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Detecting genotyping errors and describing American black bear movement in northern Idaho

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Abstract: Non-invasive genetic sampling has become a favored tool to enumerate wildlife. Genetic errors, caused by poor quality samples, can lead to substantial biases in numerical estimates of individuals. We demonstrate how the computer program DROPOUT can detect amplification errors (false alleles and allelic dropout) in a black bear (*Ursus americanus*) dataset collected in 2003 from northern Idaho, USA, and detect scoring and other database errors (misreads, shifts in scoring, and transcription errors). Removing errors from our sample via computer techniques reduced our minimum number alive index from 187 to 146 bears and was less expensive than commonly used multi-tube approaches. We subsequently estimated gene flow between our 2 study areas (Purcell and Selkirk Mountains), which are separated by a large, open, agricultural valley. Gene flow data suggested that, although this valley was not a complete barrier to movement, its effects on population substructure were not inconsequential. We documented a low level of substructure ($G'_{ST} = 0.097$) between study areas. Assignment tests confirmed this, as assignment to the population where the animal was captured was 74% for the Purcell Mountains and 89% for the Selkirk Mountains.

Key words: allelic dropout, American black bear, genotyping error, microsatellite, population genetics, *Ursus americanus*

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Non-invasive genetic sampling has become a popular tool for wildlife biologists and managers to detect species presence (Farrell et al. 2000, Mills et al. 2001) and hybridization (Adams et al. 2003, Miller et al. 2003, Schwartz et al. 2004), evaluate relatedness or paternity in a population (Gerloff et al. 1999, Constable et al. 2001), and assess movement (Flagstad et al. 2004). However, the most prominent application has been to evaluate the population size of rare and elusive species or those species for which traditional capture methods are expensive, dangerous, or difficult (Taberlet et al. 1997, Kohn et al. 1999, Paetkau 2003).

Non-invasive genetic sampling to evaluate abundance uses unique genotypes, primarily derived from DNA microsatellite markers, to determine the number of individuals in a population. Abundance is commonly assessed either through the direct count of unique genotypes or by using these genotypes as molecular tags in capture–mark–recapture (CMR) models (Woods et al. 1999, Bellemain et al. 2005). However, non-invasive genetic sampling has several problems that must be resolved before unbiased population estimates can be generated (Mills et al. 2000, Waits and Leberg 2000, Creel et al. 2003), the foremost being that DNA data, like many types of field data (such as GPS and telemetry error), are subject to error. These errors can lead to overestimates in abundance >5.5 fold (see Creel et al. 2003, McKelvey and Schwartz 2004).

Non-invasive genetic samples usually are of lower quality and have lower quantities of DNA than tissue samples. Thus, the original low copy number of DNA strands, coupled with DNA degradation can, upon amplification, lead to either the production of spurious bands that look like alleles (false alleles), or the amplification of only one allele in a heterozygous individual (allelic dropout). Fortunately, the solution to this problem has been aggressively addressed by molecular ecologists (Morin et al. 2001, Miller et al. 2002, Valiere 2002, Bonin et al. 2004), and, in general, has taken one of 3 forms. First, many laboratories have used the "multi-tube" approach, where DNA samples

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are analyzed multiple times to ensure accuracy (Taberlet et al. 1996, Bellemain et al. 2005). This approach has proven successful in many published studies, but suffers from several problems: amplifying samples multiple times is expensive, and DNA is often limited when collected using non-invasive means (Paetkau 2003). A second approach is to quantify the amount of extracted DNA and only analyze samples with adequate yield (Morin et al. 2001). A limitation of this approach is that the equipment needed for precise quantification of target DNA is expensive. A third approach is to use computer algorithms to detect samples containing genotyping errors and re-run samples that might contain errors (Ewen et al. 2000, Miller et al. 2002, Valiere 2002, Van Oosterhout et al. 2004, McKelvey and Schwartz 2005).

We contend that the first 2 approaches, while valid for detecting errors, do not demonstrate that a dataset is error-free. We have commonly read that dataset errors have been removed through application of a multi-tube or quantification approach, but this confuses process with product. In other words, both multi-tube and quantification approaches fail to differentiate the rigor of the protocol from the results due to the application of the protocol. Multi-tube and quantification approaches also do not detect errors commonly created when scoring gels or transcribing data into a database. In contrast, screening the datasets with computer algorithms provides statistical evidence that the data have few or no errors. Furthermore, some of these algorithms can detect important scoring and transcription errors.

McKelvey and Schwartz (2004, 2005) proposed 2 screening methods, the examining bimodality (EB) and difference in capture history (DCH) tests, to detect genotyping errors, both of which can be found in a computer package called DROPOUT. The EB test formalizes tests presented in Paetkau (2003), but has a longer genetic tag (more loci). This test takes advantage of bell-shaped relatedness distribution in an ideal population produced from genetic transfer to offspring. If errors occur in a dataset, under most circumstances they appear as a second mode. Thus, a genetic sample analyzed with a sufficient number of highly variable markers will produce a bimodal distribution, the first mode being error and the second mode being samples without error (see McKelvey and Schwartz 2004, 2005 for details and limitations). This test identifies samples and loci containing errors, allowing for both error removal and demonstration that the dataset has reduced errors to trivial levels.

