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A technique for rapidly quantifying mammal hair morphology for zoological research

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Abstract. Within the zoological disciplines the study of mammalian hair has mostly been limited to cross-species comparisons, but there is also considerable intraspecific variation in hair characteristics that may be biologically meaningful and deserving of study, though it can be tedious to manually measure hundreds of hairs under a microscope. Here a method is presented for assessing a variety of morphological characteristics of mammalian hairs that is fast, nearly fully-automated, does not require a microscope, and that could easily be used by wildlife biologists or researchers studying museum skins. Using hair samples from 6 captive white-tailed deer (*Odocoileus virginianus*) hairs were placed in groups of ten on white 3 x 5 inch index cards and covered with clear packing tape. Cards were scanned with a standard flatbed scanner at high resolution (1200dpi) and the images imported into a computer image analysis program. The program automatically selected and measured each hair, relayed the data to a text file, and cycled through all images so that the 120 deer hairs examined (20 per animal) were all measured within 5 minutes. The data returned included the length of each hair (even if it was curly), the width (the average width of the entire shaft), the 2-dimensional surface area, as well as the colour of the hair, measured with hue and brightness scores averaged over the entire shaft. These data are well-suited for examining questions regarding factors influencing the morphology or colour of mammalian pelage, or for using hair morphology to assess the nutritional status of individuals, as is done with humans. When measurements are completed, cards can be conveniently stored, either in an index card box or ringed binder, and they can even be re-scanned (at higher resolutions, for example) if needed. Alternatively, the index card step could be skipped and hairs could be scanned loosely in batches. Either way, this method should allow zoological researchers to pursue a wide variety of questions relating to mammalian hair morphology.

Key Words: fur, pelage, image analysis, white-tailed deer, *Odocoileus virginianus*

Introduction

The fine-scale morphology of mammalian hairs is often of interest to zoologists, especially for use in species identification where the animal itself was not captured (e.g. Tumilson 1983, De Marinis & Asprea 2006, Pocock & Jennings 2006). By examining features such as hair width, length, and other variables on sets of hair samples it is often possible to determine what species the hairs came from, especially if multiple variables are used (Sahajpal et al. 2008). In addition to species identification, hairs have historically been measured for taxonomic purposes (Hausman 1920, Nason 1948). Aside from these purposes, however, mammalian hairs are rarely

measured in research projects that focus on single species, even when many animals are captured and other vital data (weight, sex, condition, etc.) is obtained. This is an area where there is much work to be done, since research with humans and mammalian lab animals suggests that there is considerable intraspecific variation in hair features which is often biologically important, and that can be useful to wildlife biologists. For example, in humans and lab rats, hair diameter declines with age (Wynkoop 1929, Naruse & Fujita 1971, Funk et al. 1989). For the wildlife researcher then, this phenomenon could be used to estimate the age of captured animals. Hair morphology can also signal the nutritional

status of individuals (Bradfield 1972, 1974), which would also be of importance to wildlife researchers. Clearly, detailed measurements of hair morphology can be of use to zoologists, though this area is sadly devoid of research. Perhaps the largest impediment to researchers is the time needed to gather the necessary data on individual hairs. Indeed, hair measurements have traditionally been performed under a microscope (Pocock & Jennings 2006, Sahajpal et al. 2008), and this approach can be tedious. Here a method is described for quickly measuring batches of hair samples that is nearly fully-automated and that does not require a microscope. It would therefore be ideally-suited for use in zoological research projects where many animals are examined.

Material and Methods

Sampling hair

On December 9, 2008, hair samples were obtained from a set of captive white-tailed deer (*Odocoileus virginianus*) at the Whitehall Deer Research Facility at the University of Georgia (Athens GA, USA). Six deer in total were sampled, 3 males and 3 females, all varying in age from 0.5 to 4 years old. All deer were tame enough so that they could be approached

and each could be sampled while they were eating or distracted. From each individual a small tuft of hair (~500 hairs) was pulled from the lower part of the back of the neck with small pliers. This standardized sampling location was necessary since hair morphology (i.e. length, width, etc.) can vary with body location in deer (Kulak & Wajdzik 2006). Hairs were placed in bags labeled with the deer identification and brought to the lab for processing.

