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TECHNICAL NOTE

Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates

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Abstract Noninvasive population genetics has found many applications in ecology and conservation biology. However, the technical difficulties inherent to the analysis of low quantities of DNA generally tend to limit the efficiency of this approach. The nature of samples and loci used in noninvasive population genetics are important factors that may help increasing the potential success of case studies. Here we reviewed the effects of the source of DNA (hair vs. faeces), the diet of focal species, the length of mitochondrial DNA fragments, and the length and repeat motif of nuclear microsatellite loci on genotyping success (amplification success and rate of allelic dropout). Locus-specific effects appeared to have the greatest impact, amplification success decreasing with both mitochondrial and microsatellite fragments' length, while error rates increase with amplicons' length. Dinucleotides showed best amplification success and lower error rates compared to longer repeat units. Genotyping success did not differ between hair- versus faeces-extracted DNA, and success in faeces-based analyses was not consistently influenced by the diet of focal species. While the great remaining variability among studies implies that other unidentified parameters are acting, results show that the careful choice of genetic markers may allow optimizing the success of noninvasive approaches.

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Introduction

Noninvasive population genetics is a set of field, laboratory and analytical techniques that allow studying the biology of natural populations without having even to observe individuals. It has thus been put forward as a chance for investigating the biology of elusive, rare and/or endangered species (Piggott and Taylor 2003). Conservation biologists in particular have shown interest in these techniques, which for instance are now routinely used for the monitoring of brown bear populations in North America (Woods et al. 1999; Paetkau 2003). This example, however, is the exception rather than the rule, in particular because noninvasive population genetics is linked to numerous potential problems, especially at the stage of data production in the laboratory. In conservation genetics, most studies that use noninvasive samples rely on individual identifications based on genotypes at 5–15 hypervariable microsatellite loci (Taberlet and Luikart 1999). But in order to get accurate multilocus genotypes, DNA typing must be error free at all assessed loci. Unfortunately, noninvasively collected samples usually provide DNA extracts characterized by low target DNA concentration, low target DNA quality (degradation) and/or contamination by alien DNA. Microsatellite typing has proven to be sensitive to all these kinds of problems, leading to genotyping errors and/or reduced amplification success. Thus, despite an undisputed attractiveness, noninvasive population genetics may not always be the most appropriate approach (Taberlet et al. 1999; Piggott and Taylor 2003). In particular, care should be taken

regarding the financial and technical challenge involved in noninvasive genetics. Taberlet et al.'s (1999) advice is to perform a pilot study in order to estimate genotyping error rates. These rates can, in turn, be used to predict the number of times each sample must be amplified before accepting its genotype at a particular locus (Miller et al. 2002; Valière et al. 2002). Such pilot studies, however, do not address a number of important questions related to the potential success of surveys based on noninvasive sampling. Parameters such as fragment length, the repeat motif of microsatellite loci, the source of DNA or even the diet of the focal species may influence amplification and genotyping success. Evaluating their relative influence on genotyping and amplification success is not easy in pilot studies, because it requires sample sizes or experiments that are unavailable in such surveys. Another possibility, adopted in this review, is to use published results to test specific hypotheses in a comparative analysis.

DNA can be retrieved from various kinds of noninvasive samples (Höss et al. 1992; Sloane et al. 2000; Valière and Taberlet 2000; Vigilant et al. 2001), most studies being conducted with DNA extracted from shed hair or faeces. Shed hair are collected in the field either opportunistically (Vigilant et al. 2001) or using hair-traps (Mowat and Strobeck 2000; Sloane et al. 2000), and DNA is generally extracted from a single hair (e.g. Sloane et al. 2000) or from very few hair (1 to 3 hair: Constable et al. 2001) to avoid mixing DNA from different individuals (see Alpers et al. 2003 for discussion). Faeces are usually collected opportunistically and DNA is also extracted from a single sample (Vigilant et al. 2001). These two sources of DNA differ in three main characteristics that are relevant to subsequent genotyping results. First, much more DNA may be extracted from faeces (e.g. 38.4 ng per extract, Morin et al. 2001) than from single hair (1 ng per single hair, Gagneux 1997), and amplification success and genotyping errors have been found to be sensible to template DNA concentration (Goossens et al. 1998; Morin et al. 2001). Second, besides template DNA, faeces contain a very large amount of alien DNA (from bacteria and from the diet). Amplification from alien DNA, however, can in a large extent be avoided by using specific primers, and will not be considered any further here (but see Bradley and Vigilant 2002). Finally, various molecules that can inhibit the polymerase chain reaction (PCR) may be present in faeces, a problem that is less likely with hair samples when using only hair roots (but see Nievergelt et al. 2002; Roon et al. 2003). Inhibition levels can vary with diet (Murphy et al. 2003), and most difficulties are met when working with faeces that contain plant remnants (Monteiro et al. 1997, D. Vallet, E. Petit and N. Ménard, unpublished manuscript).