The second test, DCH, takes advantage the genetic uniqueness of individuals. Changes in individual identification due to increasing the number of genetic markers are therefore either a shadow effect (see Mills et al. 2000) or an error. The DCH test, used with a long tag, estimates the number of genetic markers required to eliminate shadow effects (Evett and Weir 1998, Mills et al. 2000, Waits et al. 2001), then adds additional loci, one at a time, to determine if there is an increase in numbers of individuals generated by adding these additional loci. Rotating the loci such that every locus is, in effect, the additional locus allows an efficient screen to determine which loci are erroneously adding new individuals (McKelvey and Schwartz 2004).

McKelvey and Schwartz (2004) evaluated the efficacy of these methods using computer simulations; however, the application of these methods with field collected non-invasive genetic samples has not been published. We use new data from a non-invasive genetic sampling study on black bears (*Ursus americanus*) in the Purcell and Selkirk Mountains of the Idaho Panhandle (Fig. 1) to test both computer algorithms and examine bias produced by genotyping error. We chose the black bear to test these methods because sampling for bears using non-invasive genetic sampling has become common (Woods et al. 1999, Proctor 2002, Paetkau 2003, Boersen et al. 2003, Boulanger et al. 2004), and this project dovetailed with ongoing wildlife habitat research being conducted on bears in northern Idaho (Cushman and McKelvey, unpublished data). In addition to testing the effectiveness of these error-checking mechanisms, we used these data to examine the genetic relationships between bears in the Purcell Mountains and the Selkirk Mountains.

Methods

Study area

The study area consists of an approximately 3,000 km^2 area of the Idaho Panhandle National Forests, located in the northern tip of Idaho, USA (Fig. 1). The area comprises parts of the Selkirk and Purcell Mountains, and ranges in elevation from approximately 700 m to 2000 m. The topography is mountainous, with steep ridges and narrow valleys at the highest elevations. The Kootenai River trench extends through the middle of the study area, separating the Selkirk Mountains to the west from the Purcell Mountains to the east, with an 8 to 11 km wide un-forested agricultural valley and large river, potentially acting as a barrier to movement (Fig. 1).

Genetic sampling

Genetic materials were obtained using non-invasive hair snaring between June and August 2003. Hair

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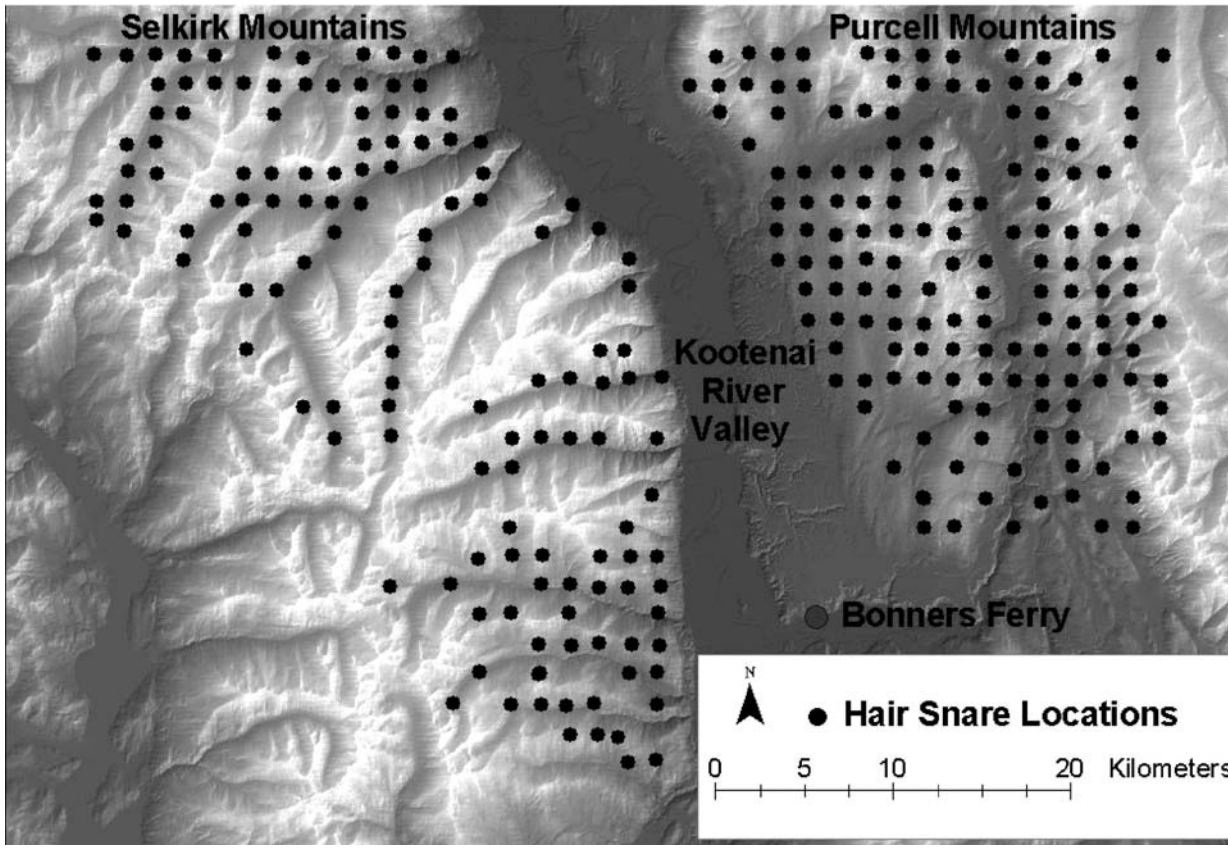


