STARCH GEL ELECTROPHORESIS FOR THE STUDY OF POPULATION GENETICS IN WHITE-TAILED DEER

by
MICHAEL N. MANLOVE
Savannah River Ecology Laboratory, Aiken, South Carolina 29081
JOHN C. AVISE
Department of Zoology, University of Georgia, Athens, 30602
HILBURN O. HILLESTAD
Institute of Natural Resources
University of Georgia, Athens, 30602
PAUL R. RAMSEY
Department of Zoology
Louisiana Tech University, Rushton, 71270
MICHAEL H. SMITH
Savannah River Ecology Laboratory, Aiken, South Carolina 29801
DONALD O. STRANEY
Department of Zoology
University of California at Berkeley, Berkeley, 94720

ABSTRACT

Methods for the collection, preparation and extraction of tissues of white-tailed deer (*Odocoileus virginianus*) are given. Electrophoretic technique, buffers, gel preparation and banding patterns for 27 proteins encoded by 28 structural loci are described. In a survey of 400 deer from the Savannah River Plant, nine loci were shown to be polymorphic. The potential use of population genetics information for wildlife management programs is discussed.

INTRODUCTION

Wildlife management models and programs should be based on functional biological characteristics of the species under consideration rather than on geographical boundaries. The management unit should consist of a biological population or a group of populations lumped together because of similar biological attributes (e.g., reproductive rate and/or age structure). It is now possible to identify local populations on the basis of their genetic constitution (Harris, Huisman and Hayes 1973), and since populations are groups of interbreeding individuals this may be one of the best ways to characterize them.

Development of starch gel electrophoresis has provided a valuable technique for the study of the genetics of natural populations. Data on gene frequencies may be used to delineate biologically meaningful management units, as well as supply information of potential use in wildlife management.

Our primary purpose is to describe electrophoresis as applied to white-tailed deer and to give some examples of biochemical variability found within this species. Secondarily, we will discuss the meaning of differences in allele frequencies and their possible use in the formation of a management plan.

Research was supported by Contract AT(38-1)-819 between the U. S. Energy Research and Development Administration and the University of Georgia and the Institute of Natural Resources and School of Forest Resources, University of Georgia. We also appreciate the cooperation of the U. S. Forest Service, Savannah River Project in the collection of samples. S. E. Fuller and P. E. Johns, Savannah River Ecology Laboratory, and R. L. Marchinton and graduate students, School of Forest Resources, University of Georgia, assisted with field collections.

COLLECTION, PREPARATION AND EXTRACTION OF TISSUES

The equipment and procedures described below have been used successfully at our laboratory for the electrophoretic demonstration of enzymes and other proteins in tissue extracts of deer. Similar methods have been used with rodents and other mammals (e.g., Selander et al. 1971; Straney et al. In Press).

Collection of Tissues

It is important that tissue samples be taken as soon as possible after death of the animal to prevent denaturation of proteins. Proteins regularly assayed are derived from blood, heart, kidney and liver tissues. Tissues such as testes and muscle have been used in other species and may be useful if other gene loci are studied.

Blood may be collected from the heart or body cavity in 25ml vacuum tubes containing 100mg heparin, or in heparinized capillary tubes if only small amounts can be obtained. Capillary tubes are immediately plugged at one end, labeled and placed on ice. When using capillary tubes several blood samples from each individual should be taken in case of breakage.

One or two grams each of heart, liver and kidney tissue are also taken, and may be kept in the same vial until further processing. Samples may be kept on wet ice in the field for short periods of time but dry ice should be used for extended periods.

Preparation of Extracts

Whole blood collected in vacuum tubes should be mixed with an equal volume of 85 percent physiological saline and centrifuged at 2500g for 10 minutes. Plasma is then ready for electrophoresis. The remaining erythrocytes are resuspended and centrifuged three times in approximately 10 volumes of 85 percent saline. The supernatent is then discarded and an equal volume of deionized water and a half volume of toluene added. Agitation will serve to lyse the erythrocytes. The solution is then centrifuged for 20 minutes at 50,000g. Hemolysate usually requires further dilution with deionized water (1:5) prior to electrophoresis. Although both hemolysate and plasma may be stored for several months or longer at −40°C to −70°C, it is imperative that they be separated by centrifugation within a day or two after obtaining blood samples to prevent contamination of plasma by lysis of erythrocytes. Hemolysate electrophoresed before freezing yields best results for staining hemoglobin.

Blood collected in capillary tubes should be centrifuged for 10 minutes in a clinical hematocrit centrifuge. It may then be kept in a refrigerator until electrophoresis for up to a week, although samples processed immediately will yield better results.

Heart and kidney tissues are combined in equal amounts to comprise about 0.5g for homogenizing. This amount is also adequate for liver samples. Tissue samples should be finely cut and put in centrifuge tubes with an equal volume (ca. 1-2 ml) of buffered grinding solution (0.1 M tris, 0.001 M ethylene-diamine-tetraaceate (EDTA), and 5×10^{-5} M NADP with pH adjusted to 7.0 with hydrochloric acid). Mesentery and other connective tissues should be removed to facilitate grinding. In some cases, extracts may be too concentrated or dilute for certain enzymes. This may be corrected by adding more or less grinding solution. Homogenize the samples (cooled in an ice bath) for one to two minutes with a ground glass or Teflon tissue grinder. Centrifuge at 50,000g at 0 to 4°C for 25 minutes and transfer the supernatent to labeled test tubes for cold storage. Fat in liver supernatent may be dissolved by adding 0.5ml toluene, agitating, and recentrifuging.

