range, and compare them to 19 *U. a. vancouveri* bears from Nimpkish. The highest proportion of white bears was observed on Gribbell Island, followed by Princess Royal Island, Roderick Island, and the mainland north of Roderick Island.

2) Coastal localities and particularly Gribbell Island demonstrate reduced within-population diversity compared to inland samples, and low to moderate levels of gene flow in among-population comparisons. These observations suggest a strong role for random genetic drift and population isolation in maintaining the white polymorphism.

3) The most likely mode of inheritance of the white pelage is recessive inheritance at an autosomal locus, and the frequency of white bears observed may result in part from assortative mating. We have been unsuccessful in determining the molecular basis of Kermodism thus far, but have been able to rule out a number of possible mutations identified from the literature or in discussions with other researchers. This effort is worth pursuing, as knowledge of the molecular basis of the mutation may lead to identifying the age and geographic origin of the mutation, as well as more accurately predicting the frequency of Kermodism if an assay for heterozygotes can be devised.

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Tables and Figures

Table 1. Summary of microsatellites profiles analyzed in this study, including incidence of black and white phenotypes sampled in the range of *U. a kermodei* in each of three sampling years, and black bears included from a *U. a. vancouveri* locality.

Locality	Samples Extracted			Successful Samples			Number of Bears			Repeat Profiles		Total
	97/98	98/99	99/00	97/98	98/99	99/00	97/98	98/99	99/00	Among-Creek	Among-Year	
U. a. kermodei												
Mainland Locations												
Terrace	99	-	-	37	-	-	17b	-	-	-	-	17b
Don Peninsula	-	65	64	-	34	45	-	13b	10b	0	0	23b
E. of Princess Royal	14	120	1	0	71	0	0	21b	0	1 (Dome /Aaltanhash)	0	21b
N. of Princess Royal	6	-	-	0	-	-	0	-	-	-	-	0
W. of Hawkesbury	-	-	47	-	-	26	-	-	6b	2 (Keesil /Kishkosh)	-	6b
E.of Gribbell	32	5	-	3	1	-	0	0	-	-	-	0
N. of Roderick	22	16	112	3	8	86	1b	2b	10b 1w	0	1 (2/3)	12b 1w
Princess Royal Island												
Northwest/Surf Inlet	149	22	80	44	13	40	10b 2w	3b	9b 1w	1 (Wales /Evinrude)	1 (1/2)	21b 3w
Laredo Inlet	178	83	-	37	46	-	6b 1w	15b 2w	-	1 (Packe/Nias)	0	21b 3w
East	14	16	-	0	10	-	0	1b 1w	-	1 (Canoona /Nomel)	0	1b 1w
Southeast	-	-	2	-	-	0	-	-	0	-	-	0
Other Island Locations												
Yeo Island	-	-	44	-	-	31	-	-	10b	0	-	10b
Pooley Island	14	49	57	2	12	37	2b	6b	5b	1 (Duthie /Windy)	3 (1 1/3, 2 2/3)	10b
Roderick Island	29	26	42	1	10	20	1b	3b 1w	6b 1w	1 (Watson /Bottleneck)	1 (2/3)	9b 2w
Gribbell Island	63	31	25	27	17	13	5b 6w	1b 4w	2b 1w	0	2 (1 1/2, 1 1/2/3)	8b 8w
Hawkesbury Island	93	-	86	21	-	55	6b	-	14b	1 (Fishtrap /Vesney)	0	20b
Sub-Total	713	433	560	176	222	353	48b 9w	65b 8w	72b 4w	9	8	179b 18w
U. a. vancouveri												
Nimpkish	-	-	22	-	-	19	-	-	19	-	-	19
Sub-Total	-	-	22	-	-	19	-	-	19	-	-	19

