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Genetic tagging of free-ranging black and brown bears

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Abstract Identification of individuals in a free-ranging animal population is potentially hampered by a lack of distinguishing features (e.g., scars, unique color patterns), poor visibility (e.g., densely forested environments), cost and invasiveness of physical capture, and mark loss. Advances in DNA-analysis technology offer alternative methods of individual identification that may overcome several of these problems. We investigated the genetic variability of American black bears (*Ursus americanus*) and brown (grizzly) bears (*Ursus arctos*) in the Columbia River basin of British Columbia, Canada, and developed a method to obtain genetic samples from free-ranging bears. We established the background genetic variability using microsatellite genotyping at 9 loci using tissue and blood samples from captured bears. In 3 field trials, we tested methods to obtain hair from free-ranging bears. Although all methods collected hair suitable for DNA analysis, the barbed-wire enclosure hair-trap was superior. We extracted DNA from hair roots and identified sample species with a species-specific mitochondrial DNA (mtDNA) test and sample sex from a Y-chromosome test. Using 6 microsatellite loci from nuclear DNA (nDNA), we screened all hair samples for individual identity and developed match probability functions based on scenarios of random sampling (P_{random}), the likely presence of parent-offspring groupings in the samples ($P_{par\text{-}offs}$), and the likely presence of siblings in the samples (P_{sib}). We applied the P_{sib} to each hair sample (match criteria at $P_{sib}<0.05$) and illustrated how these microsatellite genotypes can be used as genetic tags in mark–recapture bear censuses. The ability to identify species, sex, and individuality of free-ranging bears has numerous potential applications in field studies.

Key words black bear, brown bear, DNA, hair, mark–recapture, microsatellite, *Ursus americanus*, *Ursus arctos*

Wildlife researchers use various forms of ear tags, colored bands, neck collars, radiotransmitters, and natural markings to identify and track individual animals under field conditions (Nietfeld et al. 1994). Each method has advantages and limitations. The ideal mark would be non-invasive, highly visible, clearly read, inexpensive, and permanent. Genetic "tags" in the form of microsatellite genotypes have the potential to meet several of these criteria, and advances in technology are making DNA methods accessible at the field level (Parker et al. 1998). In addition to individual identification, DNA samples can be used to confirm sex (Taberlet et al. 1993), species, genetic population structure, and individual genealogies (Haig 1998).

Whole blood and tissue biopsies obtained from captured animals have been routinely used as a source of DNA. Several recent studies have

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obtained DNA from free-ranging animals using alternative tissue sources: skin samples from humpback whales (*Megaptera novaeangliae*, Palsbøll et al. 1997); feces from brown bears (*Ursus arctos*, Höss et al. 1992), black bears (*Ursus americanus*, Wasser et al. 1997), and seals (*Pinnipedia*, Reed et al. 1997); and hair from American marten (*Martes americana*, Foran et al. 1997), brown bears (Taberlet and Bouvet 1992, Taberlet et al. 1993, Taberlet et al. 1997), and chimpanzees (*Pan troglodytes*, Morin et al. 1994).

Roots of mammalian hair contain sufficient DNA for analysis when genetic material at specific loci is amplified using the polymerase chain reaction (PCR) (Higuchi et al. 1988). For free-ranging bears, hair is an attractive DNA source because bears frequently leave hair on rub trees, in beds, and at foraging sites (Taberlet and Bouvet 1992). Because bears are readily attracted by scent lures, methods to obtain hair samples from free-ranging bears permits systematic sampling regimes necessary for many ecological studies, such as animal censuses.

In mark–recapture studies, an initial population sample is captured, marked, and released. The population is then resampled during ≥1 additional sessions. The ratios of newly captured animals to recaptures is then used to compute a population estimate (White et al. 1982). Genetic tags can replace conventional marks in these studies if the tags reliably identify individuals during a series of sampling sessions. Palsbøll et al. (1997) demonstrated such an application in a census of North Atlantic humpback whales.

We conducted field trials to determine the effectiveness of genetic tags derived from hair as part of a black bear and brown bear mark–recapture census. Using hypervariable microsatellites from nuclear DNA, we addressed the questions: 1) can genetic tags consistently and accurately identify individual bears? and 2) can we effectively gather hairs from free-ranging bears suitable for DNA analysis?

Study area and methods

Study area

The West Slopes Bear Research Project study area was centered near Golden, British Columbia, Canada (51 18'N, 117 00'W), in the upper Columbia River basin. The area was about 5,000 km^2 and included parts of 3 major physiographic regions: the Rocky Mountains, the Rocky Mountain Trench, and the Columbia Mountains. About half of the study area was contained within national parks (Glacier and Yoho) and half in multiple-use provincial lands.

Preliminary genetic analysis of captured bears

Before using a genetic approach to identify individuals, it is necessary to demonstrate that the variability of alleles at the available loci is sufficient in the study population to be useful in identifying individuals. To a certain extent, increasing the number of loci used can offset lower levels of variation. However, in populations with low genetic variability, such as the brown bears of Kodiak Island (Paetkau et al. 1998*b*), the number of genetic loci required to make individual identifications could make identification prohibitively difficult and expensive.

