HAIR DETERMINATION AND IDENTIFICATION FROM BIRD NESTS

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ABSTRACT
The aim of our study was to test a new noninvasive method, the bird-nest analysis in urban environment. The study area (Merzse-swamp) is located in the south south-west of Budapest. The area is bordered by the M0 motorway (from East), the Ferihegy Airport (from South) and the suburb of the 17th district (from East). We have collected 13 nests and we have found mammalian hairs in 9 nests (69,23%). From one nest an average of 5,31 (SE=5,31) hairs were found, from this 3,77 (SE=4,17) were able to be prepared and 2,85 (SE=2,91) were categorized. We have created 13 categories from the data. 5 of these were species categories (*Talpa europea*, *Mustela nivalis*, *Homo sapiens*, *Lutra lutra* and *Myoxus glis*), 3 of them were twin-species (*Rattus rattus-Rattus norvegicus*, *Muscardinus avellanarius-Dryomis nitedula* and *Oryctolagus cuniculus-Lepus europaeus*). These species cannot be exactly identified just by hair morphology (supplementary data is needed, e.g.: area of distribution). 3 genera were identified (*Canidae* spp., *Chiroptera* spp. and *Apodemus/Microtus* sp.). Finally, there are 2 categories for unidentifiable hairs („not hair": revealed during the microscope study, „unidentifiable": data deficient). The most common species were *Homo sapiens* and *Mustela nivalis*. In the case of one species (*Lutra lutra*) we think it would be necessary to confirm the presence with other observations (visual observation, footprints and remains of preys). According to our study it has been demonstrated that the nest-analysis can be a useful technique to researchers and urban wildlife management experts. References from hairs and practice are necessary to get familiar with the method.

Keywords: hair identification, urban environment, non-invasive method, Merzse-swamp, bird nest

INTRODUCTION

It has always been a problem to examine animals with reclusive lifestyle, due to this and the rarity of some species the uses of non-invasive techniques are growing increasingly (MACKAY et al., 2008; CASTRO-ARELLANO et al., 2008 http1).
The essence of these indirect methods is that the observers and the examined animals will avoid direct contact. Because of this, researchers' presence will not bias the result and the animals can avoid stressful situations. The usage of non-invasive techniques probably dates back to the origin of humans (MACKAY et al,. 2008), the knowledge about traces and scats can be considered noninvasive methods, but we can gather useful information from mammalian hairs too.
Numerous mammalian species can be indentified from their hairs. Basically, there are two types of identification, one is based on the morphological characters of the hair (qualitative and/or quantitative features) (TÓTH, 2002, 2008; TEERINK, 1991; SEILER, 2010; MARINIS & ASPREA, 2006; DEMARINIS & AGNELLI, 1993), and another based on the mtDNA collected from hair shafts (DOMINGO-ROURA et al., 2006; AMENDOLA-PIMENTA et al., 2010; BALESTRIERI et al., 2010).
Basically, there are two groups of methods are in use for collecting hair samples. Hair-traps can be baited or unbaited (passive) (KENDALL & MCKELVEY, 2008). The former ones are usually artificial hair-traps (KENDALL & MCKELVEY, 2008; CASTRO-ARELLANO et al.,

2008), the latter are natural hair-traps like fox earth, surrounding of a trace, scratching surfaces and also bird nests (TÓTH et al., 2010A).

Bird-nest analysis (TÓTH, 2008) is a new, internationally accepted method. Certain bird species use mammal hairs for lining or structural strengthening of their nest. After the fledging of chicks these nest can be gathered and analyzed to receive faunistical data from the area. Since the growing process of urbanization (PATTERSON et al., 2003) and the urban dwelling animals with reclusive lifestyle (e.g.: *Martes foina*) (TÓTH et al., 2007; TÓTH et al., 2010B; SZŐCS & HELTAI, 2010) this method can give a new surveying technique to wildlife researchers and urban wildlife management experts.

The aims of our study were to (1) find out if there are enough nests to an appropriate assessment, (2) find out what lining materials do birds use in a semi-urban environment and to collect hair samples if there is any, (3) find out what species can be detected.