Fig. 1. Map of northern Idaho and study areas in the Selkirk and Purcell mountains, separated by the Kootenai River Trench, a large, open, agricultural valley. Circles represent hair snare locations.

snaring followed the protocols of Proctor et al. (2002), with sampling stations consisting of single strands of 4-prong barbed wire approximately 50 cm above ground in a closed ring encircling 4–6 trees. In the center of the ring, 1 L of lure consisting of 2 parts decayed fish to 1 part decayed blood and 1 part glycerine was poured over a pile of decayed wood. Each sampling station was visited at approximately 14-day intervals. During the visit, hair was collected from the barbs. Each barb was considered a single sample (Woods et al. 1999, Mowat and Strobeck 2000) and collected in 50-ml centrifuge tubes containing approximately 10 ml of silica desiccant crystals. Tubes were labeled such that the location and adjacency of samples on the wire could be determined. The corrals were set at 266 National Vegetation Pilot plots (Morgan et al. 2004; the National Vegetation Pilot is a grid of permanent vegetation plots at 1.6-km spacing located in the study area established to monitor vegetation change).

Laboratory methods

Samples consisted of either clumps of hair, single hairs, or sometimes simply hair fragments (usually without root cells); lower quality samples were only analyzed if they were the only samples from a station. Samples were extracted within 3 months of collection using the Qiagen DNeasy Tissue Kit (Qiagen Inc., Hilden, Germany) with modifications for hair samples as outlined in Mills et al. (2001). We identified black bear samples using modified 16S rRNA universal primers (Hoelzel and Green 1992) similar to those used in Mills et al. (2001).

We separated the black bear samples from other species and, if more than 1 bear sample was associated with a station, chose the first sample analyzed, thus maximizing the spatial distribution of bear samples. We analyzed these samples at 9 microsatellite markers: *G1A, G10D, G10B* (Paetkau and Strobeck 1994), *G10H, G10J, G10M, G10X, UarMu59* (Paetkau et al. 1998),

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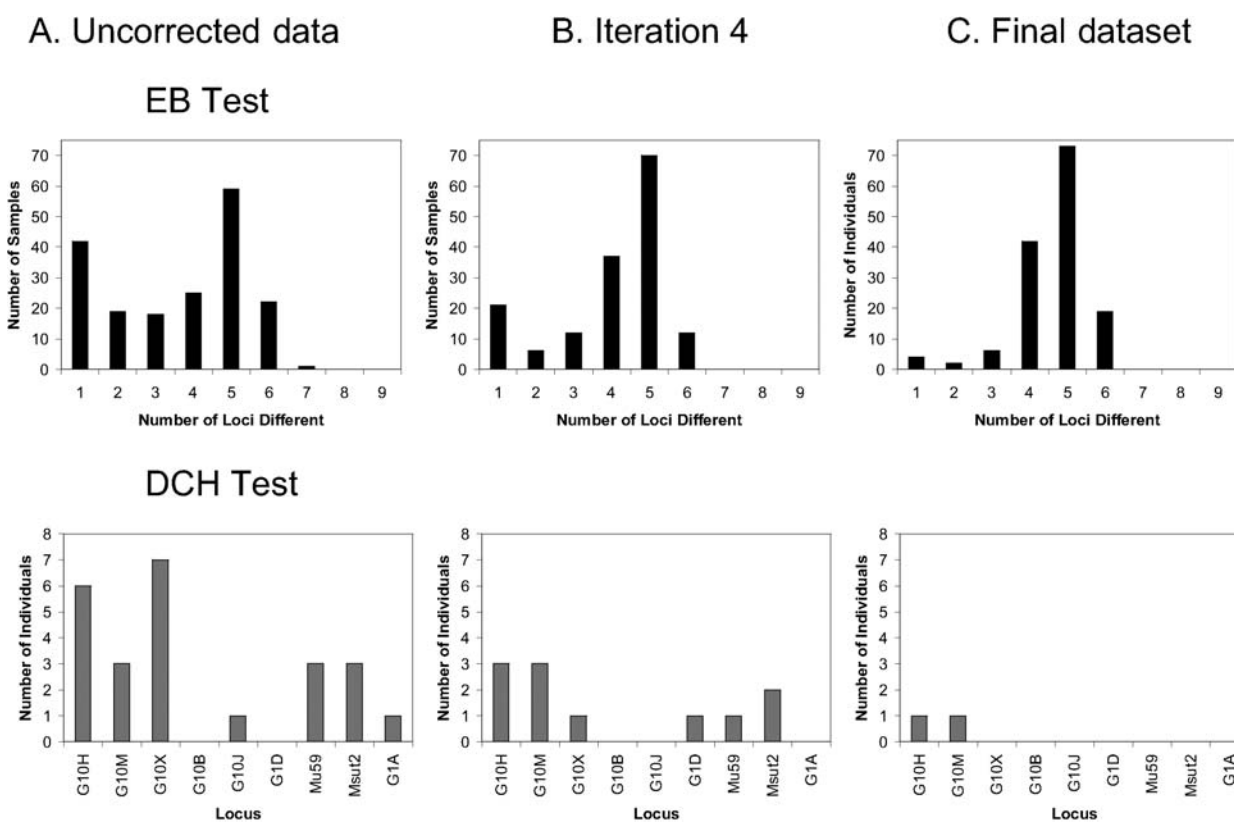