Table 2. Average expected unbiased heterozygosity within each locality

Locality	Heterozygosity	Standard Error
Mainland		
Terrace	0.793	0.021
W. of Hawkesbury	0.724	0.027
E. of Princess Royal	0.673	0.055
N. of Roderick	0.747	0.041
Don Peninsula	0.667	0.048
Average	0.721	0.018
Island		
Hawkesbury	0.699	0.038
Gribbell	0.664	0.024
Princess Royal	0.707	0.036
Pooley	0.692	0.060
Roderick	0.668	0.053
Yeo	0.725	0.029
Average	0.693	0.017
U. a. vancouveri		
Nimpkish	0.621	0.046

Table 3. Pairwise estimates of genetic differentiation (F_{ST}; below diagonal) and gene flow (*Nm*; above diagonal) among localities

Locality	T	WH	EP	NR	DP	H	G	PR	PO	R	Y	N
Terrace	-	4.2	2.5	3.7	2.2	2.9	1.4	2.3	2.3	2.1	2.5	2.0
W. of Hawkesbury	0.056	-	1.8	2.0	1.7	1.8	1.1	2.0	2.2	1.8	2.7	1.5
E. of Princess Royal	0.092	0.121	-	2.9	2.5	3.0	1.6	7.8	2.9	3.5	1.9	1.7
N. of Roderick	0.063	0.110	0.079	-	2.4	2.8	1.5	5.4	2.8	3.4	2.8	1.6
Don	0.104	0.134	0.092	0.096	-	2.9	1.2	2.7	3.3	6.9	6.2	1.0
Peninsula Hawkesbury	0.080	0.121	0.077	0.083	0.149	-	1.3	2.4	1.8	1.8	1.7	1.5
Gribbell	0.152	0.180	0.132	0.142	0.168	0.157	-	2.3	1.4	1.6	1.2	0.9
Princess Royal	0.098	0.111	0.031	0.044	0.084	0.094	0.097	-	3.2	4.7	2.3	1.9
Pooley	0.099	0.102	0.080	0.082	0.070	0.120	0.149	0.072	-	4.9	3.3	1.2
Roderick	0.107	0.124	0.066	0.069	0.035	0.125	0.136	0.051	0.049	-	3.8	1.1
Yeo	0.090	0.085	0.115	0.081	0.039	0.131	0.173	0.099	0.070	0.061	-	1.1
Nimpkish	0.109	0.142	0.128	0.134	0.203	0.139	0.224	0.116	0.171	0.179	0.190	-

Table 4. Estimates of Genetic Differentiation (F_{ST}) and Gene Flow (Nm) Among Groups of Localities

Comparison	F_{ST} (standard error)	***Nm***
Among all *U.a. kermodei* localities	0.092 (0.006)	2.5
Among island localities	0.098 (0.006)	2.3
Among mainland localities	0.093 (0.011)	2.4
Island versus mainland localities	0.014 (0.002)	18.0
White versus black localities	0.025 (0.004)	9.8
U. a. kermodei versus *U. a. vancouveri*	0.101 (0.030)	2.2
White Bears versus Black Bears	0.041 (0.005)	5.8

Table 5. Probable Recent Immigrant Ancestry of 21 Bears

Bear ID, Colour and Sex	Sample Locality	Source Locality	Generations of Mixture
4918e, black male	Don Peninsula	E. of Princess Royal	0 or 1
4910d, black female	Don Peninsula	Terrace	0 or 1
4953-1r, white male	Gribbell	Roderick	0 or 1
3-174, black male	Gribbell	E. of Princess Royal	0
3-319, black unknown sex	Hawkesbury	Terrace or Don	0 or 1
692, black unknown sex	Hawkesbury	Gribbell or Princess Royal	0 or 1
4930e, black male	E. of Princess Royal	Terrace or N. of Roderick	0 or 1
4917a, black female	N. of Roderick	Hawkesbury or Gribbell	0 or 1
3-465, black male	N. of Roderick	Terrace or Nimpkish	0 or 1
3-407, black unknown sex	N. of Roderick	Hawkesbury	0
A200, black male	N. of Roderick	Roderick	0 or 1
4923d, black male	Roderick	Pooley	0
774, black unknown sex	Princess Royal	Terrace	0
4954i1(?), black male	Princess Royal	Gribbell	0
330, white unknown sex	Princess Royal	Gribbell	0 or 1
Q84, black male	Princess Royal	E. of Princess Royal	0 or 1
A158, black unknown sex	Princess Royal	Roderick	0
4946i, black male	Princess Royal	Roderick	0 or 1
4946c, black male	Princess Royal	Yeo	1
A85, black female	Terrace	W. of Hawkesbury or N. of Roderick	0 or 1
A105, black unknown sex	Terrace	N. of Roderick	1

