Int J Legal Med (2005) 119: 370–373
DOI 10.1007/s00414-005-0521-4

TECHNICAL NOTE

Eva Hedmark · Hans Ellegren

Microsatellite genotyping of DNA isolated from claws left on tanned carnivore hides

Received: 18 October 2004 / Accepted: 7 January 2005 / Published online: 3 February 2005

Abstract Tanned hides, a common form of preservation of mammalian specimens, are usually resistant to DNA analysis. However, we show that DNA isolated from the pulp of claws of tanned hides amplifies well for microsatellite markers. For eight wolverine and eight lynx hides tanned 5–20 years ago, 93–98% of replicate amplifications gave distinct PCR products. Genotypes obtained in analysis of tissue samples of the same individuals were in all cases in agreement with those obtained by analysis of claws. We thus conclude that the use of claws from tanned hides offers new possibilities to genetic studies of preserved mammalian specimens, for instance, in the monitoring of illegal trade.

Keywords Tanning · Microsatellites · Museum specimen · Allelic dropout · Illegal trade

Introduction

Preparation of hides through tanning is a common form of preservation of mammalian specimens, both in museum collections and as trophies. While in theory it should be possible to obtain sufficient amount of DNA from hair or skin tissue to allow genetic analysis, the chemicals involved in tanning cause DNA degradation and act to inhibit enzymatic reactions needed in DNA isolation and amplification (Hall et al. 1996). As a consequence, attempts to perform DNA analysis of tanned hides commonly fail, and this is unfortunate for several reasons. First, illegal killings with the aim of obtaining skin trophies and the associated trade of such hides pose a threat to many endangered mammalian species, e.g. carnivores. The possibility to analyse hides genetically, including individual identification and determination of population origin, would facilitate forensic work. Second, analyses of historical specimens offer a possibility to study temporal variation in genetic diversity and structure (Westemeier et al. 1998; Pertoldi et al. 2001; Flagstad et al. 2003) and may even allow genetic studies of extinct animals (Higuchi et al. 1984). Knowledge about the patterns of temporal variation is important in several ecological and population genetic contexts, as well as in conservation. For many mammals the only access to historical specimens may be in the form of tanned hides.

Although tanning often precludes DNA analysis of skin, claws left on hides may potentially be a more useful source. It is not unlikely that DNA from blood cells of the pulp of claws is more resistant to degradation from tanning as it is encapsulated by hard tissue. To test this possibility, we analysed tanned hides of two carnivore species, the lynx (*Lynx lynx*) and the wolverine (*Gulo gulo*). We amplified polymorphic microsatellites from claw extracts and evaluated the results by comparison to data from fresh tissue samples of the same individuals.

Materials and methods

Eight wolverines and eight lynxes of Swedish origin collected between 1981 and 1998 and stored at the Swedish Museum of National History were used in the study. Both adult and juvenile individuals were represented (Table 1). Hides from these animals had been prepared by the same tannery shortly after collection. Tanning processes typically involve treatment with salt, aluminium, soda, formic acid and tannic acid. A sample from one of the mid claws of each animal was taken by first removing the outermost keratinized layer underneath the claw with a scalpel and then drilling with a dentists' drill into the pulp. The released material was collected and as the claw remained attached to the hide throughout sampling destructive interference was thus minimised. For all wolverines and for four of the lynxes, tissue samples (stored in ethanol) were available.

E. Hedmark (✉) · H. Ellegren
Department of Evolutionary Biology,
Uppsala University,
Norbyvägen 18D,
752 36 Uppsala, Sweden
e-mail: eva.hedmark@ebc.uu.se
Tel.: +46-18-4716462
Fax: +46-18-4716310

Table 1 Information on the tanned hides used for claw sampling

Sample	Year of hide preparation	Size of the animal (weight, kg)	Sex
Wolverine samples			
A975001	1997	Adult (15)	Male
A975113	1997	Juvenile (2)	Male
A975126	1997	Adult (not known)	Female
A975137	1997	Adult (10)	Female
A975141	1997	Juvenile (6)	Female
A905004	1990	Adult (15)	Unknown
A855103	1985	Juvenile (4)	Female
A815004	1981	Adult (12)	Male
Lynx samples			
A985011	1998	Adult (19)	Male
A975127	1997	Adult (15)	Female
A975039	1997	Juvenile (10)	Male
A965130	1996	Juvenile (3)	Female
A955188	1995	Adult (19)	Male
A965063	1995	Adult (Not Known)	Female
A955164	1995	Adult (20)	Male
A965021	1996	Adult (not known)	Female

