HAIR MOUNTING TECHNIQUE: HELPFUL IN CONSERVATION OF CARNIVORES

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Abstract

Mammalian hair is the best source to solve the biological problems like species identification and diet analysis of endangered large carnivores because it is difficult to examine the intestinal contents of the large carnivores. Mammalian hair has the three basic parts cuticle, cortex and medulla. These parts undergo labs for the mounting technique. In mounting technique hairs are placed on glass slide and mount is pasted on them then observed under photomicroscope to examine hair cuticle and medullary structure under the high magnification. 100x to 400x magnification is considered best for hair mounting technique. Take the sketch under photomicroscope then compare it with reference book. This technique can be applied on large and small carnivores, rodents and birds. By this study we can explore not only diet but species identification, habitat analysis, predator-prey relationship and human-carnivores conflict can also be assessed.

Introduction

Analysis of contaminants in hair, fur, nail clippings and feathers has been used to determine exposure to heavy metals (Airey, 1983a, 1983b; Eaton and Ferrant, 1982; Obrusnik and Paukert, 1984; Matsubara and Machida, 1985; Tavares et al., 1989; Agahian et al., 1990; Ahmed et al. 1990; Ahmed and Elmubarak, 1991; Folin et al. 1991; Pfeiffer et al., 1991; Roelke et al., 1992). Hair is an important morphologic characteristic of mammals and hair identification has been used in the food habit studies of predators, forensic sciences, textile testing, archeological studies and mammalians identification (Mayer 1952; Mcfadden 1968; Brunner and Coman, 1974; Kennedy, 1982; Valente, 1983; Oli, 1993; Walis, 1993; Meyer et al., 1995). Hair structure of mammalian species has been the focus of many scientific investigations dating back to the mid 1800s (Quay, 1970). However the taxonomic importance of hair structure has been a topic of some debates. Mammal hairs differ among species and may therefore be used for diagnoses at the species level (Cavia et al., 2008). Morphological examination of hair samples is the first step in forensic hair comparisons. Themain medico-legal concerns with hair examination include identification of the species of origin, ascertainmnet of the hair’s provenance from the body and, finally, comparison of the control hair sample from the victim to the hair sample from the crime scene (Gaudette, 1982). Though it is not possible to definitely identify a sample of hair originating from a particular animal body, unequivocal determination can be established based on microscopic examination of the hair’s cuticle, cortex, medulla and pigment granules. Mammal hair structure presents characters that differ among species and may therefore be used for diagnoses at species level (Day, 1966;Gurini, 1985).

Hair biology: All hair is comprised of three major portions-cuticle, cortex, and medulla. The cuticle or covering of individual hairs is made up of overlapping scales, the distal edges of which are free. The patterns made by these scales have been classified by (Hausman, 1920) into two type’s coronal (scales surrounding the hair shaft) and imbricate (scales not surrounding the hair shaft). The shape of the individual scale is also an important feature. An inverse relationship between the width of a hair and the proximodistal length of each scale has been reported by (Hausman, 1930; Noback, 1951). The primary function of the cuticle is protection of the hair (Rudall, 1941; Azzola and Shurmann, 1969). After treatment with trypsin, the structural elements of the cuticle are found to be a network of what appears to be fibers of various widths, which appear randomly oriented (Lundgren and Ward, 1963). Another structural component of the cuticle can be seen in some overlapping scales where small projections from the under scale protrude into the overlying scale (Rogers, 1959). The development of scanning electron microscopy and other techniques have allowed detailed study of the ultrastructure of cuticular scales. Each scale is composed of an epicuticle, an exocuticle, and an endocuticle. The outermost layer, the epicuticle, has received much attention (Haly et al. 1970; Bradbury and Leeder, 1970; King and Bradbury, 1968; Leeder and Bradbury, 1968). This layer influences the surface properties of wool, as well as probably other types of hair, including wettability and frictional characteristics.

