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TECHNICAL ARTICLE

Effects of genotyping protocols on success and errors in identifying individual river otters (*Lontra canadensis*) from their faeces

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Abstract

In noninvasive genetic sampling, when genotyping error rates are high and recapture rates are low, misidentification of individuals can lead to overestimation of population size. Thus, estimating genotyping errors is imperative. Nonetheless, conducting multiple polymerase chain reactions (PCRs) at multiple loci is time-consuming and costly. To address the controversy regarding the minimum number of PCRs required for obtaining a consensus genotype, we compared consumer-style the performance of two genotyping protocols (multiple-tubes and 'comparative method') in respect to genotyping success and error rates. Our results from 48 faecal samples of river otters (*Lontra canadensis*) collected in Wyoming in 2003, and from blood samples of five captive river otters amplified with four different primers, suggest that use of the comparative genotyping protocol can minimize the number of PCRs per locus. For all but five samples at one locus, the same consensus genotypes were reached with fewer PCRs and with reduced error rates with this protocol compared to the multiple-tubes method. This finding is reassuring because genotyping errors can occur at relatively high rates even in tissues such as blood and hair. In addition, we found that loci that amplify readily and yield consensus genotypes, may still exhibit high error rates (7–32%) and that amplification with different primers resulted in different types and rates of error. Thus, assigning a genotype based on a single PCR for several loci could result in misidentification of individuals. We recommend that programs designed to statistically assign consensus genotypes should be modified to allow the different treatment of heterozygotes and homozygotes intrinsic to the comparative method.

Keywords: allelic dropout, blood, multiple alleles, multiple-tubes, noninvasive genetic sampling, population estimates

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Introduction

The increased use of noninvasive genetic sampling has led to mounting concern that genotyping errors may affect estimates of animal abundance (McKelvey & Schwartz 2004a, b; Lukacs & Burnham 2005a ,b). When genotyping errors are high and recapture rates are low, misidentification of individuals can lead to overestimation of population size (Mills *et al*. 2000; McKelvey & Schwartz 2004a, b; Lukacs & Burnham 2005a, b). Such overestimation can negatively influence the management of threatened and endangered species (Mills *et al*. 2000; Palomares *et al*. 2002; Creel *et al*. 2003), for which noninvasive genetic sampling may be the most viable method for population monitoring (Mills *et al*. 2000). Estimation of genotyping errors should therefore become an integral part of noninvasive genetic sampling (McKelvey & Schwartz 2004a, b).

Estimation of genotyping errors requires multiple amplifications of each sample at multiple loci (Taberlet *et al*. 1996; Broquet & Petit 2004; Paetkau 2004; Waits & Paetkau 2005), because the number of loci must be sufficient to