MATERIAL AND METHOD

Merzse-swamp (located in the 17th district of Budapest) and its surroundings were the study area. The swamp is surrounded by agricultural areas, shelterbelts and planted forests. The area is bordered by the M0 motorway (from East), the Ferihegy Airport (from South) and the suburb of the 17th district (from East).

The field survey was done in 17 February 2011 which occasion we have found 13 bird nests. All nests have been photographed, coded (M1, M2… Mn) and GPS coordinates have been recorded. After a few days drying, the nests were placed under an UV disinfection equipment to avoid potential zoonoses. Then the nests were took into pieces on a white paper. Mammal hairs were placed into labeled reclosable polyethylene bags.

It was necessary to record the macroscopic features of the hairs (color, shape, length). Before the preparation to microscopic analysis, the hairs were soaked into 70-80% alcohol for a few hours and then placed into ethyl-ether for a few seconds to remove all grease and contamination (TÓTH, 2008).

For the microscopic analysis we used the guidance of TEERINK (1991), TÓTH (2008) and LANSZKI (personal comm.). The cuticula impressions were made in 5-10% gelatin solution with a few thymol crystals. After the solution cooled a bit hairs were put into the solution, with special attention not to be covered by gelatin. After the gelatin solidified the hair can be dig out with an insect pin (minimal damage is unavoidable) and scale pattern will be seen in the gelatin. Then the hair was put on another microscope slide and fixed with transparent nail polish, after this a scalpel was used to cut the hair and lastly, paraffin oil was applied to replace the air in the medulla (structure will be revealed). According to our guides and personal experiences five features can be important at the identification, (1) the cuticula scale pattern at the shaft and (2) at the thickest part of the shield, the medulla structure at the thickest part of the shield without oil penetration (3) and with oil penetration (4) and macroscopic features (5). The microscopic features (1-4) were recorded with a digital microscope camera, thus as far as the preparation was optimal 4 pictures were taken from one hair. 178 pictures were taken from 13 bird nest.

Since this technique requires a lot of practice we have created a reference material from 22 species. The references were gathered both from live animals and from prepared ones. We have used 3 hairs from one species, altogether 269 pictures were taken (in some cases more than 4 pictures were taken from a hair). To help the identification reference books, papers, websites (TEERINK, 1991; TÓTH, 2002, MARINIS & ASPREA, 2006; TÓTH, 2008, http2, http3, http4) and our personal reference materials were used.

RESULTS

Nests' lining materials

We have found mammal hairs in 9 nests (out of 13) (69,23%). Artifical nesting materials were found in 3 cases (M5, M7 and M13) (25%). The artificial materials' quantitative features were not examined in this study, due to their irrelevance (*Table 1.*).

In 4 cases we thought we found hairs but during the microscopic analysis it turned out that the found objects were artificial materials. From one nest an average of 5,31 (SE=5,31) hairs were found, from this 3,77 (SE=4,17) were able to be prepared and 2,85 (SE=2,91) were categorized (*Table 1.*).

***Table 1.* Hairs and articifal linig materials found in bird nests**.

Nest code	Hairs found (db)	Hairs prepared (db)	Hairs categorized (db)	Artifical nesting material
M1	11	11	9	-
M2	5	5	5	-
M3	3	3	3	-
M4	18	12	7	-
M5	0	0	0	fishing line, synthetic liner
M6	0	0	0	-
M7	10	8	5	fishing line
M8	7	4	2	-
M9	6	1	1	-
M10	0	0	0	-
M11	5	2	2	-
M12	0	0	0	-
M13	4	3	3	thread
x	5,31	3,77	2,85	-
SE	5,31	4,17	2,91	-

Identified hairs

We have created 13 categories from the data (*Figure 1.*). 5 of these were species categories (*Talpa europea, Mustela nivalis, Homo sapiens, Lutra lutra* and *Myoxus glis*), 3 of them were twin-species (*Rattus rattus-Rattus norvegicus*, *Muscardinus avellanarius-Dryomis nitedula* and *Oryctolagus cuniculus-Lepus europaeus*). These species cannot be exactly identified just by hair morphology (supplementary data is needed, e.g.: area of distribution). 3 genera were identified (*Canidae* spp., *Chiroptera* spp. and *Apodemus/Microtus* sp.). Finally, there 2 categories for unidentifiable hairs („not hair": revealed during the microscope study, „unidentifiable": data deficient).