To determine background genetic variability of bears within our study area, we used nDNA extracted from blood and ear biopsy samples collected from bears captured as part of a radiotracking study. We measured genetic variation at 9 microsatellite loci in both species of bears (Paetkau et al. 1998*b*, unpublished data for locus G10H, primers in Paetkau et al.1998*a*).

We used hair samples from a subset of captured bears to test the suitability of hair as an alternate DNA source (see Microsatellite analysis). Because animals frequently leave hair samples on barbed-wire fences, we pretested the effectiveness of wire as a hair-trapping device by passing a short strand of barbed wire through the body hair of several immobilized bears. This procedure had no adverse effects on the bears, and small clumps of hairs readily stuck between the prongs of the barbed wire. Similarly, we pretested a wire brush and a dog brush on immobilized bears to ensure that they would gather hair without harming the bear. Animals were captured and handled under permits issued by the British Columbia Ministry of Environment, Lands, and Parks and by Parks Canada.

Hair-trapping trial 1: preliminary experiment to compare hair-trapping methods

In June and July 1995, we installed 20 hair-trapping stations in the Beaver River valley, Glacier National Park. They were set out at 1-km intervals along the valley bottom, except for a gap of 8 km between stations 10 and 11. Care was taken to

locate the hair-trapping stations at least 200 m from active hiking trails. Game trails were avoided to reduce the risk of ungulates moving through the site.

At each station, free-ranging bears were simultaneously presented with 4 hair-trap designs (described below). All stations were operated for approximately 28 days and scented with commercial liquid fish fertilizer. We visited the stations at approximate 7-day intervals, removed hair samples, and refreshed the scent lure. In the case of barbed-wire hair-traps, each cluster of points was individually examined by placing a hand or white card behind it. All hairs from a cluster of points became a sample. For brush samples, all hairs within the entire brush were removed and considered to be a sample. Each sample was placed in a small paper envelope and kept dry until it could be frozen. At the end of trapping, we removed all wire, obvious scent lure residues, and warning signs.

We built an enclosure hair-trap by running a single strand of barbed wire (2-strand wire, 4 points, 15-cm spacing between barbs) around several trees about 5 m out from a central tree and uniformly about 50 cm above the ground (Figure 1). If necessary, we filled terrain irregularities with woody debris to ensure a uniform wire height. Standard fencing staples fastened the wire to the outside of each perimeter tree, and we hand-tensioned the wire. We scented the hair-trap with 125 ml of fish fertilizer splashed on the bark of the central tree about 2 m above the ground.

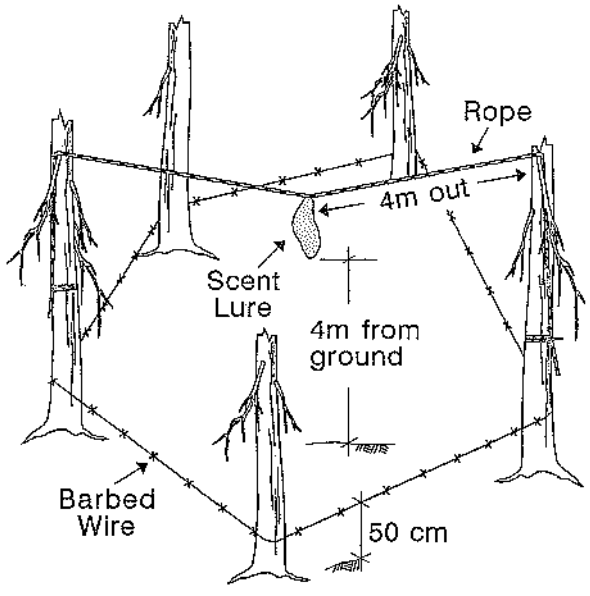

Figure 1. Barbed-wire enclosure hair-trap used to collect hair from bears. Scent was placed about 2 m above the ground on a central tree at hair-traps without cameras in 1995 instead of suspended as shown. Conducted in the Columbia River basin near Golden, British Columbia, Canada, 1995 and 1996.

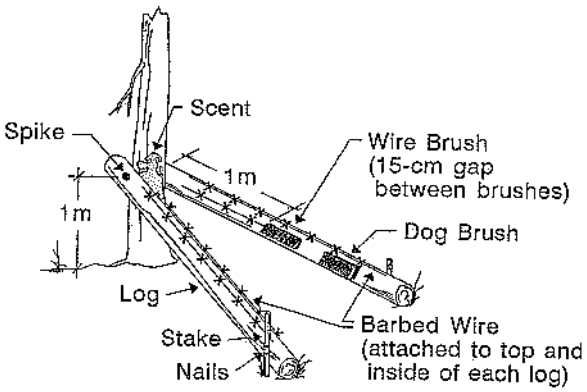

Figure 2. Cubby with barbed wire, wire brush, and dog-grooming brush hair-traps used to collect hairs from bears in the Columbia River basin near Golden, British Columbia, Canada, 1995.

