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Scanning electron microscopic study of different hair types in various breeds of rabbits

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The microarchitecture of the cover hairs, wool hairs and tactile (sinus) hairs of feral, New Zealand White and Angora rabbits was studied by means of scanning electron microscopy. The morphology and variability of the cuticular scale patterns, hair cortex, medullary arrangement and profile of the hairs are described, illustrated and compared with findings resulting from conventional light microscopy, cuticular casting and medullary impregnation. All parameters examined in cover hairs presented a considerable variation along the length of the hair shaft. In wool hairs, in contrast, only the cuticular scale pattern was subject to manifest segmental variation, whereas the shaft diameter, cortical profile and medullar composition changed little over the entire length of the hair. The tactile hairs of the head were characterised by a round profile of the hair shaft, a cylindrical central medullar canal, and a thick cortex covered by cuticular scales that were arranged in a waved pattern and oriented transversally in relation to the longitudinal axis of the hair. It was concluded that the scanning electron microscopic observation of hair samples is a fast and valuable method for identifying hair types with useful applications in different disciplines such as mammalian biology, the textile industry and forensic medicine.

key words: morphology, hair, wool, cuticular scales, rabbit, Angora

INTRODUCTION

Scientists from different disciplines, including veterinary anatomy [3,8], wildlife biology [14,16], the textile industry [1–2,6–7,11–12,15,17] and forensic medicine [9], have used the micromorphologic characteristics of the various hair types of mammals to identify hair samples. Determination keys to reach correct differentiation are based on macroscopic features such as the shape, size, profile and colour of the hairs, as well as on microscopic characteristics of the cortical cuticular and medullary patterns. However, the identification is complicated by the presence of considerable variation caused by differences of species, breed and gender of the animals, by environmental conditions (climate, habitat, nutrition) and possibly also by the body region from which the hairs are derived [1,4,5,9,15]. Nevertheless, a correct determination of hairs is often possible by a detailed study of their morphologic features, which are quite distinct and characteristic in several species, including rabbits [3,6,14,17].

Procedures for the examination of the cuticular scale and medullary patterns of hairs include conventional histologic sectioning and specific techniques such as cuticular casting and medullary impregnation [14–15]. More recently, the hair patterns of various species have also been documented by scanning electron microscopic (SEM) studies. This technique is not only relatively simple and fast, but it also gives unique three-dimensional information

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about cuticular patterns, cortical thickness, medullary arrangement and profile of the hairs. It therefore offers a distinct advantage for studying particular hair types such as the wool fibres of rabbits that possess complex and multiple scale patterns from which casts are difficult to make and inadequate [15]. However, the number of SEM pictures of rabbit hairs that are available in the literature is too small to cover completely all the characteristics and variations that are found in this species [2,6–7,12,17].

Therefore the present study was conceived in order to provide amply illustrated SEM information about the features that are important for the identification of the dominant hair types in the rabbit, viz. the cover hairs and wool hairs. The long and coarse *cover hairs,* which are most important for taxonomic purposes, are named *capilli* in official veterinary anatomical nomenclature [10], but in numerous studies they have been designated by various other terms such as *overhairs* and *guard, beard, principal, primary* or *kemp hairs*. The cover hairs in many species, including rabbits, consist of various subtypes that differ in size and rigidity. The larger and most sturdy cover hairs are often described by alternative terms such as *central primary hairs* indicating that they arise from the centre of the hair follicles, or as *shield fibres* because they present a distinctly enlarged and flattened shield-like segment in the apical segment of their shafts. In contrast, the shorter and more slender cover hairs are designated as *lateral primary hairs* because they emerge more peripherally from the follicles. The thin and undulating *wool hairs*, which are the predominant hair type in the coat, are called *pili lanei* in official veterinary anatomical nomenclature [10] but they are also indicated by numerous alternative terms such as *underhairs*, *underfur* and *secondary* or *auxillary hair fibres*. The other hair types of the rabbit consist of tylotriches [13] and the eye lashes (*cilia*), nostril hairs (*vibrissae*), auricular hairs (*tragi*) and tactile or sinus hairs (*pili tactiles*) [10]. The morphologic characteristics of the latter hair type will briefly be described below for comparison with the patterns observed in the shielded cover hairs and the wool hairs.

