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David Oates

Ken Pearson

Nancy Dent

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DIFFERENTIATION OF WHITE-TAILED AND MULE DEER BLOOD AND TISSUE BY ISOELECTRIC FOCUSING[1]

by

David Oates, Ken Pearson and Nancy Dent

ABSTRACT

Blood and tissue samples from 66 white-tailed deer (*Odocoileus virginianus*) and 69 mule deer (*Odocoileus hemionus*) were examined by isoelectric focusing on polyacrylamide gel slabs in the pH range 6-9. Blood was differentiated via a general protein stain. Tissue differentiation required use of esterase patterns rather than general protein patterns. Esterase patterns of white-tailed and mule deer tissue differed considerably from each other in both number and location of esterase bands. Protein patterns for blood were not as distinctive as the esterase patterns were for tissue, but white-tailed and mule deer could still be differentiated from each other. Some variation in individual patterns of blood and tissue was observed in both white-tailed and mule deer.

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In recent years the range of the white-tailed deer has spread into areas once considered the primary range of the mule deer. With changes in land use, increased hunting pressure, and the great adaptability of white-tailed deer, mule deer are at a distinct disadvantage. As a result, some states have considered it necessary to separately manage the two populations. Such a management strategy requires ways and means for enforcement of regulations. For years studies have been conducted to develop techniques to identify the meat or blood of game animals. These include immunological, chromatographic, and electrophoretic methods. The precipitin test, one of the earliest techniques, was utilized initially for bacteriological studies. Its zoological and forensic capabilities were soon recognized. Employing this technique, Nuttall (1904), Gay (1908), Clarke (1914), Brohn and Korschgen (1950) and Keiss and Morrison (1956) were successful in differentiating several big-game species from common domestic animals. A survey by Oates et al. (1974) reported that the technique is still used today in several states and provinces.

Other techniques have also shown great promise for species indentification. Electrophoretic techniques, utilizing various media and assorted protein and enzyme systems, are commonly employed. Jackson (1962) used paper chromatography to identify the tissue of game animals. Starch gels were employed by Giles (1962) to observe species differences in sarcoplasmic proteins of domestic mammals. Dilworth and McKenzie (1970) used starch gels, but they also observed muscle esterases and lactic dehydrogenases (LDH). Their LDH patterns for pig and moose (Alces

Americana) appeared similar, but the muscle esterases were obviously different. Patterns of total protein for beef and venison were also similar but muscle esterase patterns differed considerably. Cummings (1972) utilized transferrin bands, separated on starch gel, to differentiate California deer. Munday et al. (1974) examined muscle LDH's of fallow deer (Dama dama) sheep, swine, and cattle and could successfully differentiate them by electrophoresis on polyacrylamide gels.

Oates and Weigel (1976) used immuno diffusion and immuno-electrophoresis on agar gels to differentiate 26 game and domestic mammals. No distinction between white-tailed deer and mule deer blood or tissue could be determined by this technique. Attempts to differentiate blood and sarcoplasmic proteins by electrophoresis on cellulose acetate and step-acrylamide gels were also unsuccessful. Morgan et al.'s (1976) differentiation of mallard (Anas platyrhynchos) and black duck (Anas rubripes) by muscle esterases, and the previous success of Dilworth and McKenzie with muscle esterases of mammals provided sufficient incentive for us to compare the muscle esterases of white-tailed and mule deer.

Isoelectric focusing was the electrophoretic technique selected for this study due to its high resolution capabilities. Proteins are separated by surface charge and hence isoelectric point differences. Vesterberg and Svensson (1966) found variation of isoelectric point differences as small as 0.02 pH units could be resolved. This technique was used by Jeppsson and Berglund (1972) to isolate variations in human hemoglobin, and by Bunch et al. (1976) to differentiate hemoglobins of

Utah's big-game species. In the pH range 6 to 9 we would differentiate white-tailed and mule deer blood using a general protein stain.

During November, 1976, blood and tissue samples were collected at several deer check stations across Nebraska. Small meat samples were taken from the flank. This area was selected for sampling convenience and because of the willingness of hunters to part with meat from that location. Blood was taken as a clot from the juglar vein when possible. Samples were placed in plastic bags or in vials, frozen, and labeled as to species, age, sex, and harvest location. The blood was air dried on glass plates after arrival to the laboratory.

Dry blood was reconstituted with distilled H_2O, centrifuged and the clear supernatant used for analysis. Muscle tissue extracts were prepared by first cutting the frozen sample into small pieces. Approximately, 0.5g was placed in a tissue grinding tube and refrozen. After adding distilled water, the sample was macerated with a tissue grinder. The resulting slurry was centrifuged at 3000 rpm in a refrigerated centrifuge and the clear supernatant used for analysis.