Fig. 2. The EB (examining bimodality) and DCH (difference in capture history) test on 245 black bear samples collected in the Selkirk and Purcell Mountains, Idaho, in 2003. Graphs display the original dataset, data after 3 attempts to remove errors (iteration 4), and the final dataset of hair samples from black bears in northern Idaho, 2003. Error presents itself in the EB test as a left-mode.

and *Msut-2* (Kitahara et al. 2000). Reactions were conducted in a 10 μL reaction volume comprised of 1X Applied Biosystems buffer (Foster City, California); 1 unit of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, California); 0.8 millimoles magnesium chloride $MgCl_2$; 200 micromoles (μM) of each deoxynucleotide; and 1 μM of each primer. Polymerase chain reactions (PCR) were run in a thermal cycler (MJ Research PTC-200, Waltham, Massachusetts, USA) under the following conditions: 94°C for 10 min; followed by 45 cycles of 94°C for 1 min, 56–60°C (see initial primer papers for details) for 1 min, and 72°C for 1 min and completed with a step of 72°C for 10 min. The subsequent products were visualized on a LI-COR DNA analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA). Allele sizes were visually estimated by comparing the allele to both lane size standards and samples with known allele sizes. We scored all genotypes only once (in contrast to our typical procedure of scoring by 2 independent observers) to determine if our error-checking protocol could detect scoring errors.

Error-checking protocol

All hair samples were analyzed once at each microsatellite locus and entered into an interim database. Samples that did not amplify were left blank in the database. Next, we ran program DROPOUT's (McKelvey and Schwartz 2005) DCH test to determine if any locus had a high error rate. Subsequently, program DROPOUT's EB test was executed to determine which samples contained putative errors. Using a 9-locus genotype allowed us to have 2 clear modes, an error mode and a non-error mode (Fig. 2). Samples in the error mode were reamplified 3 times, the genotypes were rescored, and the database was updated. Additionally, we reanalyzed samples that failed to amplify and added the results to the database. We then re-applied the EB

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test and derived a new error mode. The process of iteratively re-running samples and executing the EB and DCH tests continued until no error mode was detected in the EB test and the DCH test confirmed that no new false individuals were being generated by genotyping errors. During the error-checking process, we categorized each type of genotyping error encountered per iteration into 2 groups, scoring errors and amplification errors (Paetkau 2003). Scoring errors were of 2 types: shifts and misreads. An error was classified as a shift if it was read as one mutation away from the actual score (such as calling an allele 140 when it was 142), whereas a misread was any other type of scoring error (for example, transcription errors). Amplification errors were false alleles and allelic dropout events. In addition to classifying errors, we calculated the minimum number of bears alive at each iteration, and a final minimum number alive with genotyping errors removed. We also calculated the number of laboratory DNA runs required to conduct our analysis using the EB and DCH protocol and compared this to the minimum number required using other standard error-checking protocols.

Statistical analysis

We tested for deviations from Hardy-Weinberg (HW) proportions, heterozygote excess, and deficiency with program GENEPOP (Version 3.1d; Raymond and Rousset 1995). We used sequential Bonferroni tests to correct for multiple tests (Rice 1989). We also tested for linkage disequilibrium using FSTAT 2.9.3 and Bonferroni corrections (Goudet 1995, 2001). We estimated genetic variability for each locus within a population by calculating the mean number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and allelic richness. Lastly, we used several analytical approaches to estimate gene flow between samples collected on the Selkirk and Purcell sides of the Kootenai River Trench, the most likely barrier to movement dividing our study area. We calculated indices of population substructure F_{ST}, R_{ST}, and G'_{ST} (Hedrick 2005) with program FSTAT 2.9.3. F_{ST} (also called G_{ST} when more than 2 alleles exist at a locus; Nei 1977) is a measure of genetic divergence among subpopulations that ranges from 0, when subpopulations have equal allele frequencies, to 1, when subpopulations are completely different (Allendorf and Luikart 2007). R_{ST} is similar to F_{ST}, but accounts for allele length by assuming a stepwise mutation model of microsatellite evolution. Finally, G'_{ST} is a standardized form of G_{ST} scaled by the maximum possible value given the expected homozygosity (Hedrick 2005). We also used the Bayesian-based assignment test of Rannala and Mountain (1997) to index movement directly using program GENECLASS2 (Piry et al. 2004, Paetkau et al. 2004). Finally, the genetic relationship among individuals was visually evaluated using principal component analysis with program PCAGEN (Goudet 1999).

