An optimized hair trap for non-invasive genetic studies of small cryptic mammals

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TECHNICAL NOTES

An optimized hair trap for non-invasive genetic studies of small cryptic mammals

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Abstract As sample quality and quantity is a crucial factor in non-invasive genetics, we focused on the improvement of sampling efficiency of glue hair traps. We invented an optimized hair trap with moveable parts which enhanced sampling of high-quality genetic material. With the aid of the optimized hair trap, we were able to remotely pluck a sufficient amount of hair bulbs from our study animal the common hamster (*Cricetus cricetus*) with a trapping success of 49.3% after one survey night. The number of collected hairs with bulbs ranged between 1 and 50, with an average of 20.7±14.8. Subsequently, the use of the hair trap in combination with a simplified laboratory routine allowed us to amplify species-specific microsatellites with an amplification success of 96.2% and ADO of 4.6%. This optimized trap may find usage for species identification or could be used as an instrument for long-term genetic monitoring of mammal populations.

Keywords *Cricetus cricetus* · Remote collecting · Microsatellites · Non-invasive genetic sampling

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Introduction

Non-invasive sampling techniques for genetic studies on mammals become increasingly important due to ethical, logistical, and scientific restrictions. Those methods allow researchers to collect samples without any observer bias or negative consequences for individuals due to handling or stress. Therefore, they are most frequently applied in studies with rare, cryptic, or endangered species (Piggott and Taylor 2003; Waits and Paetkau 2005). The source of sample material together with DNA extraction methods and PCR conditions is among the most crucial sources of error (Beja Pereira et al. 2009; Broquet et al. 2007; Taberlet et al. 1999). Hair samples are a widely used, non-lethal source of DNA, and recent advantages in molecular techniques provide reliable results through the application of strict guidelines and high-performance analysis techniques (Beja Pereira et al. 2009; Mullins et al. 2009). In contrast, very little attention is directed towards the invention, construction, and application of effective traps that facilitate the collection of high-quality sample material. When using hairs, in particular their bulbs, as a source for DNA, these are needed in sufficient amounts containing enough follicular tissue. A minimum of three to ten hair bulbs are needed to reduce genotyping errors significantly (Gagneux et al. 1997). The use of hair traps with mechanical devices (e.g., barbwire) or attached glue-like substances to collect plucked hairs is not ideal as it rarely collects high proportions of hairs with roots (Ebert et al. 2010; Valderrama et al. 1999). Here, we present a new, cost-efficient, optimized hair trap for non-invasive sampling that uses movable parts to collect efficiently hairs with

bulbs. We tested the effectiveness for non-invasive genetic studies by quantifying the amount of hair bulbs, microsatellite amplification success, and genotyping error rates in the endangered common hamster *Cricetus cricetus* L., 1758.

Materials and methods

Study area

This study was conducted between July and September 2009 in Hesse (Germany). In three different study areas, 223 ha of arable fields were surveyed for the presence of common hamster burrows.

Hair trap

The hair trap is built out of a 5-cm-long PVC pipe with a diameter of 7.5 cm to match the average diameter of common hamster burrows. Through two holes at the upper periphery of the pipe, a 7.5-cm-long steel nail is placed. For trapping hairs, a plastic roll with a diameter of 1.2 cm was attached using the nail as axis, and double-faced adhesive tape was then wrapped around the roll (Fig. 1a and b). When animals went through the pipe, the plastic roll with the adhesive tape rotates which then results in a vertical extraction of hairs. Hair traps were placed in burrow entrances in the late afternoon and surveyed for hairs early in the next morning (Fig. 1c and d). For each applied trap, the amount of hairs was documented (Fig. 1e and f).

Sample storage

The appropriate storage and preservation of non-invasive samples is crucial (Beja Pereira et al. 2009). As DNA comes in small quantities and low quality, it should be protected against heat stress or sunlight (Taberlet et al. 1999). As freezing and refrigeration result in physical stress, we used dry preservation. We stored samples in 125-ml vials with 5.5-cm height and airtight closure (Sarstaedt®). To dry samples, we attached two packages of Silica Gel (Wisepac®) to each vial. After successful application in the field, the whole glue–plastic roll was moved to a vial.