MATERIAL AND METHODS

Skin and hair samples from the dorsal, lumbar, lateral abdominal, inguinal and femoral regions were collected immediately after death from 2 feral rabbits (*Oryctolagus cuniculus*), 2 New Zealand White rabbits and 2 Angora rabbits.

Skin samples for histologic examination were fixed for 12 hours in 4% phosphate-buffered formaldehyde, embedded in paraffin and sectioned into 8 μm thick sections that were coloured with haematoxylin-eosin and Van Gieson stainings. Skin samples for SEM observation were fixed for 12 hours in a sodiumcacodylate-buffered solution of 2% paraformaldehyde and 2.5% glutaraldehyde, rinsed three times for 30 min in distilled water, postfixed for 2 hours with 1% osmiumtetroxide, and rinsed again three times in distilled water. Subsequently the samples were dehydrated in graded alcohols, dried in a critical point dryer (Balzers CPD 030, Liechtenstein), mounted with double-sided adhesive tape on aluminium stubs, sputter-coated with platinum (Auto Fine Coater JFC-1300 JEOL, Tokyo, Japan) and examined by means of a JEOL JSM 5600 LV (Tokyo, Japan) scanning electron microscope in high vacuum mode at 10 kV. High resolution digital images were stored and processed using the Ulead® Photoimpact® (Taipei, Taiwan) image editing program.

Hairs were extracted with tweezers and stored without any cleaning or fixation in sealed containers before further processing into SEM samples, cuticular casts and medullary slides. Preparation of the hair samples for SEM observation consisted of mounting, sputter-coating and SEM examination using the same procedure as described before. For studying hair profiles clear-cut cross-sections of hair shafts were made by means of a razorblade. Cuticular casts were made on glass slides coated with a film of a 20% solution of gelatine in water. Medullar slides were produced by immersing cover and wool hairs on glass slides in a clearing agent composed of 20 g lactic acid, 20 g phenol, 10 ml picric acid and 10 ml distilled water for 8 and 4 hours, respectively. Histologic sections, cuticular casts and medullar slides were observed by means of a Leitz Diaplan light microscope (Wetzlar, Germany) and photographed using a Wild MPS 45 Photoautomat (Heerbrugg, Switzerland).

The various cuticular and medullary patterns were described using the nomenclature and classification keys presented by Wildman [15], Appleyard [1] and Teerink [14]. Unless mentioned otherwise, all cover and wool hairs illustrated were sampled from the dorsal region of the skin.

RESULTS

General arrangement

The cover and wool hairs of rabbits were arranged in compound follicles containing one or more cover

hairs surrounded by a much larger number of wool hairs (Fig. 1A, E). The cover hairs consisted of a large central primary hair that was easily recognisable by its distinctly enlarged shield-segment, and a few smaller lateral primary hairs that were hardly distinguishable from the secondary wool hairs. The latter were characterised by a thin and fairly uniform diameter and prominent cuticular scales along their shafts.

The profile, the cortical thickness and the scale cuticular and medullar patterns of both hair types had a number of characteristics that could be used for species identification. However, in cover hairs in particular, each of these features presented different patterns over the length of the hair shaft. This variation is described and illustrated in detail below.

Cover hairs

At the base of the shaft, i.e. near the follicular opening from which the hair emerges, the profile of the hair shaft was oval. The most basal part of the hair shaft was characterised by barely visible thin scales (Fig. 2A). The more distal part of this basal segment was relatively thick and covered by cuticular scales arranged in a waved pattern (Fig. 2B). The number of medullar cells on cross section increased distally from 1 to 3 cells that were regularly organised into a multiserial ladder type arrangement.

Further distally and in the mid-portion of the shaft a longitudinal groove appeared on one side of the hair, causing the hair profile on cross-section to change from oval to bean-shaped. The cortex became thinner and was overlaid by cuticular scales