Results

We collected 663 hair samples from 169 of the 266 stations. We extracted DNA from 515 of these, of which 90% produced species identification. Three hundred and fifty-two (75.7%) of those samples that produced species identification were from black bears and 2 (0.4%) were from brown bear (*Ursus arctos*). Avoiding adjacent samples to minimize sample redundancy, we genotyped 245 black bear samples, from across the study area, at 9 microsatellite loci.

Error-checking

Initial analysis produced 187 unique genotypes. The probability of identity (P_{ID}) was 1.82×10^{-11} and probability of identity assuming siblings (P_{SIB}) was 1.26×10^{-4} for a 9-locus genotype. This allowed sufficient power to conduct the DCH test (McKelvey and Schwartz 2004) and provided a well defined error mode with the EB test (Fig. 2). The DCH test revealed that no locus had significantly more errors than any other locus, thus we did not drop any loci from further analyses. However, the DCH test revealed that when locus *G10X* was in the L_{base+1} location (L_{base} is the base number of loci needed to achieve sufficient power to differentiate individuals and remove shadow effects; L_{base+1} is the inclusion of 1-locus to the L_{base}, which allows the evaluation of genetic errors; McKelvey and Schwartz 2004, 2005), it artificially added the most new individuals (7). Running all loci through the L_{base+1} position produced 24 new individuals, signifying genotyping errors in the database (Fig. 2, uncorrected data, DCH).

We subsequently used the EB test to determine which samples were likely causing the errors (Fig. 2, uncorrected data, EB). We found 2 modes, the lower of which is likely caused by genotyping error. We re-amplified all samples in the first mode of the EB test on the 2 loci deemed most problematic by the DCH test (*G10H* and *G10X*) and locus *G10J* (which only showed 1 new individual added) and re-scored all gels. We also re-amplified samples that initially failed to amplify at particular loci.

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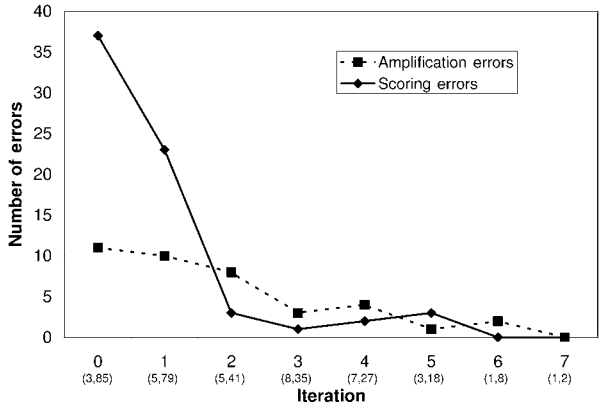


Fig. 3. Errors in a data set containing 245 black bear samples collected in the Selkirk and Purcell Mountains in 2003. Errors shown are those detected after each iteration of amplification and analysis. We could not determine the nature of errors in the initial dataset until samples were rerun, thus we could only categorize error after 8 iterations (A–H), despite running the programs 9 times. We categorized genotypic error into amplification errors (allelic dropout, false alleles) and scoring error (shifts, gross misreads) for this graph. The numbers below the iterations are the number of loci re-run, followed by the number of samples.

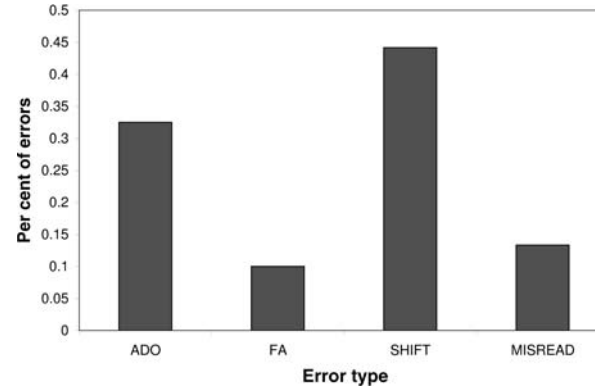


Fig. 4. Types of errors encountered during the error checking procedure. ADO = allelic dropout; FA = false alleles. Data are from 245 black bear samples collected in the Selkirk and Purcell Mountains in 2003.

After this first iteration we again ran the EB and DCH tests. A total of 179 unique genotypes were identified; however, there were still 2 apparent modes in the EB test. Thus, we continued the process of error identification and reanalysis for 8 iterations. After iteration 7 the DCH test revealed only 1 individual added for locus *G10H* and 1 individual added for locus *G10M*. We reran the associated samples a total of 7 times each, and no dropout, false alleles, or other genotyping errors were detected. Thus, despite the final DCH test producing 2 changes in capture history ($n = 1$ on each), we concluded that these were not associated with errors but rather associated with 2 very closely related bears.