Slab gels were prepared in a manner similar to Karlsson, et. al. (1973). The gel solution consisted of (1) 10ml of 29.1% (w/v) acrylamide (Eastman melting point 84°-86° C), (2) 10ml of 0.9% (w/v) NN-Methylenebisacrylamide (Eastman Reagent grade), (3) 36.6ml of distilled H_2O containing 7.5g of dissolved sucrose, (Mallinckrodt Analytical Reagent), (4) 1.5ml of Brinkman's pH 6-8 pHisolyte, (5) 1.5ml of Brinkman's pH 7-9 pHisolyte, and (6) 0.4ml of 0.004% (w/v) riboflavin (lactoflavin). The riboflavin was added after the solution

had been degassed for several minutes. Then, riboflavin was mixed in thoroughly with mimimal agitation. (Even better results were obtained by changing the concentrations of the acrylamide and Bisacrylamide to 40.7% (w/v) and 1.3% (w/v), respectively. This produced not only a more easily manageable gel, but more distinct patterns). The solution was then transferred into LKB's gel mold via a pipette. Fluorescent daylight bulbs were used for photopolymerization of the gel. After the gel polymerized, it was refrigerated for about 15 min. to facilitate removal of the glass plates from the mold. Prepared samples were placed on the gel via 5-x 10-mm strips of Whatmann 3MM Chromatography Paper. This strip was dipped into the sample extract and positioned on the gel with the aid of a template. A single gel could accomodate 24 samples. Normally, 12 samples and 12 duplicates were run. For the electrode buffer strips, a 1.0 M NaOH solution was used for the cathode (-) and a 1.0M H_3PO_4 solution was used for the anode (+).

Isoelectric focusing was then carried out on LKB's Multiphor 2117 in conjunction with ISCO's Model 493 power supply without a prerunning step. Gels were focused vertically for 90 min. at a constant power of 10 watts and for 30 min. at a constant 1,000 volts. After focusing, the nonspecific muscle esterases were identified by the method of Morgan et al. (1976). Gels were developed in a solution containing 20ml of 0.20 M Tris-HCl buffer (pH 7.4), 470ml of distilled water, 10ml of alpha-napthylacetate (1% in acetone) and 250mg of Fast Blue RR. Inhibitor reactions were not employed. The protein components of the blood were stained by the method of Karlsson et. al. (1973). Gels were stained for 15 minutes at 60° C with Coomassie Brilliant Blue R - 250 (0.75g) dissolved in 225 ml of

methanol. This was added to 465 ml H_2O. Sulphosalicylic acid (22.5g) and trichloroacetic acid (75.0g) were added with continuous stirring. The stain was used the same day.

Twelve duplicate samples were run simultaneously so patterns could be compared directly. The resultant patterns were retained for permanent records by two methods: (1) The gels were photographed using 35 mm high contrast black and white film and enlarged to 8 x 10 photos. (2) Gels were dried between 2 layers of cellophane in a manner similar to Work and Work (1970). We modified their technique by drying the gels on a Teflon-coated piece of aluminum (a cookie sheet cut to the size of the gel), instead of a glass plate, to prevent the cellophane from adhering to the plate. The plasticizers preserved gels stained with Coomassie Blue extremely well as mentioned by Work and Work (1970) but the plasticizers removed our esterase bands. A 5% solution of glycerin proved satisfactory for our needs.