Large enough data sets have offered the opportunity to test for locus or allele-specific effects, but results from surveys on single species appear contradictory. Frantz et al. (2003) found in a study on badger (*Meles meles*) that the different loci they used had heterogeneous error rates, but that allele length had no effect on these rates. A similar pattern was observed on Yellowstone grizzly (Miller and Waits 2003). In other studies, amplicon size has been shown to impact amplification success as well as allelic dropout rate (Morin et al. 2001; Buchan et al. 2005; Hoffman and Amos 2005). Locus specific effects could be explained, among other causes, by differences in the repeat motifs, dinucleotide microsatellites being for example more prone to slippage than tetranucleotides (Kruglyak et al. 1998).

In this comparative analysis, we specifically addressed the following hypotheses: First, we tested whether DNA extracted from shed hair is less easily amplified by PCR and leads to more genotyping errors than faecal DNA does. Second, we tested whether faecal DNA is less amplifiable when faeces contain plant material. Finally, we looked at locus specific effects, testing the hypotheses that longer amplicons amplify less often and yield more genotyping errors than shorter amplicons, and that microsatellite repeat motif affects amplification success and rates of genotyping error.

Methods

Literature survey

This review was based on population genetics studies involving noninvasive sampling of wild ranging species. We limited our search to studies based on hair or faeces samples, as other noninvasive sources of DNA (such as buccal swabs, urine, feathers ...) are more scarcely employed and would not allow comparing their respective efficiencies. We performed a search in the *Current Contents* database for any such paper published before 2004 to achieve an as exhaustive literature survey of the subject as possible (complete list of references available on request).

The focal species of each study was recorded, and was classified according to its diet either as a strict herbivore, a strict carnivore, or an omnivore. This classification was adopted in order to identify the cases where plant material was likely present in faeces. We then recorded the published genotyping success data associated to noninvasively collected hair or faeces. Mitochondrial DNA (MtDNA) and/or nuclear DNA (nucDNA, i.e. microsatellite) amplification success was recorded as the percentage of successful PCR (number of

successful PCR relative to the total number of amplification attempts). Two kinds of genotyping errors may then be considered: allelic dropout (ADO), corresponding to the non-amplification of one allele of a heterozygous individual during a positive PCR, and false alleles (FA) resulting from slippage artefacts during the first cycles of the PCR, from cross-contaminations, or from other causes. Because different methods were used to calculate genotyping error rates among studies, we retained only ADO rates calculated as the number of observed drop-outs (false homozygous genotypes) divided by the total number of successful amplifications of heterozygous genotypes, following Broquet and Petit (2004). When adequate raw data were available, ADO rates computed using other methods were recalculated using published data such as to allow for a comparison between studies. False alleles were not included in genotyping errors as their frequency may not reliably be compared among studies (see discussion in Broquet and Petit 2004). Hence in this paper genotyping errors refer to ADO only. When available, the length of the target DNA fragments (either mitochondrial or microsatellite sequences) and the repeat motif of microsatellite loci were also recorded. All literature data used in statistical analyses are presented in Appendix Tables 1–3.

If hair samples were used as DNA source, the sampling method was determined and only the data associated to the analysis of shed hair were retained (i.e. hair samples directly plucked on animals were not included here). We also retained genotyping data based on hair ''trapped'' on barbed wire (Roon et al. 2003), on glue tape (Sloane et al. 2000), on tree bark (Kohn et al. 1995) or other systems, assuming that such remote systems are more likely to collect hair at the end of their growth cycle. Such hair presenting less germinal tissue attached to their root would therefore be similar to naturally shed hair. Some hair-trapping systems could potentially allow collecting high-quality samples (i.e. similar to plucked hair), but we assumed that they would not do so constantly, and that hair containing less DNA would therefore be routinely used in downstream genotyping experiments (see Discussion).

Conditions of sampling, DNA extraction and genotyping procedures employed in each study were thoroughly surveyed, and all data potentially producing pseudo-replicates were eliminated from subsequent analyses. For instance only one data set was retained from studies involving a comparison between various laboratory protocols based on the same samples. In such cases, only the results obtained with the best method were included in our analyses. Similarly, genotyping success data based on a very small sample size were discarded (the smallest total number of independent amplifications used for quantifying genotyping success in the studies retained for the analyses was 9). Some published papers presented several datasets (i.e. different sets of samples corresponding to distinct species, or distinct studies involving the same focal species). In such cases the genotyping success data associated to each dataset were independently considered in the analyses. Each data set used in the analyses presented here were therefore obtained following a unique protocol, using one type of samples (either faeces or hair), and were generally directed at the study of a single species (with the exception of three studies of closely related species for which average genotyping success data were reported (Reed et al. 1997; Adams et al. 2003; Valière et al. 2003).

