Journal of the Arkansas Academy of Science

Volume 30 Article 19

1976

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Greg S. Jackman
University of Arkansas at Little Rock

Phyllis J. Garnett
University of Arkansas at Little Rock

Electrophoretic Patterns of Serum Proteins in Two Subspecies of *Odocoileus virginianus*

GREG S. JACKMAN and PHYLLIS J. GARNETT
Department of Biology, University of Arkansas, Little Rock, Arkansas 72204

ABSTRACT

Cellulose acetate electrophoresis revealed six monomorphic forms of serum protein in natural populations of two subspecies of white-tailed deer, *Odocoileus virginianus virginianus* from Tennessee and *Odocoileus virginianus macroura* from Arkansas. The fixed pattern of serum proteins in the two populations indicates a lack of genetic variation in the loci controlling these proteins.

However, electrophoresis revealed different hemoglobin phenotypes in the two subspecies. This finding indicates that further study is needed to determine whether or not there are genetic differences in the hemoglobin forms.

INTRODUCTION

Two subspecies of white-tailed deer, *Odocoileus virginianus macroura* and *Odocoileus virginianus virginianus*, are present in Arkansas and Tennessee, respectively (Hall and Kelson 1959). The range of *O. v. macroura* includes Missouri, Arkansas, eastern Kansas and Oklahoma, northern Louisiana, and a small area of northeastern Texas. The range of *O. v. virginianus* includes virtually all of Tennessee, Kentucky, the inland areas of Mississippi, Alabama, Georgia, South Carolina, North Carolina, Virginia, and most of West Virginia. The zone of contact between the two subspecies is the Mississippi River.

Electrophoretic analysis of serum proteins has been used as a valid method of determining genetic variation between natural populations of various mammals (Zimmerman 1975, Kilpatrick and Zimmerman 1976, Selander and Yang 1969). The purpose of this study was to analyze the serum proteins of *O. v. macroura* from Arkansas and *O. v. virginianus* from Tennessee to assess the levels of genetic similarity between the two subspecies.

MATERIALS AND METHODS

Samples of blood were collected from deer shot by hunters which were brought to Wattensaw Wildlife Management Station, Prairie County, Arkansas, and the Natches Trace Wildlife Management Area, Dyer County, Tennessee, during the respective hunting seasons. Samples were taken from 25 deer selected from 160 tagged at Natches Trace and 23 selected from approximately 200 tagged at Wattensaw. Selection was based on the time of death and the condition of the deer. Most samples were taken from deer killed within one hour of their arrival for tagging. Approximately 10 cc of blood was collected from the pleural cavity of each deer. Care was taken to collect clear serum; however, in most cases hemoglobin contamination could not be avoided. A sample of blood also was collected from a live deer at the Little Rock Zoo to be used as a control. Samples were placed in glass vials, stored on ice, and transported to the laboratory where the serum was separated by centrifugation, frozen, and stored until electrophoretic analysis.

Serum proteins were separated on cellulose acetate membranes by use of Shandon Electrophoresis Apparatus Model U77. A minimum of two runs was made of each sample. After an application of 2.5 μl of serum on a 10-cm cellulose acetate strip, the samples were subjected to electrophoresis in barbital buffer, pH 8.6 and ionic strength 0.075, for 2 hr at 175 v with a mean current of 2.5 ma per strip. At the conclusion of the run, strips were transferred to 5% trichloroacetic acid fixing solution, then to Amido 10B staining solution for 10 min. Strips were washed in methyl alcohol until the last wash remained colorless. Visual inspection was carried out on each strip.

RESULTS

Electrophoretic analysis revealed six bands of serum protein which appeared to be monomorphic with no detectable differences in occurrence or intensity between the two populations. No sexual variation was detected. For two bands of hemoglobin, variation of occurrence between the two subspecies was detected. Migrating toward the anode, the fastest component, albumin, was followed by alpha$_1$ globulin, hemoglobin A, hemoglobin A$_1$, alpha$_2$ globulin, beta$_1$ and beta$_2$ globulin, and gamma globulin (Fig. 1).