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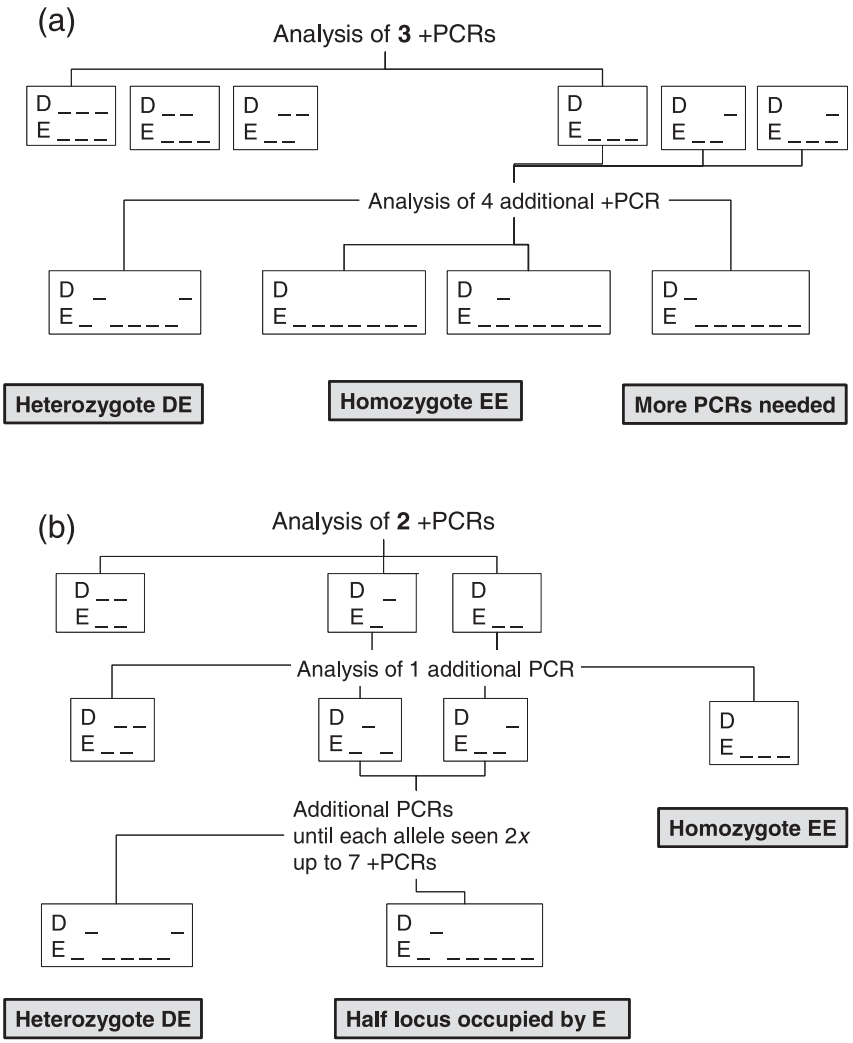

Fig. 1 Flow charts representing the differences in genotyping protocols. The multiple-tubes approach (a) based on Taberlet *et al*. (1996) requires three initial positive PCRs (+PCR), and the amplification of a homozygote up to seven times. The comparative method (b) is based on two initial positive PCRs and amplification of homozygotes three times (Frantz *et al*. 2003). Dashes represent a positive PCR product for the specific allele. For example, in (a) a minimum of three PCRs are required to obtain a consensus genotype for heterozygote DE, whereas the same genotype can be obtained with only two PCRs in (b) if both alleles occur twice. In both methods additional PCRs are required when an allele is observed only once.

obtain a high probability of identity (Mills *et al*. 2000; Creel *et al*. 2003). The multiple-tubes approach (Taberlet *et al*. 1996; Fig. 1A) is a standard conservative protocol, widely used to obtain reliable genotypes from noninvasive genetic samples (Goossens *et al*. 2000). Nonetheless, conducting multiple polymerase chain reactions (PCRs) is time-consuming and the cost can become prohibitive (Paetkau 2004; Waits & Paetkau 2005). In addition, because noninvasive genetic sampling usually results in low volume and yield of extracted DNA, reducing the number of PCRs per locus should be a priority (Taberlet *et al*. 1996). Recently, Frantz *et al*. (2003) proposed a modification of the multiple-tubes approach (termed the 'comparative method') that may reduce the number of amplifications required to obtain reliable genotypes. In this protocol, consensus genotypes are determined after two initial positive PCRs for heterozygotes and three for homozygotes (Frantz *et al*. 2003; Fig. 1B) compared with initial three PCRs and additional four required with the multiple-tubes approach (Fig. 1A). In this study, we compared the performance of the conventional multiple-tubes approach with the modification proposed by Frantz *et al*. (2003) on success and error rates in identifying individual river otters (*Lontra canadensis*) from their faeces.