The middle layer of a hair shaft, the cortex, is made up of fusiform or spindle-shaped cells, which interdigitate with each other along the long axis of the shaft. In some kinds of hair there are air spaces or fusi
between the cells (Hausman, 1932). Each cell contains a nucleus and pigment granules. As keratinization takes place in a growing hair, the cytoplasm is replaced by fibers of protein (alfakeratin). A keratinized cortical cell contains rounded subunits (microfibrils) 3,000 A in width. Within each of these units are still smaller units (microfibrils) 60-80 A, which are separated and delineated by thin layers of dense matrix (Anderson and Lyeder, 1971; Anderson and Lipson, 1970; Rogers, 1959a, 1959b). A microfibril contains “protofibrils” 20 A wide and is composed of smaller filaments, possibly single protein molecules (Johnson and Speak-man, 1965). Another division of the cortex is that of the ortho and paracortex in some wools. Chapman and Bradbury (1968) concluded that the differences between the two areas are based on (1) the configuration of the microfibrils, (2) the microfibril to matrix ratios, and (3) possible variation in amino acid sequences in the two areas.

The central portion of the hair shaft, the medulla, is made up of cells of various shapes, which are often interspaced with air pockets. The presence and patterns of these cells have been used to distinguish various kinds of hair (Hausman, 1920, 1944; Dearborn, 1939; Day, 1966; Mayer, 1952). Bradbury and O’Shea (1969) analyzed amino acid components of the medulla in various monotremes, marsupials, and placental and discovered remarkable similarity of composition in all medullas. Pigment is normally concentrated in the medulla cell, but may be lacking entirely.

**Hair morphology is important in species identification:** Hair morphology is especially useful to identifying predator pellets, pelt remains found in burrows (Day, 1966; Cavia et al., 2008). To identify hairs at the species level based on hair length and width and scale morphology observed in specific sections of dorsal guard hairs. Mammal hair has four structural components: (1) the cuticle (epidermiclea) or outside cover, with scales; (2) the cortex or inner sheath, with strongly keratinized cells disposed lengthwise; (3) the medulla or central region with groups of keratinized cells or their skeletons, with intercellular spaces that may be filled with air; and (4) pigment granules located in the cortex and medulla (Meyer et al., 2002). These hair components show differences among species and may be used for species identification (Meyer et al. 2002; Day, 1966; Gurini, 1985)

**Scientific Basis for Microscopic Hair Examinations:** All organisms differ widely in many dimensions, including morphological appearance, physiology, and genetic makeup. Some groups of organisms clearly are more similar to some groups than to others. For instance, a monarch butterfly is more similar to a tiger swallowtail butterfly than either is to a ladybird beetle. Biologists seek to identify these differences and use them to organize and classify the world around them. They use these differences to generate classification schemes that can be used for many purposes, from examining how traits evolve to solving crimes.

These classification schemes have their roots in the field of taxonomy. Taxonomy is the practice of classifying biodiversity, and it has a long and venerable history. In 1758, Carolus Linnaeus proposed a system that has dominated classification for centuries. He proposed a system of binomial nomenclature to describe living organisms. However, the term taxonomy is now applied in a wider, more general sense and refers to a classification of things, as well as to the principles underlying such a classification. Almost anything—animate objects, inanimate objects, places, concepts—may be classified according to some scheme.

Evaluation of shared and distinguishing characteristics is essentially the process used in forensic hair examinations. The microscopic characteristics allow for hair to be categorized into smaller groups, such as human or animal, racial group, body area, color, phase of growth, etc. This is considered the identification phase, for example, classifying the hair as being human, exhibiting Caucasian characteristics, coming from the head, being brown in color, and possessing a telogen root.

Species identification by means of their hair structure, the discriminant functions and the dichotomic key. Discriminate function analysis requires fewer measurements and allows faster identification of samples than the use of the key. To identify the species employing the first method, hair morphometric values must be introduced in the functions corresponding to each species. The sample is assigned to the species whose function gives back the greatest score (Huberty, 1994). In addition, the researcher can use a program to make the calculations easier. The key is presented for those which are more familiar with its use, as well as to provide a description of hair characters of most small rodent species occupying the Pampean region. Both the key and the discriminate functions must be applied only when guard hairs from the dorsal part of the animal (the back) are available, because hair characteristics may change among different portions of the body (Gurini, 1985).