Storage of Tissues and Extracts

Most proteins will not denature for several weeks or months in intact tissues kept below −20°C. Tissue samples should be frozen in vials or plastic bags (preferably in 85 percent saline) to prevent dessication. Extracted proteins, however, denature rapidly unless stored at or below −40°C. All grinding, centrifuge, and storage tubes containing intact tissues or tissue extract should be kept on wet ice.

ELECTROPHORETIC PROCEDURE

Apparatus for horizontal starch-gel electrophoresis employs a variable-voltage power supply to provide direct current through a gel supported across electrode bridges which contain the appropriate buffer solution. Standard electrolytic cells with platinum electrodes are used. Gels are prepared with electrostarch (Otto Hiller, Madison, Wisconsin), at a concentration of 12.4 percent (approximately 45g per 400ml buffer depending upon the lot). The gel solution is "cooked" over a flame with constant agitation until the solution boils vigorously and then aspirated for 1 minute. The solution is then poured into a 9×180×210mm plexiglass mold and left to cool for 30 minutes and then covered with plastic wrap.

Samples of tissue extracts are absorbed on 9×5mm pieces of filter paper, blotted to remove excess liquid, and inserted in a vertical slit (the "origin") cut across the gel 6cm from one end. Gels are rested longitudinally on the electrode bridges (buffer trays) with the origin nearest the cathode. DuPont

cellulose sponge cloth (180×70mm) is placed in each electrode buffer tray and over the terminal 6cm of the gel. Cathodal to the origin, the plastic wrap should be evenly doubled over leaving 3cm of exposed gel. The cathodal sponge is placed over both gel and plastic wrap, parallel with and adjacent to the origin. The anodal sponge is placed in direct contact with the gel and covered with the plastic wrap.

During electrophoresis, gels may be cooled by a pan of ice supported above the gel on a plate of glass or may be placed in a refrigerator at 0 to 4°C for the duration of the run. Following electrophoresis, 3mm slices are cut transversely from each gel and incubated in plastic trays with appropriate staining solutions. Slices are made by transferring the gel to a 3mm recessed plate and passing a wire transversely through the gel. The top portion is lifted from the slice, and the slice may then be lifted into the stain tray.

Protein Stains

Stain recipes for assaying proteins in white-tailed deer are standard for our laboratory as modified from Selander et al. (1971), and are presented in appendix 1. Buffer systems employed for specific proteins are described in appendix 2 and referred to by number in appendix 1.

PROTEIN SYSTEMS AND BIOCHEMICAL VARIATION

Banding patterns and allelic frequencies described in this section are based primarily on a survey of 400 white-tailed deer sampled during fall public hunts operated by the U. S. Forest Service on the Savannah River Plant (SRP) near Aiken, South Carolina.

Twenty-seven protein systems encoded by 28 loci were assayed, nine of which were found to be polymorphic (possess more than one allele) in the SRP deer. Three additional (two polymorphic) proteins were observed but could not be consistently demonstrated for all individuals. Variants at the glutamate oxalate transaminase-1, α-glycerophosphate dehydrogenase and glutamate dehydrogenase loci were also detected, but these loci were considered monomorphic in the SRP population since they were observed in only single individuals for *GOT*-1 and *α GPD* and two individuals for *GDH*. We assume the quaternary structure of each protein to be similar to that reported by Selander et al. (1971).

Table 1. Summary of buffer types and tissues used for electrophoresis in white-tailed deer. Voltage and duration for each type are also indicated. Proteins are listed in order of slices taken from each gel, the last listed being from the bottom slice. Where only one protein is assayed from a given gel, a middle slice gives best results.

Gel Buffer	*Tissue*	*Voltage (D. C.)*	*Time (hours)*	*Proteins Stained**
Lithium hydroxide	Liver	300-350	4	PGI ES-2
	Plasma	300-350	4	ALB and TRF ES-1
Poulik	Liver	250-300	3½	G-6-P PGM/IPO SDH α-GPD
Continuous tris-citrate	Liver	130	4½	AP MDH and ME GOT GDH
	Heart and Kidney	130	4½	LDH and IDH
Tris-hydrochloric acid	Hemolysate	250	2	Hb-α and -β
	Liver	250	2	ES-4 and ES-2
Tris-maleate	Hemolysate or Liver	100	5	6-PGD
	Hemolysate	100	5	ES-3

* Many of the proteins may also be assayed from different tissues and buffer types resulting in banding patterns of varying degrees of quality. Abbreviations are as given under Protein Systems and Biochemical Variation.

Polymorphic Proteins

In the following account the abbreviations for the proteins and the tissues from which they were demonstrated are indicated in parentheses following the name of the protein. Tissue sources, and buffer types for demonstrating these systems are also summarized in Table 1. All proteins migrate anodally unless otherwise noted. Except for the esterases, numerical designations refer to relative migration distance, with the lowest number being the faster migrating form (e.g., *PGM*-1 migrates more anodally than *PGM*-2). Alleles are designated by letter superscript.