Categorizing errors and effort

For iterations 2–8, we categorized the type and number of errors removed by comparing results with those of the previous iteration. The number of scoring errors differed per iteration ($\chi^2 = 98.05$, 5 df, $P < 0.0001$; Fig. 3), declining markedly after the first 2 rounds of error-checking and rescoring. Genotyping errors also differed per iteration and also declined ($\chi^2 = 29.57$, 6 df, $P < 0.0001$; Fig. 3). Overall, we detected 120 genotyping errors. The number of errors differed by type ($\chi^2 = 37.67$, 3 df, $P < 0.0001$; Fig. 4), with shifts in gel reading producing the most errors.

Using the algorithms in program DROPOUT required 3,090 single locus runs (an amplification and scoring at a locus) to obtain a sample with errors removed (Table 1). Without any error-checking, running 245 samples at 9 loci would have required a minimum of 2,205 (9 x 245) runs.

Numbers of bears

Before error-checking with program DROPOUT, the number of unique genotypes was 187 and the number of recaptures was 58 (Fig. 5). During the error-checking process we discarded 2 additional samples, 237-2A and 376-1LP. The first was discarded because we suspected more than one bear to have left the sample (3 or more alleles appeared at several loci), and the second was

Table 1. Number of runs required to conduct our non-invasive sampling effort on 245 samples compared to estimated minimum number of runs required using other protocols to detect genotyping errors and evaluate population structure for 2 black bear population in northern Idaho, 2003; DCH = difference in capture history; EB = examining bimodality.

Protocol	Runs conducted or estimated
No error checking, no re-running blanks	2,205
Error checking with DCH and EB test	3,090
Running all samples 3 times	6,615
Running all samples 7 times	15,435

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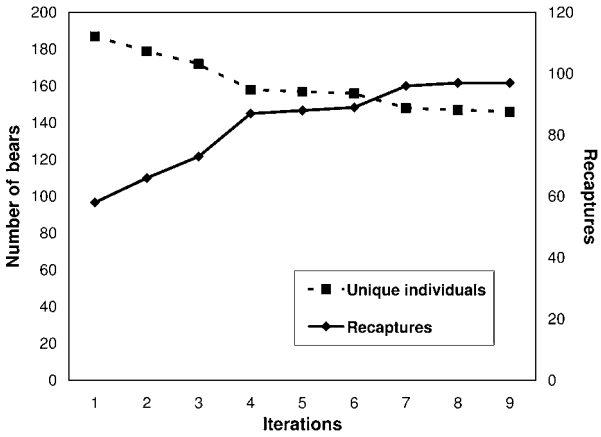


Fig. 5. Effect of removing error each iteration on the number of unique individuals and recaptures. Data are from 245 black bear samples collected in the Selkirk and Purcell Mountains, Idaho, in 2003.

discarded because it produced inconsistent genotypes throughout the analysis. After completing the error-checking, we found 146 unique genotypes and 97 redundant samples (Fig. 5). Thus, failure to check and correct errors would have produced a 28.1% over-estimate of unique genotypes.

Population genetics

Global tests show that both populations were in Hardy-Weinberg equilibrium, although in the Purcell samples, locus *G10H* had a significant deficit of heterozygotes ($P = 0.042$, $F_{IS} = 0.075$; Table 2) and locus *G10X* had a significant excess of heterozygotes ($P = 0.005$, $F_{IS} = -0.099$; Table 2). The overall F_{IS} values for the Purcell and Selkirk samples were 0.024 and 0.003, respectively. The Purcell sample had 5 loci with a positive F_{IS} and 4 with a negative F_{IS}; the Selkirk sample had 6 loci with a positive F_{IS} and 3 with a negative F_{IS}. There was no evidence of gametic disequilibrium, suggesting that the markers were independent.

We were interested in black bear movement between the Selkirk and Purcell areas. F_{ST} was 0.022 (SE = 0.005) with values ranging between 0.0022 and 0.043. R_{ST} was 0.0452 with values ranging from 0.00 to 0.129 and G'_{ST} was 0.097. Assignment tests showed that 54 bears (74.0%) sampled in the Purcell Mountains were assigned to the Purcell Mountains, whereas 19 (26.0%) were assigned to the Selkirk Mountains. Alternatively, 65 bears (89.0%) sampled in the Selkirk Mountains were assigned to the Selkirk Mountains and 8 bears (11.0%) sampled in the Selkirk Mountains were assigned to the Purcell Mountains. The PCA conducted using individual bear genotypes identified no discrete clusters (Fig. 6).

Discussion

Error detection and removal

The iterative use of algorithms EB and DCH and re-running questionable samples effectively detected genotyping errors using a 9-locus genotype with an empirical dataset from a wild population. It required a maximum of 40% more laboratory effort to reduce errors to trivial levels than if no error-checking was done (even in cases where errors are not sought, usually some samples need to be re-run to confirm ambiguous or missing scores). Some investigators contend that only a 5- or 6-locus genotype is required to uniquely identify most bears. However, with a 5- or 6- locus tag, many error-free samples would only differ at 1–3 loci, meaning that a larger proportion of the total samples would need to be re-amplified to achieve similar levels of error removal (Paetkau 2003 describes short tag protocols). Further, tags consisting of only 5 or 6 loci are unlikely to uniquely identify all bears; even with a tag of 9 we had 2 bears that differed at only a single locus due to the shadow effect (see Waits et al. 2001).