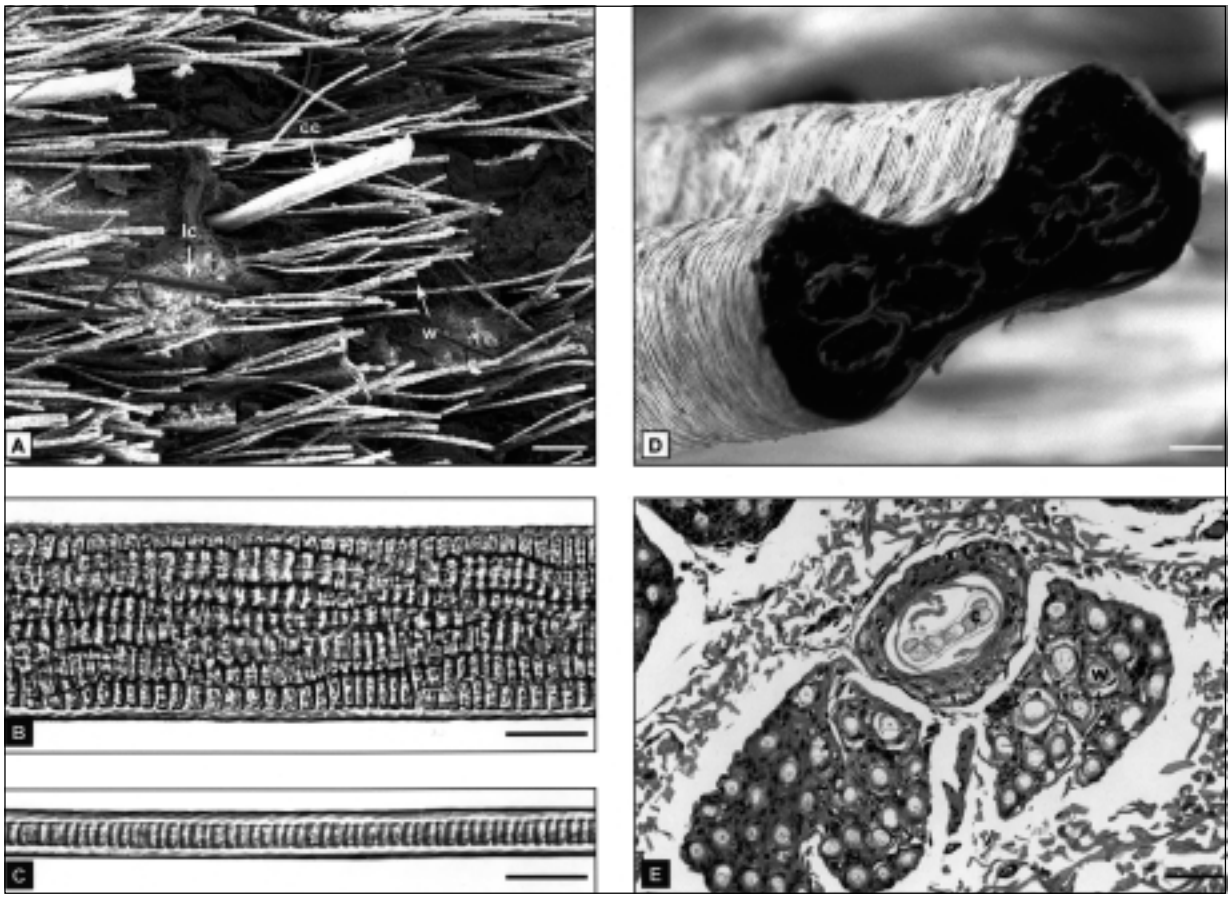

Figure 1. General characteristics of cover and wool hairs of the rabbit.
A) SEM view of a large central cover hair (cc), a presumptive small lateral cover hair (lc) and numerous fine wool hairs (w) in the dorsal skin area of a New Zealand White rabbit. Scale bar = 100 μm. B) Multiserial ladder type arrangement of the medulla in the mid-portion of a cover hair shaft of a New Zealand White rabbit. Cleared specimen; scale bar = 50 μm. C) Uniserial ladder type arrangement of the medulla in the mid-portion of a wool hair shaft of a New Zealand White rabbit. Cleared specimen; scale bar = 50 μm. D) SEM view of the shield region of a cross-sectioned cover hair of a feral rabbit showing the regular waved pattern of the cuticular scales, the dumb-bell profile and the broad multicellular medulla. Scale bar = 10 μm. E) Histologic section of the dorsal skin area of an Angora rabbit showing a compound hair follicle containing a large central cover hair (c) surrounded by numerous fine wool hairs (w). Van Gieson staining. Scale bar = 50 μm.

