Analysis of Animal Fibres

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Fraudulent adulteration of cashmere with cheaper animal fibres has long been a problem for both the consumer and the manufacturer. Because all animal fibres share the same basic chemistry, providing reliable evidence of any such substitution was always difficult and uncertain, since it had to rely on observation of differences in scale structure by expert microscopists examining hundreds of fibres. Now, by application new techniques to examine the genetic material in the fibre, it is at last possible to provide positive identification that will stand up in court.

Animal hair fibres are based on cross-linked proteins known as keratins. The major fibre in this group is sheep's wool but other fibres of considerable commercial importance are those obtained from animals such as goats (cashmere and mohair), rabbits (angora), the bactrian camel and the South American camelids. The latter are known as speciality or luxury fibres due to their softness, lustre and scarcity relative to other fibres. Commercially, the most important speciality fibre is cashmere which commands some of the highest prices in the world of textiles. For example, the cashmere sweater illustrated in Fig. 1 retails for around GB£1000.

Accurate identification of animal fibres is important to help combat adulteration or false declaration of content and ensure adherence to international trading agreements. Cashmere, in particular, has frequently been found to be adulterated with much cheaper fibres, such as fine wool, angora, yak hair and occasionally mohair. Angora and mohair are luxury fibres in their own right but their lower prices, relative to cashmere, make them suitable candidates for substitution. Stringent garment labelling requirements within the EU underline the need for exact analytical methods but these can be difficult to achieve, particularly for materials of biological origin.

Traditionally, accurate fibre identification has depended on expert microscopists, working with high-powered optical or electron microscopes, utilising their subjective experience. Chemically, there is little difference between cashmere and other animal fibres. Therefore, microscopists have to rely on physical parameters such as internal morphology, fibre diameter, and cuticle scale height. Detailed measurements have to be made on hundreds of individual fibres for each sample received for analysis.

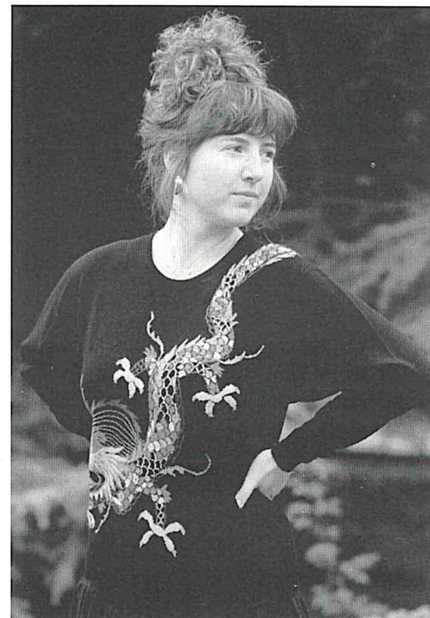


Fig. 1 Cashmere sweater.

Even skilled personnel working in this area, however, may have difficulty in positively identifying the components of certain fibre blends. Some examples of fibres viewed under the microscope are given in Figs 2–4. The scale patterns are clearly visible in these pictures but can be more difficult to see following fibre processing, because of mechanical damage and chemical erosion.

A breakthrough in speciality fibre analysis was made in the late 1980s when it was demonstrated that DNA (deoxyribonucleic acid), the chemical molecule that carries the hereditary information, was not only present in hair roots but could easily be extracted from animal hair shafts. This was an important development since some fibres, such as wool, are shorn rather than combed. DNA of sufficient quality to be used as a template in DNA hybridisation studies has been successfully isolated from scoured, bleached, and dyed fabrics, as well as from raw fibre samples, leading to the development of DNA fibre profiling techniques at BTTG for the specific identification of animal fibres.

Location of DNA in fibres

Animal hair fibres are constructed from cellular material. In the living cells of animals the DNA is contained inside spherical bodies, known as nuclei, and smaller cellular bodies, called mitochondria. Nuclei contained within individual immature hair follicles can be directly visualised using the technique of *in situ* DNA hybridisation and a high-powered microscope. As the follicle matures, the nuclei become elongated but the signal does not extend into the more mature parts of the fibre where keratinisation has taken place, Fig. 5. During the process of keratinisation the cells become metabolically inactive, however, remnants of the nuclear material containing DNA remain trapped inside the fibre. The recalcitrant and hydrophobic nature of fibres helps to protect the DNA from chemical and biological degradation.