BLOOD DIFFERENTIATION

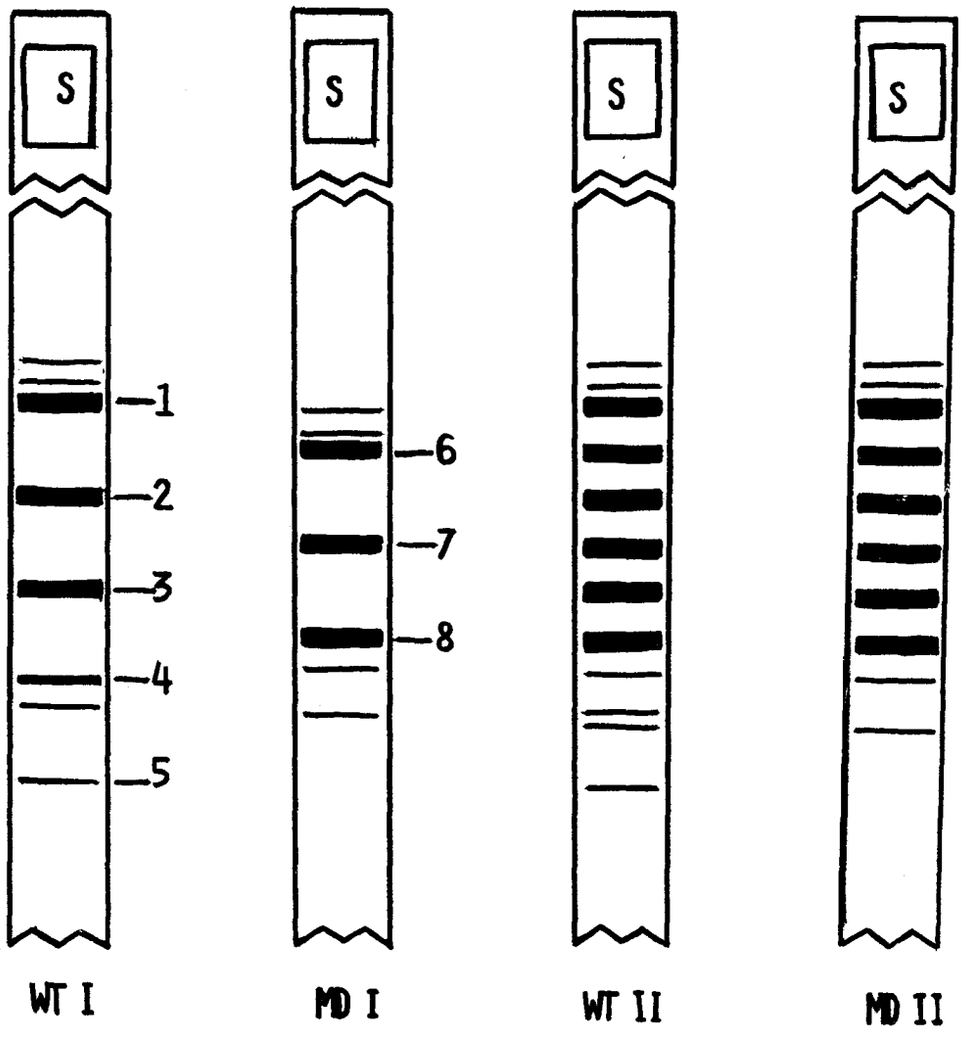
Protein patterns of dry blood were not always as obviously different for mule deer and white-tailed deer as were the muscle esterase patterns. However, they could be differentiated by isoelectric focusing on a pH 6-9 gel.

Essentially four types of patterns were observed (Fig. 1). We classified them as type I white-tailed deer (WT I), type II white-tailed deer (WT II), type I mule deer (MD I) and type II mule deer (MD II). The WT I and the MD I patterns differed distinctly from one another. Approximately 80% of the deer examined were classified as type I. Differences in the type I patterns were observed in the anodic half of the gel where usually 8 to 15 bands were present. Both WT I and MD I patterns were characterized by 3 or 4 relatively strong bands. Differences in these patterns were (1) relative positions of the strong bands, (2) absence of band #5 in MD I and (3) MD I patterns were more compact than WT I patterns.

Type II patterns were more difficult to differentiate for they seemed to be a combination both the MD I and WT I patterns. There appeared to be no difference in the stronger bands of WT II and MD II. Minor differences were observed in the patterns in that WT II patterns possessed two weak bands that appeared to correspond to bands 4 and 5 observed in WT I patterns.

Type I and II white-tailed and mule deer were found throughout the state. Occurrence appeared to be related to geographic location rather than age or sex. The majority of the type II deer were from areas of the state cohabited by both white-tailed and mule deer. This suggested that the type II deer were the result of hybridization of white-tailed and mule deer.

FIGURE 1. Diagram of protein patterns obtained from dried blood of white-tailed and mule deer.



S - Sample application site

WT I - White-tailed deer Type I, WT II - White-tailed Type II

MD I - Mule deer Type I, MD II - Mule deer Type II

TISSUE DIFFERENTIATION

Esterase patterns of white-tailed and mule deer differed considerably. For the most part, mule deer esterase patterns were weak (Fig. 1). Usually, only 1 or 2 bands were observed but occasionally no bands or as many as 5 were visible. Esterase patterns for white-tailed deer were always characterized by 1 to 3 strong bands (Fig.2), however, as many as a dozen bands were occasionally observed. Esterase bands for mule deer were more anodic than most of the white-tailed bands and were usually found in close proximity to sample applicaton site.

Effects of storage, handling, and sample location were also examined. White-tailed and mule deer tissue could be differentiated when (1) fresh, (2), refrigerated for a month, (3) recently frozen, (4) frozen up to 5 years (older sample not available), and (5) partially decomposed (had been refrigerated but obviously spoiled). Esterase bands were not as sharp and distinct for the spoiled or older meat samples but white-tailed and mule deer tissue could still be easily distinquished from each other. Cooked meats could not be identified by this technique due to esterase denaturization. Examination of 12 different skeletal muscles from a white-tailed doe were found to produce identical muscle esterase patterns.