Table 6. (A) Log-likelihoods of recessive vs. dominant inheritance of coat color, combined with the pattern of mating, based on the analysis that fits the sharing of coat colors to relationship. (B). Number of bootstraps (of 100) in which one model was more likely than another.

A.

	Likelihood
No inheritance	-2612.45
Recessive	
no assortative mating	-2604.07
assortative mating	-2601.47
Dominant	
no assortative mating	-2603.86
assortative mating	-2604.47

B.

Recessive, assortative mating vs. no assortative mating	97 ($p<0.05$)
No assortative mating, dominance vs. recessive	58 (ns)
Assortative mating, dominance vs. recessive	48 (ns)
Recessive assortative vs. dominance non-assortative	96 ($p<0.05$)

Figure 1. Map of Study Area

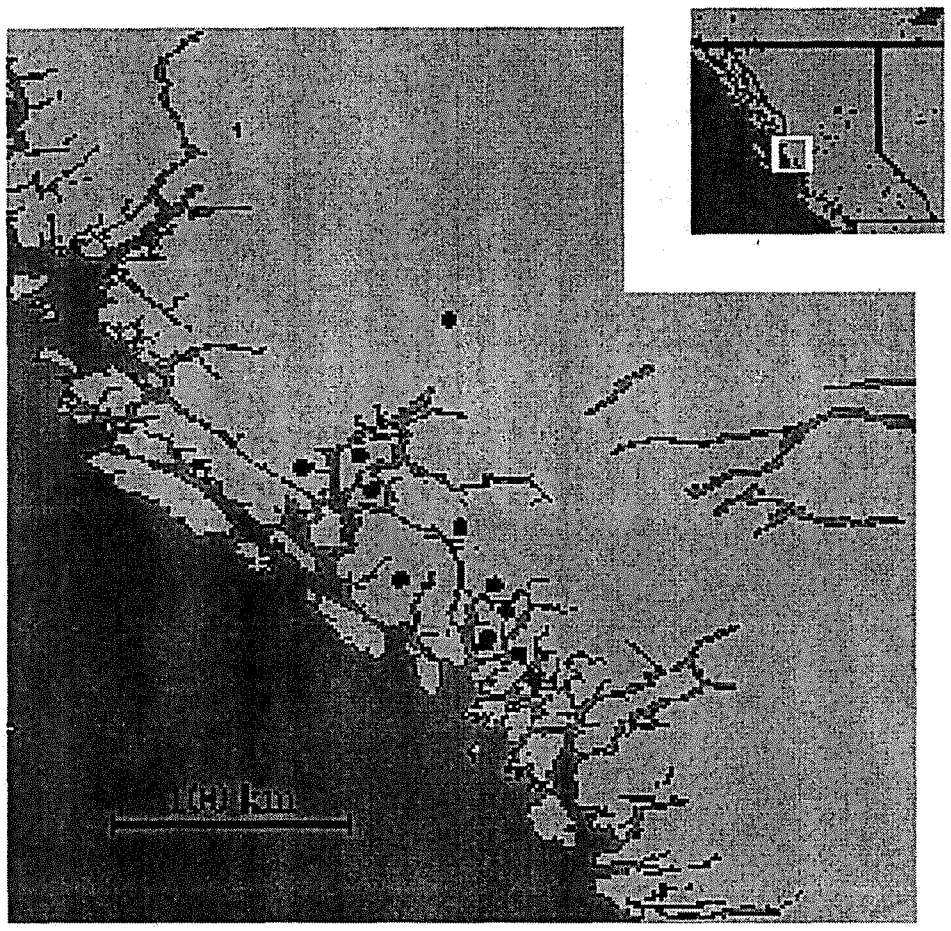


Figure 2. Percentage of successful and unsuccessful samples by number of roots. (Series 1- successful; Series 2 - unsuccessful)

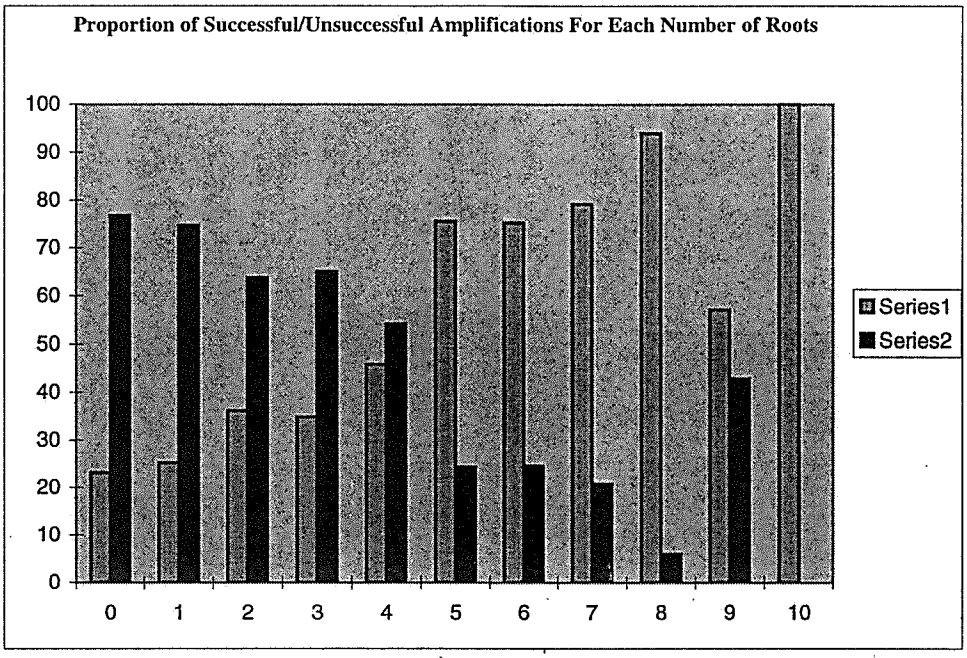


Figure 3. Percentage of successful and unsuccessful samples according to type of collection. (s - snare; i - investigation; Series 1- successful; Series 2 - unsuccessful)