Although it would be convenient to be able to carry out DNA analysis on intact fibres, our investigations have indicated that *in situ* DNA analysis of fibre shafts is not practicable, since the DNA is encapsulated inside the waterproof environment of the keratinised cells. Attempts to get at this DNA lead to the dissolution of the fibres. Therefore, DNA has to be extracted from each sample received for analysis. Improved DNA extraction procedures have been developed at BTTG such that only 20 milligrams of a fibre sample are required for routine analysis (originally 20 grams were needed).

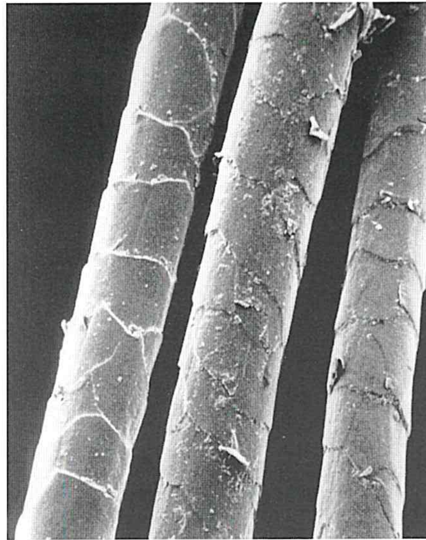


Fig. 2 Chinese white cashmere fibre visualised by scanning electron microscopy (1800×).

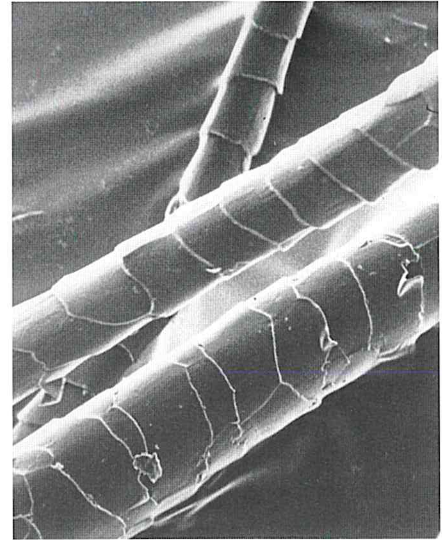
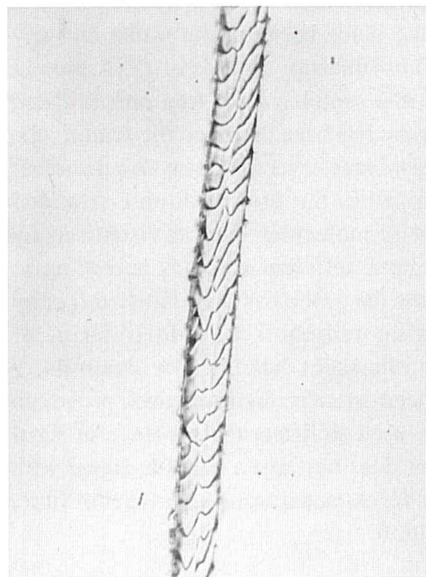


Fig. 3 Fine wool fibre visualised by scanning electron microscopy (1800×).



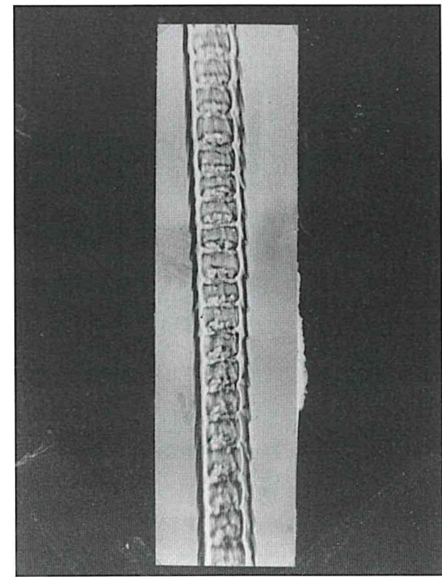


Fig. 4 Cast of the scale patterns of an Angora rabbit fibre viewed under the light microscope (400×). Angora differs from the other fibres mentioned in this article in having a distinctive chevron (i.e. V-shaped) pattern of scales. After Appleyard, 1978.