Although faeces can be relatively easy to find, and in many cases may be the only indication of the presence of secretive and elusive animals (Kohn & Wayne 1997; Ernest *et al*. 2000; Palomares *et al*. 2002), they often have high quantities of nontarget DNA (i.e. prey, bacteria), low quantities of amplifiable target DNA, and high amounts of PCR inhibitors. This results in susceptibility to genotyping error, contamination, amplification of nontarget DNA, and high laboratory costs (Gerloff *et al*. 1995; Bradley & Vigilant 2002; Waits 2004). In addition, success of faecal DNA extraction and amplification seems to vary among species due to the quantity and quality of target faecal DNA (Frantzen *et al*. 1998). For example, Taberlet & Luikart (1999) found that wolf (*Canis lupus*) faeces provided more

target DNA with higher amplification success than did bear (*Ursus* spp.) faeces using the same extraction protocol. Otter faeces are notorious for low quality and quantity of extracted DNA, with overall genotyping success ranging from 20 to 40% (Dallas *et al*. 2003; Prigioni *et al*. 2006). Thus, comparing the performance of the two genotyping protocols on such samples provides a conservative cost–benefit analysis. To evaluate the performance of the multiple-tubes and comparative genotyping approaches, we (i) compared genotyping success and errors from these two genotyping protocols for faecal samples collected in the Green River, Wyoming, using primers developed for Eurasian (*Lutra lutra*; Dallas & Piertney 1998) and North American otters (Beheler *et al*. 2004); and (ii) evaluated the rates of genotyping errors for blood samples of captive individuals used as positive controls.

Materials and methods

Sample collection and preservation

In 2003, we collected 48 fresh river otter faeces at identified river otter latrine or den sites along the Green River in south-central Wyoming in Seedskadee National Wildlife Refuge, and downstream of the Flaming Gorge Dam in northeastern Utah. Because river otter faeces are easy to distinguish from those of other species, the risk of collecting nontarget faecal samples was nearly nonexistent. We collected only faeces which had a glossy appearance and a characteristic smell indicating freshness. In the field, we stored whole faeces individually in Whirl-Pak bags filled with 100% ethanol (EtOH), and for long-term storage at 4 °C, we transferred samples to sterile vials with 100% EtOH.

We collected blood samples from 15 adult male river otters that were live-captured in northwestern Prince William Sound, Alaska, USA, in spring 1998, and held in captivity for a companion study (Ben-David *et al*. 2000, 2001; Blundell *et al*. 2002, 2004). We randomly chose samples from five of these otters to serve as positive controls for this study.

DNA extraction

Prior to DNA extraction, we sieved each faecal sample through fine mesh stainless steel, autoclavable sieves to ensure the removal of all hard parts of prey material. This reduces extraction and amplification of nontarget DNA and homogenizes the distribution of shed target cells (Kohn *et al*. 1995). Excess EtOH was evaporated from each sample after sieving in a closed hood. We isolated DNA from whole blood of captive otters using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, http://www.sigmaaldrich.com). DNA from 180 to 220 mg of faeces was extracted using QIAamp DNA Stool Mini Kit (QIAGEN, http://www1.qiagen.com), and we included at least one negative control in each extraction. We extracted each faecal sample only once.

PCR amplification

We selected two polymorphic primers from a battery of microsatellite primers developed for Eurasian otters (Dallas & Piertney 1998) and two developed for river otters (Beheler *et al*. 2004), based on observed heterozygosities and number of alleles. Primers Lut701 and Lut801 are both tetranucleotide repeats (Dallas & Piertney 1998), whereas RIO05 is a trinucleotide repeat and RIO10 is a dinucleotide repeat (Beheler *et al*. 2004). Amplification followed protocols detailed by Dallas & Piertney (1998), and Beheler *et al*. (2004), with slight temperature modifications to adjust for differences in atmospheric pressure associated with high altitude. Positive (blood from captive otters) and negative (PCR blanks) controls were included with each PCR run to ensure the reliability of PCRs and to monitor contamination. Reactions were performed using a PTC-0200 DNA Engine Peltier Thermal Cycler (MJ Research). PCR products were resolved on a LI-COR DNA Analyser Gene Readir 4200 (LI-COR Biosciences) and alleles were sized and analysed using version 3.00 GENE IMAGIR software. Only one person scored the gels and determined the correct bands.