Near each barbed-wire enclosure hair-trap, we built a log "cubby" similar to the type used to live-trap bears using leg-hold snares (Figure 2). The cubby consisted of 2 log poles (2 m long, 8–12 cm diameter) with one end spiked about 1 m above ground to opposite sides of a standing tree trunk (about 30 cm diameter) and the other ends on the ground about 60 cm apart. We attached 2 strands of barbed wire to each cubby pole. One strand ran along the top of the pole in a position where it would likely brush against a bear climbing over the sides of the cubby. The other strand ran along the inside of the pole in a position where it would likely brush against a bear investigating the cubby from the front entrance. Finally, we piled sticks and logs on the outer sides of the cubby poles to discourage bears from climbing over or ducking under the sides. We scented the trap with 65 ml of fish fertilizer splashed on the attachment tree about 50 cm above the ground and an additional 65 ml poured into a shallow hole in the ground at the base of the tree inside the cubby poles.

At each hair-trap cubby, we nailed 1 dog-grooming brush and 1 wire paint-scraping brush (15 cm × 2 cm) to the inside of a cubby pole 1 m out from the attachment tree and about 15 cm apart. Each wire brush was oriented so its length was parallel to the side logs and the wires pointed inward to the other side pole (Figure 2).

Hair-trapping trial 2: hair-traps at remote camera sites

Following the general methods described by Mace et al. (1994), we set up remotely triggered automatic cameras at 49 stations within a 70 × 70-km grid at a density of approximately 1 camera/100 km^2. We scented these stations with about 2 kg of rotten meat and 250 ml of liquid fish fertilizer placed in a burlap sack suspended 4–5 m above the ground. In general, this arrangement prevented bears from receiving a food reward. Starting 10 June 1995, we activated the cameras. We then selected 22 of these stations based on bear activity and, starting 20 June, added barbed-wire enclosure hair-traps as described above in trial 1 (Figure 1). We visited each station at approximate 7-day intervals, ending 25 July to replace camera film and to collect hair samples.

Hair-trapping trial 3: large-scale experiment

In 1996, we conducted a large-scale hair-trapping experiment over a 64 × 64-km study grid at the density of 1 hair-trap/8 × 8-km cell (Figure 3). Based on our 1995 experience, we installed barbed-wire enclosure hair-traps with suspended scent lures at each station (as in trial 2 without the cameras, Figure 1). We collected hair and moved the hair-traps to new positions within the same grid cell at approximate 10-day intervals (4 moves within each cell). We activated the first traps on 10 June and removed all traps by 23 July. Actual dates and intervals varied slightly in all 3 trials because daylight and weather constraints limited helicopter operation.

We used data from this trial to compute an estimate of bear numbers using the program CAPTURE (White et al. 1982).

Macroscopic and molecular analysis of hair samples

We examined each hair sample under a dissecting microscope and classified it as potentially usable if it contained ≥1 visible roots. Hairs also were tentatively classified to species on the basis of color: light-tipped samples as brown bear, entirely black samples as black bear, and hair of questionable color as unknown. Although visible hair characteristics can be used to identify some species (Oli 1993), we know of no microscopic technique that reliably separates black and brown bears. Hair samples for extraction consisted of 1–4 guard hair roots

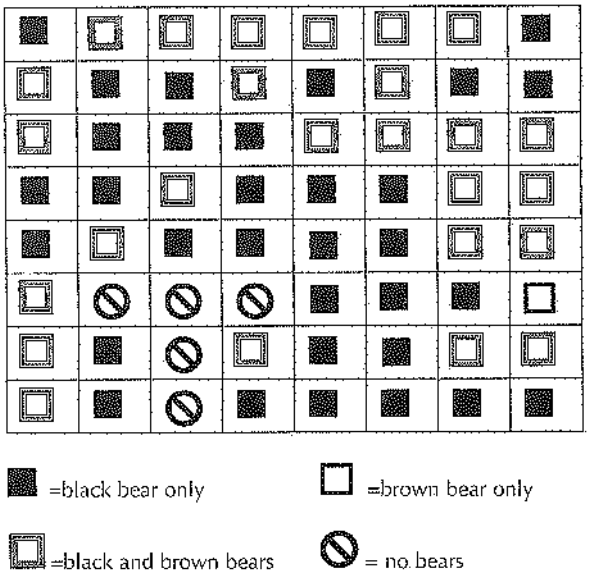

Figure 3. Distribution of black bears and brown bears determined by mtDNA species determination from hair samples in the Columbia River basin near Golden, British Columbia, Canada, 1996. Diagram represents 64 x 64-km study area divided into 8 x 8-km sampling cells.

(maximum chosen when available). DNA was extracted in 200 µl of 5% Chelex solution (Walsh et al. 1991).