Sorbitol dehydrogenase (SDH; liver). The *SDH* locus possesses three relatively common alleles and one rare fourth allele in SRP deer. *SDH* is a tetramer, with homozygotes appearing as single bands and heterozygotes having five bands, which decrease in intensity away from the center band (Fig. 1). Subbanding tends to occur in some samples and bands are often blurred. *SDH* appears to be one of the more thermally unstable systems in deer. It is, however, easily scored in fresh samples that have been kept cold.

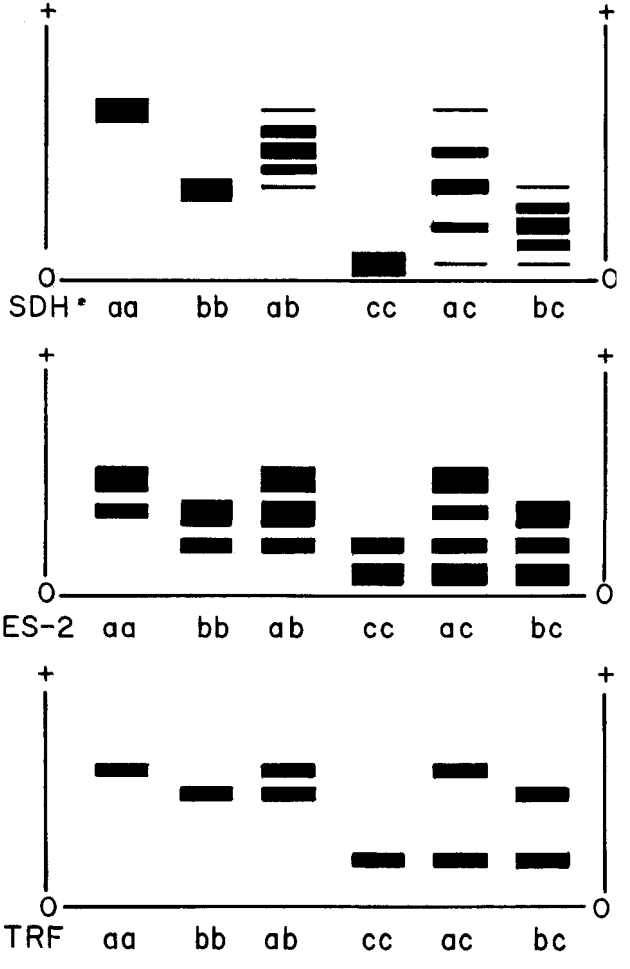

Figure 1. Banding patterns of three polymorphic loci in white-tailed deer. Allelic designations are given below the origin (0—0). Top: Sorbitol dehydrogenase from liver. Center: Esterase-2 from liver. Cathodal subband for ES-2^c is not illustrated. Bottom: Transferrin from plasma. Anodal subbands are not illustrated.

Esterases (ES; plasma, liver, kidney). A number of esterases have been observed, four of which can be consistently demonstrated and scored. The slowest anodally migrating esterase (*ES*-2) is polymorphic and can be demonstrated in liver extract with lithium hydroxide or tris-hydrocholoic acid gel buffer. Other esterases banding in the region of *ES*-2 are eserine sensitive, thus making the *ES*-2 protein easier to score when eserine is used. Three alleles are present at this locus in SRP deer. Homozygotes appear with three or four bands, usually with one of the bands being most intense. Banding patterns are essentially identical to those of *ES*-4 in *Peromyscus* (Selander et al. 1971). Apparently the products of this locus subband cathodally although *ES*-2^C also subbands anodally (Fig. 1). The subbands of *ES*-2^b and *ES*-2^c each respectively appear at the same position as the other allele. Phenotypes show variability in intensity of staining depending upon the degree to which proteins have subbanded, thus in some cases, heterozygotes and homozygotes can be difficult to distinguish. A fast migrating esterase assayed from kidney and liver with a Poulik buffer is variable but not consistently scorable.

Transferrin (TRF; plasma). There are three alleles present at the transferrin locus in white-tailed deer (Fig. 1). This protein is monomeric and heterozygotes have two bands; homozygotes have one band. Anodal subbanding gives the appearance of two bands for the homozygote and three or four bands in heterozygotes (see Miller et al. 1965). If it is necessary to confirm the identity of the transferrin bands, the plasma may be treated with rivanol (Sutton and Karp 1965; Chen and Sutton 1967) which precipitates non-transferrin proteins. If red cell lysis occurs before separation of the plasma, transferrin bands will be obscured by those of hemoglobin.

Phosphoglucomutase (PGM; liver and muscle). Two primary zones of activity appear on gels stained for *PGM*. Only the slowest anodally-migrating set of bands has been scored. This system is the darkest staining on the gel and has three alleles in SRP deer. *PGM*-2 is monomeric in structure, with two-banded heterozygotes (Fig. 2). The slowest migrating allele (*PGM*-2^C) in this system migrates to the same position as indophenol oxidase. A more anodal migrating and apparently polymorphic system appears on some over-stained gels. This locus (*PGM*-1) is variable with at least three alleles. It bands faintly, however, and we have been unable to score it consistently.