Hair comparisons are a combination of a pattern-recognition process and a step-by-step analysis of a questioned hair and a known sample. An example of the pattern-recognition process is the manner in which we identify a friend in a crowd of people. It is an instant recognition, based on our experience with that person. It is not conducted in a logical, step-by-step process, evaluating first the height, hair color, skin color, eye color, and other characteristics. It is an almost instantaneous evaluation of all of these characteristics together. The identification of our friend does not carry any less weight based on the mechanism we used to identify him or her (Oien, 2009).
Animal hair: Hair identification is not employed solely by forensic scientists. Hair identification is an important tool used by wildlife biologists, archeologists, anthropologists, and textile conservators. Many researchers have investigated the morphological characteristics of hair, devised keys, and reviewed the science of animal-hair identification (Appleyard, 1960; Day, 1966; Mathiak, 1938; Mayer, 1952; Moore, 1974; Oyer, 1939; Stains, 1958, 1962; Wildman, 1954, 1961; Williams, 1938). These works have aided in ecological studies, food-habit studies, and law enforcement investigations by providing descriptions, keys, and photographs of the microscopic characteristics of animal hairs. Hair characters used in the respective study are scale length, scale width, hair length, and scale forms (Benedicts, 1957; Elgmork and Riiser, 1991). There are some example from the literature i.e. (Brown, 1942) attempted to develop a technique for identifying hairs and wools from various types of materials recovered from archeological works. Hausman (1930) used hair examination in his laboratory to perform archeological work, examine stomach remains, identify fur, and conduct legal proceedings. Animal-hair studies also have been conducted within the field of forensic science. Peabody et al. (1983) determined that the medullary fraction could be used to reliably distinguish between dogs and cats. Hicks (1977) and Deedrick and Koch (2004) described the microscopic characteristics that can be used to discriminate between animal hairs that are most likely to be encountered in forensic casework. The purpose of study to use various method to identify the animal hair.

Types of hairs: There are three types of hair usually seen in animals:
- **Vibrissa.** These are the whiskers of many animals. They are normally tactile and sensitive, such as the whiskers of a cat.
- **Bristle.** This is the coarse bristle that provides an animal with a protective coat. These guard hairs can readily be identified by their distinctive appearance and morphology between various animal families.
- **Wool.** Wool or fur provides insulation from wet and cold. These fine hairs cover the bodies of all mammals (David and Katz, 2005)

Materials and Methods

For preparation of slide, dry mount and wet mount are implemented.

The dry mount is convenient in terms of preparation. However, degree of curl and twist cannot be observed due to constraints in the mounting process. It may be useful before the wet mounting to study the exterior texture and the overall color of the hair. Usually, several hairs are placed in parallel on slide, so that their texture and color can be compared easily. The dry mounting technique is nothing fancier than the words it describes. Samples can be fixed with melted Kronig cement, but it is unnecessarily if the cover slip can be fixed firmly on the slide. (www.bergen.org, 2003)

The wet mount is essential in hair analysis because of the refractive index of Canada Balsam – a special resin used to prepare slides – is close to that of the keratin in the hair. If the dry mount provides a view of texture and color, wet mount provides the interior structure of the hair, such as inclusion and pigment granules. Because the refractive index of mounting medium plays the most significant role in viewing internal details, it is suggested that if other reagents than Canada Balsam is used, it is chosen carefully. (www.bergen.org, 2003) Wet mount having two types (1) whole mount method and (2) scale replicate method.

Whole mount method: In whole mount method (1) several strands of hair were placed in parallel on a microscope slide. (2) Two drops of carbon tetrachloride were added over the hairs in order to hold them in place. (3) A cover slip was placed over the hairs and they were scanned along their length at 100x and 400x under a compound microscope to observe the morphological characteristics of the cuticle and medulla, and the distribution of pigment in the cortex (Robertson et al. 1999).