Data analyses

All analyses were performed using generalized linear models (GLM) implemented in R 2.2.0 (R Development Core Team, 2005). Error distribution in GLMs was always described using the binomial family (logit link function). Potential interpretation biases due to over-dispersion were avoided by systematically using *F*-tests to select significant variables among models (e.g. Crawley 2005, p. 530).

The effect of the source of DNA (hair vs. faeces) was tested against mitochondrial or nuclear DNA amplification success and microsatellite genotyping error rate (models based on data reported in Appendix Table 1). We then tested for the effect of the diet (presence vs. absence of plant material in faeces, i.e. carnivore vs. herbivore+omnivore) on amplification success (data in Appendix Table 1). The amount of DNA contained in hair and faeces of different focal species may be influenced by the phylogenetic relationship among taxa. For this reason the analyses described above were performed using generalized estimating equations (GEE), which allow correcting for the non-independence of the data among taxa in a GLM framework (Paradis and Claude 2002). These analyses were performed in R using the package ape (Paradis et al. 2005).

Next we tested for the effect of MtDNA fragment length on amplification success using six studies in which fragments differing in length had been used (data in Appendix Table 2). Finally, the effects of microsatellite fragment length and motif on genotyping success were conjointly tested because of their potential relationship (data in Appendix Table 3). The origin of the data sets used in the analyses was also included as a factor in all models involving locus-specific data (i.e. tests based on Appendix Tables 2 and 3), therefore controlling for the effect of data clusters corresponding to distinct case studies usually

involving different species and different genotyping methodologies.

Results

Amplification success of both MtDNA sequences and nuclear microsatellites appeared to vary greatly among studies, and was not influenced by the nature of the samples used for DNA extraction ($P > 0.05$ in both cases), hair and faeces yielding comparable amplification rates (Fig. 1A). There may be a trend towards greater rates of ADO in hair-extracted DNA (Fig. 1B), but there is also a great variation in the frequency of ADO among studies, and the effect of the source of DNA was not statistically significant.

Similarly, the diet of focal species did not consistently affect the rate of successful PCR among studies (Fig. 2). Faecal samples collected from herbivore and omnivore species (i.e. containing plant material) did not provide less amplifiable DNA for microsatellite or MtDNA analyses ($P > 0.05$).

MtDNA and microsatellite amplification success were both correlated with the length of target DNA sequences ($P < 0.001$ in both cases, Fig. 3A and B). Fragment length also appeared to significantly affect ADO rates when genotyping microsatellites ($P < 0.001$, Fig. 3C). Independently of amplicon size, the effect of the repeat motif of microsatellite loci on amplification success was marginally significant (P=0.067, Fig. 4A), while its effect on ADO rates was significant ($P < 0.001$). Shorter repeat motifs lead to higher amplification success and lesser allelic dropouts than longer ones (Fig. 4B).

Discussion

Amplification success showed no advantage of faeces- over hair-extracted DNA, which is surprising regarding the amount of target DNA, expected to be usually higher in faecal samples (Morin et al. 2001). However, greater inhibitor concentrations on average in faecal samples may counterbalance this effect. It is also worth noting that the amount of DNA in hair samples critically depends on the number of hair used for DNA extraction, and also on the presence of follicles in hair roots, two factors that could not be disentangled in this comparative study. Results therefore suggest that the nature of samples (i.e. hair or faeces) is less important than the thorough optimization of extraction and genotyping protocols used with particular samples.

This is consistent with the absence of a clear effect of the nature of samples on ADO rates. The rate of error was on average slightly higher using hair samples (mean= 18.7%) than using faeces (mean=11.3%), and it is possible that this difference did not appear statistically significant due to a lack of statistical power (small sample size in GEE). However the overall probability of successfully amplifying a specific nuclear locus without dropout at the first attempt is 67.1% on average for faecal-extracted DNA and 65.2% for hair-extracted DNA (computed from mean values given in Fig. 1).