Selection of target DNA sequences

A prerequisite for fibre profiling is the identification of short DNA sequences unique to each species. It is an advantage if these sequences are present as multiple copies, since this increases the sensitivity of the test. Once species-specific DNA sequences have been located (e.g. from databases containing published sequence information), complementary DNA sequences, known as oligonucleotides, can be constructed which, under carefully controlled conditions, specifically hybridise to the target DNA molecule, giving a positive signal and confirming the presence of a particular fibre type.

Oligonucleotides based on the repetitive Satellite II DNA region are available that can be used to distinguish between DNA samples from closely related species such as sheep and goats. Satellite II DNA is present in high concentrations in the nuclei of many higher animals. Evolutionary pressure has produced a series of mutations in this DNA, however, resulting in natural variations between animal species.

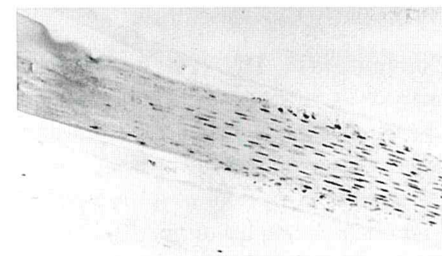


Fig. 5 Visualisation of the nuclei inside a hair follicle using the technique of *in situ* hybridisation.

Animal Fibres

Angora: fibres from Angora rabbits. The fibre diameters range from 11 to 15 microns (μm).

Cashmere: the fine underwool or 'down' from goats with a double coat. The outer coarse 'guard' hairs are separated and discarded from the fine underwool by a process called dehairing. Cashmere fibres range from 12.5 to 21 microns in diameter.

Cuticle scales: the surface layer of animal fibres, consisting of flat overlapping scales.

Mohair: fibres of the fleece of Angora goats. Angora goats have a single coat that is unique among goats in lacking guard hairs. The fibres are not as fine as cashmere and range from 24 to 46 microns.

Speciality fibres: all types of animal (keratin) fibres used in textiles other than sheep's wool.

Wool: fibres of the fleece of sheep. Wool fibre varies in diameter from approximately 17 microns (which is very fine wool) to over 70 microns.

Yak hair: the fine underwool or 'down' from the yak. The yak is employed as a beast of burden in the cashmere growing areas of Asia. Fibre diameters are around 18 to 20 microns.

Mitochondrial genes, such as cytochrome b, which are also present in high copy numbers (up to 1000 mitochondria are present for each nucleus in an animal cell) have a faster evolution rate than nuclear genes and are proving useful in the design of species-specific primers for the analysis of processed fibres.

Conventional DNA hybridisation analysis

Conventional DNA hybridisation analysis is carried out using a simple 'dot-blot' technique, but is only useful for the analysis of raw fibres. DNA extracted from the fibre samples, together with control samples of genomic DNA, is denatured by boiling to convert the native double-stranded DNA molecules into single strands and then spotted onto a nylon membrane. A single-stranded preparation of the oligonucleotide to be used as the 'probe' is then incubated with the membrane to allow hybridisation to take place between the probe and any complementary strands of DNA present in the sample. After free unhybridised probe has been removed by washing the membrane several times with a detergent solution, the hybrid double-stranded DNA molecules can be visualised by several different methods according to how the probe has been labelled (i.e. by using radioactive, colorimetric, or luminescent labels). For example, a sheep-specific oligonucleotide probe can be used to detect the presence of wool but does not give a positive signal with DNA extracted from other types of fibre, Fig. 6.