DNA isolation and genetic analyses

DNA extraction was performed using 20% Chelex following standard protocols (Walsh et al. 1991). This method is used in several other studies with small-sized mammals (Sloane et al. 2000; Moncrief et al. 2008). The roots of ten

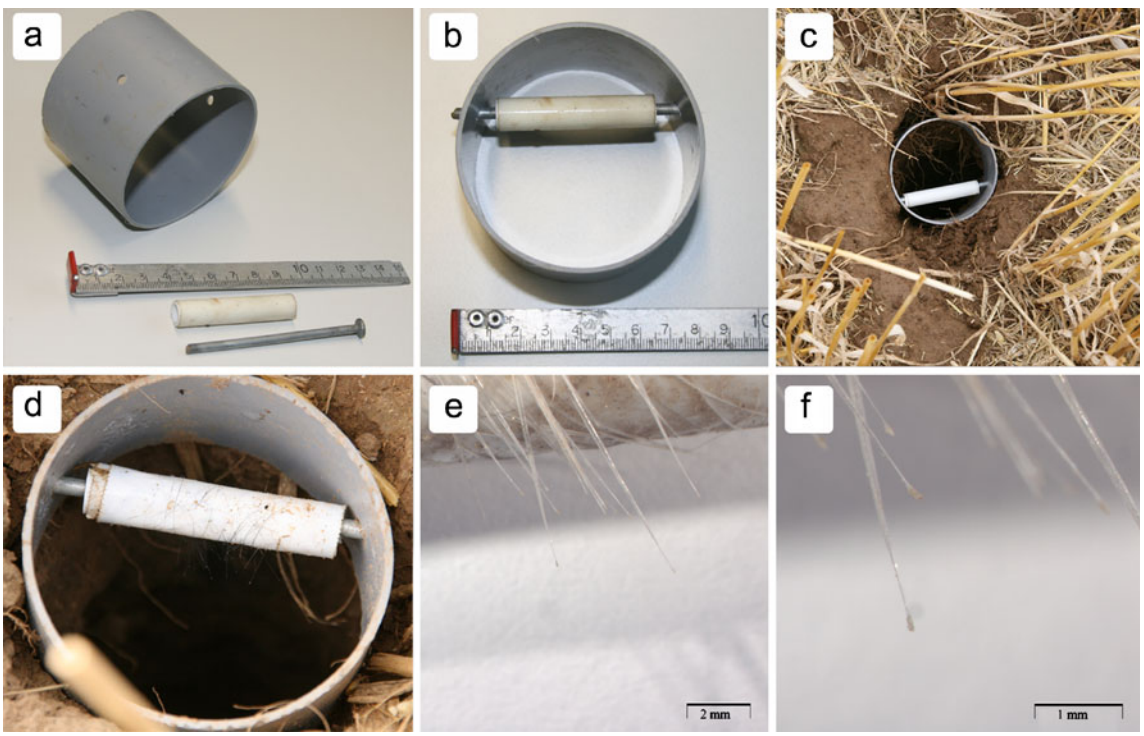

Fig. 1 Materials and application of the optimized hair trap. The trap consists of a PVC pipe with two holes, an iron nail, and a plastic roll (**a**). The plastic roll is wrapped with double-faced adhesive tape and attached inside the pipe by the nail (**b**). Traps are placed in entrances of burrows (**c**), and as an animal passes, hairs are plucked by the rolling tape (**d**). Most of plucked hair contained bulbs with a sufficient amount of follicular tissue (**e** and **f**)

Table 1 Analyzed loci of *C. cricetus* used in the study, listed with GenBank accession no., locus name, repeat motif and primer sequences (sequences in capitals stand for unmodified primers, lowercase sequences for modified primers), assignment to multiplex reaction groups, number of detected alleles, size range of alleles in base pairs (bp), expected heterzygosity (H_E), amplification success, and allelic dropout (ADO)