The analysis of an influence of diet did not support the idea that plant components negatively impact amplification success of faeces-extracted DNA. This may be accounted for by the use of adequate DNA extraction methods in most studies, which implies that working with faeces may involve additional lab work to identify what is the best

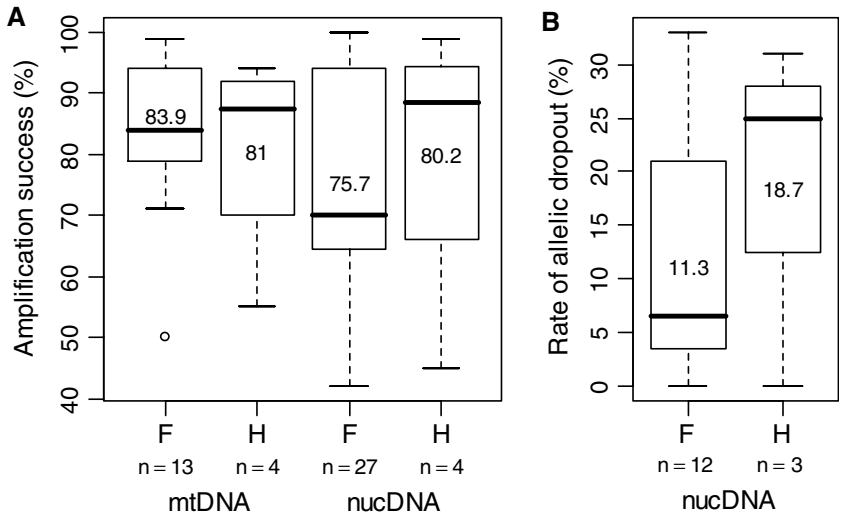

Fig. 1 (A) Amplification success of MtDNA and nucDNA (microsatellite loci) extracted from faeces (F) vs. hair (H) samples. (B) Rate of allelic dropout in faeces- (F) vs. hair-based (H) microsatellite genotyping. Numbers correspond to mean values

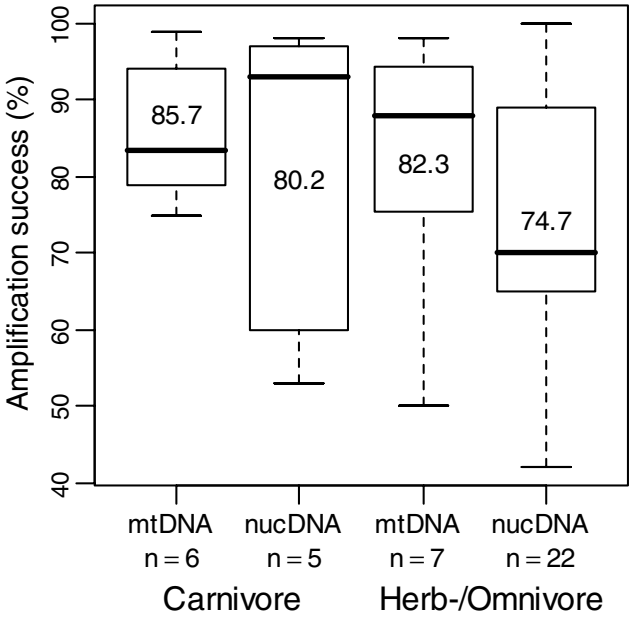

Fig. 2 Amplification success of MtDNA and NucDNA (microsatellites) extracted from carnivore vs. herbivore or omnivore species. Mean amplification rates are indicated