DNA from claws was extracted by proteinase K digestion and the use of QIAquick columns (QIAGEN) as described for bone fragments by Yang et al. (1998). Briefly, claw material (generally less than 0.2 g) was dissolved in 1 ml extraction buffer (1 ml 0.5 mol/l EDTA, pH 8, 0.5% SDS and 100 μg proteinase K) and incubated at 55°C for 24 h. After centrifugation, 0.4 ml of the supernatant was mixed with 1.8 ml QIAquick PB buffer and spun through a QIAquick column in 0.75-ml batches. QIAquick PE buffer (0.75 ml) was then added to the column and spun through. In a final step, DNA was eluted into 1.5-ml tubes using 100 μl QIAquick EB buffer. Extractions were performed in a special room devoted to the analysis of low-copy number DNA samples and negative extraction controls were included to check for contamination. DNA concentration was measured by a spectrophotometer. Microsatellite amplification was attempted for five species-specific markers in wolverines (*Gg14*, *Gg454*, *Gg465*; Walker et al. 2001; *Gg-7*; Davis and Strobeck 1998; *Ggu234*; Duffy et al. 1998) and four in lynxes (*FCA001*, *FCA506*, *FCA559*, *FCA031*; Menotti-Raymond et al. 1999). Amplification reactions (10 μl) contained 3.0 mmol/l $MgCl_2$, 0.2 mmol/l of each dNTP, 3.2 pmol of each primer, 0.5 μg of bovine serum albumine (BSA), 0.45 U of HotStar DNA polymerase (QIAGEN) and 10–100 ng template DNA. A 15-min pre-denaturation step at 95°C was followed by 35–37 cycles of amplification with 30 s at 94°C, 30 s at 52°C and 1 min at 72°C. A final 10-min extension step was added after the last cycle. Three independent amplification replicates were conducted for each sample and locus. One of the primers of each pair was fluorescently labelled allowing detection on an ABI 377 instrument (Applied Biosystems). Subsequent allele scoring was done using GENESCAN and GENOTYPER software (Applied Biosystems). DNA from tissue samples was extracted following a standard phenol/chloroform protocol (Sambrook et al. 1989), and was amplified and analysed using the same markers and procedures as described above for claw samples (though the numbers of PCR cycles were reduced to 32–34).

Results and discussion

DNA concentration of the claw extracts from wolverines and lynxes ranged between 5 and 60 ng/μl. This is far higher concentrations than in low-copy number amplifications, although we cannot ignore that the DNA in these extracts has been subject to degradation or cross-linking, or that the extracts contain PCR inhibitors. However, all samples of both species amplified very well. For wolverines, 112 out of 120 (three replicates for five markers in eight samples) amplification attempts gave distinct PCR products (93%; Table 2) and for lynxes, 94 out of 96 (98%) attempts were

Table 2 Microsatellite genotypes (bp) obtained from wolverine claws (three replicates) and the corresponding tissue samples (bold). The single inconsistency detected in claw amplifications is underlined

Sample		*Ggu234*	*Gg465*	*Gg14*	*Gg454*	*Gg-7*
A975001	**Tissue**	**92/92**	**173/183**	**199/201**	**134/138**	**169/171**
	Claw 1	92/92	173/183	199/201	134/138	169/171
	Claw 2	92/92	173/183	199/201	134/138	169/171
	Claw 3	92/92	173/183	199/201	134/138	169/171
A975113	**Tissue**	**98/98**	**183/183**	**191/201**	**140/140**	**169/171**
	Claw 1	98/98	183/183	–	–	169/171
	Claw 2	98/98	183/183	191/201	140/140	169/171
	Claw 3	98/98	183/183	191/201	140/140	169/171
A975126	**Tissue**	**92/98**	**183/183**	**191/191**	**136/140**	**169/171**
	Claw 1	92/98	183/183	191/191	136/140	169/171
	Claw 2	92/98	183/183	191/191	136/140	169/171
	Claw 3	92/98	183/183	191/191	136/140	169/171
A975137	**Tissue**	**92/98**	**173/183**	**201/201**	**136/136**	**169/171**
	Claw 1	92/98	173/183	201/201	136/136	169/171
	Claw 2	92/98	173/183	–	136/136	169/171
	Claw 3	92/98	173/183	–	136/136	169/171
A975141	**Tissue**	**92/98**	**183/183**	**201/201**	**136/140**	**171/171**
	Claw 1	92/98	183/183	201/201	136/140	171/171
	Claw 2	92/98	183/183	201/201	–	171/171
	Claw 3	92/98	183/183	201/201	136/140	171/171
A905004	**Tissue**	**92/98**	**173/173**	**201/201**	**136/136**	**171/171**
	Claw 1	92/98	173/173	201/201	136/136	171/171
	Claw 2	92/98	173/173	201/201	136/136	171/171
	Claw 3	92/98	173/173	201/201	136/136	171/171
A855103	**Tissue**	**92/98**	**173/183**	**201/201**	**140/142**	**169/171**
	Claw 1	92/98	173/183	201/201	140/142	169/171
	Claw 2	92/98	173/183	201/201	–	169/171
	Claw 3	92/98	173/183	201/201	140/142	169/171
A815004	**Tissue**	**92/98**	**173/183**	**201/201**	**138/142**	**169/169**
	Claw 1	92/98	173/183	201/201	138/138	169/169
	Claw 2	92/98	173/183	–	138/142	169/169
	Claw 3	92/98	173/183	–	138/142	169/169

