Differentiating hair samples of the European mink (*Mustela lutreola*), the American mink (*Mustela vison*) and the European polecat (*Mustela putorius*) using light microscopy

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Abstract

The European mink *Mustela lutreola* is a threatened species that is being monitored throughout its range, and innocuous methods of detection are needed as an alternative to live trapping, which may result in fatalities. A method is described for the identification of European mink hair by light microscopy, and its differentiation, on the basis of cuticular and medullary patterns of guard hairs, from hairs of similar mustelids, the introduced American mink *Mustela vison* and the European polecat *Mustela putorius*. When used in conjunction with hair trapping of living animals, this could prove to be the ideal way of conducting monitoring programmes on the European mink.

Introduction

The European mink *Mustela lutreola* is one of the most threatened carnivorous species (International Union for Conservation of Nature and Natural Resources, 2004). The current distribution of this mustelid is estimated to be only 20% of the original population and its decline continues at an accelerating rate. Periodic monitoring campaigns are currently underway to obtain information about the changes taking place in the distribution area of the mink. Owing to a number of circumstances (populations with low densities, the fact that these animals are difficult to observe and the impossibility of identifying their excrements and trails), the most commonly used detection technique is live trapping (Maizeret *et al.*, 2002). This method offers unequivocal observations of the species. However, live trapping presents the same drawbacks as any capture method in that it is always associated with a mortality rate, which is a factor that must be taken into account when studying a threatened species. Moreover, in order to conduct regular updates on the distribution of this species by means of live trapping in a large area (several thousand km^2) the assistance of many people is required, which entails either ample funding or the collaboration of volunteers. In view of these drawbacks, alternative methods of detection are needed. In recent years, hair trapping has become widely used in mammal distribution studies (Mills *et al.*, 2002; Sanecki & Green, 2005). Because of the use of a combination of different techniques to collect hair samples of live animals and prepare identification keys based on the pattern and structure of micro-scopic hair characteristics (medulla and cuticular scales), it is now possible to update inventories and determine the distribution of a large number of species without harming the animals or interfering with their behaviour. This method is also less costly and does not involve as many people as live trapping, but it cannot be used with the European mink, as identification keys, based on macroscopic or microscopic characteristics that provide the unequivocal identification of the hair of this species, are not yet available. The atlas published by Debrot *et al.* (1982) contains drawings and photographs with the characteristics of European mink hair, but it does not include any characters to differentiate it from the hair of other morphologically similar species. After a comparison of the information reported by Faliu, Lignereux & Barrat (1980), Keller (1981), Debrot *et al.* (1982) and Teerink (1991), it can be seen that among the species whose distribution overlaps with that of the European mink, there are two with hair that is morphologically very similar: the introduced American mink *Mustela vison* and the European polecat *Mustela putorius*. This paper examines the hair morphology of these three mustelids with the aim of determining if there are differences that will allow for the identification of a hair sample collected by means of non-invasive techniques, such as hair trapping.

Materials and methods

Hair samples of 34 European minks, 26 American minks and 21 European polecats were collected between 2000 and 2005. Samples were taken from dead specimens (run over by

vehicles) and from animals caught during the European mink monitoring campaign and the American mink control surveys carried out in the north of Spain in recent years. A sample of full-grown hair was collected from the different parts of each specimen's body – the back, abdomen and head – and stored in 96% ethanol. Although underhair is the most abundant type of hair in the coats of these animals, it is of little use for identification purposes (Teerink, 1991). Therefore only guard hairs were used. The techniques and terminology put forth by Teerink (1991) were used to observe and describe hair characteristics.

The hairs were cleaned and degreased with xylene. It is sufficient to bathe the hairs for only a few seconds. The cuticular pattern was obtained by using transparent nail polish as the printing medium. First a fine coat of nail polish was spread on the slide. Before the nail polish hardened, the hair was placed on the slide. Once the nail polish had hardened, the hair was carefully removed with tweezers, and a print of the cuticular pattern was left on the slide. This can be examined under an optical microscope with a magnification of $\times 200$–400. The process lasts only a couple of minutes and, if performed carefully, the hair will not be damaged and can be used to observe the medulla.