Lactate dehydrogenases (LDH; kidney, liver and muscle). Two *LDH* systems are evident. Both migrate anodal to the origin. In deer the faster migrating *LDH*-1 is monomorphic and the slower *LDH*-2 is polymorphic with two alleles. Polypeptides of *LDH*-1 and *LDH*-2 are tetrameres and combine their gene products randomly to produce a five banded pattern (Fig. 2). Only the three fastest migrating of the five bands of *LDH* are consistently demonstrated from kidney tissue due to the higher activity of *LDH*-1. Only the bottom two bands are consistently present from muscle tissue.

Malate dehydrogenases (MDH; kidney, liver and muscle). *MDH* exhibits two isozymes in deer. The anodal protein, *MDH*-1, is polymorphic with two alleles (Fig. 2). Homozygotes appear as single bands and heterozygotes are three banded; the molecule is a dimer.

Glutamate oxalate transaminases (GOT; liver, heart, kidney and muscle). Two forms of this enzyme are detectable in deer. A cathodally migrating mitochondrial form, *GOT*-2, is variable and possesses two alleles. Homozygotes are represented by single bands and heterozygotes are three banded. Bands tend to be less distinct than is illustrated in Fig. 2.

Hemoglobin β (Hb-β; hemolysate). *Hb-β* is the most variable system demonstrated in deer, with seven alleles occurring in southeastern deer (Fig. 3). Detailed discussion of geographic variation of hemoglobin in deer is given by Harris, Huisman and Hayes (1973). Products of the II, III, V, and VII allele have been demonstrated in SRP deer. This protein migrates anodally. Homozygotes show single bands and heterozygotes have two bands.

Hemoglobin-$^{II}\alpha$ (Hb-$^{II}\alpha$; hemolysate). Gene products of Hb-$^{II}\alpha$ migrate anodally to the origin but cathodal to *Hb-β* and appear as single bands. Migration distances vary as a function of the mobility of the associated Hb-β allele. We have scored these bands as absent, faint or dark (Fig. 3). "Faint" bands are considered as heterozygotes but may be difficult to differentiate from dark homozygotes. This locus represents a duplication of, and is linked with a $^{I}\alpha$ gene and apparently segregates in a Mendelian fashion in deer populations (Huisman et al. 1968; Taylor et al. 1972). Products of the $^{I}\alpha$ locus could not be distinguished by our techniques.

Monomorphic Proteins

The following protein systems exhibit no variation in our samples or show a variant in only one or two individuals.

Isocitrate dehydrogenase (IDH; liver, heart and muscle). Two forms, (*IDH*-1 and -2) exist, each having a single band.

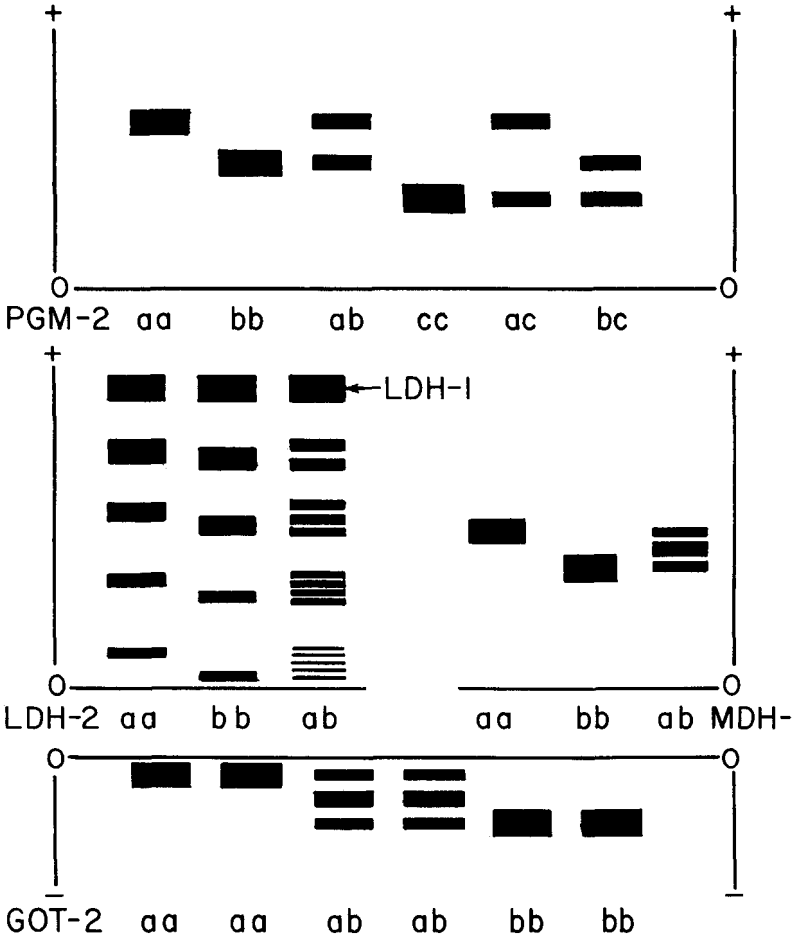

Figure 2. Banding patterns of four polymorphic loci and one monomorphic locus in white-tailed deer. Allelic designations are given below the origin (0—0). Top: Phosphoglucomutase-2 from liver. Center left: Lactate dehydrogenase-1 and -2 from kidney. *LDH*-1 (top band) is monomorphic. *LDH*-2 (bottom bands) is polymorphic. Bands between those of *LDH*-1 and -2 are inter-locus heterotetrameres and show heterogeneity and polymorphism in *LDH*-2. Center right: Malate dehydrogenase-1 from liver. Bottom: Glutamate oxalate transaminase-2 from liver.