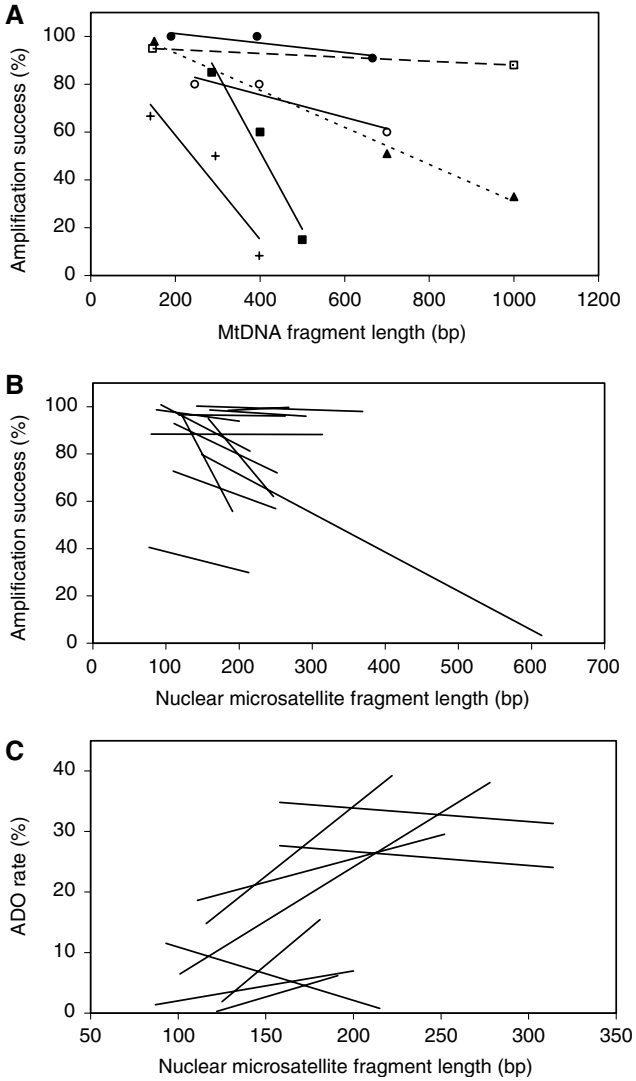

Fig. 3 (A) Relationship between amplification success and MtDNA fragment length in six noninvasive studies (● *Papio ursinus*, feces, Frantzen et al. 1998; ▲ *Ursus Arctos*, feces, Murphy et al. 2000; ■ *Pan troglodytes verus*, hair, Vigilant 1999; + *Ursus arctos*, feces, Kohn et al 1995; □ *Ursus arctos*, hair, Roon et al. 2003; ○ *Ursus americanus*, feces, Wasser et al. 1997). (B) Relationship between amplification success and microsatellite fragment length in 13 noninvasive studies (identified by superscript c in Appendix Table 3). For clarity purpose only regression lines are shown (each line corresponds to a study). (C) Relationship between rate of allelic dropout and microsatellite fragment length in 10 noninvasive studies (identified by superscript d in Appendix Table 3)

extraction method (Wasser et al. 1997) in contrary to shed hair that may be used as source of DNA in all species using slight variations of a simple extraction protocol (Walsh et al. 1991, but see Vigilant 1999 for alternatives). For instance, mitochondrial amplification success varied from 17 to 96% when extracting DNA from bat droppings using various methods (S. Puechmaille, G. Mathy and E. Petit, unpublished data).

Amplification success decreased with increasing amplicon length, except in one study in which a 245 bp MtDNA fragment repeatedly appeared less amplifiable than a 418 bp fragment in three species (Whittier et al. 1999). This case, however, seems to remain an exception and it may be partly resolved considering that the smaller fragment was amplified 1 year after the longer fragment. We thus discarded this study from our analyses. As the risk of ADO also increases with amplicon's length, using longer fragments may therefore in some cases result in a drastic decrease of overall genotyping success (see also Sefc et al. 2003; Buchan et al. 2005). Smaller MtDNA sequences may therefore be preferred in noninvasive genetic studies, provided enough variability is retained. When selecting from available microsatellite markers, ''short'' loci should also generally be preferred. The sensitivity of the rates of amplification and the rates of ADO to the length of a DNA fragment (independently of other factors) was calculated from the logistic models: for instance an amplification success of 73% (average rate of mtDNA amplification reported in the papers reviewed) is expected to drop to 64% as a result of an increase of 100 bp in MtDNA sequence length. Similarly, a rate of microsatellite amplification of 80% would decrease to 71%, and a rate of ADO of 20% would increase to 26% when microsatellite fragment length is increased by 100 bp.

Our comparative study allowed us disentangling the effects of fragment length and repeat motif for microsatellites. Results indicate that dinucleotides should be preferred to longer repeat units: both the amplification

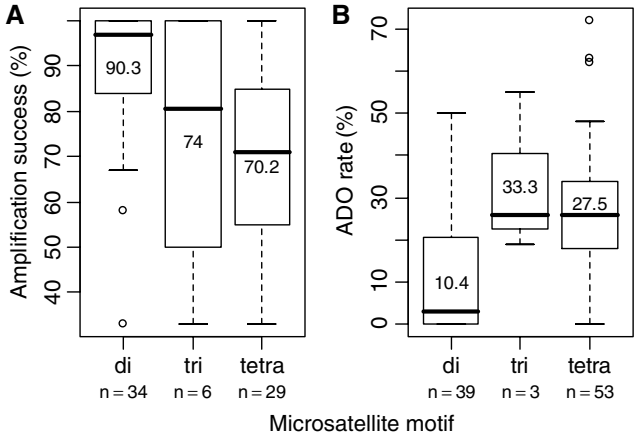

Fig. 4 Microsatellite amplification success and rate of allelic dropout of di-, tri- and tetra-nucleotides in noninvasive genetic studies. Mean rates are indicated

success and the genotyping error rates are best for these markers, the probability of amplifying a heterozygote genotype without dropout at the first attempt being 81% on average for dinucleotides versus about 50% for tri- and tetranucleotides (computed from values given on Fig. 4).