Several different muscle esterase patterns were observed for both white-tailed and mule deer. Mule deer patterns were usually weak but appeared to be more distinct, when the sample was applied near the cathode or if gels containing 40.7% acrylamide and 1.3% bisacylamide were used.

Samples positioned toward the top of the gel (anode) gave more distinct white-tailed deer patterns. Tailing was noted in white-tailed deer patterns when the sample was placed near the cathode. Several different patterns were discerned for both white-tailed and mule deer. However, they didn't necessarily correspond to age, sex, or geographical differences. Such patterns still might be useful in court cases for determining whether more than one deer is involved.

White-tailed and mule deer tissue can easily be differentiated by this technique. However, until esterase patterns have been examined for more species, a tissue sample may first have to be identified as being from a deer.

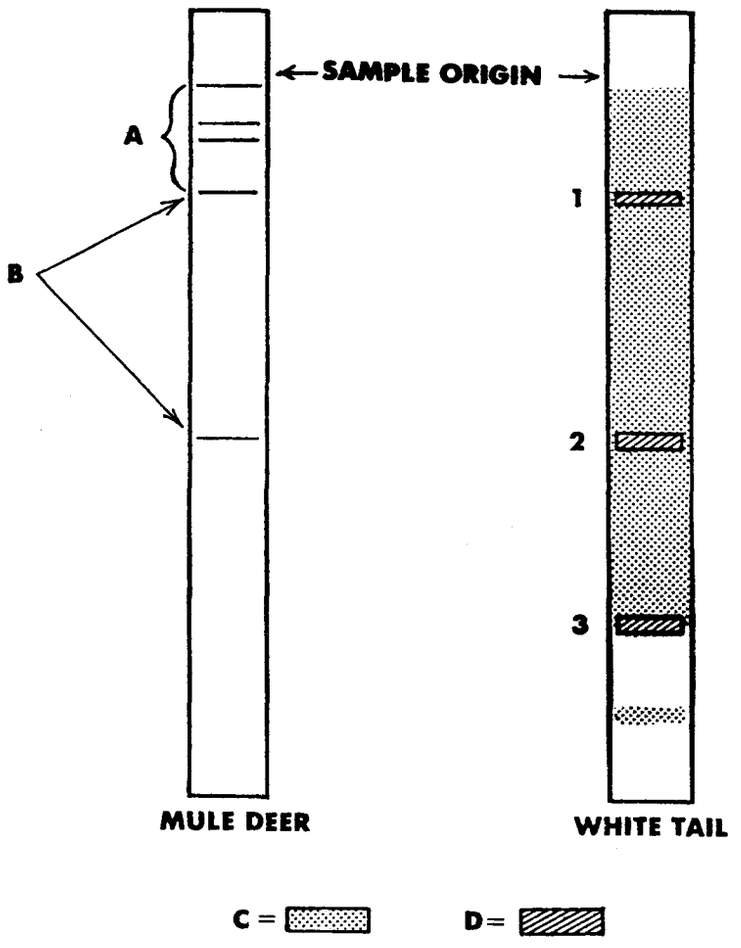
Differentiating white-tailed or mule deer from other species may require extensive examination of the esterase patterns of many species. A preliminary investigation of esterase patterns was made on tissue samples from 27 mammalian species including herbivores (ungulates and rodents) and carnivores. Results indicated that tissue from white-tailes deer may be differentiated from other species examined by this technique. The results of this preliminary investigation also suggest the distinct possibility of differentiating some of the other species examined from each other.

This technique has potential as a management tool for law enforcement. Several states have both white-tailed and mule deer while others may have only one or the other. The great adaptability of the white-

tailed deer has led to concern by some states and mule deer may, therefore, be managed separately from white-tailed deer. We have employed this technique several times in law enforcement cases, but the defendent has always pleaded guilty prior to an actual court case. This technique should meet the criteria necessary to be admissible in court. The difference between the esterase patterns of white-tailed and mule deer is so distinct that one could even allow a jury to decide whether the meat was from a white-tailed or mule deer.

Laboratories with electrophoresis capabilities should be able to distinquish the difference between tissue from a white-tailed and a mule deer by examining muscle esterases. Differentiation of white-tailed or mule deer dry blood can be attained by isoelectric focusing, but it may also be possible on starch gels or on gradient acrylamide gels.

FIGURE 2. Diagram of observed white-tailed and mule deer muscle esterase patterns.



A Weak bands - usually from one to four bands observed in this area.

B Weak bands occasionally observed in mule deer patterns - commonly strong bands in white-tailed deer patterns.

C One or more of these strong bands were observed for all white-tailed deer patterns (occasionally occurring as doublets).

D Fine or weaker bands occur in these areas with the majority being located between bands 1 and 2.

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