Comparison of genotyping profiles

To reduce time spent trying to amplify poor quality samples, each sample that did not amplify after four PCR runs with our two most reliable markers (Lut701 and RIO05) was discarded (Morin *et al*. 2001; Paetkau 2003). Samples that did not amplify in three or more of seven PCR reactions were classified as 'incomplete' (Inc). Samples for which genotypes could not be assigned after seven positive PCRs were classified as 'unknown' (UK; Murphy *et al*. 2002). For the multiple-tubes approach, we used three initial positive PCRs to assign consensus heterozygous genotypes, and alleles were not accepted until observed at least twice (Fig. 1A). Homozygote consensus genotypes were scored only if the same allele was documented at least seven times (Fig. 1A). Genotypes manually obtained with the multiple-tubes approach were verified using GIMLET 1.0.1 (Valière 2002) with the threshold of number of allele recurrences set between 2 and 4. For the comparative method, we evaluated consensus genotypes after two initial positive PCRs (Frantz *et al*. 2003). Loci that amplified the same heterozygous individual twice were recorded and homozygote genotypes were accepted after three amplifications (Fig. 1B). When a locus amplified as a heterozygote only once in seven runs, with the other six runs resulting in the same homozygous genotype, the locus was designated as a half-genotype (Fig. 1B; Miller *et al*. 2002; Frantz *et al*. 2003).

Calculating error rates

Similar to Murphy *et al*. (2002), we distinguished among the following: (i) false homozygote (FH), where only one allele was observed in a heterozygote due to allelic dropout (Gagneux *et al*. 1997); (ii) false allele (FA), where alleles were detected out of range of the primer or deviated from the consensus genotype (Goossens *et al*. 2000). In this category, we only considered those bands that had clear microsatellite characteristics (i.e. typical stutter bands); or (iii) multiple alleles (MA), where more than two alleles were observed or band pattern was too noisy to score due to stuttering. We calculated error rates as the number of alleles that did not match the consensus genotype per locus for each sample, divided by the number of positive PCRs per locus per sample (Broquet & Petit 2004). We then calculated the average and standard deviation for each error type for each locus as well as an overall average error rate for all loci. Incomplete genetic profiles were not included in determining error rates. For comparing genotyping errors between the two protocols we used Z-tests for two proportions (Zar 1999).

Results

We successfully amplified river otter DNA in 948 of 1386 (68%) PCRs for faecal samples. None of the negative controls (n = 740 blanks) for extractions or PCRs produced results, and positive PCR controls (n = 365; river otter blood) always amplified, indicating successful and uncontaminated extraction and PCR amplifications. Of the 48 samples collected on the Green River, DNA was obtained from 41 samples (85%) as indicated by at least one successful amplification in any of the four loci. Twenty-seven of the 48 samples (56%) generated consensus genotypes for RIO05 (Table 2). Twelve samples of 48 (44%) had consensus genotypes for three of the loci tested, but only four samples (8%) had consensus genotypes for all four loci. Those samples for which we were unable to assign a consensus genotype for RIO05 (21 samples of 48) were discarded from further analyses.

Overall, we had more success in amplifying DNA with RIO05 and Lut701 than with the other two primers (Table 1). Consensus genotypes obtained from the multiple-tubes approach were similar to those obtained from the comparative approach (Table 1). GR01 was the only sample to result in a discrepancy at locus Lut801 between the two protocols (Table 1). Five samples at locus RIO05 resulted in varying genotypes between the multiple-tubes and comparative approach; only one of which had a conflicting heterozygous genotype (GR10; Table 1). In all other cases, the two approaches were in complete agreement. For the 27 faecal extracts from the Green River, fewer PCRs were necessary to obtain consensus genotypes for each of the four loci using the comparative approach than with the multiple-tubes approach (Fig. 2). On average, 4.5 (± 0.5) positive PCRs were needed with the comparative approach compared to an average of 6.8 (± 0.6) for the multitubes protocol. In addition, different loci converged on consensus genotypes at different rates (Fig. 2).