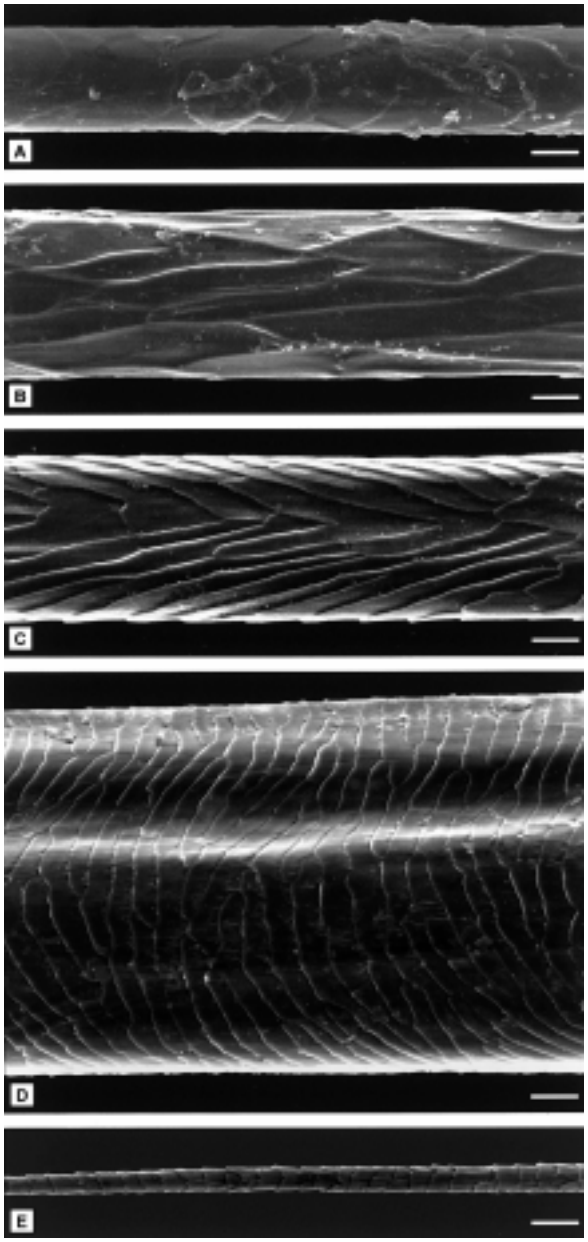

Figure 2. SEM pictures of cuticular scale patterns along a dorsal cover hair of a New Zealand White rabbit. Scale bars = 10 μm.
A) Transition between root segment and base of the shaft showing barely visible thin scales. B) Interrupted streaked waved pattern at the base of the shaft. C) Double chevron pattern at the mid-portion of the hair shaft. D) Regular waved pattern at the shield. Observe the deep longitudinal groove in this segment. E) Coronal pattern at the apex.

that were mostly arranged in a double chevron pattern which locally alternated with an interrupted streaked waved or single chevron pattern (Fig. 2C, 3A). This pattern was subject to some individual as well as interbreed variation (Fig. 4 A–C). The medulla in this hair segment consisted of 3–5 rows of medullar cells that were arranged in a multiserial ladder type (Fig. 1B).

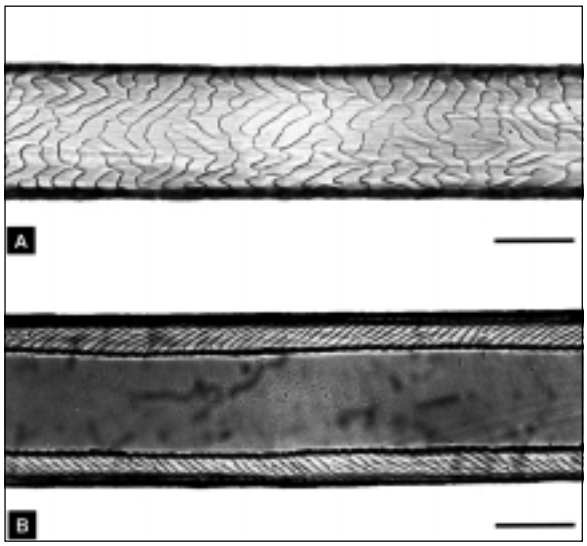

Figure 3. Gelatine casts of cuticular scale patterns of dorsal cover hairs. Scale bars = 50 μm.
A) Single chevron pattern at the transition between the base and the mid-segment of a New Zealand White rabbit. B) Absence of impressions in the centre of the cast at the level of the longitudinal groove in the mid-portion of the hair shaft of a feral rabbit.