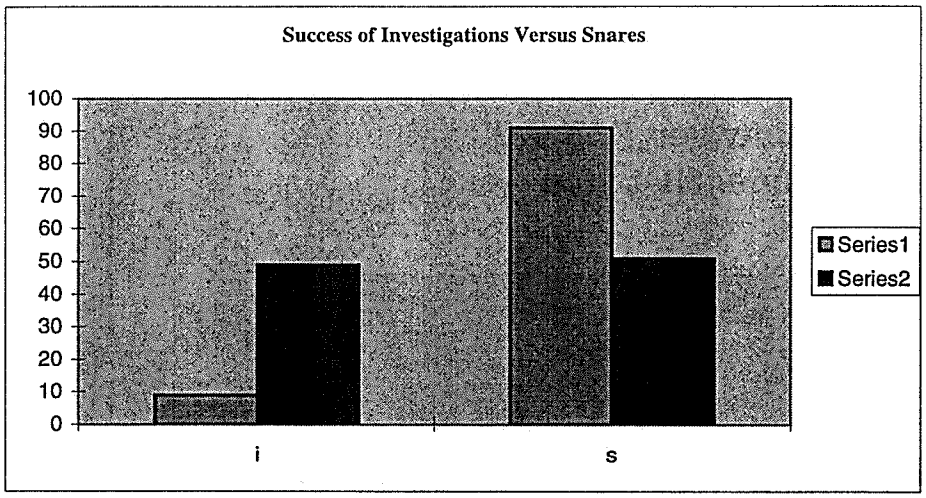
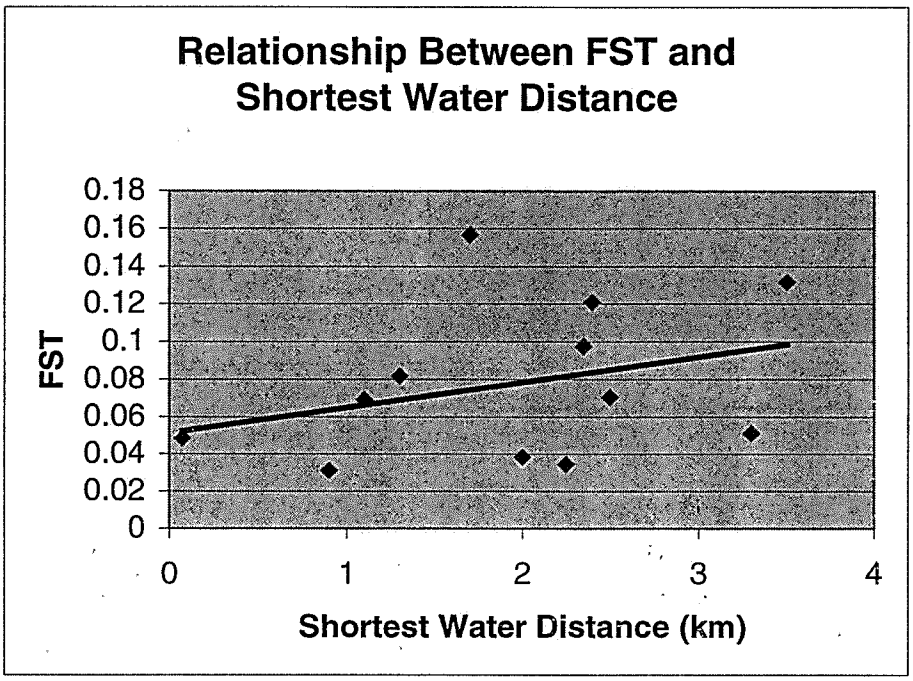


Figure 4. Relationship between F_{ST} and shortest water distance separating two sampled populations (comparisons between populations on the same island, or both on mainland, excluded).



Appendix 1

Pink-eyed Dilution Gene Sequence Alignment for Human, White Bear, and Black Bear

Note: // indicates the end of one exon and the beginning of the next.
"pun dupl'n" and pJ del'n" are the duplication and deletions, respectively,
associated with two white coat mutations in mice
dash (-) indicates sequence that is deleted or duplicated in the above mutations
oca indicates positions altered in oculocutaneous albinism type 2 (OCA II) in humans
white - white bear,. black - black bear
? indicates sequence yet to be obtained