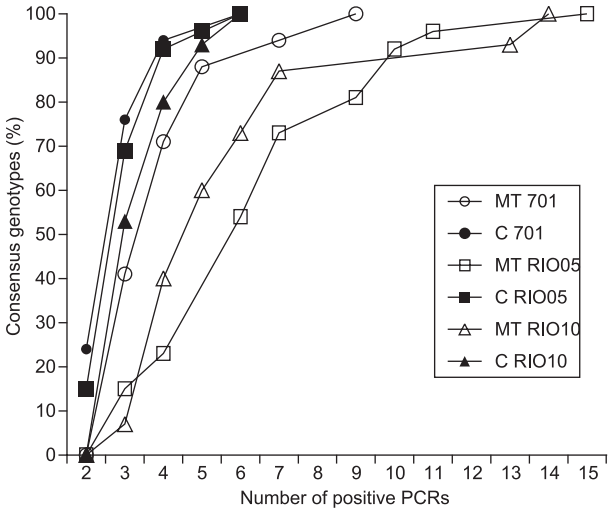

Fig. 2 Cumulative percent of faecal samples that achieved consensus genotype with increasing PCRs obtained by the comparative approach (C; filled symbols) and the multiple-tubes approach (MT; open symbols) for three of four loci tested. Samples were collected on the Green River in Wyoming and Utah in 2003. Two features emerge: first, the comparative approach converges on consensus with fewer PCRs than does the multiple-tubes approach; second, different loci converge at different rates.

Types and rates of genotyping errors in faecal samples differed between loci. For example, false homozygotes were significantly more frequent in Lut801 (39.2%) than in the other loci, while RIO10 had the highest rate of false alleles (19.8%; Table 2). For all loci, error rates were consistently lower for the comparative approach (Table 2; one-tailed Z-test, FA – P = 0.002, FH – P = 0.038). Although positive PCRs were more reliably obtained for blood samples, genotyping errors occurred even with this tissue type. Especially prevalent was the occurrence of false alleles, and locus RIO05 was especially prone to all types of genotyping errors (Table 3).

Discussion

Our results indicate that the number of PCRs per locus can be minimized by following the comparative genotyping protocol proposed by Frantz *et al*. (2003). For the majority of samples, the same consensus genotypes were reached with fewer PCRs and with reduced error rates using this approach as compared with the multiple-tubes protocol. This finding is reassuring, because our data also indicate that genotyping errors can occur at relatively high rates,

Table 1 Consensus genotypes obtained for 27 Green River faecal samples and for captive river otter blood for both the multiple-tubes (*M-T*) and comparative (*C*) approaches. Discrepancies in consensus genotypes are highlighted in bold italics. Genotypes containing 'F' are those cases in which an allele was observed only once in a series of replications and was scored as a half-locus. Incomplete (Inc) genotypes are those that had PCR product but did not have sufficient replication. Unknown (UK) genotypes are those that had varying repeats for which a true genotype could not be assigned. A dash indicates no amplifiable DNA for that sample at a particular locus