The shield of the guard hairs contains a dark pigment that makes it difficult to see the medullar pattern clearly. The hair was therefore rinsed with paraffin oil. This product serves to replace, either partially or in full, the air in the intercellular chambers, thus improving the observation of the structure and position of the cells. This was done by coating the slide with transparent nail polish. Before the nail polish dried, the clean, degreased hair was placed on the slide. Once the nail polish had dried, the hair was cut transversally with a sharp razor blade in several places on its longitudinal axis in the area where the medulla was to be observed. Next, a drop of paraffin oil was poured onto the hair and the slide was warmed a little to facilitate the penetration of the oil. The medullar pattern could then be examined under an optical microscope with a magnification of $\times 200$–400. In slides of cuticula and medulla, the flat side of the shield was placed against the glass. The entire process can be completed in roughly 5 min.

Results

A combination of two characteristics make it possible to identify the hair of the species under study: the cuticular pattern of the shaft and the medullar pattern of the shield. The discriminatory features observed were valid regardless of the body part of the animal from which the guard hair was taken. Morphological variance within species was not observed. All the hair samples were classified correctly according to the medullar and cuticular patterns.

In the guard hairs of the European polecat, the shaft scales are triangle-shaped with divergent edges. Both the mink species exhibited a similar cuticular pattern when compared with each other, but different from the pattern observed in the European polecat. The scales are long and narrow and the edges are almost parallel (Fig. 1).

With regard to the medullar pattern of the shield, in the European polecat and European mink both the cells and the air-filled spaces lie mostly at right angles to the longitudinal axis of the hair. The air-filled spaces are large, narrow and some actually take up most or all of the width of the medulla. In the American mink, the pattern is different. The air-filled spaces separating the cells are smaller, having a round shape, and give the medulla a reticulated appearance (Fig. 2).

Discussion

López-Giráldez *et al.* (2005) described a molecular method for the genetic identification of the hair of these three species on the basis of DNA analysis. The authors of this study used a minimum of 15 hair roots per specimen, reporting that in this type of analysis, in order for the sample to produce a
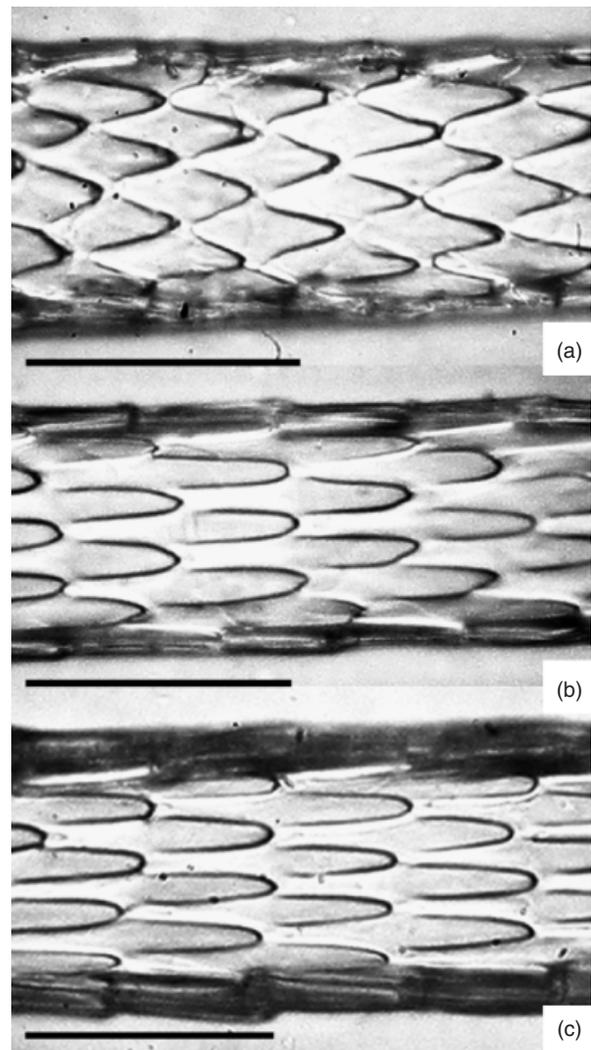