GenBank accession no.	Locus	Repeat motif		Primer sequence (5′–3′)	Multiplex group	Allele numbers	Size range (bp)	H_E	Amplification success (%)	ADO (%)
AM167541	IPK01	$(TC)_{22}N_4(TG)_{14}$	F	CTGTGGGCTGATCTCTCTC	1	9	99–129	0.425	97.8	2.7
			R	gcaagagagcaaaagtgtg						
AM167544	IPK05	$(TC)_{19}(AC)_7$	F	gttccatagcccttcactc	1	6	153–165	0.608	97.8	2.7
			R	ccctatttgcaaagaatcc						
AM167545	IPK06	$(CT)_{24}$	F	TGACCTGAGTTTGATTTCC	1	3	80–84	0.505	97.8	0.0
			R	ATTTGTGCATGTGCATGAC						
AM167546	IPK07	$(CT)_{22}N_{29}(CT)_7N_1(TC)_8$	F	tccaggtagattttgagtgc	1	8	154–170	0.434	97.2	2.0
			R	tgaaaacatagtgagagaggg						
AM167547	IPK09	$(GT)_{21}$	F	ctcaacagtccctgaatacg	1	5	177–185	0.510	96.1	0.0
			R	gaggaaacaaagtggaaaag						
AM167548	IPK12	$(GT)_{22}$	F	gttagggtccattgagtgag	1	4	100–106	0.522	98.9	0.0
			R	agaccagcaaatcaaaagag						
AJ532554	Ccrμ4	$(GT)_{18}$	F	tgagatgtatgcatgtgtgc	2	4	150–164	0.197	95.5	13.5
			R	tttcatatgtgtgccaagac						
AJ532556	Ccrμ10	$(CA)_{23}$	F	tatatgcacatcatgtcacg	2	5	99–107	0.196	92.9	14.8
			R	ggtcttaagaatcaggtgtg						
AJ532557	Ccrμ11	$(GT)_{22}$	F	gcatttgccactatcgttc	2	4	148–162	0.400	93.9	7.0
			R	tccaactccttcaagttgtc						
AJ532563	Ccrμ20	$(TC)_{23}(CT)_9(CT)_6$	F	cacttggatctgactttgtg	2	7	136–142	0.681	94.4	13.8
			R	tggggtatttggatgattac						

hairs were incubated in 200 μl of a 20% Chelex solution for 2 h. To test if extracts are sufficient for genetic studies, ten species-specific microsatellites were used (Jakob and Mammen 2006; Neumann and Jansman 2004). Primers were modified, respectively new designed, following the guidelines for non-invasive sampling (Broquet et al. 2007; Taberlet et al. 1999) (Table 1). For PCR, the Qiagen® Multiplex PCR Kit was used, arranging primers into two multiplex reactions (Table 1). All reactions were carried out in a separate laboratory with an Eppendorf® MasterCycler Gradient using a 10-μl reaction volume with 5 μl 2× QIAGEN® Multiplex PCR Master Mix combined with the touchdown-PCR technique to increase annealing specificity (Hecker and Roux 1996).

Assessment of amplification success and error rates

Amplification success was measured by counting the amount of failed amplifications for each locus. To test for allelic dropout (ADO), reactions (n=77) and isolations (n=41) were repeated randomly. ADO was calculated using the equation presented in Broquet and Petit (2004).

Results

During the study, we applied 199 hair traps to common hamster burrows. At 144 burrows, the trap contained hairs in 98 cases resulting in a 49.3% trapping success after one survey night. The number of collected hairs with bulbs per trap was between 1 and 50, with an average of 20.7±14.8 (n=98) bulbs. The proportion of successful amplifications for the species-specific microsatellites over all loci was 96.2%. The rate of ADO over all loci was 4.6% (Table 1). False alleles were not detected in the dataset. Our approach provided enough material to successfully genotype 88 different individuals.

Discussion

Recent developments in molecular techniques and genotyping pronounce the importance of non-invasive sampling (Waits and Paetkau 2005). But most non-invasive techniques are still expensive, because reliable and replicated samples of genetic material are only available using costly methods (Navidi et al. 1992). With our approach, we will reduce costs significantly by improving a basal technique for non-invasive genetic sampling. Our study showed that the newly developed trap is easy to apply and provides a high gain of genetic material. Additionally, the glue-containing part is fast and easily replaceable, and samples can be directly conserved for further analyses.

One of the major drawbacks of non-invasive sampling, the generally small amount and low quality of genetic material (Piggott and Taylor 2003), has been overcome by our current approach. As demonstrated, we were able to amplify species-specific microsatellites with high amplification success (96.2%) and comparably low error rate (ADO, 4.6%). These rates are markedly better than the mean amplification success of 80.2% and mean allelic dropout rate of 18.7% reported from a review of non-invasive studies using hair samples as source for genetic material (Broquet et al. 2007).

The effectiveness of the hair trap with the glue roll was only shown for one burrow-using species. Our approach may also find use in studies with other species as comparable studies using tubes for species identification without genetic sampling were already conducted (Mortelliti and Boitani 2008; Ruiz-Gonzalez et al. 2008; Sanecki and Green 2005). As a conclusion, this trap is well suited for short- and long-term monitoring studies in population genetics of small mammals and provides a suitable technique for highly replicated studies on relevant, large spatial scales.

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