The most common species were humans (*Homo sapiens*) (7) and *Mustela nivalis* (7). 5 *Mustela nivalis* hairs were found in one nest (M4). 16 hairs were unidentifiable (43,27%) ("not hair" or "unidentifiable") from the total of 37. 2 rarer species were found during the identification, *Lutra lutra* (2) from M3 and *Myoxus glis* (1) from M7.

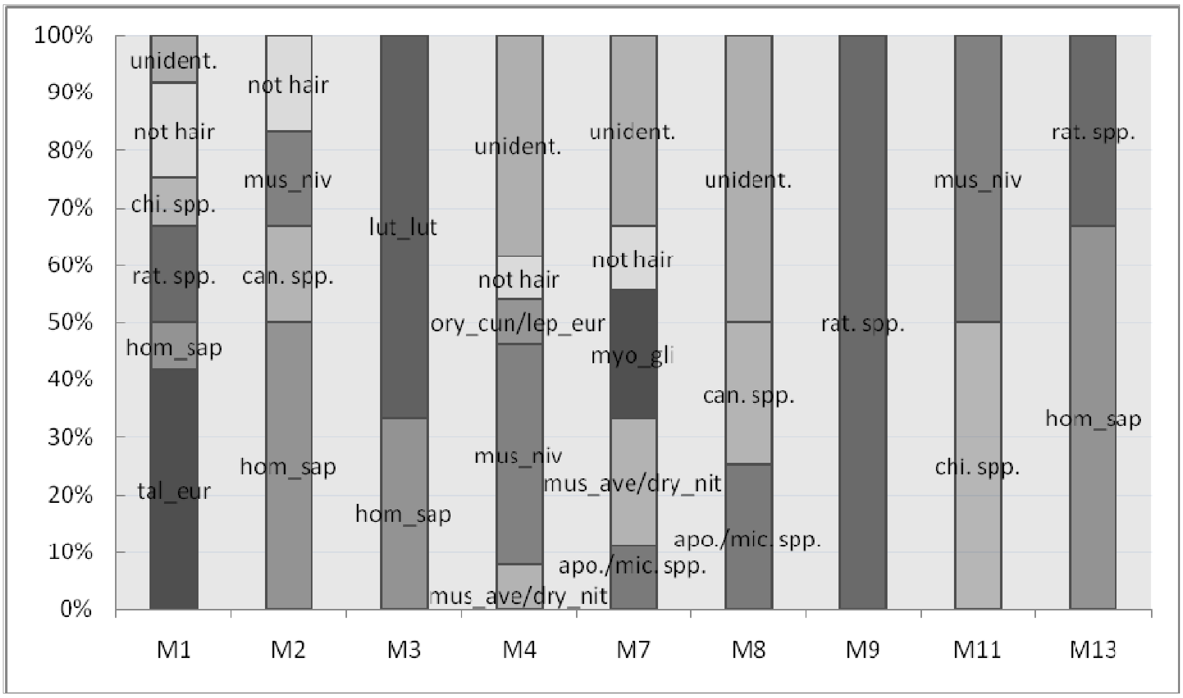

***Figure 1.* Species and species groups found in bird nests.** (Legend: unident=unidentifiable, chi. spp.=*Chiroptera* spp., rat spp.=*Rattus* spp., hom_sap=*Homo sapiens*, tal_eur=*Talpa europea*, mus_niv=*Mustela nivalis*, can. spp.=*Canidae* spp., lut_lut=*Lutra lutra*, ory_cun/lep_eur=*Oryctolagus cuniculus*/*Lepus europaeus*, mus_ave/dry_nit=*Muscardnius avellanarius*/*Dryomis nitedula*, apo./mic. spp.=*Apodemus*/*Microtus* sp.)