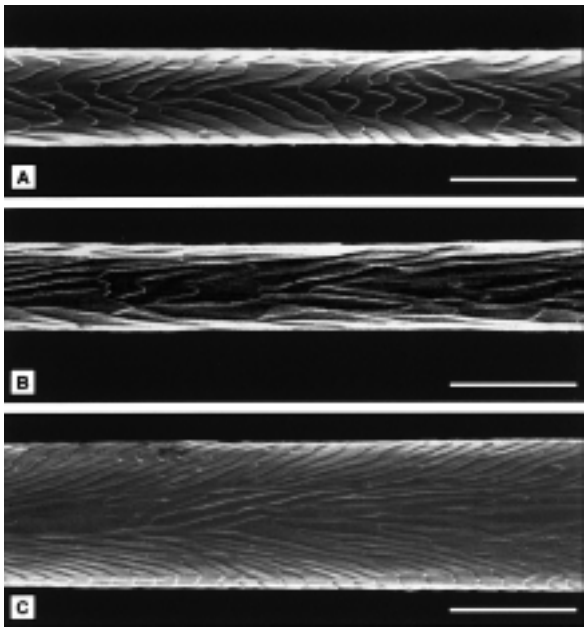

Figure 4. Variability of cuticular scale double chevron patterns in the mid-portion of the shaft of dorsal cover hairs in different breeds. Scale bars = 50 μm.
A) Feral rabbit. B) New Zealand White rabbit. C) Angora rabbit.

In the distal part of the shaft the hair became distinctly broadened and somewhat flattened, forming a widened *shield* portion. This segment was indented by a deep and wide longitudinal groove on one side and a smaller shallow longitudinal groove

on the opposite side, resulting in a dumb-bell shaped profile in this area (Fig. 1D). The cuticular scale pattern in the shield could be studied better on SEM pictures than by means of cuticular cast slides because the latter were often devoid of cast impressions at the level of the deep groove (Fig. 3B). The scales were mainly arranged in a regular waved pattern with smooth scale margins, which sometimes alternated with a more irregular waved pattern (Fig. 2D). The cortex of the shield segment was relatively thin in comparison with the broad medulla, which consisted of 8 or more rows of cells.

In the apical portion of the hair the profile changed from oval to circular and the shaft became progressively thinner owing to a decrease in thickness of both the cortex and the medulla. The latter was progressively reduced from 5 to 3 cell rows toward a single solid medullary pith at the most apical part of the shaft. The cuticular scales were mostly arranged in a regular waved pattern with smooth scale margins, but the scale pattern became coronal at the very tip of the shaft (Fig. 2E).

Wool hairs

The diameter of the wool hairs in rabbits was fairly constant over the major part of the shaft; it was only slightly decreased in the basal and terminal apical segments, and moderately widened in the apical half of the shaft. Furthermore, the profiles of the hair shaft, cortex and medulla on cross-sections showed little variation and ranged from angular-oval to rectangular.

The medulla over the entire length of the wool hair was composed of a single column of cells that were neatly arranged in an uniserial ladder or lattice type of pith (Fig. 1C).

The cuticular scales of wool hairs were manifestly protruding and arranged in different patterns presenting considerable segmental, individual and interbreed variation (Fig. 5A–C; Fig. 6A–C). The scales in the basal portion of the wool hairs formed mostly a petal pattern that often shifted into a double chevron or an interrupted streaked waved pattern (Fig. 7A, 8A). More distally, the wool hairs presented a single or double chevron pattern which was present over the major portion of the shaft (Fig. 7B, 8B). Because of the small diameter of the wool hair shaft, however, this pattern appeared sometimes as a torsion pattern on SEM images that were observed under a specific angle, namely tangentially instead of perpendicularly (Fig. 9A–B). In the apical portion of the wool hairs the double chevron pattern changed progressively into a single chevron and hence into a regular waved pattern with smooth scale margins (Fig. 7C, 8C). The very tip of the wool hair presented a coronal scale pattern with smooth and parallel scale borders (Fig. 7D, 8D).