Scale replicate method: It may be necessary to make a scale cast of the hair specimen in order to see the scale pattern more clearly, particularly in the identification of some animal hairs. It is often very difficult to directly observe scale patterns from hair strands on a slide. Hence, (1) a cast was made using nail polish to obtain the impression of the scales. (2) A thin layer of nail polish was spread on a microscope slide and a hair was placed in the middle of the slide. (3) It was allowed to stand for 15 minutes so that the nail polish could harden and the hair was then gently removed using forceps, (4) the scale pattern was observed under a compound microscope at 100x and 400x. Cubicular scale patterns were observed on casts (Day, 1966; David and Katz, 2005). Castscan be made by placing hairs over a thin layer of vinyl adhesive on a slide and left until the adhesive became dry (approximately 30 min). Then the hair was removed leaving a visible pattern of cuticular scale (Gurini, 1985).

Polaroid print coater method: Ogles and Mitschinka (1973) devised a quick and easy method of making a scale cast with the use of a Polaroid film-print coater. A thin layer is applied to a glass microscope slide with two or three passes of the Polaroid print coater. The hair specimen is lightly pressed onto the film and allowed to stand until the film is dry. The hair is then pulled from the film and the cast remains.
**Crocker method:** A method developed by Crocker (1998) at the Centre of Forensic Sciences in Toronto, Canada, uses clear tape as a mounting medium and cover slip together, which allows for quick observation of such surface features as the scale pattern.

![Fig. Medulla patterns from different types of hair. (a) Human head hair (450x), (b) dog fur (450x), (c) Deer hair (100x), (d) Rabbit fur (450x), (e) Cat fur (450x), (f) Mouse hair (450x). (Reproduced from Prentice, 1995).](image)

**Results and Discussion**

The colour of the tip: Moore *et al.* (1974) defined the tip as the opposite of the basal part of hair (Kennedy, 1982) interpreted it as the region most distal from the basal part of the hair and has one proximal colour demarcation. Teerink (1991) used the expression ‘tip’ mainly for the most distal zone from the base without medulla. The exact measurement of length of this region is difficult, because it often shows a gradual transition; nevertheless, colour is regarded important.

The number of bands. Moore *et al.* (1974) defined the band as the region of the hair that shows distinct proximal and distal colour bands.

The pattern of cross-section of thickest part of shield. The methods were given previously (Mathiak, 1938; Kennedy, 1982; Teerink, 1991). Making cross-sections is a problem since the exquisite fixation of hair is difficult. Hair often curves, giving deformed contours, so require a lot of samples and cutting. Another method was probing fitting the hair in the middle of elder pith or other flexible twigs. After freezing (or without it) samples were cut by razors. This method has the same problem but easy to apply for field works.

The pattern of cuticula and medulla of shaft (the proximal part of the hair = near to the base of hair).

The pattern of cuticula and medulla in the transitional part between the shaft and shield.

The pattern of cuticula and medulla in the shield (the distal part of the hair = near to the tip of hair)

Once a hair has been determined to be suitable for microscopic comparison, it is compared with an appropriate known hair sample. Head hairs must be compared with known head-hair samples, and pubic hairs must be compared to known pubic-hair samples. The comparison process involves the side-by-side analysis of a questioned hair and known hair samples using a comparison microscope. This allows for a direct comparison of the microscopic characteristics of the questioned hair within the same relative area of the known sample, at the
same time and in the same field of view. This comparison must occur over the entire length of the hair. Usablyve used point sampling to identify hair types present in each scat (Ciucci et al., 2004). The closest hairs to 10 random points on a 6 mm by 6 mm grid were identified by comparison to a reference collection assembled. Unidentified hairs were labeled as unknown.

The hair mounting technique is the best and simplest technique in conservation of small and large carnivores. It also helps us to species identification of small rodents. It helps investigating diet and foraging ecology of carnivore species. Aids determining magnitude of competition and coexistence among sympatric species. Helps evaluate nature and magnitude of human-carnivores conflicts. Helps identifying key resources in an ecosystem, thus aids effective management of natural resources.

References