These general statements do not preclude the importance of other locus characteristics (variability, readability, multiplexing possibilities) when planning genotyping experiments. It is worth noting here that, for instance, the higher slippage propensity of dinucleotides (Kruglyak et al. 1998) leads to increased levels of PCR-generated shadow bands and hence, false alleles, which in some cases make dinucleotide microsatellite amplification patterns less easily and reliably read than amplification patterns of tri- or tetranucleotides (Taberlet and Luikart 1999). Also, as suggested by Hoffman and Amos (2005), the variability of microsatellite loci (expressed as gene diversity *He*) appeared to have a small but significant positive effect on the risk of allelic dropout (but not on amplification success) in the studies reviewed here, independently of amplicon length and repeat motif (a 10% increase in *He* would increase ADO rate from 20 to 22.2%, data not shown). There is no obvious reason why the variability of a locus should affect its rate of dropout. One hypothesis (that remains to be tested) is that if smaller DNA fragments are more consistently amplified than bigger ones (as suggested by the short allele dominance hypothesis: Wattier et al. 1998, and by our results), then an increase in the difference in allele size within heterozygous individuals will result in an increased risk of ADO. Since the expected difference in size among pairs of alleles at any given locus depends on its variability (namely its distribution of allelic frequencies), ADO rates may appear indirectly correlated to gene diversity *He*.

Overall, our results show that both the effects of diet and DNA source are limited when compared to locus characteristics, which have quantitatively a more important influence on the success of noninvasive genetic surveys. Variability of genotyping success among studies was also significant in all models tested, suggesting that other parameters specific to each study and not identified here are also acting.

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Appendix

Table 1 Literature review of estimates of amplification success and error rates (formulas according to Broquet and Petit 2004)

Reference	Species (or group)	Diet	Source of DNA	MtDNA pcr success (%)[a,b]	NucDNA pcr success (%)[a,b]	ADO rate (%)[a,b]
Adams et al. (2003)	*Carnivora*	C	F	83.0		
Bayes et al. (2000)	*Papio cynocephalus*	O	F		70.0	*10.3*
Bradley et al. (2000)	*Pan troglodytes verus*	O	F		82.0	
	Gorilla gorilla gorilla	H	F		56.0	
Constable et al. (2001)	*Pan troglodytes schweinfurthii*	O	F		71.0	*33.3*
	Pan troglodytes schweinfurthii	O	H		90.0	*25.3*
Fernando et al. (2003)	*Elephas maximus*	H	F		99.6	*0.4*
Flagstad et al. (1999)	*Ovis aries*	H	F		*96.5*	*5.9*
	Rangifer tarandus	H	F		*95.0*	*4.2*
Frantz et al. (2003)	*Meles meles*	O	F		~61	
	Meles meles	O	F			*26.8*
Frantzen et al. (1998)	*Papio ursinus*	O	F	97.0	42.0	
Gagneux (1997)	*Pan troglodytes*	O	H			31.3
Gerloff et al. (1995)	*Pan paniscus*	O	F	66.0		
Goossens et al. (2000)	*Pongo pygmaeus abelii*	H	F		65.0	4.2
Huber et al. (2003)	*Lepus europaeus*	H	F		96.3	
	Cervus elaphus	H	F		97.4	

Table 1 continued

Reference	Species (or group)	Diet	Source of DNA	MtDNA pcr success (%)[a,b]	NucDNA pcr success (%)[a,b]	ADO rate (%)[a,b]
Idaghdour et al. (2003)	*Otis tarda*	O	F	*70.6*		
Kohn et al. (1999)	*Canis latrans*	C	F	79.0	96.6	
Kohn et al. (1995)	*Ursus arctos*	O	F	*50.0*		
	Ursus arctos	O	H	*93.7*		
Lathuillière et al. (2001)	*Macaca sylvanus*	H	F		70.0	3.0
Launhardt et al. (1998)	*Presbytis entellus*	O	F		70.0	~6.8
Lucchini et al. (2002)	*Canis lupus*	C	F	84.0	53.0	18.0
Morin et al. (2001)	*Pan troglodytes verus*	O	F		79.0	24.0
Murphy et al. (2000)	*Ursus arctos*	O	F	98.0	89.0	
Murphy et al. (2002)	*Ursus arctos*	O	F	92.0	88.0	
Murphy et al. (2003)	*Ursus arctos*	O	F	88.0	65.0	
Palomares et al. (2002)	*Lynx lynx*	C	F	99		
Parsons (2001)	*Tursiops truncatus*	C	F		98.1	0
Reed et al. (1997)	*Halichoerus grypus/ Phoca vitulina*	C	F	~75	~60	
Roon et al. (2003)	*Ursus arctos*	O	H	~90	~99	
Sloane et al. (2000)	*Lasiorhinus krefftii*	H	H		*87.4*	0
Smith et al. (2000)	*Papio cynocephalus*	O	F		53.0	
Valière et al. (2003)	*Canis lupus, C. familiaris, Vulpes vulpes*	C	F	94.2		
	Canis lupus, C. familiaris, Vulpes vulpes	C	H	55		
Vege and McCracken (2001)	*Eptesicus fuscus*	C	F		93.0	0
Vigilant (1999)	*Pan troglodytes verus*	O	H	85.0	45.0	
Vigilant (2002)	*Pan troglodytes verus*	O	F		~64	
Wasser et al. (1997)	*Ursus americanus*	O	F	80.0	67.0	