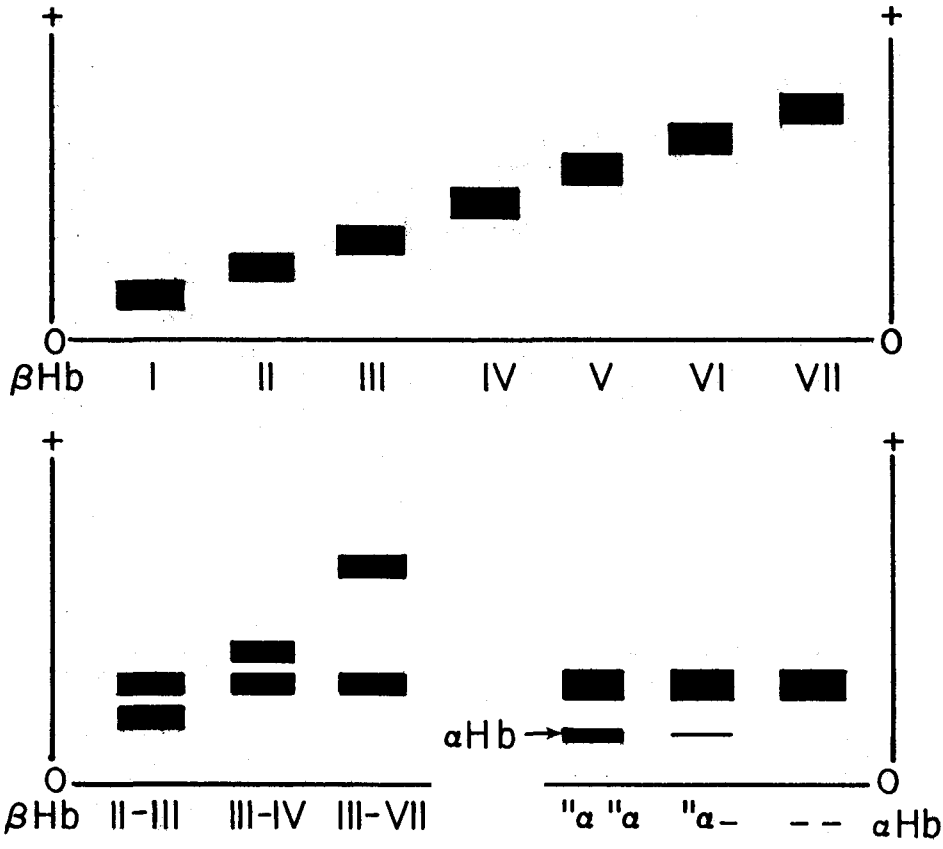

Figure 3. Banding patterns of two polymorphic loci in white-tailed deer. Allelic designations are given below the origin (0—0). Top: Hemoglobin -β chain from hemolysate. Alleles I, IV, and VI occur in other populations in the southeast but have not been found in deer from the Savannah River Plant. Bottom left: Common *Hb*-β heterozygotes. Bottom right: *Hb*-$^{II}\alpha$ chain phenotypes from hemolysate. *Hb*-$^{II}\alpha$ phenotypes occur with other *Hb*-β phenotypes but are not illustrated. The dash in the legend indicates absence of the $^{II}\alpha$ chain.

Phosphoglucose isomerase (PGI; liver and muscle). Three bands are present which decrease in intensity anodally. Since *PGI* is dimeric, we hypothesize that two loci *PGI*-1 and *PGI*-2) are involved which interact randomly to give the appearance of a heterozygous phenotype. *PGI*-2 is probably the more active. This interpretation is tentative, since no heterozygotes have been seen at either locus. Heterozygotes should appear as in some fish (see Avise and Kitto 1973).

Indophenol oxidase (IPO; liver). As noted in appendix 1, this protein is most clearly demonstrated on gels stained for *PGM*. A single band appears as a clear, non-stained area on a darker background below the band corresponding to *PGM*-2^b.

Albumin (ALB; liver and plasma). Albumin forms the most anodal band on gels stained for general proteins.

α-Glycerophosphate dehydrogenase (α-GPD; liver and muscle). Two loci (*α-GPD*-1 and *α-GPD*-2) have been demonstrated in white-tailed deer. *α-GPD*-2 forms a primary band with two more anodally migrating subbands. One heterozygote was observed for *α-GPD*-2 having a second, slow allele in SRP deer.

Glutamate dehydrogenase (GDH; liver). The presence of a second, slower allele was observed in heterozygous condition in one individual and homozygous in one other. It appears to be a dimer.

6-Phosphogluconata dehydrogenase (6-PGD; hemolysate and liver). *6-PGD* migrates as a single band with low mobility.