Figure 1 Cuticular pattern. Guard hairs (shaft region). Print in nail polish: (a) European polecat *Mustela putorius*; (b) European mink *Mustela lutreola*; (c) American mink *Mustela vison*. Scale bars = 50 μm.
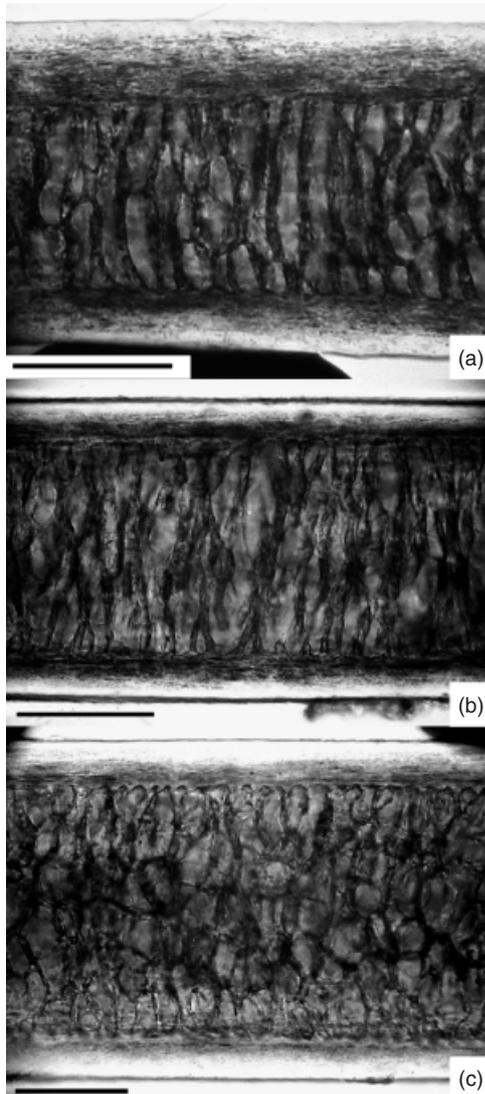
Figure 2 Medullar pattern. Guard hairs (shield region). Whole mounts: (a) European polecat *Mustela putorius*; (b) European mink *Mustela lutreola*; (c) American mink *Mustela vison*. Scale bars = 50 μm.

reliable identification, it must contain no fewer than 10 hair roots. In addition to the drawback of needing a minimum sample size, this method also requires costly equipment and material for processing, not to mention qualified personnel.

With regard to the technique proposed in this paper, identification by light microscopy is a method that has proved to be simple, quick and inexpensive, requiring only one hair per specimen to obtain a reliable datum. The problem with this method is that it requires guard hairs that are well-formed and complete, whereas genetic identification can be carried out using only the roots of any kind of hair.

In the present study, all samples were collected from the north of Spain. It would be interesting to test the discriminatory features with a sample of broader geographic origin, in order to account for possible morphological variance

within the same species. Hence, we recommend testing identification by light microscopy in known species before applying it in other countries where the European mink is also threatened.

The possibility of identifying the hair of these species opens up new prospects of being able to use hair trapping as an alternative to the techniques generally employed in monitoring programmes targeting the European mink, such as live trapping (Birks & Kitchener, 1999; Maizeret *et al.*, 2002) and camera trapping (González-Esteban, Villate & Irizar, 2004). Moreover, the differences between the two hair identification methods, molecular and light microscopy, mean that they can be used to complement each other in the different sampling situations that may arise. However, it is necessary to design hair traps specifically targeting minks, similar to those used with other mustelids (Foran, Minta & Heinemeyer, 1997; Mowat & Paetkau, 2002), and to test a sampling protocol that will allow the monitoring of the distribution and abundance of this species.

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