[a]Values preceded by ''~'' are not exact values but were evaluted from published graphics

[b]Values in italic were recalculated using published data

Table 2 Literature review of estimates of MtDNA amplification success

Reference	Species or group of species	Source of DNA	Length of fragment (bp)	MtDNA pcr success (%)[a]
Adams et al. (2003)	Carnivora	F	200	83
Frantzen et al. (1998)	*Papio ursinus*	F	190	100
			393	100
			666	91
Idaghdour et al. (2003)	*Otis tarda*	F	452	71
Kohn et al. (1995)	*Ursus arctos*	F	141	67
			295	50
			398	8
		H	398	94
Kohn et al. (1999)	*Canis latrans*	F	398	79
Lucchini et al. (2002)	*Canis lupus*	F	404	84
Murphy et al. (2000)	*Ursus arctos*	F	150	98
			700	51
			1000	33
Murphy et al. (2002)	*Ursus arctos*	F	146	92
Murphy et al. (2003)	*Ursus arctos*	F	146	88
Reed et al. (1997)	*Halichoerus grypus/Phoca vitulina*	F	520	~75
Roon et al. (2003)	*Ursus arctos*	H	146	~95
			1000	~88
Vigilant (1999)	*Pan troglodytes verus*	H	286	85
			400	60
			500	15
Wasser et al. (1997)	*Ursus americanus*	F	246	80
			398	80
			700	60

[a]Values preceded by ''~'' are not exact values but were evaluated from published graphics

Table 3 Literature review of estimates of nuclear microsatellite loci genotyping success (formulas according to Broquet and Petit 2004)

Reference	Species	Source of DNA	PCR success (%)	Length of fragment (bp)	Motif	ADO rate (%)[a,b]
Bayes et al. (2000)[c]	*Papio anubis*	F		163	Tetra	*4.3*
				125	Tetra	*3.9*
				181	Di	*22.7*
				161	Di	*9.4*
				174	Tetra	*6.7*
				170	Di	*6.1*
				167	Tetra	*15.8*
				169	Di	*21.4*
Bradley et al. (2000)[d]	*Pan troglodytes verus*	F	62	247	Tetra	
			95	158	Tetra	
Constable et al. (2001)[c]	*Pan troglodytes schweinfurthii*	H		218	Tetra	*14*
				177	Tetra	*32*
				190	Tetra	*42*
				158	Tetra	*26*
				290	Tetra	*30*
				239	Tetra	*20*
				255	Tetra	*15*
				259	Tetra	*21*
				283	Tetra	*39*
				314	Tetra	*35*
				283	Tetra	*24*
				290	Tetra	*15*
				165	Tetra	*29*
				271.5	Tetra	*30*
				271	Tetra	*13*
				213	Tri	*26*
Constable et al. (2001)[c]	*Pan troglodytes schweinfurthii*	F		218	Tetra	*24*
				177	Tetra	*48*
				190	Tetra	*25*
				158	Tetra	*33*
				290	Tetra	*29*
				239	Tetra	*19*
				255	Tetra	*40*
				259	Tetra	*45*
				283	Tetra	*39*
				314	Tetra	*15*
				283	Tetra	*29*
				290	Tetra	*24*
				165	Tetra	*18*
				271.5	Tetra	*63*
				271	Tetra	*21*
				213	Tri	*55*
Fernando et al. (2003)[d]	*Elephas maximus*	F	100	144	Tri	
			100	220	Tri	
			100	246	Tetra	
			97.5	369	Tetra	
			100	149	Di	
			100	142	Di	
Flagstad et al. (1999)[c,d]	*Rangifer tarandus*	F	*90*	171		*0*
			80	215		*0*
			100	93	Di	*0*
			100	97	Di	*0*
			100	106	Di	*50*
			100	94	Di	*0*
Flagstad et al. (1999)[c,d]	*Ovis aries*	F	*92.9*	137		*5.3*
			97.6	87	Di	*0*
			100	118	Di	*0*
			90.5	184	Di	*7.1*
			100	120	Di	*6.7*
			97.6	200	Di	*5.3*