Species identification

In trials 1 and 2, we attempted individual identification for all samples (see Microsatellite analysis). However, in trial 3, most hair samples were from black bears, whereas the primary research interest was in brown bears. In this case, we first determined the species of each sample by amplifying a short region of the mitochondrial control region that has a 13–15 bp (some length variation exists within species) deletion in brown bears relative to black bears (Shields and Kocher 1991; Paetkau and Strobeck 1996; light strand primer TCTATTTAAACTATTCCCTG and heavy strand primers GCTTATATGCATGGGGC and ATTTTTAGCTTATATGCATGGGC, which differ by 7 base pairs at the 5′ end). We chose PCR primers for the short length of their amplicon (169 bp or 176 bp in the reference brown bear) because we felt that longer products might be difficult to amplify from a small and potentially degraded source of DNA. We labeled the heavy strand primers with HEX and TET fluorescent dye groups (Perkin Elmer-Applied Biosystems, Foster City, Calif.; multiple dyes and different sizes allowed 2

samples to be typed/lane/gel loading) to facilitate analysis on an automated sequencer. PCR conditions were as described in Paetkau and Strobeck (1996), except that the reaction volume was 15 μl and 5 μl of the Chelex extract was used as template. Every run included reference samples from captured black and brown bears and control reactions with no target DNA. In the third trial, we ceased further analysis after 2 unsuccessful attempts at species recognition or confirmation that the sample was from a black bear. Only 1 sample from each individual was typed with the species recognition marker for the first 2 trials (i.e., after the microsatellite analysis instead of before it).

Microsatellite analysis

We used a suite of 6 microsatellite loci to identify individuals (Paetkau et al. 1995; loci G1A, G1D, G10B, G10C, G10L and G10X). These loci detected high variation in study-area brown bears (4–8 alleles [6–13 in black bears], Paetkau et al. 1998*b*). These microsatellites were not excessively long (all alleles <200 bp), they amplified strongly from small amounts of DNA, they could be analyzed in a single gel lane, and they could be amplified in a total of 3 PCR reactions, making efficient use of the limited amount of DNA available. Reaction conditions were as described (Paetkau et al. 1998*a*), except that the volume was increased to 30 μl so that 20 μl of Chelex extract could be used as template. The genetic analysis was performed without knowledge of collection date or location, and genotypes were scored using GENOTYPER software (Perkin Elmer-Applied Biosystems, Foster City, Calif.), but confirmed by independent visual scoring.

To avoid biases during scoring, we searched for matches after all microsatellite analyses were complete. Whenever a particular multi-locus genotype was identified from a single sample, that genotype was compared to all available brown bear genotypes from the study area (i.e., genotypes from this study and from physically captured research bears) to identify similar genotypes. When the genotype from 1 of these "unique" samples matched another genotype except for a single allele designation, the unique sample was typed again at the locus that caused it to be unique.

Statistical basis for match declaration

Excluding errors in genetic analysis (see Discussion), a difference in genotypes between individuals can be taken as evidence that they originate from different animals. However, when samples have identical genotypes, the possibility always remains that they came from individuals with identical genotypes at the loci examined. This means that a statistical basis for genetic match declarations must be developed.

In human forensics, it is often assumed that samples are drawn at random from the population (Avise 1994) and that the samples being compared do not come from relatives. In this "random" case, the probability of drawing a given genotype (P_{random}) at a particular locus from the population at large can be taken as p_i^2 for homozygotes and $2p_ip_j$ for heterozygotes where p_i and p_j are the frequencies of the ith and jth alleles (A_i,A_j). These values can be multiplied across all loci analyzed to give an overall probability of a match (P_{random}).

In the type of study described here, however, samples are not expected to be random or to come from unrelated bears. For example, a female and her cubs could visit a hair trap as a family unit and independent young may share overlapping home ranges with both parents and siblings (Mace and Waller 1997). We therefore developed formulae to calculate the probability that the parent or offspring of a particular individual ($P_{par\text{-}offs}$) or their sibling (P_{sib}) would have the same observed genotype:

for homozygotes,

$$P_{random} = p_i^2,$$

$$P_{par\text{-}offs} = p_i \text{ and}$$

$$P_{sib} = (1+2p_i+p_i^2)/4;$$

and for heterozygotes,

$$P_{random} = 2p_ip_j,$$

$$P_{par\text{-}offs} = (p_i+p_j)/2 \text{ and}$$

$$P_{sib} = (1+p_i+p_j+2p_ip_j)/4.$$

If individuals X and Y are related as parent–offspring, then 1 gene of X is identical by descent to 1 gene in Y. Thus the probability that both X and Y are homozygous for a particular allele ($A_i A_i$) is p_i^3. Because the probability that X is homozygous ($A_i A_i$) is p_i^2 (P_{random}), the conditional probability that Y is homozygous ($A_i A_i$) given that X is homozygous ($P_{par\text{-}offs}$) is p_i. Similarly, the probability that both X and Y are heterozygous ($A_i A_j$) is $p_ip_j^2+p_jp_i^2$. Therefore the conditional probability that Y is het-

erozygous ($A_i A_j$) given X is heterozygous is $(p_i+p_j)/2$ ($P_{par\text{-}offs}$).