Sequence name	DNA sequence	Nucleotide number
exons	exon 1 // exon 2 coding	
human	cactcctggagaaagatctgcaagtgcgcagagagaagactggcagtggagcatgcatct	60
white	??	
black	??	
pun dupl'n		
pJ del'n		
oca		
exons	sequence starts	
human	ggagggcagagacggcaggcggtaccccggcgcgccggcggtggagctcctgcagacgtc	120
white	??	
black	??	
pun dupl'n		
pJ del'n		
oca		
exons		
human	cgtgcccagcggactcgctgaacttgtggccggcaagcgcaggcttcctcggggagccgg	180
white	??	
black	??	
pun dupl'n		
pJ del'n		
oca		
exons		
human	tgga----------gctgacccctcgcactcctgccccaggggggctgccgg	222
white	??	
black	??	
pun dupl'n		
pJ del'n		
oca		
exons	//	
human	gcagagctcttgggctcctgcaggccaggagtttgcttcattcctcacaaaagggaggtc	282
white	??	
black	??	
pun dupl'n		
pJ del'n		
oca		

```
exons       exon 3
human       tcactcttctttgccccagatgtccagctccaggtctaaagattcctgctttacagaaaa 342
white       ??????????????????????????????????????????????????????????
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca

exons                                              // exon 4
human       cactcctttgctgaggaattccttacaggagaaagggtcacggtgcatacctgtttacca 402
white       ??????????????????????????????????????????????????????????
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca

exons
human       tccagagttcatcactgctgaagagtcttgggaagacagctctgctgactgggagcgaag 462
white       ??????????????????????????????????????????????????????????
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca

exons
human       atacctgctaagcagggaggtgtctggtctgtctgcatctgcctcctccgagaagggaga 522
white       ??????????????????????????????????????????????????????????
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca

exons                                                    // exon 5
human       ccttctggacagcccgcacatccgactccgtctttccaagctgaggcgctgtgtgcagtg 582
white       ??????????????????????????????????????????????????????????
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca

exons                                                  // exon 6
human       gctgaaagtcatgggcctgtttgcctttgtggtgctgtgttctattttgttcagcctata 642
white       ??????????????????????????????????????????????????????????
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca

exons                                                              //
human       tccggatcaaggaaagctctggcagctgttggccttatcaccgctggagaactactccgt 702
white       ??????tcaaggaaagttctggcagttgttggctgtgtcacccctgcaaagctactcttc 54
black       ??????????????????????????????????????????????????????????
pun dupl'n
pJ del'n
oca
```

```
exons       exon 7
human       gaaccttagcagccacgtggactccacgctgctgcaggtggacctggcaggggccctagt 762
white       aaacctcaccagccacgtggactccacactactccaggtggatctggcaggggccctggt 114
black       ??????????????????????????????????????????????????????????
pun dupl'n                  ---------------------------------------------
pJ del'n
oca

exons
human       ggccagtgggccgagtcgtcctgggagggaagagcacatcgtggtggagctgacccagga 822
white       ggccggtggcccaagtcataccgggagagaagaacatgtcgtggtggaggtgacccaggc 174
black       ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons                                                 // exon 8            22
human       tgacgctttgggctccaggtggcggcggccacagcaggtcactcacaactggacggtgta 882
white       tgatgttccaggctccaggcgacggcggccacagcaggtcactcagaattggacagtatt 234
black       ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons                                                                   /
human       tttaaatccgaggagaagcgagcactcagtgatgagcaggacctttgaggtactgaccag 942
white       tttaaatccgagaagaagtgagcgcttggtggtgagcaggacctttgagatactgagcag 294
black       ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons       / exon 9                                                    23
human       agagacggtgtccatcagcatccgggcctccctgcagcagacccaggctgtccctctttt 1002
white       ggatactgtttctgtgagcattcgagcctccttccagcagacacaggcagttcctctttt 354
black       ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons
human       gatggctcatcagtacctccgcggaagtgtagaaacccaggtgaccatcgcgacggccat 1062
white       gatggctcatcagtacctccgtgcaagcatagaagcacaagtgaccattgctgtggtaat 414
black       ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons                                             // exon 10