Table 3 continued

Reference	Species	Source of DNA	PCR success (%)	Length of fragment (bp)	Motif	ADO rate (%)[a,b]
Frantz et al. (2003)[c]	*Meles meles*	F		196	Di	*47.7*
				143	Di	*25.6*
				222	Di	*29.7*
				116	Di	*10.6*
				134	Di	*20*
				122	Di	*14*
				183	Di	*26.1*
Frantzen et al. (1998)[d]	*Papio cynocephalus*	F	86.4	149		
			31.8	365		
	ursinus		9.1	614		
Gagneux et al. (1997)	*Pan troglodytes*	H		220	Tri	*32.1*
					Tri	*22.6*
					Di	*43.5*
				204	Di	*25.3*
				178	Di	*27.5*
				143	Di	*26.8*
					Tetra	*34.6*
					Tetra	*31.4*
					Tetra	*36*
					Tetra	*29.2*
					Di	*42.6*
Goossens et al. (2000)	*Pongo pygmaeus abelii*	F	65		Tetra	*4.2*
Huber et al. (2003)[d]	*Lepus europaeus*	F	*97.2*	117		
			96.6	182		
			96.4	263		
			95.2	172		
Huber et al. (2003)[d]	*Cervus elaphus*	F	*95.6*	291.5	Di	
			98.2	160	Di	
			98.2	220.5	Di	
Lathuillière et al. (2001)[c,d]	*Macaca sylvanus*	F	100	122	Di	0
			75	148	Di	3
			58	191	Di	6
Launhardt et al. (1998)	*Presbytis entellus*	F		124	Di	~6.8
Lucchini et al. (2002)[c]	*Canis lupus*	F		101	Di	3
				208	Di	29
				206	Di	33
				278	Tetra	31
				115	Tetra	15
				102	Tetra	0
Morin et al. (2001)[c,d]	*Pan troglodytes verus*	F	75	244	Tetra	15
			74	191	Tetra	31
			84	182	Tetra	35
			88	138	Tetra	12
			80	252	Tetra	34
			97	158	Tetra	26
			71	196	Tetra	27
			80	180	Tetra	19
			65	247	Tetra	32
			85	132	Tetra	20
			92	111	Tri	19
			87	162	Tetra	23
			92	180	Tetra	21
Murphy et al. (2000)	*Ursus arctos*	F	89	200	Di	
Murphy et al. (2002)	*Ursus arctos*	F	88	190	Di	
Murphy et al. (2003)	*Ursus arctos*	F	65	190	Di	
Roon et al. (2003)[d]	*Ursus arctos*	H	~100	268	Di	
			~99	185	Di	
			~98	214	Di	
Sloane et al. (2000)[c,d]	*Lasiorhinus krefftii*	H	*88*	193	Di	*0*

Table 3 continued

Reference	Species	Source of DNA	PCR success (%)	Length of fragment (bp)	Motif	ADO rate (%)[a,b]
			88	200	Di	*0*
			100	99	Di	*0*
			92	145	Di	*0*
			96	159	Di	*0*
			92	126	Di	*0*
			84	158	Di	*0*
			84	207	Di	*0*
			84	138	Di	*0*
			76	80	Di	*0*
			80	303	Di	*0*
			96	314	Di	*0*
Smith et al. (2000)	*Papio cynocephalus*	F	47		Tetra	*72*
			55		Tetra	*47*
			67		Di	*28*
			52		Tetra	*46*
			44		Tetra	*62*
Vigilant (1999)[d]	*Pan troglodytes verus*	H	33	90	Di	
			50	77	Tri	
			33	213	Tri	
			33	138	Tetra	
			33	128	Tetra	
Vigilant (2002)[d]	*Pan troglodytes verus*	F	~69	110	Tri	
			~74	135	Tetra	
			~70	160	Tetra	
			~69	175	Tetra	
			~65	250	Tetra	
			~57	245	Tetra	
			~53	247	Tetra	
			~52	205	Tetra	
Wasser et al. (1997)	*Ursus americanus*	F	~67	256	Di	

[a]Values preceded by ''~'' are not exact values but were evaluated from published graphics

[b]Values in italic were recalculated using published data

[c]Studies represented in Fig. 3C

[d]Studies represented in Fig. 3B

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