CONCLUSION

The aim of our study was to test a new noninvasive method, the bird-nest analysis (TÓTH, 2008) in urban environment. We have found 13 nest in the study area (Merzse swamp) and in its surroundings. Hairs were found in the 69,23% of nests (9 out of 13). From one nest an average of 5,31 (SE=5,31) hairs were found, from this 3,77 (SE=4,17) were able to be prepared (hairs were damaged during the preparation) and 2,85 (SE=2,91) were categorized. An average of 2,85 hairs were categorized, in our opinion this number can increases with practice. We think that the number of the found nests and the number of hairs in a nest can be a proof of the usage of this technique in a semi-urban environment. *Table 1*. also shows us that it is relatively few artificial lining materials were used in the nests, although all nests were find in a semi-urban environment.

Humans were one of the most common species. We have found 7 hairs in 4 nests. This is not an exceptional result because Merzse-swamp is used by several people for recreation and relaxation, and also these hairs are bigger and easier to find for birds. We have also found 7 hairs of *Mustela nivalis*, although 5 of these were found in a nest on the ground (M4). This nest could be a victim of a nest predation (HELTAI & LANSZKI, 2007) and this might explain the relatively large amount of *Mustela nivalis* hairs in it.

The above mentioned species can be problematic during the identification. *Mustela nivalis* and *Mustela erminea* are so called twin-species, thus differentiation from their hairs' qualitative features cannot be done. In this case supplementary data is needed for example area of distribution or additional surveys (or quantitative features). *Mustela erminea* is probably one of the least spread mustelid in Hungary which also avoid anthropogenic effects (LANSZKI & HELTAI, 2007), that is why we excluded this species. In this way we can exclude *Qryctolagus cuniculus* (the twin pair of *Lepus europaeus*). In our field studies we saw *Lepus europeus* at several times, while from *Qryctolagus cuniculus* it is known that RHD, myxomatosis and a few extreme cold winters suppressed its distribution area (KATONA & ALTBÄCKER, 2007). It is also just a few places in Hungary where *Rattus rattus* can be found (HORVÁTH, 2007), thus it is more likely that *Rattus* sp. hairs are belong to *Rattus norvegicus*. Distribution maps can help really lot to separate twin pairs, but some of these species can also be differentiated from hairs' quantitative features.

In the case of exact identification there are more problems that we have to mention. In a study at Northern Australia experienced trainees' identifications were examined, they indentified 23 taxon categories and from these 19 were species. In 18 cases identification involved at least some level of error. Several factors influenced the accuracy of identifications in the study, principally the need to identify samples to species level, rather than not making identification. The lack of samples locality was also a problem (they did not know the area of distribution) (LOBERT et al., 2001). SPAULDING et al. (2000) received the same result when examining *Canis lupus* scats. However, the authors agree on that practice and reference materials are make identification more accurate. Thus, we think reference materials are needed for practice and for make identification more accurate. This is why we have created the reference from 22 species (269 pictures), and also this is why the unidentifiable categories' percentage can be considered relatively high (43,27%).

In the case of one species (*Lutra lutra*) we think it would be necessary to confirm the presence with other observations (visual observation, footprints and remains of preys). In the study area *Lutra lutra* presence has not been demonstrated so far. However, according to literature *Lutra lutra* population in Hungary is considered stabile and widespread in the country (LANSZKI et al., 2007; LANSZKI, 2008), thus it can be that this species will appear sooner or later at Merzse-swamp. Despite that, *Lutra lutra* is a strictly protected species in Hungary, it is a possibility that the hairs can came from a fur.

Finally, in our opinion bird-nest analysis is a good technique in urban or semi-urban environment, but references from hairs and practice are necessary to get familiar with the method. Our future goal is to test this method at a city park (Gödöllő) to find out if there is any usage of the technique there.

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