Glutamate oxalate transaminase-1 (*GOT*-1; liver and muscle). One faster migrating allele has been seen in a heterozygous state for one individual from the SRP. The protein is a dimer as in other mammals.

Lactate dehydrogenase-1 (*LDH*-1; kidney and liver). See Figure 2.

Malate dehydrogenase-2 (*MDH*-2; heart, liver and muscle). This form migrates just cathodal to the origin and subbands anodally.

Malic enzyme (ME; liver). This enzyme appears as a single band anodal to *MDH*-1 in deer.

Glucose-6-phosphate dehydrogenase (G-6-P; liver). *G-6-P* appears as a single anodal band.

Esterases (ES-1, plasma; *ES*-3, hemolysate; and *ES*-4, liver). *ES*-1, assayed on lithium hydroxide consists of a single band and is eserine sensitive. *ES*-3, the fastest migrating band on tris-maleate gels is not eserine sensitive. *ES*-4 appears anodal to *ES*-2 on tris-hydrochloric acid gels treated with eserine.

DISCUSSION

Most proteins are thermally unstable to varying degrees, breaking apart at temperature-dependent rates. Denaturation products show up on gels as either subbands or, in extreme cases, as broad smears with no well defined zones of activity or complete lack of bands. Since systems become more difficult to score with denaturization, it is imperative that samples be stored at appropriate low temperatures and assayed while still relatively fresh. In addition, if gels are not kept sufficiently chilled during electrophoresis, Coulomb heating can totally denature the samples being assayed.

Errors in the preparation of buffer solutions are evidenced in several ways. Proteins will fail to migrate appropriate distances from the origin and fail to separate sufficiently if the pH of the solutions is not accurate. The voltages listed in Table 1 for each buffer type can be achieved rather quickly (at most 10-15 minutes) at less than 100 milliamperes. Abnormally high amperage readings usually indicate problems with buffer solutions or, secondarily, with electrical connections.

Banding pattern anomalies are perhaps the most frequent problems encountered. Sponges should be properly aligned and it is imperative that solutions be freshly prepared and uncontaminated. Stain solutions should not be prepared until they are needed, however, most stain ingredients (e.g., NAD,NADP, PMS, MTT, NBT, etc.) may be prepared in small stock quantities if turnover time is rapid. Use of old stains or substrates results in faint or no banding patterns. Care should be exercised with chemicals used in stain solutions as some are metabolic poisons or carcinogens.

It is important that side by side comparisons be made of questionable alleles. Particularly in polymorphic systems, checks should be made of alleles present on different gels to confirm a consistent identification of alleles across samples. Photographs of each stained slice provide a direct record of results. However, scoring should be done immediately after staining since some systems fade quickly after staining. Slices stained for other systems (esterases and general proteins) may be tightly wrapped in plastic and stored for future reference.

The buffer specifications and staining procedures we have used are generally suitable to demonstrate these protein systems in many species, hence the potential applicability of these techniques extends beyond that discussed for white-tailed deer. Other systems not considered herein are

potentially demonstrable for many species from a technical standpoint. Studies of biochemical variation in humans (Harris 1975) provide examples of other systems which may be assayed. Notable examples of the use of electrophoresis for studying other wildlife species include the study of genetic correlates with density in blue grouse (Redfield 1973) and a systematic study of the eastern and New England cottontails (Chapman and Morgan 1973).

An important question is how information on population genetics can be used in the management of wildlife species, especially white-tailed deer. There are three primary ways in which these data might be useful: 1) identify the spatial extent of local populations, 2) determine the breeding structure of the populations, and 3) assay population quality from genetic indices. If two or more populations that differ in their characteristics are included within one management unit, the management program may not be appropriate for each population. A preliminary analysis of the SRP herd has revealed genetic differences between swamp and upland populations (i.e., in the frequency of heterozygotes, *h*, at a given locus) which correspond with major differences in their demographic characteristics (i.e., reproductive rates and age distribution; Urbston 1967). For example, while gene frequencies in swamp male and female fawns are not significantly different at the SDH locus, the males show greater heterozygosity at this locus compared to swamp females ($p < .05$, Table 2). This disparity is not observed in upland fawns. Further studies may reveal more than two populations in the area and hunting pressure may be modified according to the productivity of the identified populations. Thus management decisions can be made on the basis of precise information on the populations to be managed.

Table 2. Allelic frequencies and heterozygosity *(h)* at the *SDH* locus in fawns from two subpopulations on the Savannah River Plant, Aiken, South Carolina. *h* is the proportion of individuals heterozygous for a given locus.

Allele	*Frequency*			
	Swamp		*Upland*	
	Males (N=24)	*Females (N=32)*	*Males (N=75)*	*Females (N=85)*
a	0.042	0.031	0.040	0.070
b	0.375	0.313	0.213	0.200
c	0.583	0.656	0.747	0.730
h	0.750	0.531	0.400	0.435

Information concerning the breeding structure of a population can be important in a variety of ways. It allows an assessment of which animals are most responsible for the secondary productivity of the population, the extent to which inbreeding is occurring and gives a base from which to evaluate the possible effects of selection on animals of different genotypes. In addition there are genetic correlates which may prove useful in evaluating the quality of a population. For example, reproductive effort and aggressive behavior are correlated with genetic heterozygosity (Smith et al. 1975). Since deer appear to occur in spatially subdivided populations even within local areas, management programs that disrupt this structure may be useful in temporally increasing heterozygosity within these subdivided populations. An inbreeding coefficient can be calculated from the information gathered by the use of electrophoresis (Falconer 1960). The higher the coefficient the greater the inbreeding and the likelihood of a depression of fitness. Other measures associated directly with single loci may also prove useful in assaying population quality. Deer and other game species may represent an ideal opportunity to conduct long term temporal and geographical studies of gene frequencies and to apply this information to the management of their populations.