Hair measurement procedures

From each hair sample ten hairs were haphazardly selected, taking care to ensure that all were in telogen (dormant) phase which is characterized by a prominent root bulb (Montagna & Van Scott 1958). These hairs were arranged in parallel on a white 3 x 5 inch index card and a piece of clear packing tape (Scotch Brand storage tape, 3650 series) was placed over them, which effectively 'mounted' the hairs to the card (Fig. 1). Care was taken to ensure that hairs did not overlap one another. In addition, hairs of deer (like many mammals) are slightly flattened (i.e. the shaft is not perfectly cylindrical), so care must be taken during this step to place the hairs in a consistent manner with respect to the shaft profile. Through trial

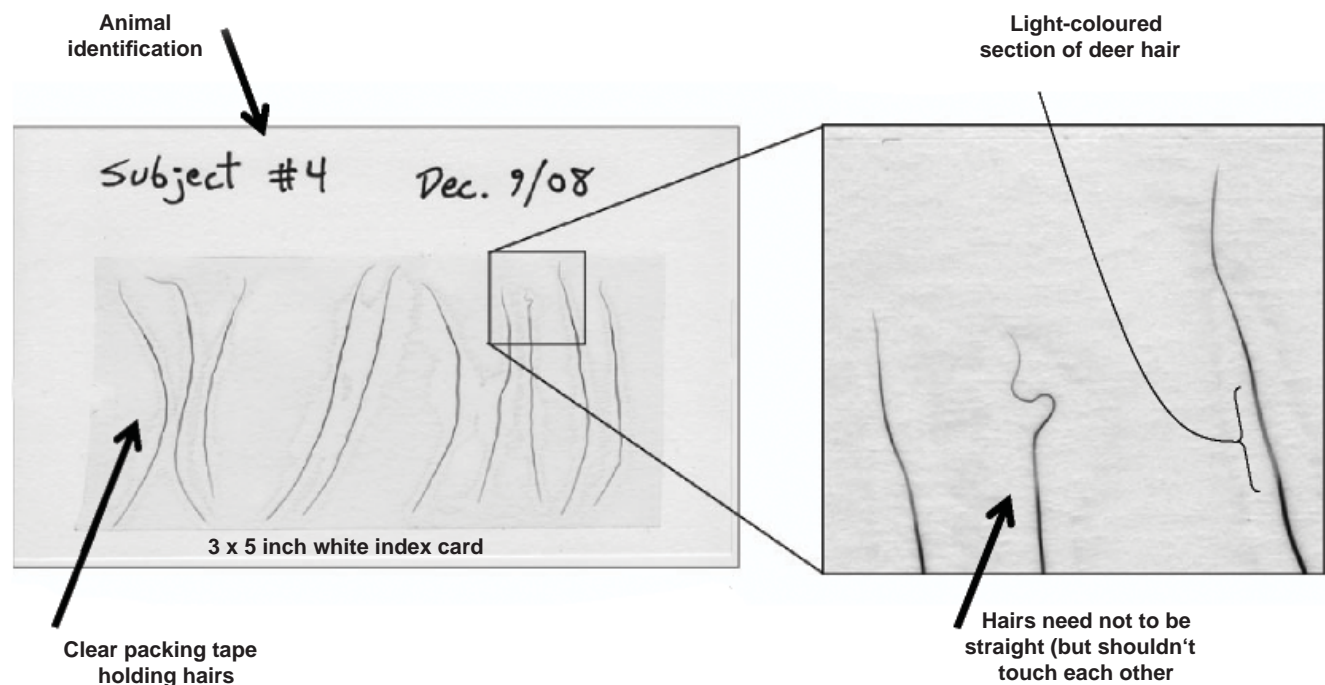


Fig. 1. Typical hair sample card used to scan into image analysis program. Ten hairs (from captive white-tailed deer) are arranged on a standard 3 x 5 white index card and permanently 'mounted' to the card with clear packing tape. The hairs need not be straight, but should not overlap. The card is scanned with a flatbed scanner at high resolution (1200 dpi).

and error, it was determined that ten hairs could be easily fit this way onto one card. In the space above the tape the animal identification and sampling date was written. For the purposes of this project two cards were made for each animal (twenty hairs total), though more could have been made. Each card took approximately two minutes to make.

When the hair sample cards were completed each was *scanned* with a standard flatbed scanner (Canon Canoscan LiDE 600F) connected to a computer, with the scanner set to its highest resolution (1200dpi), which allowed for each hair to be visualized on the computer screen with minimal pixelation (Fig. 1, inset). At this resolution, most images were 5000 x 2000 pixels in dimension, and when saved as jpegs, were approximately 1 MB in file size. These image files were then imported into an image analysis program (FoveaPro, www.reindeergraphics.com) to obtain the hair measurements. There are several image analysis software packages that could be used, though the author has used this particular program for a variety of projects where features of animals are of interest (Davis & Grayson 2007, Davis & Maerz 2007, Todd & Davis 2007, Davis et al. 2008, Davis & Grosse 2008), or rapid enumeration of on-screen objects is sought (Davis et al. 2004a, Davis 2007). The advantage of FoveaPro is the ability to automate the measurement routines. The drawback to this program is the cost (~800.00 US). A free software package that can perform most of the measurements used here is called 'ImageJ' and can be downloaded at <http://rsbweb.nih.gov/ij>, although it does not have all of the automation capabilities of FoveaPro.