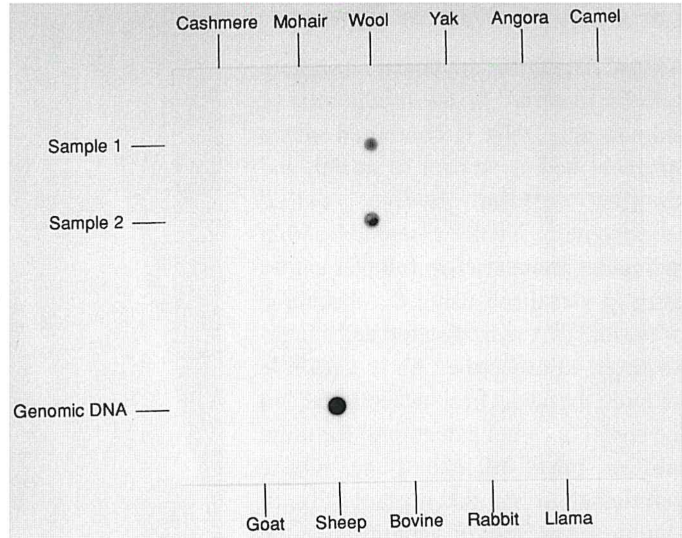


Fig. 6 Hybridisation of a sheep-specific oligonucleotide probe to DNA extracted from various animal fibres. A positive signal was obtained only with wool DNA.

DNA amplification technology

With processed materials (scoured, bleached, or dyed) and finished garments the quality and quantity of DNA present within the fibre is much reduced and it is not possible to use conventional methods of DNA analysis. Only small quantities of DNA can be extracted from processed fibres, typically less than 1ng DNA per gram of sample. However, the development of *in vitro* DNA amplification technology, known as the polymerase chain reaction (PCR), allows for the analysis of minute quantities of DNA. In essence, PCR reproduces what happens when the DNA inside a cell is replicated during cell division. By using an automated temperature cycling block, Fig. 7, this 'enzymatic copying system'

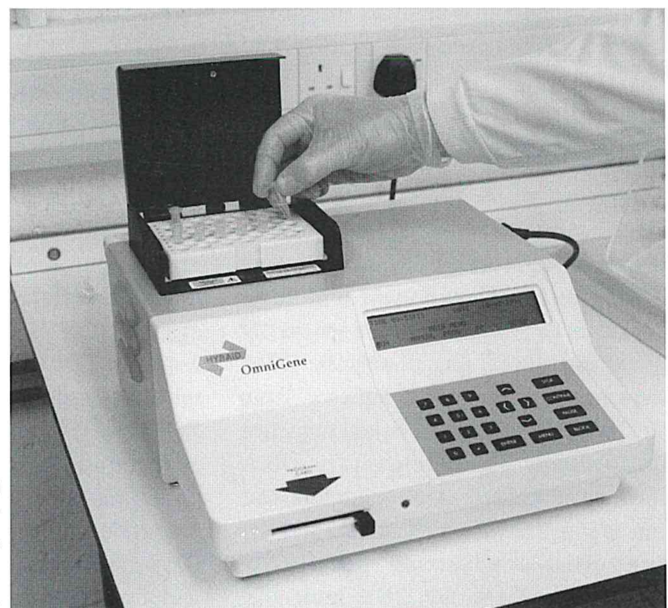


Fig. 7 Thermal cycling machine used for carrying out the polymerase chain reaction.

can be used to produce millions of identical copies of a specified DNA sequence after only 1–2 hours, Fig. 8. These copies provide sufficient DNA for further analysis (e.g. sequencing), or can simply confirm the presence of the target sequence in the sample.

Species-specific primers have been designed to give different sized amplification products with wool, yak hair, and cashmere, and these can be visualised by ultraviolet fluorescence after resolution on an agarose gel containing ethidium bromide, Fig. 9. Thus the presence or absence of an adulterant can be quickly determined and this result can later be confirmed by DNA sequencing. The resulting DNA sequence can be compared to published sequences providing courts with evidence of an exact match that leaves no room for doubt. Because of the prodigious sensitivity of PCR it is essential that negative controls are included with the samples and go through the same extraction process.