human       cctcgcgggcgtctacgcgctgatcatatttgagatcgtgcacagaactctggcggccat 1122
white       ccttgctggggtctacatgatgattatatttgagattgttcacagaaccctggcagccat 474
black       ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca
```

```
exons                                                    // exon 11
human      gctgggttcccttgcagcactggcagcactggctgtgattggcgatagacccagcctgac 1182
white      gttgggttcccttgcagcattagcagcgctggctgtgattggagatagacccagccttac 534
black      ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons                                                          // exon
human      ccatgtggtggagtggattgattttgagacgctggccctgctgtttggcatgatgatctt 1242
white      ccatgtggtggagtggattgattttgagacct?????????????????????????????? 566
black      ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons      12                                                  // exon 13
human      agtagccatattttcagaaacgggatttttcgattattgtgctgtaaaggcataccggct 1302
white      ??????????????????????????????????????????????????????????
black      ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca                                          oca

exons
human      ctcccggggacgggtgtgggccatgatcatcatgctctgtctcatcgcggccgtcctctc 1362
white      ??????????????????????????????????????????????????????????
black      ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca                              oca               oca

exons                                                          // exon
human      tgccttcttggacaacgtcaccaccatgctcctcttcacgcctgtgaccataaggttgtg 1422
white      ??????????????????????????????????????????????????????????
black      ??????????????????????????????????????????????????????????
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons       14
human      tgaggtgctcaaccttgatccaagacaagtcctgattgcagaagtgatcttcacaaacat 1482
white      ???????????ccttgatccaagacaagtcctgattgcagaagtgatcttcacaaatat 614
black      ??????????????????????aagacaagtcctgattgcagaagtgatcttcacaaatat 39
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons
human      tggaggagctgccactgccatcggggaccctccaaatgtcattattgtttccaaccaaga 1542
white      tggaggagctgccactgccattggggacccaccaaatgtcattattgtttccaaccagga 674
black      tggaggagctgccactgccattggggacccaccaaatgtcatt---------------- 82
pun dupl'n ------------------------------------------------------------
pJ del'n
oca             oca                    oca                      oca
```

```
exons                    // exon 15
human      gctgaggaagatgggcctggactttgccggattcactgcacacatgttcattgggatttg 1602
white      gttgaggaagatgggcctggacttcgcaggatttactgcacacatgtttgttgggatttg 734
black      ------------------------------------------------------------
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons
human      ccttgttctcctggtctgctttccgctcctcagactcctttactggaacagaaagcttta 1662
white      cttcattctcctgttctcctttccactcttcagactccttttctggaacaaaaagcttta 794
black      ------------------------------------------------------------
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons                                // exon 16
human      taacaaggaacccagtgagattgttgaactgaagcacgagattcacgtctggcgcctgac 1722
white      taacaaggaacccagtgagattgttgaactgaagcacgagatccacgtctggcgcctgac 854
black      -----------------------------------cgagatccacgtctggcgcctgac 106
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons
human      tgctcagcgcatcagcccggccagccgcgaggagacagctgtgcgccgcctgctgctggg 1782
white      tgcacagcgcatcagcccggacagccgagaggagacggcggttcggggcctgctcctggg 914
black      tgcacag??catcagcccggacagccgagaggagacggcggttcggggcctgctcctggg 164
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons                                                        // exon
human      gaaggtgctggcactggagcacctgctcgcccggaggctgcacaccttccacagacagat 1842
white      gaaggtgcgggcactggagcacctgctggcccgaaggctgcacaccttccacagacaaat 974
black      gaaggtgcgggcactggagc??ctgct---------------------------------- 189
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons       17                                                // exon
human      ctcacaggaggacaaaaattgggagaccaatatccaagaactccaaaaaaagcataggat 1902