		Locus							
		Lut701		Lut801		RIO05		RIO10	
		M-T	*C*	*M-T*	*C*	*M-T*	*C*	*M-T*	*C*
Faecal samples	GR01	202202	202202	***224224***	***224F***	349361	349361	Inc	Inc
	GR02	198202	198202	224228	224228	349355	349355	237237	237237
	GR03	198202	198202	224228	224228	***355358***	***355355***	237237	237237
	GR04	198198	198198	224228	224228	349361	349361	227237	227237
	GR05	194198	194198	—	—	352364	352364	227237	227237
	GR06	198202	198202	—	—	***UK***	***349364***	Inc	Inc
	GR07	198198	198198	—	—	349361	349361	Inc	Inc
	GR08	198198	198198	—	—	349364	349364	227237	227237
	GR09	Inc	Inc	—	—	352355	352355	Inc	Inc
	GR10	Inc	Inc	—	—	***355364***	***355358***	237237	237237
	GR11	194198	194198	224228	224228	349352	349352	237237	237237
	GR15	198F	198F	—	—	***355355***	***355358***	Inc	Inc
	GR17	Inc	Inc	224228	224228	***355367***	***355355***	237237	237237
	GR19	190198	190198	Inc	Inc	349355	349355	237237	237237
	GR20	198198	198198	Inc	Inc	352355	352355	235235	235235
	GR21	194198	194198	Inc	Inc	355358	355358	Inc	Inc
	GR22	Inc	Inc	—	—	Inc	Inc	235239	235239
	GR23	198F	198F	—	—	349355	349355	237237	237237
	GR26	Inc	Inc	Inc	Inc	358358	358358	—	—
	GR27	198202	198202	Inc	Inc	355364	355364	—	—
	GR28	Inc	Inc	—	—	361361	361361	—	—
	GR30	194198	194198	Inc	Inc	352355	352355	237237	237237
	GR33	Inc	Inc	—	—	352355	352355	Inc	Inc
	GR34	198202	198202	Inc	Inc	349355	349355	Inc	Inc
	GR37	Inc	Inc	Inc	Inc	352355	352355	Inc	Inc
	GR42	Inc	Inc	—	—	352355	352355	235235	235235
	GR43	Inc	Inc	—	—	352355	352355	237237	237237
Blood samples	EP07	198198	198198	224228	224228	364370	364370	239239	239239
	NI02	198206	198206	224228	224228	364364	364364	229237	229237
	UI03	198198	198198	224228	224228	361364	361364	227237	227237
	WB02	198198	198198	228228	228228	361361	361361	237237	237237
	WB07	194198	194198	224228	224228	361361	361361	231237	231237

even in tissues such as blood. In addition, different loci may be prone to different types and rates of genotyping errors. Moreover, loci that amplify readily and yield consensus genotypes, may still exhibit high error rates (e.g. RIO05). Thus, assigning a genotype based on a single PCR for several loci could result in misidentification of individuals, because prescreening of samples with the most reliable primers (Paetkau 2003), does not guarantee reduced errors in others.

Our success of 56% amplifiable DNA for all samples was near the reported average of 69% for faeces (Frantzen *et al*. 1998; Goossens *et al*. 2000). It was greater than that reported in other studies using otter faecal DNA analyses (20% — Dallas *et al*. 2003; 40% — Prigioni *et al*. 2006). Our error rates (overall 2.7–19.4% using the comparative approach) were comparable to those of other studies using faeces as the source for DNA. Creel *et al*. (2003) reported allelic dropout rates between 0% and 39% for wolf faeces, while Lucchini *et al*. (2002) reported 0–33% error rates for the same species. Morin *et al*. (2001) reported an average dropout rate of 24% from DNA extracted from known wild chimpanzee (*Pan troglodytes verus*) faeces. Frantz *et al*. (2003) reported an average allelic dropout rate of 27%, with the highest of 47.7% at one locus, for faecal DNA extracted from wild

Table 2 Summary of DNA amplifications and errors observed in river otter faecal samples collected on the Green River, Wyoming, in 2003. Data are from 27 faecal samples at four loci. Positive PCRs ($P^+ \pm SE$), positive PCRs resulting in consensus genotype (CG ± SE), false allele (FA ± SE), false homozygote (FH ± SE), and multiple alleles (MA ± SE) were calculated using the number of observed P^+ from PCR attempts for both genotyping approaches (multiple-tube and comparative). Total PCR attempts and P^+ are reported in parentheses. Only samples with complete genotypes were included in the error calculations