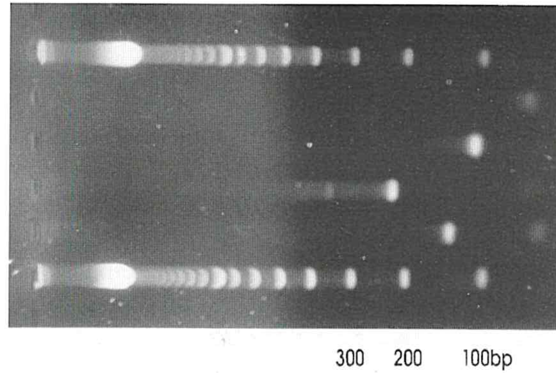


Fig. 9 DNA amplification products obtained from wool, yak hair, and cashmere using different primer sets.

Application

Although this type of analysis is not quantitative, it is a simple and rapid test providing useful criteria for the confirmation of fraudulent substitution. In a recent press release, Karl Spilhaus, President of the Cashmere and Camel Hair Manufacturers' Institute, Boston, USA, observed that 'mis-statement of fibre content in cashmere and cashmere blend garments constitutes a multi-million dollar fraud on the consuming public and damages legitimate cashmere producers and the image of the fibre. The new technology allows us to state with absolute certainty whether contamination by the other fibres is present at any stage of processing from raw material to finished garment.'

Commercially, the present position at BTTG is that fibre blend analysis is carried out using the projection microscope for quantification (i.e. the different types of fibres in the blend are identified, their diameters measured and used to calculate the percentage by weight of the components of the blend) and DNA analysis is employed to confirm, objectively, the nature of those fibre types. This joint approach provides an objective speciality fibre analysis service currently available to our customers. ■

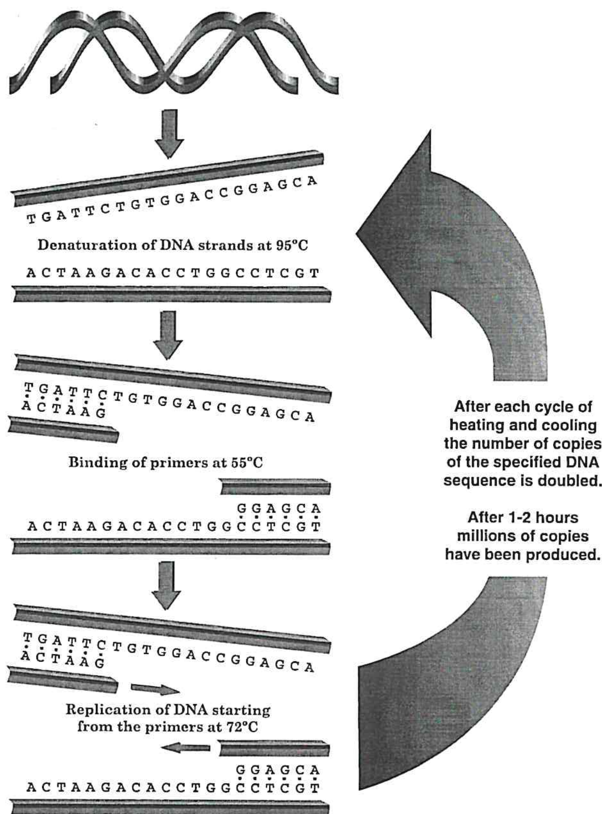


Fig. 8 Amplification of DNA using the polymerase chain reaction. The primers are synthetically prepared oligonucleotides having sequences complementary to the DNA on either side of the segment of DNA to be amplified.

Further reading

B. McCarthy. 'Speciality animal fibres', *Textiles Magazine*, 1991, **20**, No.1. 6–8.

H. M. Appleyard. *Guide to the Identification of Animal Fibres*. Wira, Leeds, UK, 1978.

Dr Paul Hamlyn joined the British Textile Technology Group (BTTG) in 1982, following postgraduate work on the genetics of fungi at the University of Nottingham. Previously, he had worked as a microbiologist in the pharmaceutical industry. Section head of molecular biology and fermentation at BTTG, his current research work is concerned with applications of biotechnology to the textile industry.