LITERATURE CITED

Avise, J. C., and G. B. Kitto. 1973. Phosphoglucose isomerase gene duplication in bony fishes: An evolutionary history. Biochem. Genetics 8(2):113.

Chapman, J. A., and R. P. Morgan, II. 1973. Systematic status of the cottontail complex in Western Maryland and nearby West Virginia. Wildl. Monogr. No. 36:5-54.
Chen, S., and H. E. Sutton. 1967. Bovine transferrins: Sialic acid and the complex phenotype. Genetics 56:425-430.
Falconer, D. S. 1960. Quantitative Genetics. Ronald Press, New York, 365 + vi pp.
Harris, H., 1975. Multiple allelism and isozyme diversity in human populations. In Isozymes, Vol. IV, C. L. Markert (ed.), Academic Press, New York. pp. 131-147.
Harris, M. J., T. H. J. Huisman, and F. A. Hayes. 1973. Geographic distribution of hemoglobin variants in the white-tailed deer. J. Mammal. 54:270-274.
Huisman, T. H. J., A. M. Dozy, and M. H. Blunt, 1968. The hemoglobin heterogeneity of the Virginia white-tailed deer; A possible genetic explanation. Arch. Biochem. Biophys. 127:711-717.
Miller, W. J., A. O. Haugen, and D. J. Roslien. 1965. Natural variation in the blood proteins of white-tailed deer. J. Wildl. Mgmt. 29(4):717-721.
Redfield, J. A. 1973. Demography and genetics in colonizing populations of blue grouse. Evolution 27:576-592.
Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse *(Peromyscus polionotus)*. Studies in Genetics. VI. Univ. Texas Publ. 7103:49-90.
Smith, M. H., C. T. Garten, Jr., and P. R. Ramsey. 1975. Genic heterozygosity and population dynamics in small mammals. In Isozymes, Vol. IV, C. L. Markert (ed.), Academic Press, New York. pp. 85-102.
Straney, D. O., M. H. Smith, I. F. Greenbaum, and R. J. Baker. Biochemical variation and genic similarity of *Myotis velifer* and *Macrotus californicus*. Comp. Biochem. Physiol. (In press).
Sutton, H. E., and G. W. Karp, Jr. 1965. Absorption of rivanol by potato starch in the isolation of transferrins. Biochem. Biophys. Acta 107:153-154.
Taylor, W. J., C. W. Easley, and H. Kitchen. 1972. Structural evidence for heterogeneity of two hemoglobin α chain gene loci in white-tailed deer. J. Biol. Chem. 247:7320-7324.
Urbston, D. F. 1967. Herd dynamics of a pioneer-like deer population. Proc. XXI Ann. Conf. Southeastern Assoc. Game and Fish Comm. pp. 42-57.

APPENDIX 1

PROTEIN ASSAY SOLUTIONS

The following abbreviations are used in descriptions of stains: NAD=β−nicotinamide adenine dinucleotide; NADP=nicotinamide adenine dinucleotide phosphate; NBT=nitro blue tetrazolium; MTT=MTT tetrazolium; and PMS=phenazine methosulfate. These are made up as 1% stock solutions and kept refrigerated. While staining, gel slices are incubated in the dark at 37°C, unless otherwise noted. When sufficiently stained, gel slices are washed and fixed in a 1:5:5 mixture of acetic acid, methanol, and water.

1. Esterases *(ES)*. Tissues: plasma (buffer system: 1), liver (buffer system: 1, 2 and 5). 4ml sodium phosphate buffer (stock solution=1:1 mixture of 0.1m monobasic sodium phosphate, pH 4.4, and 0.1 M dibasic sodium phosphate, pH 8.7) in 45ml of water. To inhibit certain esterases, add 35mg eserine sulfate and incubate 20 minutes in light prior to staining. To stain add 1.5ml of 1% β−naphthyl proprionate in acetone (1g βNP to 100ml acetone) and 25mg Fast Blue RR salt to phosphate buffer. Incubate 10 to 30 minutes in light at room temperature.
2. Hemoglobin *(HB)*, transferrin *(TRF)*, albumin *(ALB)*, and other "general" proteins. Tissues: hemolysate (buffer system: 1). Plasma and liver (buffer system: 2). Stain: 2% solution of Buffalo Black NBR (naphthol blue black) in fixing solution. Stain for 20 minutes at room temperature. Stain may be re-used; wash gels in fixing solution several times until background is pale.
3. Glutamate oxalate transaminases *(GOT)*. Tissues: liver, heart, kidney and muscle (buffer system: 4). Stain: 0.5mg Pyridoxal - 5' - phosphate, 200mg L - Aspartic acid, 100mg α - Ketoglutaric acid, 150mg Fast Blue BB in 50ml 0.2 tris-hydrochloric acid buffer (pH 8.0). Incubate 10 to 15 minutes or until green bands appear. Do not add buffer until ready to stain.
4. α - Glycerophosphate dehydrogenase *(GPD)*. Tissue: liver and muscle (buffer system: 3). Stain: 1ml 0.1 M magnesium chloride, 50mg disodium α - DL - glycerophosphate, 20mg NAD, 13mg NBT, and 5mg PMS in 50ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). Incubate in the dark for 1 to 2 hours.