For the purposes of the current study, the FoveaPro program was adapted to the measurement of hairs on the index cards. First, the program was set to automatically select the hairs based on the contrast between them and the white background. If light coloured hairs are of interest, a non-white background should be used (see Results and Discussion). Next, a measurement routine was run, which performs a suite of measurements on each selected object (the ten hairs in this case). By default, this routine returns a large number of parameters, though only a select few were of importance here. These included the length of each hair, which was the length of the line running down the center of the hair, and which followed the curvature of the hair (so that the hair need not have been straight on the card). The surface area (i.e. the two-dimensional area) was also measured for each hair, as well as the width of each hair, which was the *average width of the entire shaft*.

All size measurements were returned in millimeters, since the program was calibrated beforehand using a scan of a standard ruler. Next, the program returned several variables that describe each hair's *colour*, the average hue, saturation and brightness. In computer images, each pixel on the screen has a hue, saturation and brightness score associated with it, and the program returned the average score within each hair (which were usually made up of 10000-20000 pixels each). For this project, only the hue and brightness scores were of interest. The hue score is what most people think of as 'colour' (i.e. red, blue, orange, etc.), which is measured in degrees, and the brightness score is a measure of how dark an on-screen object is in the absence of colour and is measured from 0 (perfect black) to 255 (perfect white) (Davis et al. 2004b, Davis et al. 2005). Importantly, the software recorded all data

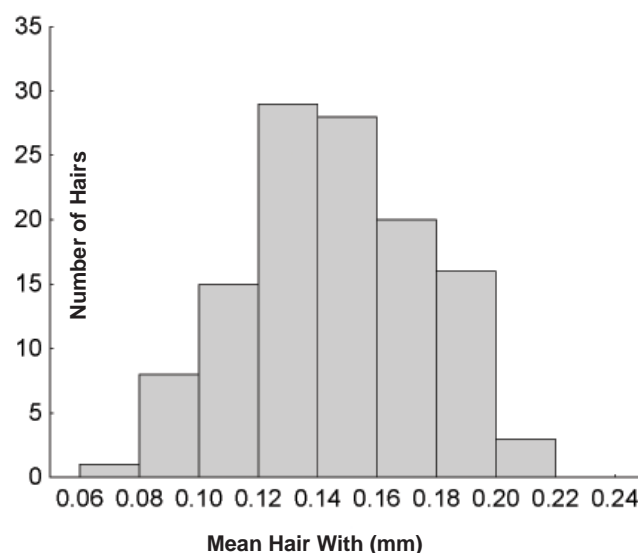


Fig. 2. Distribution of hair width measurements (the mean width of the entire shaft) observed in a collection of hairs from six captive white-tailed deer (twenty hairs per animal).

on a user-specified text file, so that no recording or transcription errors were possible. Further, the selection and measurement routines were automated so that the program processed all images one after the other. The time required to process all images (twelve in this case, or 120 hairs) was less than five minutes.

Results and Discussion

Using a standard flatbed scanner and an image analysis software program, a number of key

morphological measurements were obtained on 120 hairs from 6 deer in a matter of minutes. These data are summarized for each deer in Table 1. Further, the data obtained represent a considerable advancement over manual measurements. For example, the diameter of hair shafts is often of importance to investigators, though since the hair shaft tends to bulge at the middle and taper at the ends (Pocock & Jennings 2006), researchers have traditionally selected a single, standardized, position along the shaft to measure the hair ‘width’. The ‘width’ measurement here represents the average width along the entire shaft, which is a completely objective measure. The widths of all hairs in this study ranged from 0.08 mm to 0.21 mm and displayed a normal distribution (Fig. 2). This figure also demonstrates another important component to this method, in that it can discern the extremely minute variation in morphology that is typical of hair. This is critical since the differences in mean hair diameter among individual deer can be as little as 0.02 mm (Table 1). An even more useful parameter to gauge the hair width might be the ‘surface area’ measure, which encompasses not only the length of the shaft but the width as well.

In addition to the size-based measurements, the method used here also simultaneously allowed for the measurement of individual hair *colour*, which to the author’s knowledge, has never before been examined in mammals to this level of detail. The ability to measure individual variation in hair colours of mammals allows for a wealth of questions to be addressed, such as how individual nutritional status, or hormonal variations affect pelage colour,

for example, or how hair colour varies among genetically different individuals (Zima & Cenevová 2002). It should be noted though that this method would not necessarily allow for comparisons of hair colours *between* investigators, since flatbed scanners would differ in the degree of lighting given off, exposure, etc., and these variables would affect the hue and brightness of scanned objects. However, if the scanning is done so that the same settings are maintained throughout a given study, the *relative* differences among individuals (or even among individual hairs) would easily be observed (Davis et al. 2004b, Davis et al. 2007, Davis & Grayson 2007), thus this method would be most appropriate for examining intraspecific variation in hair colour.