		P^+	CG	FA	FH	MA
Lut701	Multiple tubes	38 ± 4% (251)	77 ± 5% (91)	5.6 ± 3.2% (91)	21.6 ± 6.9% (91)	2.1 ± 2.1% (91)
	Comparative	45 ± 5% (190)	77 ± 6% (73)	3.3 ± 3.3% (73)	16.7 ± 6.0% (73)	2.2 ± 2.2% (73)
Lut801	Multiple tubes	26 ± 6% (164)	51 ± 9% (49)	10.7 ± 5.5% (49)	39.2 ± 11.3% (49)	0.0 ± 0.0% (49)
	Comparative	25 ± 6% (141)	62 ± 10% (34)	0.0 ± 0.0% (34)	36.7 ± 11.1% (34)	0.0 ± 0.0% (34)
RIO05	Multiple tubes	62 ± 4% (290)	46 ± 5% (176)	18.5 ± 4.7% (176)	31.7 ± 5.6% (176)	9.5 ± 3.7% (176)
	Comparative	62 ± 5% (165)	65 ± 6% (87)	11.8 ± 3.0% (87)	16.7 ± 4.4% (87)	7.3 ± 3.2% (87)
RIO10	Multiple tubes	41 ± 5% (243)	73 ± 6% (106)	19.8 ± 7.0% (106)	8.2 ± 4.6% (106)	1.8 ± 1.8% (106)
	Comparative	42 ± 6% (191)	74 ± 6% (73)	12.5 ± 5.6% (73)	7.3 ± 4.1% (73)	1.4 ± 1.4% (73)
Overall	Multiple tubes	42 ± 8% (948)	62 ± 8% (422)	13.7 ± 3.4% (422)	25.2 ± 6.7% (422)	3.4 ± 2.1% (422)
	Comparative	44 ± 8% (687)	70 ± 4% (267)	6.9 ± 3.1% (267)	19.4 ± 6.1% (267)	2.7 ± 1.6% (267)

Table 3 Summary of DNA amplifications and errors observed in captive river otter blood samples. Data are from five known individuals at four loci. Positive PCRs ($P^+ \pm SE$), positive PCRs resulting in consensus genotype (CG ± SE), false allele (FA ± SE), false homozygote (FH ± SE), and multiple alleles (MA ± SE) were calculated using the number of observed P^+ from PCR attempts and the comparative genotyping approach. Total PCR attempts and P^+ are reported in parentheses

	P+	CG	FA	FH	MA
Lut701	100% (112)	97 ± 5% (112)	2.2 ± 1.4% (112)	3.6 ± 3.6% (112)	1.8 ± 1.8% (112)
Lut801	100% (71)	79 ± 9% (71)	18.8 ± 3.6% (71)	3.3 ± 1.9% (71)	1.1 ± 1.1% (71)
RIO05	100% (104)	76 ± 8% (104)	11.7 ± 4.7% (104)	16.8 ± 5.6% (104)	10.1 ± 6.7% (104)
RIO10	100% (78)	63 ± 6% (78)	37.4 ± 6.7% (78)	3.3 ± 1.7% (78)	0.0 ± 0.0% (78)
Overall	100% (365)	78 ± 7% (365)	17.5 ± 7.4% (365)	6.8 ± 3.4% (365)	3.3 ± 2.3% (365)

Eurasian badgers (*Meles meles*). A range of 0.3–11% false alleles rates occurred in wolf faeces (Creel *et al*. 2003), and 13.5% and 16% in European hares (*Lepus europaeus*) and red deer (*Cervus elaphus*), respectively (Huber *et al*. 2003).