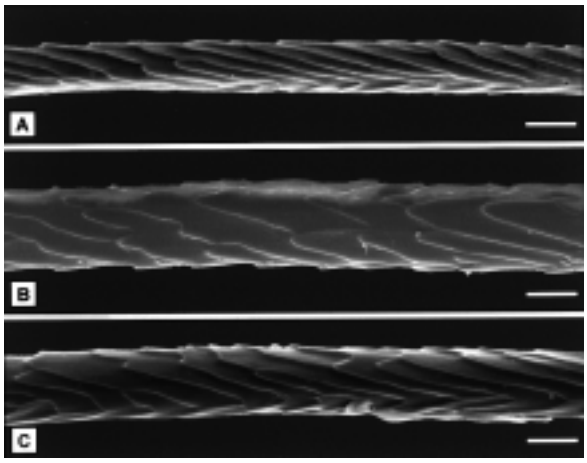

Figure 5. Variability of cuticular scale double chevron patterns in the mid-portion of the shafts of dorsal wool hairs in different breeds. Scale bars = 10 μm.
A) Feral rabbit. B) New Zealand White rabbit. C) Angora rabbit.

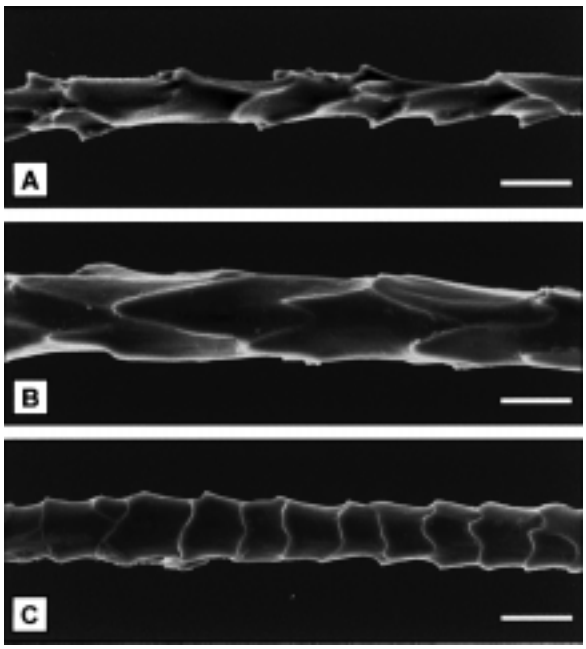

Figure 6. Variability of cuticular scale petal patterns at the base of wool hairs in different breeds. Scale bars = 10 μm.
A) Feral rabbit. B) New Zealand White rabbit. C) Angora rabbit.

Tactile hairs

The tactile or sinus hairs of the head are long and stiff hairs whose root is surrounded by a blood-filled

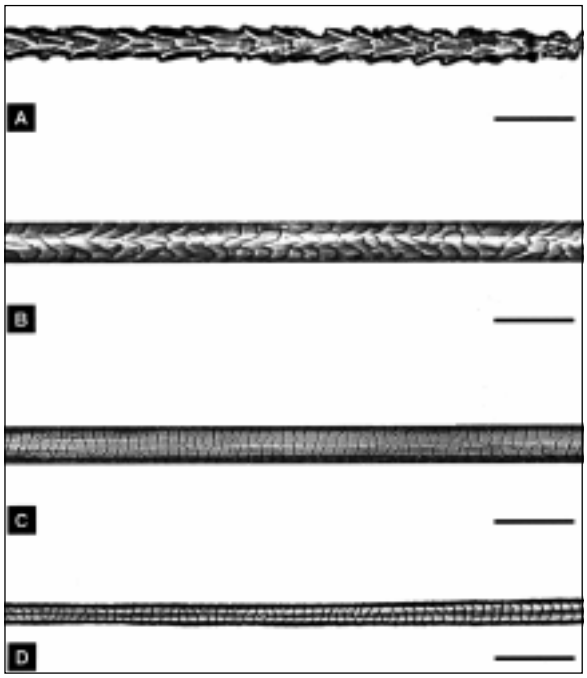

Figure 7. Gelatine casts of cuticular scale patterns of abdominal wool hair in a New Zealand White rabbit. Scale bars = 50 μm.
A) Petal pattern at the basal segment of the hair shaft. B) Single chevron pattern at the mid-segment of the hair shaft. C) Regular waved pattern at the transition between the apical portion and the mid-segment of the hair shaft. D) Coronal pattern at the apex of the hair shaft.