It should also be kept in mind that the digital colour parameters obtained here represent averages of the entire hair shaft, including the light-coloured bands near the distal ends (Fig. 1). However, the dimensions of these banded sections could also be examined separately with this digital approach, which might provide additional data of interest to researchers. To test this idea, 20 hairs from one deer in this study were examined further to obtain these data. It was found that by selecting the banded portions manually, these sections could then be measured separately from the main hairs, with the program returning the same parameters (length, width, hue, etc.). For example, the data from the one deer examined here indicated the average length of colour bands was 2.2 mm (0.65 mm SD).

Also on the subject of hair colour, while many mammals tend to have brown coloured hair fibers (like the deer used here), which stands out easily on

Table 1. Summary of hair morphology variables measured using image analysis program (FoveaPro). Twenty hairs per animal (white-tailed deer) were measured and the mean of each parameter (and standard deviation) is shown. See methods for details of each parameter.

Hair Measurement	Deer 1		Deer 2		Deer 3		Deer 4		Deer 5		Deer 6	
Surface Area (mm ²)*	7.04	(1.74)	8.41	(2.43)	4.46	(0.96)	7.74	(1.72)	4.44	(0.81)	6.75	(0.96)
Length (mm)	39.59	(4.81)	42.15	(6.82)	34.89	(4.15)	40.72	(5.34)	30.36	(3.45)	38.96	(3.60)
Width (mm)	0.15	(0.03)	0.17	(0.03)	0.11	(0.01)	0.16	(0.02)	0.13	(0.01)	0.15	(0.01)
Width/Length Ratio	0.0038	(0.0004)	0.0041	(0.0004)	0.0031	(0.0002)	0.0041	(0.0003)	0.0041	(0.0002)	0.0039	(0.0002)
Mean Hue (degrees)	15.51	(5.35)	7.24	(0.91)	3.69	(1.12)	8.10	(1.18)	9.86	(1.36)	8.63	(0.98)
Mean Darkness†	179.28	(11.60)	175.55	(3.39)	174.16	(4.34)	174.18	(3.33)	179.63	(4.54)	178.39	(3.31)

* Surface area of one side of the hair
† Measured on a scale of 0-255, with 0 being completely black and 255 being completely white

a white background, others may have light-coloured hair fibers, which may not. This is an important point to consider with the computer-based approach used here, especially if an auto-select procedure is desirable. In other words, the researcher must ensure there is a strong colour contrast between the hair samples and the background in the scanned image in order for the software (or the user) to recognize the edges or boundaries of the hair. Thus for light-coloured hair one should use non-white index cards or some other background material that differs in colour from the hair colour, such as light green or blue. Although hair from white-tailed deer was measured in this study, this method should be useful for measuring hairs from a large range of mammalian species, with little to no modifications. If especially small hairs are of interest, such as those of bats, which are short and extremely fine (Nason 1948), these could be scanned using a slide scanner attachment that comes standard with many flatbed scanners, or a stand-alone slide scanner that connects directly to a computer. For long hairs (whiskers, or mane hairs, for example), larger index cards might need to be used, or even full-sized card-stock paper, perhaps with self-sticking laminating paper instead of packing tape. It should also be noted that creating 'hair sample cards' for scanning purposes by default also allows for easy storage of all hair samples, since they can be stored in a standard index-card storage box, or even hole-punched and kept in a ring-binder. And, the cards can be easily re-scanned if the original file is accidentally deleted, or if higher resolution images are needed. Alternatively, if archived

samples are not necessary, the card step could be skipped altogether and hairs from individual animals could simply be placed loosely on the scanner and scanned in large batches, which no doubt would save additional time, and would also eliminate the issue of finding the right card size for the hair samples.

To be fair, there are certain drawbacks associated with this technique, most notably that there are some hair features that are not well-visualized in the computer images. One is the root morphology; at the resolution used here (1200 dots per inch), the hair roots are visible, but their fine-scale features are not. This could be alleviated with higher resolution scans, though most retail scanners are only capable of 1200dpi. Plus, there are often other characteristics of hair that are of interest, and which this approach can not measure, such as the interior detail of the hair medulla (Hausman 1920), and the shapes of the cuticular scales on the outer surface of the shaft (Sahajpal et al. 2008). However, considering the many advantages of this method over manual microscope measurements, it is clear that this method for rapidly measuring hair samples should allow for greater depth of research into mammalian hair morphology.

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