Overall, we needed 5 times less effort to obtain a 9-locus genotype using EB and DCH than multi-tubing samples 7 times, approximately 2 times less effort than multi-tubing samples 3 times; and 3.3 times less effort than obtaining a 6-locus genotype and multi-tubing each sample 7 times. Furthermore, there are other reasons to prefer a 9-locus genotype as an end-product. For

Table 2. Descriptive statistics of the 245 black bear samples collected in the Purcell Mountain and Selkirk Mountains, Idaho, in a 2003 black bear dataset. *A* is mean number of alleles per locus (SE is standard error), A_{adj} is allelic richness, which is *A* adjusted for sample size, H_o is observed heterozygosity, H_e is expected heterozygosity, and F_{IS} is a measure of the departure from Hardy-Weinberg proportions within local subpopulations usually caused by nonrandom mating or population subdivision.

	A (SE)	*A* (adj)	H_o	H_e	F_{IS}
Purcell Mountains ($n = 72$)	9.00 (0.75)	8.94 (0.71)	0.76 (0.04)	0.78 (0.02)	0.024
Selkirk Mountains ($n = 73$)	9.33 (0.76)	9.26 (0.74)	0.80 (0.02)	0.80 (0.02)	0.003

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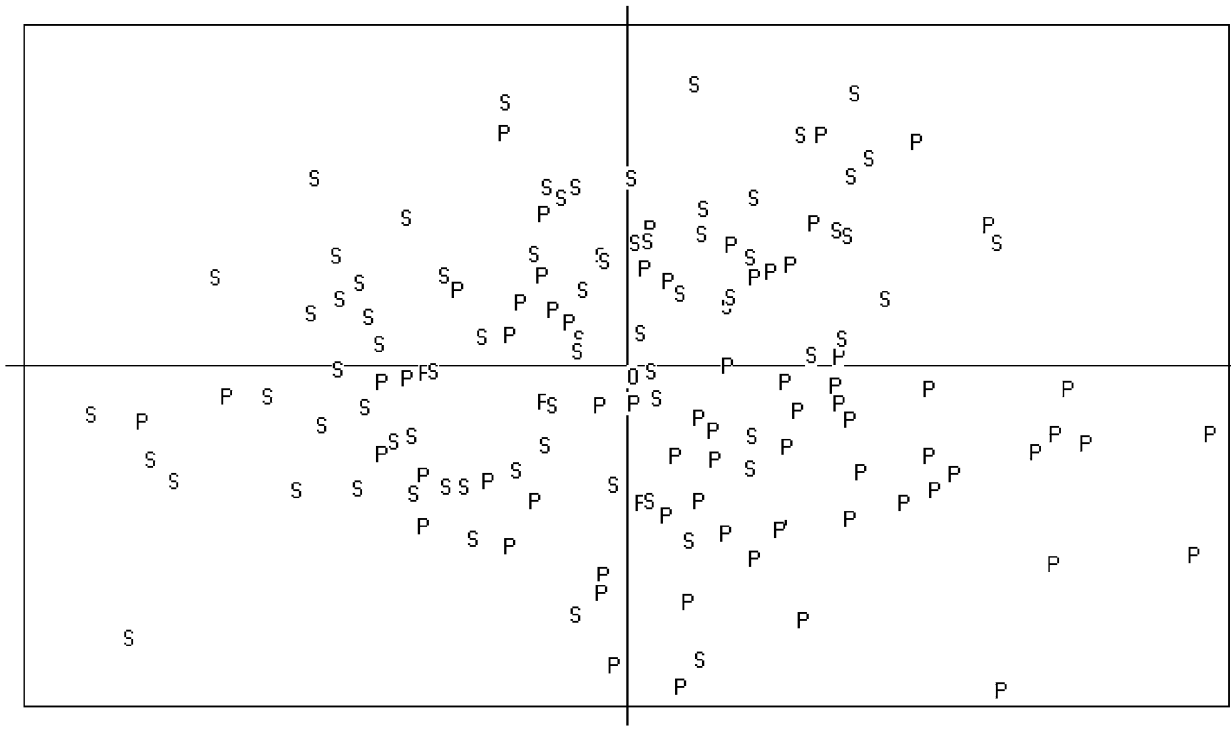


Fig. 6. Principle components analysis on bears from the Selkirk Mountain Range (S) and the Purcell Mountain Range (P). Data are from 245 black bear samples collected in 2003.

instance, if these data are ultimately used to answer questions regarding relatedness, gene flow, effective population size, or genetic bottlenecks, the use of 9 loci will provide more precise estimates.

The majority of errors in this study were due to scoring errors. A simple shift in scoring a gel can cause multiple samples on that gel to be mis-scored by a single repeat. Thus, errors due to shifts are not independent and are often the cause of multiple errors at one time. Paetkau (2003), who used a method similar to the EB test to check for errors, also noted that scoring errors were more abundant than amplification errors. The EB and DCH tests detect errors of any type, whether shifts, misreads (scoring errors), false alleles, or allelic dropout (amplification errors). We rescored loci after the first and second iteration (3 of the loci during the first iteration and the remainder during the second iteration), after which shift errors were greatly reduced.