If individuals Y and Z are related as siblings, then 25% of the time the 2 genes contributed to Y and Z by the mother and the 2 genes contributed to Y and Z by the father are identical by descent, 50% of the time the genes contributed by the mother or by the father (but not by both mother and father) to Y and Z are identical by descent, and 25% of the time neither of the genes contributed by the mother or by the father to Y and Z are identical by descent (Jacquard 1974). Thus the probability that both Y and Z are homozygous at a particular allele ($A_i A_i$) is $(p_i^2+2p_i^3+p_i^4)/4$. Because the probability that Y is homozygous ($A_i A_i$) is p_i^2 (P_{random}), the conditional probability that Y is homozygous given Z is homozygous ($A_i A_i$) is $(1+2p_i+p_i^2)/4$ (P_{sib}). Similarly, the probability that both Y and Z are heterozygous ($A_i A_j$) is $2p_ip_j+2p_ip_j^2+2p_jp_i^2+4\ p_i^2p_j^2)/4$. Therefore the conditional probability that Z is heterozygous ($A_i A_j$) given Y is heterozygous (A_i Aj) is $(1+p_i+p_j+2p_ip_j)/4$ (P_{sib}).

The general relationship between these probabilities is $P_{sib}>P_{par\text{-}offs}>P_{random}$ (because p_i and $p_j< 1$). We chose P_{sib} as the most conservative estimator given the high probability of sibling pairs in our samples. Note that for any given locus, P_{sib} can never be <0.25. Because we arbitrarily chose our statistical criterion for declaring a match as $P_{sib}<$ 0.05, at least 3 loci had to be scored to declare a match. In practice, 4 to 6 loci were required to achieve this standard for brown bear genotypes within our study population. This standard is not very conservative, given that an individual female might be traveling with 3 cubs, all of which might get sampled. However, we decided the standard was acceptable because most samples were typed at >4 loci ($P_{sib}<0.01$ for 91% of black bears and 75% of brown bears) and because such large family groups were uncommon (unpublished data, this study). We abandoned samples not meeting this statistical requirement.

It is important to realize that the statistical basis for a match must be made on an individual basis, not simply by calculating a mean expected probability of exclusion for the study population. This is because allele frequencies can vary dramatically, such that some genotypes will be very common and therefore likely to match genotypes from other individuals, whereas other genotypes will be extremely rare. In practice, this means different individuals will require different numbers of loci to achieve the desired level of statistical confidence.

Gender identification

In the third trial, we used a single sample from each individual to determine gender. The system involved co-amplification of a ZFX/ZFY fragment (genes found on the X and Y chromosomes and included as a positive control for amplification) and an SRY fragment (a Y-chromosome locus). The strategy was to amplify short products in both cases, but to make the SRY product shorter to eliminate any length-based amplification bias that might cause the ZFX/ZFY genes, but not the SRY gene, to amplify in some weak samples from males. The SRY primers were Taberlet et al.'s (1993) 41F and 121R. They should amplify a 119-bp product based on their data (we did not confirm this length precisely). One ZFX/ZFY primer was Aasen and Medrano's (1990) P1-5EZ, which they showed to be conserved in humans, mice, cattle, sheep, and goats. The other ZFX/ZFY primer was based on sequence that was conserved between mice (*Mus musculus*, Mardon et al. 1990) and hyenas (*Crocuta crocuta*, Schwerin and Pitra 1994, CTCCTTTTTCCTTATGCACC) and such that a 130-bp product should be produced (the length was not confirmed, but was very close to 130 bp). We labeled 1 primer from each pair with TET to allow use of an automated sequencer for analysis. PCR conditions were as used with microsatellites. Three known males, 3 known females, and 3 negative controls were included in each PCR experiment. PCR reactions were set up by a female technician, and great care was taken not to bring amplified DNA into the room where PCR reactions were set up.

Results

In our study of background genetic variability within the study area, we observed a mean expected heterozygosity of 0.81 in black bears and 0.69 in brown bears. This was sufficient variation to distinguish individuals with a small number of loci.

Trial 1: preliminary experiment to compare hair-trapping methods

Although all trap configurations collected bear hair, the barbed-wire enclosure design produced 74% of the usable samples (Table 1) and only this design was used in trials 2 and 3. In no case was there evidence of injury to bears passing under or over the wire or entanglement in the wire by bears or other animals.

Table 1. Comparison of four bear hair-trapping techniques at 20 sites in the Columbia River basin near Golden, British Columbia, Canada, 1995.

Hair-trap design	Total samples	Samples with roots	Samples with genotypes[a]	Unique genotypes[b]
Barbed-wire enclosure	227	128	118	25
Cubby with barbed-wire	42	36	32	10
Cubby with wire brush	16	8	5	3
Cubby with dog brush	8	2	5	1

[a] $P_{sib}<0.05$.