white      ctcacaggaagataaaaattgggagaccaatatccaggaactacaaaaaaagcacaggat 1034
black      ------------------------------------------------------------
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons      18
human      atctgacgggattctgctcgccaaatgcctgacagtgttgggatttgttatcttcatgtt 1962
white      atcagacaagattctgctcaccaaatgcctgatggtgttgggatttgttatcttcatgtt 1094
black      ------------------------------------------------------------
pun dupl'n ------------------------------------------------------------
pJ del'n
oca
```

```
exons                                                  // exon 20
human      tttcctcaattcgtttgtccctggcattcatcttgatcttggatggattgctattctggg 2022
white      ctttctcaattcatttgtccctggtgttcatcttgatcttgggtggattgctattctggg 1154
black      ------------------------------------------------------------
pun dupl'n ------------------------------------------------------------
pJ del'n
oca

exons
human      tgccatctggttgctaattttagctgatattcatgattttgagataattctacacagagt 2082
white      tgccatctggttgctaattttagctgatattcatgattttgaggtaattctacaccgagt 1214
black      ----------ttgctaattttagctgatattcatgattttgaggtaattctacaccgagt 239
pun dupl'n -
pJ del'n
oca          oca

exons                                                        // exon 21
human      ggaatgggcaacccttctgtttttgcagcgctctttgttctgatggaggcattggcaca 2142
white      ggaatgggcaactcttctattctttgcagcactctttgttctgatggaggcactggcaca 1274
black      ggaatgggcaactcttctattctttgcagcactctt----------------------- 275
pun dupl'n
pJ del'n
oca                              oca

exons                                                        // exon 22
human      tctccacttaatagaatatgttggagaacaaactgctttgctaataaagatggtcccaga 2202
white      cctccacttaatagaatatgttggagaacagactgctttgctaataaagatggtcccagA 1334
black      ------------------------------------------------------------
pun dupl'n
pJ del'n           ----------------------------------------------------
oca

exons
human      ggagcagcgcctcatagccgccattgtcctggtggtgtgggtctcagccctggcgtcgtc 2262
white      Ggatcagcgcctcacaactgccatcatcttggtagtctgggtttcagccattgcatcrtc 1394
black      -gatcagcgcctcacaactgccatcatcttggtagtctgggtttcagccattgcatcrtc 334
pun dupl'n
pJ del'n   ------------------------------------------------------------
oca

exons                                          // exon 23
human      cctgattgacaacatcccgttcactgctaccatgattcccgtgctcctgaacctgagcca 2322
white      cttgattgacaacatcccattcaccgcaacaatgattcccgtgctcctgaacctcagcca 1454
black      cttgatt-----------------------------------------------ctcagcaa 349
pun dupl'n
pJ del'n   ------------------------------------------------------------
oca                      oca                    oca

exons
human      cgaccctgaggttggcctgcccgcaccgccgctcatgtatgccctggccttcggtgcttg 2382
white      agaccccgaggtcagcctgcctgcaccaccactcatgtatgccctggcccttggcgcctg 1514
black      agaccccgaggtcagcctgcctgcaccaccactcatgtatgccctggcccctt-------- 401
pun dupl'n
pJ del'n   ------------------------------------------------------------
oca
```

```
exons              // exon 24
human      cctgggaggcaacgggacactgattggcgcgtcggcaaacgtcgtgtgtgcagggattgc 2442
white      cctgggaggcaatgggacactgattggcgcatcagcaaatgtcgtttgtgctggaattgc 1574
black      ------------------------------atcagcaaatgtcgtttgtgctggaattgc 431
pun dupl'n
pJ del'n   ------------------------------------------------------------
oca

exons                                                  // exon 25
human      agaacagcatggatatgggttctccttcatggaatttttcaggctgggcttcccaatgat 2502
white      agagcagcatggatatgggttctctttcatggaatttttcaggttgggcttcccaatgat 1634
black      agagcagcatggatat---------------------------------------------- 447
pun dupl'n
pJ del'n   ------------------------------------------------------------
oca

exons
human      ggttgtgtcctgcactgttgggatgtgttatctccttgtggctcatgtggtggtgggatg 2562
white      gctcgtctcttgcacagttgggatgtgttacctccttgttgctcatgt------------ 1682
black      ------------------------------------------------------------
pun dupl'n
pJ del'n
oca

exons
human      gaattaa                                                      2569
white      -------
black      -------
pun dupl'n
pJ del'n
oca
```