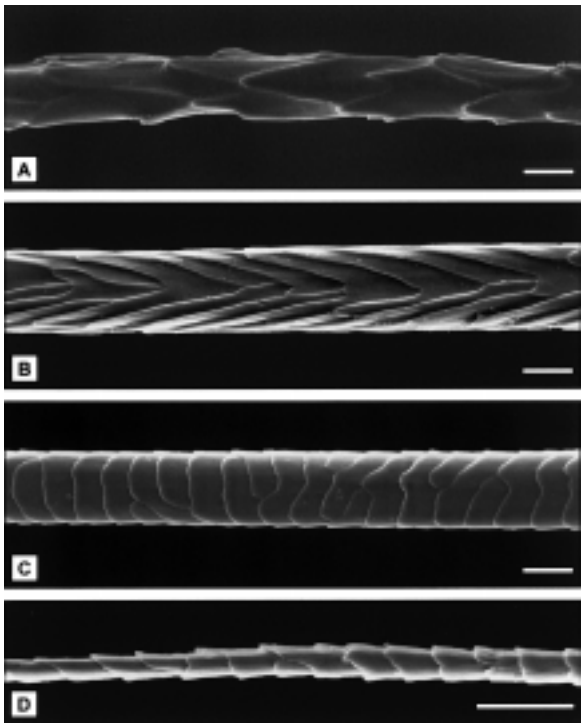

Figure 8. SEM pictures of cuticular scale patterns of wool hair in a New Zealand White rabbit. Scale bars = 20 μm.
A) Petal pattern at the basal segment of the hair shaft. B) Double chevron pattern at the mid-segment of the hair shaft. C) Regular waved pattern at the transition between the apical portion and the mid-segment of the hair shaft. D) Coronal pattern at the apex of the hair shaft.

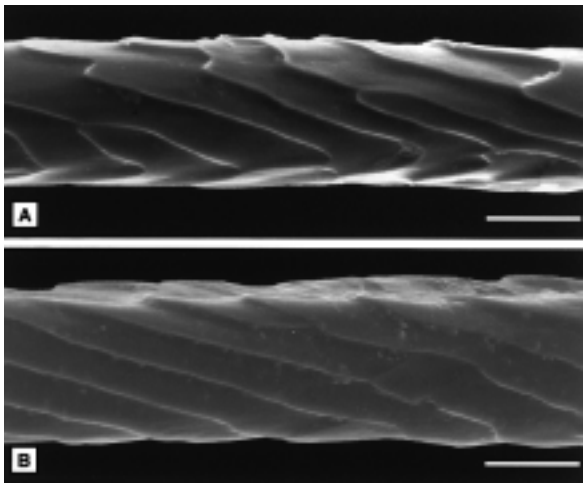

Figure 9. Different SEM images of double chevron scale pattern of wool hairs. Scale bars = 10 μm.
A) View of the crests and bottoms of the troughs formed by the scale margins when observed perpendicularly. (Angora rabbit). B) Torsion image obtained when the scale margins are observed under an oblique angle (New Zealand White rabbit).

annular sinus and sensory nerves. Cross-sections of the shaft clearly demonstrated the round profile of the shaft, cortex and central medullar canal, as well as a considerable cortical thickness which is responsible for the rigidity of the hair (Fig.10A).

The cuticular scales of tactile hairs were less prominent than in cover and wool hairs, and their impressions on gelatine casts were often not clearly delineated (Fig. 10B). In contrast, the cuticular scale pattern of tactile hairs could be defined much better by SEM observation and they ranged from a regular to an irregular waved pattern (Fig. 10C). The orientation of the cuticular scales was always transversal to the longitudinal direction of the hair. The structure of the scale margins was quite variable and ranged from smooth to crenate or rippled.