[b] All bears were black bears based on mtDNA analysis.

In trial 1, we collected 293 hair samples, of which 170 (58%) had visible roots and were used for DNA extraction. Of these, 153 (90%) had genotypes that passed our threshold of $P_{sib}<0.05$ and represented 25 individual black bears, 8 of which were known from the preliminary study of genetic variation in captured bears (i.e., recaptures). An additional sample gave a genotype that was a combination of 2 genotypes obtained from other samples at the same site. Genotypes were extended to 9 loci for 24 individuals to allow more detailed analysis of relationships between individuals and groups of individuals. All trial 1 bears were black bears, most likely due to the low elevation of these hair-traps.

Trial 2: hair-traps at remote camera sites

In trial 2, we collected 154 hair samples and extracted DNA from 118 (77%). Of these, 100 (85%) gave sufficient genotypes to identify 33 individual bears (29 black bears from 89 samples and 4 brown bears from 11 samples). An additional 5 samples appeared to contain hairs from >1 individual, some of which could be identified. Seven of the black bears and 3 of the brown bears were known from the preliminary study or from trial 1. Eight or 9 locus genotypes were obtained for 32 individuals.

During 387 days of concurrent sampling with the hair-traps, remote cameras produced 52 black bear and 9 brown bear samples. Cameras produced fewer samples than DNA hair-traps. However, a direct comparison with numbers of unique genotypes was not possible because individual bears could not be consistently determined from the photographs (lack of distinguishing features, differences in lighting, and subject orientation).

Trial 3: large-scale experiment

In trial 3, we collected 1,753 hair samples in 2,653 trap nights; 1,548 samples (88%) had roots, and it was possible to assign mtDNA species identification to 1,496 of these samples (97%). Sixteen samples produced both black bear and brown bear mtDNA bands and were abandoned. For black bears, there were 1,091 mtDNA identified samples. MtDNA species identification confirmed that 98% of the visually categorized black bear hairs were from black bears. Based on mtDNA analysis, we collected bear hair identifiable to species in 59 of the 64 cells (Figure 3). We collected black bear samples in 58 cells and brown bear samples in 27 cells.

For brown bears, there were 405 mtDNA-identified samples, of which 303 (78%) produced genotypes with $P_{sib}<0.05$. During a sampling session, a single hair-trap collected up to 12 usable hair samples from each unique genotype (median 3 samples/bear, 79% >1 sample/genotype). Further genetic analysis of black bear samples was not pursued because of constraints on laboratory time.

We initially identified 55 different brown bear genotypes among 303 hair samples. Of these, 10 genotypes were identified from only a single sample and 2 of these differed from another genotype by only 1 allele (the category where essentially all incorrect genotypes will be found if the error rate is low). We repeated the analysis of these 2 samples at the suspicious loci and found that a "false allele" (Taberlet et al. 1996) had been amplified in 1 case, reducing the total number of unique genotypes to 54.

Subsequent to the initial hair-capture session A, brown bears were recaptured during sessions B (4 individuals), C (5 individuals), and D (15 individuals) (Table 2). Because there were only 4 capture sessions, program CAPTURE's model selection routine choose the null model (M_o) followed by the individual heterogeneity model (M_h). We considered variation in individual capture probabilities to be most biologically reasonable for brown bears and therefore chose M_h to generate an estimate of 104 bears (CI 86–133, P=0.05). However, concurrent radiotracking of brown bears within the study area demonstrated individual movement in and out of the census area during trial 3 and therefore lack of complete geographic closure (unpublished data, this study).

Sex was assigned to all individual brown bears based on Y-chromosome analysis: 25 females, 29 males. In 1 case (bear 0284), we detected an error

Table 2. Captures and recaptures of brown bears based on genetic tags in the Columbia River basin near Golden, British Columbia, Canada, 1996.

	Hair-trap session[a]				
Category	A	B	C	D	Total
Bear captures	16	15	22	20	73
New bears	16	11	18	9	54
Recaptured bears	0	4	5[b]	15[c]	24
Cumulative new bears	16	27	45	54	N/A

[a] Four non-overlapping periods starting 10 June. Sixty-four hair-traps were moved within each 8 × 8-km grid cell at approximate 10-day intervals.

[b] Includes 3 bears from session A and 2 from session B.

[c] Includes 6 bears from session A, 4 from session B and 5 from session C.

in sex designation. Initial samples from this bear appeared to be male because of a strong SRY band on an overloaded gel. When diluted to produce a more normal strength gel, the SRY band was absent on 2 successive gels. Faint (false) SRY bands were often observed on female samples.

Discussion

Reproducibility of microsatellite genotypes

We provided statistical methods for declaring matches between samples that should allow researchers to control the risk of declaring samples from different individuals as the same. Using this approach, it is not possible to determine the number of loci required for a given sample *a priori*, but a preliminary survey of genetic variability in the study population will indicate the number of loci that will typically be required.