All samples clearly indicated the presence of four serum proteins including albumin, alpha$_2$, beta$_1$, and beta$_2$ globulins. Because of the light staining of alpha$_1$ globulin, this band was not always detectable. Gamma globulin, which moved only a slight distance from the origin, stained lightly and also was difficult to detect.

Visual analysis of the electrophoretic strips indicated that the concentration of each component of the serum was consistent for all deer. Bartlett (1963) observed that the optical density of proteins stained with Amido Black 10B increased linearly with increasing concentration of dye and that albumin and globulins have equal affinity for the stain on a percentage basis.

After electrophoretic separation the hemoglobin bands were visible before staining and thus could be easily identified. After staining, two forms of hemoglobin designated HbA and HbA$_1$ were observed. HbA migrated approximately midway between alpha$_2$ and alpha$_1$ globulins; HbA$_1$ migrated slightly faster than alpha$_2$ globulin. Of 23 samples of blood from *O. v. macroura*, two showed both hemo-

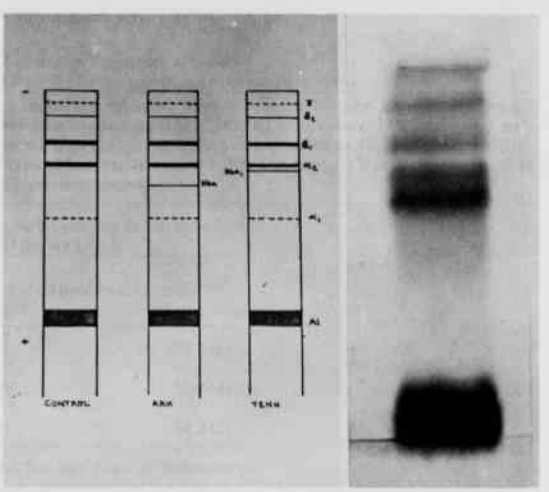

Figure 1. A. Schematic drawing showing the electrophoretic pattern of serum proteins and hemoglobin from *O. virginianus*. B. Electrophorogram showing the pattern of serum proteins and hemoglobin in *O. virginianus*.

globin forms; one sample had only HbA and another had only HbA_1. A hemoglobin band was not visible in any other samples taken from Arkansas deer.

With the exception of two samples of blood from *O. v. virginianus* for which the cellulose strips were difficult to analyze, all samples from Tennessee deer indicated the presence of HbA_1 and the absence of HbA.

DISCUSSION

The Mississippi River does not provide complete geographic isolation for the two populations because residents and officials of state agencies have reported deer crossing low-water areas of the river. Furthermore, Lowery (1974) in reporting on white-tailed deer in Louisiana stated that transplantation of deer has resulted in a genetic conglomerate of hybrids among several natural geographic races. Attempts to obtain stocking information on populations of deer in the two states were unsuccessful because of lack of accurate records maintained in the states.

Analysis of the serum proteins showed no detectable differences in the occurrence, electrophoretic migration, or intensity of staining either among individuals of a population or between the subspecies. The consistent pattern of serum proteins in the two populations indicates a lack of genetic variation in the loci controlling these proteins.

This study was intended as an analysis of only the serum proteins; however, because of hemolysis of blood before collection, hemoglobin was detected in samples of blood from both populations. *O. v. virginianus* was characterized by the presence of HbA_1 and the absence of HbA. The characterization of hemoglobin in *O. v. macroura* was more difficult as both HbA and HbA_1 were found rarely in the samples. No visible difference in hemoglobin contamination was observable in the samples of serum; however, the consistent HbA absence and HbA_1 presence observed for Tennessee deer was not observable in samples from Arkansas deer. This study indicates the presence of polymorphic forms of hemoglobin within the two populations. Further study of isolated hemoglobin from the subspecies is desirable to determine whether or not there is geographic variation in hemoglobin forms.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of the Arkansas Game and Fish Commission and Tennessee Wild Life Resources Agency in their cooperation for the procurement of samples of blood.

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