Table 3 Microsatellite genotypes (bp) obtained from lynx claw samples. Tissue samples were available for four animals and data from these are shown in bold

Sample		*FCA506*	*FCA001*	*FCA559*	*FCA031*
A985011	Claw 1	199/199	190/190	114/118	228/228
	Claw 2	199/199	190/190	114/118	228/228
	Claw 3	199/199	190/190	114/118	228/228
A975127	**Tissue**	**179/195**	**176/180**	**122/126**	**228/228**
	Claw 1	179/195	176/180	–	228/228
	Claw 2	179/195	176/180	122/126	228/228
	Claw 3	179/195	176/180	122/126	228/228
A975039	**Tissue**	**195/195**	**176/180**	**122/122**	**228/228**
	Claw 1	195/195	176/180	122/122	228/228
	Claw 2	195/195	176/180	122/122	228/228
	Claw 3	–	176/180	122/122	228/228
A965130	Claw 1	193/199	180/190	110/114	228/230
	Claw 2	193/199	180/190	110/114	228/230
	Claw 3	193/199	180/190	110/114	228/230
A955188	Claw 1	195/199	180/180	118/118	228/228
	Claw 2	195/199	180/180	118/118	228/228
	Claw 3	195/199	180/180	118/118	228/228
A965063	**Tissue**	**197/197**	**180/180**	**118/122**	**228/228**
	Claw 1	197/197	180/180	118/122	228/228
	Claw 2	197/197	180/180	118/122	228/228
	Claw 3	197/197	180/180	118/122	228/228
A955164	**Tissue**	**199/201**	**176/180**	**110/114**	**228/228**
	Claw 1	199/201	176/180	110/114	228/228
	Claw 2	199/201	176/180	110/114	228/228
	Claw 3	199/201	176/180	110/114	228/228
A965021	Claw 1	199/199	176/176	114/122	228/228
	Claw 2	199/199	176/176	114/122	228/228
	Claw 3	199/199	176/176	114/122	228/228

successful (Table 3). Of the eight wolverine replicates that failed to amplify five were from one marker and three from another, indicating some difference in the performance among markers. There was only a single case in which all three replicates did not give an identical genotype (wolverine A815004, locus *Gg454*; see Table 2). In this case, the homozygous appearance of one of the alleles present in heterozygous state in the other two replicates is consistent with allelic dropout (failure of an allele to amplify in a heterozygote individual, see, e.g. Taberlet et al. 1999). Quantitative studies indicate that allelic dropouts may occur frequently if the amount of template DNA is less than 60 pg (Taberlet and Luikart 1999; Morin et al 2001). The low rate of allelic dropout observed in our study (<1%) further indicates that sufficient amounts of DNA were obtained. There were no observations of false alleles.

A comparison of data obtained from amplification of claws and tissue samples of the same individuals showed completely identical genotypes, both for wolverines (Table 2) and lynxes (Table 3). This indicates that the reliability of microsatellite genotypes obtained from claws left on tanned carnivore hides is very high. The interpretation of an allelic dropout in the single deviant replicate in claw amplifications was confirmed in the analysis of the corresponding tissue sample (where it was recorded as a heterozygote).

Our study demonstrates that claws from tanned hides provide a useful source of DNA for genetic studies. We found a high consistency between replicate amplifications of microsatellite markers and the genotypes we obtained were in agreement with those derived from the corresponding tissue samples. As the sample set contained recently preserved hides as well as hides preserved 20 years ago, this indicates that long-term storage does not significantly affect the possibility to obtain successful results. The age of animals, and thus body (claw) size, does not seem to be critical; our sample contained juveniles (2–10 kg) as well as adults (10–20 kg). We conclude that, while tanning processes normally mean that DNA analysis of hides is not possible, or at least not straightforward, claws offer a means for the successful genetic analysis of tanned hides.

Acknowledgements Financial support was obtained from the Norwegian and Swedish Environmental Protection Agencies and the Swedish National Laboratory for Forensic Sciences. Claws were sampled from hides at the Swedish Museum of Natural History, which also provided tissue samples.

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