The preliminary data from brown bears (Paetkau et al. 1998*b*) support our contention that the chosen confidence threshold (P_{sib}<0.05) was conservative. When 6 locus genotypes were compared between all 46 of the individuals typed from blood and skin samples in that survey (1,035 comparisons), none had identical genotypes or genotypes that differed by a single allele. All 3 pairs of genotypes that differed by only 2 alleles were from known siblings or mothers and their cubs. These data also support our contention that even though few comparisons between genotypes involved relatives, it is these comparisons that are of primary concern for making mistaken declarations of matches. However, 1 of the hair samples in trial 3 had a genotype that differed from that of a previously physically captured bear by only 1 allele over 6 loci. This sample matched an animal that was subsequently physically captured. These 2 female bears had very small and highly overlapping home ranges, and they differed in age sufficiently that they could be either mother and daughter or siblings from different litters.

Errors due to mistakes introduced during genetic analysis can fall into 2 categories. First, inconsistent gel conditions, band sizing, analysis methods, accidental confusion of samples, or failure to carefully double check data could cause errors. For example, new technicians should be alerted to the problem of overloaded gels. Secondly, amplification of DNA from very small sources could result in the amplification of "false alleles" or the failure to amplify 1 allele (allelic dropout) in heterozygotes (Taberlet et al. 1996, Gagneux et al. 1997). It is essential that genotypes identified from only 1 sample, and which are very similar to other genotypes, be re-analysed. We encountered 1 case of a false allele when we did this with our samples. The barbed-wire enclosure hair-trap facilitates such reanalysis because of the frequent multiple samples collected for each bear.

We encountered lower error rates than might be expected based on other people's experiences (Taberlet et al. 1996, Gagneux et al. 1997). The difference may stem from our conservative standard for calling genotypes (we only used clean, obvious bands) or from differences in PCR conditions. For example, we extracted DNA from up to 4 hairs from a given sample and used a much larger proportion of individual DNA extractions for each PCR. Also, the commercial DNA polymerase used by others may be able to amplify smaller quantities of DNA than our polymerase, which we isolated using fairly crude purification procedures (Pluthero 1993). It also may be that the error-prone weak samples could not generally be amplified at enough loci to be included as a completed identification. Note that although use of multiple hairs per extraction occasionally resulted in mixed samples, these samples stood out due to their complex banding patterns, and we do not feel that mistakes were introduced because of this problem. In some cases the 2 genotypes present in such mixtures could be identified from unmixed samples obtained from the same strand of wire.

Using hairs plucked from alpine marmots (*Marmota marmota*), Goossens et al. (1998) examined the errors associated with microsatellite geno-

typing based on samples using a variable number of hair roots and demonstrated increased genotyping reliability as they increased the number of plucked hairs per sample. In the Upper Columbia Basin, average body weights for adult (age 5 years and older) bears were 63 kg (n=20) for female black bears, 93 kg (n=37) for male black bears, 102 kg (n=8) for female brown bears, and 168 kg (n=15) for male brown bears (this study, unpublished data) or approximately 12 to 32 times a 3.0–7.5 kg marmot (Nowak 1991). If hair size increases with body mass, there may be considerable variation in the amount of DNA per hair among species, sex, and age classes of bears, and hair from adult bears may contain more DNA than marmot hair. The relationship between body size, hair size, and quantity of DNA per hair root is of central importance to the application of these techniques across taxa, but to our knowledge has not been investigated.

In our experience, misidentifications can occur when using any tagging system. Ear tags may be lost or misread, tattoos can be distorted with time and growth of the bear, bears can change in appearance, radiotransmitters can be lost or malfunction, and radio frequencies can be confused or duplicated by overlapping projects. Despite taking great care in genetic tagging, it is clear that errors will occasionally occur and that rates will vary among laboratories and individual analysts. Thus, it is essential to scrutinize data for possible errors. We recommend that field workers evaluate error rates by including a number of "blind" samples from known individuals when sending samples away for analysis.

Species identification

Species identification based on mtDNA from hair provided a rapid screening that consumed only 2.5% of the DNA per extraction. Molecules of mtDNA are present in cells at much higher copy numbers than nDNA and therefore samples from which mtDNA cannot be amplified are unlikely to yield nDNA data. This screening eliminates weak samples from nDNA analysis.

All mtDNA samples identified as brown bears, and subsequently typed at the nuclear microsatellite loci, had genotypes that were more likely to occur in brown bears than black bears within this study area (based on allele frequencies in Paetkau et al. 1998*b*, essentially the assignment test of Paetkau et al. 1995). This nDNA-based genotype likelihood approach could be used to discriminate between species, but would be practical only if the proportion of samples from the non-study species was small and did not represent a prohibitive amount of work to type at multiple nuclear loci.

Black bear entering barbed-wire enclosure hair-trap, Columbia River basin, 1995.

Identifying black bears by visual inspection of hair was highly successful. This approach can provide an efficient way to minimize amount of genetic analysis required in areas where brown bears are the species of primary interest and black bears are sympatric. However, success may vary according to the pelage variation of the bears in the particular study area.