In this study error-checking proved to be critical. We had a 28.1% overestimate in the number of unique genotypes before error-checking and incorporating these data into a capture–mark–recapture framework. Had we used these uncorrected data to estimate population size, the degree to which we overestimated abundance and the variance around this estimate would have increased, as unique captures would have been artificially high and recaptures artificially low (McKelvey and Schwartz 2004). If these numbers were used by management to set harvest quotas or to determine protection for the species, this overestimate could lead to erroneous inferences. Although our protocol is not the only way to ensure that a sample has low error levels, it has 2 advantages over multi-tube approaches: it was more efficient (in terms of number of sample re-runs; Table 1) than the traditional multi-tubing approach, and the DCH test confirms that errors have been reduced to trivial levels. Other approaches, such as multi-tubing, can remove some errors, but do not demonstrate that the errors are ultimately removed. Furthermore, multi-tube protocols don't detect scoring errors, which have been found to be more common than amplification errors in this study and in Paetkau (2003).

Several requirements are needed for the DROPOUT tests to effectively detect errors. The genetic markers must have sufficient variability to eliminate the shadow effect and differentiate the error mode from the second, population mode, using the EB test. Lack of variability will also limit the utility of the DCH test because

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this test requires the inclusion of one additional locus past the point where the probability of identity is inconsequential; in systems with low variable markers, this point will never be reached. In addition, the sample must contain a substantial number of redundant samples for errors to be detected. While these algorithms detect errors important to CMR, if a genotype is only captured one time (i.e., no re-captures) this sample may still contain errors which are irrelevant to CMR analyses in that they produce no new individuals and do not confuse one individual with another. Thus, if overall error removal is critical to an analysis (e.g., paternity), multi-tubing all samples may be the best alternative. Finally, the EB test is time-consuming, requiring time to sort through samples that have putative errors and to run the database through DROPOUT multiple times.

Relationships among Purcell and Selkirk bears

The 245 samples analyzed in this project had 146 unique genotypes: 73 in each mountain range were identified. In the Purcell Mountains, H_o was 0.76 and H_e was 0.78; in the Selkirk Mountains both were 0.80. Overall, F_{IS} was non-significant in both populations. Although F_{IS} can be significant for a variety of reasons, the fact that F_{IS} is insignificant provides additional confirmation that genotyping errors are low (Hosking et al. 2004).

The 2 samples (Purcell and Selkirk) were drawn from 2 distinct mountain ranges separated by a large agricultural valley. F_{ST} differences indicated movement of approximately 3 migrants per generation across the valley. Although gene flow estimates based on F_{ST} can be biased (Whitlock and McCauley 1999), it is widely used as an index of movement, and F_{ST} between 1 and 10 migrants is considered to be a medium level of movement per generation (Mills et al. 2003). These results were supported by the assignment test (Rannala and Mountain 1997), where 72.6% and 89.0% of the individuals sampled in the Purcell and Selkirk Mountains, respectively, were assigned to their sampling location. If the agricultural valley acted as a complete barrier, the assignment test would have shown near 100% assignment to the population where the animal was sampled. Alternatively, if there were no substructure, assignment of individuals would have been approximately equal between the populations.

Overall, these gene flow results combined with the principal components result point to there being neither panmixia, nor complete isolation between the populations. However, *F*-statistic data have a time lag that is proportional to the effective population size. Thus, the F_{ST}, R_{ST}, and G'_{ST} estimates of population subdivision may reflect movement rates associated with past conditions when the valley was less anthropogenically modified. In 2005, a radio-instrumented male black bear crossed the agricultural valley (J. Hayden, unpublished data), confirming that the agricultural valley between mountain ranges is still not an impermeable barrier.

Our results are consistent with the literature indicating that black bears, while occasionally moving through open areas, tend to stay in mid-elevation forest types (Lee and Vaughan 2003, Lyons et al. 2003). In fact, in North Carolina, bear use was positively correlated to slope and low–mid elevation forests (Powell and Mitchell 1998).

Conclusions and recommendations

We found that DROPOUT provided an efficient tool to detect and remove genotyping errors. The DCH test used with a 9-locus genotype allowed us to statistically demonstrate that our dataset had errors reduced to inconsequential levels. In the case of the northern Idaho black bears, without error-checking we would have a 28.1% overestimate in the minimum number of bears known alive. The abundance overestimate would have been more severe had we used these uncorrected data in a mark–recapture framework (Mills et al. 2000, McKelvey and Schwartz 2004). Thus, genetic errors must not only be caught, but there must be some ability to demonstrate their removal. Given that datasets with errors reduced to inconsequential levels can be produced at reasonable cost, we recommend that studies both employ and document rigorous error checking protocols whenever accurate population estimates are required. We also caution against confusing process with product. Removal of errors through multi-tube and DNA quantification approaches certainly limit errors, but they do not demonstrate that errors have been removed.

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