



SKELETAL REMAINS IN OWL PELLETS, COLLECTIONS AND GENETIC ANALYSES: A SURVEY ON *Microtus* sp. IN CENTRAL ITALY.

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INTRODUCTION: Skeletal remains retrieved from pellets of raptors like the Long eared owl (Fig. 1), are often the main body of small mammal zoological collections, being extremely useful in the study of wildlife [1]. Pellets (Fig. 2a) are build-ups of indigested materials such as hair, feathers and bones (Fig. 2b) that are periodically regurgitated and that can be found under nests or roosts, common perches. Bones found in owl pellets can be used to do both morphometrical and molecular analyses that can help in better understanding the evolutionary trends and population structure of prey species.



Figure 1: Long eared owl (*Asio otus*)



Figure 2b: rodent remains extracted from the pellet in Figure 2a



Figure 2a: Long eared owl pellet

MATERIALS and METHODS: 33 left hemimandibles, morphologically ascribable to voles of the genus *Microtus* were isolated and used in both morphometrical and genetic analyses. DNA extraction (Fig. 3) was done using a previously tested and validate protocol [2] adding a prior washing phase of the sample in PBS (Phosphate Buffer Saline) solution to remove external contaminants and, in the meantime, to decalcify the bone matrix exposing the cells from which DNA was extracted. DNA was then amplified with a PCR reaction aimed at a fragment of the mitochondrial gene *cytb* using species-specific primers [3], that allow the amplification of DNA extracted from degraded samples.



Figure 3: DNA extraction from bone tissue

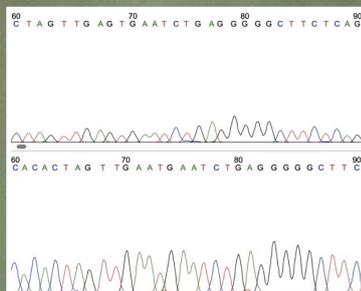


Figure 4: Comparison of two electropherograms of a first amplifications (upper) and of a reamplified fragment (lower). Is evident that the base-calling peaks of the sequencer in the reamplified fragment are higher and well-defined, allowing for a much more reliable result

RESULTS and DISCUSSIONS: DNA was extracted, amplified and sequenced successfully for all the analysed samples. Is to be noted that the reamplification of already amplified samples with internal primers can improve the quality of amplicones, enhancing the signal (Fig. 4), allowing therefore for a much more accurate investigation of DNA molecules that could have been degraded by time, by anthropogenic conservation processes or by the digestion of the prey within the gastric system of the predator. The experience described confirms, yet again, the paramount importance of naturalistic collections and their use in scientific research. The genetic analyses of specimens collected and stored within zoological collections allows to put together the evolutionary history of species, acting like a window to the past. Naturalistic collections can therefore be tools of fundamental importance in the conservation and valorisation of biodiversity.

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