Sex identification

Although faint SRY bands have been observed on female samples by ourselves and Taberlet et al. (1993), these are readily distinguishable. The ideal sex determination test for analysis of hair would amplify short regions of genes that were present on the X and Y chromosomes using the same PCR primers and would discriminate between sexes by way of a length polymorphism that caused the Y-chromosome amplification product to be shorter. We were unable to develop such a system and settled for one where the amplification control was amplified by different primers than the Y-chromosome locus, albeit in the same PCR reaction. This system was accurate in the large number of controls that were included in each PCR experiment. We conclude that this system will be acceptable until a system closer to the ideal is developed.

Using DNA sampling of free-ranging bears in ecological field studies

Many hair samples yielded DNA in sufficient quantity and quality to determine bear species, sex,

and individuality and the wire hair-trap provided an efficient way to collect many DNA samples from free-ranging bears. In refining this technique, protocols that reduce equipment weight and heighten the drawing power of the scent lure would increase efficiency. For example, substitution of a concentrated scent lure for the rotten meat would facilitate trap setup and popularity with pilots and others. Researchers in Europe have used turpentine to attract European brown bears to "wire netting" hair-traps (Camarra 1994). To our knowledge, the effectiveness of this scent lure on North American brown bears has not been reported. Because the quantity of available DNA may be limiting in some hair samples, refinements in hair-snagging devices, protocols for collection and storage of samples, and DNA extraction methods would improve the technique. This may be particularly important for studies requiring pedigree analysis (e.g., Craighead et al. 1995) or a more stringent DNA match criterion (P_{sib}).

Although field equipment (wire, scent lures, hand tools) for our experiments was inexpensive, transportation costs were considerable because helicopters were essential in our mountainous study area. Laboratory expenses included costs for extraction of the DNA from the hair samples and costs for analysis of the nDNA and mtDNA. We estimate the commercial cost of DNA extraction from hair roots at approximately $5.00 (Can.)/sample and the subsequent analysis at $50–$100 (Can.)/sample. In comparison, physically capturing brown bears in our study area cost approximately $5,000 (Can.)/bear. In areas where black and brown bears are not sympatric, mtDNA analysis would not be necessary. While the availability of academic and commercial laboratories to perform DNA analyses is likely to increase, we caution researchers to establish laboratory availability and costs before commencing a DNA based mark–recapture project.

As noted by Parker et al. (1998) and others, genetic tags provide a reliable method of repeatedly recognizing individuals and have potential applications to numerous types of field studies. DNA gathered from free-ranging bears will be useful as an alternative or compliment to capturing and marking bears in mark–recapture censuses, presence–absence studies, and home-range delineation. In addition, bear hair DNA can be used to assess genetic diversity within and between populations, family relationships, dispersal, and the genetic success of bear translocations. For example, our genotypic data suggest strongly that a group of animals we captured on 3 occasions in adjacent cells consisted of a mother (0308) and her offspring (0302 and 0311). Similarly, the combination of hair-trap and bear capture DNA data identified a group of female brown bears with extremely similar genotypes occupying overlapping or adjacent ranges (0035, 0284). By extending the genotypes of these individuals to more loci, it would be possible to confirm relatedness hypotheses with high probability, identify the mothers, and identify fathers if they have been sampled. Similar analysis has been done successfully from brown bear blood and ear tissue samples using 8 microsatellite loci (Craighead et al. 1995).

There are numerous biological and logistical challenges in applying mark–recapture methods (see White et al. 1982 and Garshelis 1992). Using genetic tags to estimate brown bear numbers may improve several aspects of census design. For example, DNA fingerprints cannot be lost (unlike ear tags or streamers) and, with appropriate analysis procedures, should be rarely misread. Snagged hair may reduce the likelihood of "trap response" because the bear is never physically restrained or surprised by a sudden event such as a camera advance or electronic flash. These tags also provide valuable additional resolution in the data, including the sex of animals sampled and confirmation of species (a potential source of error in photograph-based bear mark–recapture studies with sympatric bear species). Potential shortcomings of the genetic tag technique include under-representation of animals <50 cm high at shoulder height, the possibility of previous negative encounters with electric fencing causing trap avoidance, and the lag

Brown bear entering barbed-wire enclosure hair-trap, Columbia River basin, 1995.

between field collection and laboratory analysis.

Brown bears in forested habitats are particularly difficult to census because they are difficult to observe, exist at low densities, and have relatively large home ranges (Mace et al. 1994, Mace and Waller 1997). While genetic tags may help to address some of these issues, lack of geographic closure will be a difficulty in many study situations.

We agree with Haig's (1998) observation that the combination of molecular information with ecological data gathered from the animals in the field is a useful tool in population biology. For example, of the 54 brown bears identified by hair in trial 3, 12 also are known from live-animal captures and radiocollaring. This provides the potential to add animal age (from tooth analysis), movements, and observed relationships to the genetic information and provide a strengthened insight into bear ecology.

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