While we expected high error rates in faecal samples, we were disconcerted to observe similar error rates in blood samples. Nonetheless, it appears that our observation is not unique. For example, Johnson (2006) reported an average allelic dropout of 9.8% for blood samples collected from American martens (*Martes americana*) from Prince of Wales Island, Alaska (range 6.3–17%; five primers), and 11.3% for muscle tissues (range 0–28%). Similarly, Bayes *et al*. (2000) reported allelic dropout rates of 1% in baboon blood, while Fernando *et al*. (2003) reported similar rates of nondetection of alleles for Asian elephants (*Elephas maximus*) dung and blood (0.4%). Bonin *et al*. (2004) estimated a 17.6% rate of erroneous multilocus genotypes for muscle tissues of bears. It is interesting to note that in our study, blood samples were more prone to false alleles than allelic dropout. Allelic dropout usually results from low DNA quantity or PCR inhibitors, whereas the occurrence of false alleles suggests amplification of nonmicrosatellite products, which is likely a result of inadequate microsatellite design. Future studies of river otters should concentrate on improving the design of these microsatellite primers.

Our observation that different loci performed differently on the same samples was again in agreement with other studies. Taberlet *et al*. (1999) claimed that lower quality DNA could be hard to amplify using primers that generate relatively long fragments (between 200 and 300 base pairs). Similarly, Banks *et al*. (2002) found that amplification success for wombat faeces was negatively correlated with the length of the amplification product. In our case, locus length is unlikely to have been the sole factor in determining amplification success. All but one of the Green River faecal samples yielded consensus genotypes with RIO05 (> 330 base pairs), whereas RIO10 (< 242 base pairs) and Lut701 (< 208 base pairs) yielded consensus genotypes in only 55% and 63% of the same samples, respectively. In accord with Dallas & Piertney (1998), we found the alleles of the tetranucleotides and GATA loci (Lut701, Lut801) to be well defined and those of the dinucleotide (RIO10) more difficult to score due to stuttering. This may explain the high rates of false alleles for this locus in both faecal and blood samples. Other factors that influence the performance of particular loci merit further investigation.

Paradoxically, RIO05 that amplified readily and yielded consensus genotypes, exhibited the highest error rates in both faeces and blood. In addition, although samples that amplified well with this primer usually also amplified with the other three primers we tested, this success did not ensure lower error rates in these additional loci. For example, samples that amplified well with RIO05, yielded a rate of 36.9% of FH with Lut801. Thus, assigning a genotype based on a single PCR for any of the four loci could result in misidentification of individuals. This observation emphasizes that prescreening of samples with the most reliable primers (Paetkau 2003), does not guarantee reduced errors in others. Therefore, similar to previous researchers (Lukacs & Burnham 2005a, b) we propose that all studies using noninvasive genetic sampling make the estimation of genotyping errors an integral part of their protocols and that genotypes not be assigned based on a single PCR. We further suggest that the same approach be adopted even in studies where DNA quality is higher (e.g. blood and muscle). We also join McKelvey & Schwartz (2004a, b) in recommending that other methods (e.g. examination of bimodality and difference in capture history) be used to assess the occurrence of misidentified individuals for capture–recapture studies.

Finally, our data suggest that the number of PCRs per locus can be minimized by following the comparative genotyping protocol proposed by Frantz *et al*. (2003). That two of the primers we tested produced the same consensus genotypes for the comparative approach and the multiple-tubes approach, and that for RIO05 only five of 25 samples resulted in differing consensus genotypes, indicates that fewer replications may be necessary than previously proposed (Taberlet *et al*. 1996). The lower error rates we observed likely stemmed from the reduced number of PCRs. Because of human error and machine irregularities, DNA amplifications can result in different PCR products that translate to increased genotyping errors. Therefore, the comparative approach may not only be a more cost- and time-efficient protocol but also be less error-prone. We recommend that programs desgned to assign consensus genotypes, such as GIMLET (Valière 2002) and RELIOTYPE (Miller *et al*. 2002), be modified to allow the differential assessment of heterozygotes and homozygotes intrinsic to the comparative method (Frantz *et al*. 2003).

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