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PROCEDURES FOR DNA EXTRACTION AND AMPLIFICATION FROM QUILLS OF CRESTED PORCUPINE *(Hystrix cristata).*

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ABSTRACT - Genetic analysis in mammalian populations are useful for any conservation management. This kind of analysis utilised tissue of specimens studied involving a damages to the populations, only recently many study are indicated alternative procedure based on scales, hairs or feathers. The porcupine *(Histrix cristata)* is an elusive species with localised populations. In this work I propose one procedure of genetic analysis starting by DNA quill in order to help studies about this species, whiteout damage the population examined. The quills are easy to find because the porcupine loss them constantly.

Nucleic acids extraction was done utilising synthetic resin (chelex) and then ipervariable region of mitochondria] DNA was amplified through PCR with specific primers. The advantages of this selective amplification is the selective increase of interest DNA in spite of possible organic contamination. At this point two different procedure could be utilised to characterise of population: restriction fragment analysis or sequence analysis.

Key words: Hystrix cristata, DNA extraction, mtDNA amplification, quills.

INTRODUCTION

Histricids is a fairly ancient family with four genera and 15 recent species appearing first in the Oligocene of Europe; occur throughout most of Borneo, South Celebes, Flores, Philippines, Asia and South China, Africa and South Italy (Vaughan, 1972).

In Italy the current distribution of porcupine *(Hystrix cristata)* is restricted to South and Central part with localised populations recently spread overall North range (Tedaldi and Scaravelli, 1993). The species is elusive and with vulnerable populations, for this reasons the census is difficult.

Conservation of Italian populations represents an important aim to preserve the endemisms; in fact, in spite of its relatively wide distribution, information on this biology is scarce, being confined to partial report (Corsini *et al.*, 1995).

"One important aspect in the complex field of conservation biology is the preservation of genetic variation within populations and species" (see. Frankel and Soulé, 1981; Schonewald-Cox *et al.,* 1983; Soulé, 1987, for reviews); conservation planning of Italian porcupine must consider their population structure and genetic characteristics. Unluckily, the sampling is difficult and probably a negative impact for the wild populations.

Non destructive procedures for genetic studies are very important to asses polymorphism and population relations.

Mitochondria1 DNA analysis can be used to explain lineage differentiation (Cann *et al.*, 1987) and polymorphism (Taylor *et al.,* 1997). A very small amount of DNA can be used to genomic analysis through thermostable DNA polymerase in PCR reaction (Saiki *et al.*,

Figure 1 - Quill of porcupine (*Histrix cristata*) utilised for genetic analysis.

1988). DNA typing has been reported from a single hair and feather (Ellegren, 1994; Higuchi ***et al.***, 1988; Vigilant *et al.*, 1989) requiring a very small amount of DNA.
This paper shows that authentic DNA can be recovered from quill of Italian porcupine and describes in detail the methods used for the extractions and amplifications of the DNA to type populations.

MATERIAL AND METHODS

Sample collection

The quill (Fig. 1) collected are preserved in a cryotube with 70% ethanol, at room temperature. One quill is used for the DNA extraction and referred to one correspondent individuals of South Tuscanian porcupine.
This animal shows solitaries life or forming little familiar nucleus; the moving carried out by each porcupine individual is inside of a polygon that has a diagonal of about 3,9 kilometres, while during the night this range is reduced to 700 meters (Sonnino, 1998).
We can assume that the quills collected for genetic analysis, with good probability, belong to different individuals if they are found at the minimum distance of 4 kilometres. This caution safeguards us by possible false and not clear genetic results.
During all steps is essential to minimise contamination of the analysed material by exogenous DNA from derived by operator, the use of disposable gloves is recommended.

Genetic analysis

One quill is cut transversely 2 mm from the base and micronized with forceps. The fragment is put into an eppendorf tube containing 200 ml of 5% aqueous solution of Chelex-100 (Walsh ***et al.***, 1991). Subsequently incubated for 4 hours with Proteinase K (10 ng/ml) at 50°C with gentle mixing. The tube is boiled for 8 minutes and spinned at 13000 rpm for ***2*** minutes. The supernatant is picked and concentrated using a Crist Alpa RVC essicator.
The final volume of the extract was assayed through spectrophotometer at 260 nm and 280 nm.
DNA amplifications were done by the method recommended by Perkin Elmer Cetus in 30 ml reaction containing 1 units *Termus aquaticus* (Taq) DNA polymerase. The reaction buffer consisted of ***2*** mM $MgCl_2$, 1 mM dNTP, 67 mM TrisHCl pH 8.0.
The primers used in this procedure were the highly conserved mtDNA primers a 121bp fragment in a small non coding region (region v) containing a useful marker (primer A: ***5'-*** ATGCTAAGTTAGCTTTACAG – 3' and primer B: ***5'-*** ACAGTTTCATGCCCATCGTC – 3') (Wrischnik ***et al.***, 1987; Laurent ***et al.***, 1998).
A blank reaction containing no DNA was set up in each experiment to monitor any possible contamination of PCR reagents.
The amplification of the region V fragment consisted of 35 cycles of denaturation at 92°C (40 sec), annealing at 55°C (1 min), and extension at 72°C (1 min).
Portions (15 ml) of PCR reaction were electrophoresed on 1.5% agarose minigel and stayed with ethidium bromide to visualise the DNA fragments under ultraviolet light.
The amplification products can be typed through two way: direct sequence and restriction fragment length polymorphism.

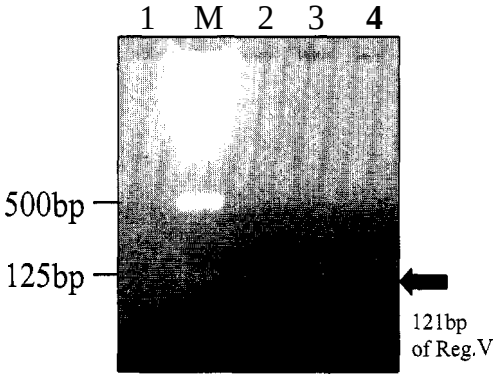

Figure *2* - Gel electrophoretic analysis of PCR products resulting from amplification of porcupine **DNA.** Line 1: blank control; M: molecular marker; Line **2,** 3 and 4: region V fragment obtained utilising primer **A** and B.

RESULTS AND DISCUSSION

A fragment about 1 cm was removed from each quill of three porcupine specimens and DNA was extracted as described above. The final volume of the extraction was assayed through spectrophotometer at 260 nm and 280 nm showing, approximately, 0.3 mg/ml amount of nucleic acids and 0.67 of purity rate.

Mitocondrial DNA (mtDNA) fragment was amplified successfully from all three quill DNA extracts. Figure 2 shows the results of PCR of the three samples with the primers A and B of Region V of mtDNA. In the agarose gel the characteristic band to 121 base pairs represent the amplified region V mtDNA of analysed porcupine.

All the blank control were negative, indicating that there was no detectable contamination of the samples during the procedure.

The next steps concern characterisation of amplified DNA to asses genetic structure. The two methods suggested, for characterisation, are the Restriction Fragment Length Polymorphism (using restriction enzyme compatible with amplified region, as *Sca*I) and direct sequence with marked ^{32}P and thermostable enzyme TH.

The results show that this is a procedure to extract genetic material from porcupine. It is particularly important in studying of genetic variability and geographic polymorphism. Moreover, by optimising this easy procedure regarding: the choice of the mitochondrial fragment and to the appropriate restriction enzyme, will open up new research possibilities in many conservation projects that can involve this species. In fact there are a lot of endemic population that have to recuperate or protect.

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