DISCUSSION

Conventional methods for the identification of various hair types combine macroscopic data with light microscopic evaluation of histologic, casting and impregnation preparations. These techniques are elaborate and sometimes inadequate, e.g. when studying fine, curled hairs with different circumferential patterns. This is the case in rabbit hairs of which cast preparations are often inadequate due to the complexity of the cuticular patterns, the grooves in the cover hairs and the angularity of the wool hairs [15]. Direct SEM observation is a fast and valuable alternative method for examining such hair types because this technique provides a better insight into

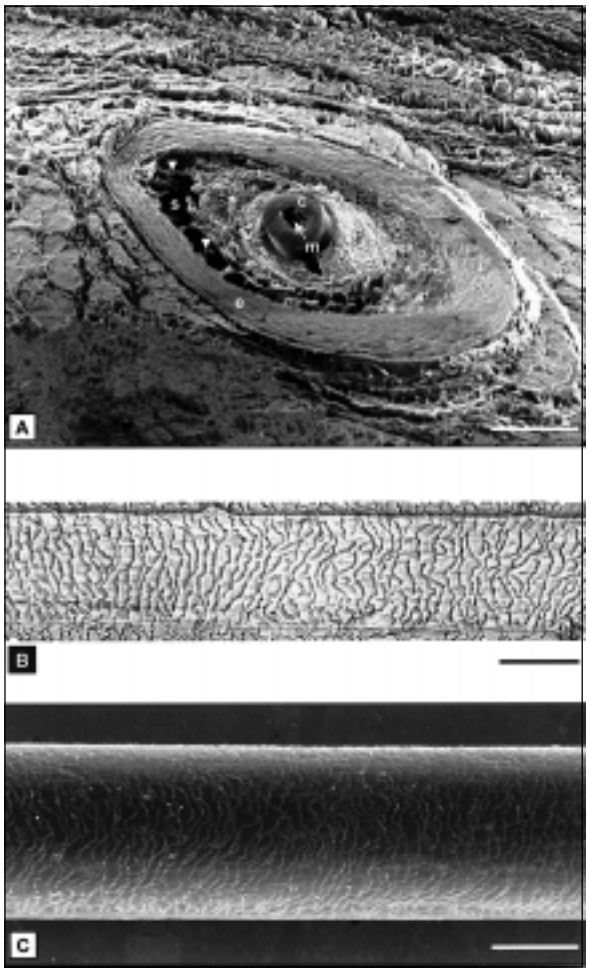

Figure 10. Characteristics of tactile hairs.
A) SEM view of a cross-sectioned tactile hair root in a New Zealand White rabbit showing the round profile of the thick cortex (c) and the relatively narrow medulla (m), surrounded by internal (i) and external (e) connective tissue sheaths which are separated by an annular blood sinus (s) and connected by trabeculae (arrowheads). Scale bar = 200 μm. B) Gelatine cast of the irregular waved cuticular scale pattern in an Angora rabbit. Observe that the central scale impressions are slightly out-of-focus because of the large depth of the specimen. Scale bar = 100 μm. C) SEM view of the irregular waved cuticular scale pattern at the base of a tactile hair in a New Zealand White rabbit. Scale bar = 50 μm.

the three-dimensional arrangement and topographic characteristics of the cuticular scales, the longitudinal and circumferential profile of the shaft, and the inner structure of the hair. The present study also demonstrates that informative SEM images can be obtained from cuticular scales that are not prominent, like those on the tactile hairs, and from complex scale patterns that change frequently in character from one part to the other of the hair shaft, as is the case for the cover and wool hairs.

The illustrated findings of this study confirm and complement previous descriptions of the hair patterns that are found in rabbits and are relevant for different fields of research. Microanatomical data of cover hairs are useful for studies in wildlife biology and ecology [14,16] whereas the wool fibres of rabbits, and in particular those of the Angora breed, have been studied in detail because of their economic value in the textile industry [2,4–7,11–12,15,17]. The present study confirms the presence of species-specific characteristics as well as individual and breed-dependent variations of the structural hair components in rabbits. This variability, which is often understated and more prominent in highly-specialised domesticated breeds than in feral mammalian species, complicates the morphologic analysis and identification of textile wool samples [6,7,12,15,17] and may interfere with a precise determination of hair samples in forensic studies [9].

Despite this limitation it is concluded that the combination of conventional gross and microscopic techniques with modern morphometric and scanning electron microscopic analysis of hair patterns is a valuable and widely useful technique for the identification of mammalian hairs.

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