5. Indophenol oxidase *(IPO)*. Tissue: liver (buffer system: 3). Stain: 15mg NBT or MTT, and 10mg PMS in 50ml 0.2 tris-hydrochloric acid buffer (pH 8.0). Incubate in the light for 1 to 2 hours or until pale bands appear against blue background. *IPO* usually appears on all gels stained for dehydrogenase activity, but for deer the best patterns are demonstrated with liver extract stained for *PGM*.
6. Isocitrate dehydrogenases *(IDH)*. Tissues: heart, kidney and muscle (buffer system: 4). Stain: 0.2ml 0.25 M manganese chloride, 2ml 0.1 M trisodium DL-isocitric acid, 8mg NADP, 4mg NBT, 4mg MTT and 5mg PMS in 30ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). Incubate for 30 to 60 minutes in the dark.
7. Lactate dehydrogenases *(LDH)*. Tissues: kidney and muscle (buffer system: 4). Stain: 6ml 0.5 M lithium DL-lactate, 13mg NAD, 3mg NBT, and 5mg PMS in 30ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). Incubate in dark for 1 hour.
8. Malate dehydrogenases *(MDH)*. Tissues: heart, liver and muscle (buffer system: 4). Stain: 5ml 2.0 M DL-malate (stock solution: 268.2g DL-malic acid, 100ml water; pH adjusted to 7.0 with NaOH) 20mg NAD and/or 10mg NADP, 20mg NBT, and 5mg PMS in 30ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). Incubate in the dark for 30 minutes (NAD-*MDH*) to 2 hours (NADP-*MDH*). Malic enzyme is NADP dependent and may be stained as for *MDH*.
9. Phosphoglucomutases *(PGM)*. Tissues: liver and muscle (buffer system: 3). Stain: 4ml 0.05 M disodium α-D-glucose-1-phosphate, 4ml 5×10^{-4} M dipotassium α-D-glucose-1, 6-diphosphate, 4ml 0.1 M magnesium chloride, 3.2ml glucose-6-phosphate dehydrogenase (10 units/ml water), 5mg NADP, 5mg MTT, and 2mg PMS in 20ml water and 4ml 0.2 tris-hydrochloric acid buffer (pH 8.0). Incubate in dark for 1 hour. Bands fade rapidly.
10. 6-Phosphogluconate dehydrogenase (6-*PGD*). Tissues: hemolysate and liver (buffer system: 5). Stain: 7ml 0.1 M magnesium chloride, 20mg barium 6-phosphogluconic acid, 1mg NADP, 4mg MTT, and 1mg PMS in 10ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). Incubate in the dark at room temperature. Bands develop fully in 1 to 2 hours, then fade rapidly when gel is washed. In preparing gel, add 1ml of a 1% solution of NADP prior to aspirating.
11. Phosphoglucose isomerase *(PGI)*. Tissues: liver and muscle (buffer system: 3). Stain: 10ml 0.1 M magnesium chloride, 1ml 0.018 M disodium D-fructose-6-phosphate, 3ml glucose-6-phosphate dehydrogenase (10 units/ml water), 3mg NADP, 5mg MTT, and 3mg PMS in 30ml 0.2 M tris-hydrochloric acid buffer (pH 8.0). Incubate in the dark for 30 to 60 minutes. Bands fade rapidly.
12. Acid phosphatase *(AP)*. Tissue: liver (buffer system: 4). Stain: 50mg Na-α-napthyl acid phosphate and 50 mg of Fast Garnet dissolved in 50 ml of 0.05 M acetate pH 5.0 (=6.8 g sodium acetate and 15 ml of 1 N HCl dilute to 1 liter with deionized water; adjust pH with 0.1 N HCl). Incubate 1 to 2 hours.
13. Glutamate dehydrogenase *(GDH)*. Tissue: liver, heart and kidney (buffer system: 4). Stain: 2.1g L-glutamic acid 50mg NAD, 35mg NBT, 5mg PMS in 30ml 0.1 M dibasic sodium phosphate, pH 8.7 in 18ml deionized water. Incubate 30 minutes to 1 hour.
14. Glucose-6-phosphate dehydrogenase *(G-6-P)*. Tissue: liver (buffer system: 3). Stain: 4ml tris-hydrochloric acid buffer, pH 7.1, 2.3ml 0.25 M G-6-P (=9.6g disodium glucose-6-phosphate in 100ml deionized water), 11mg NADP, 8mg NBT, 2mg PMS in 30ml deionized water. Incubate 1 to 2 hours.
15. Sorbitol dehydrogenase *(SDH)*. Tissue: liver (buffer system: 3). Stain: 0.5g D-sorbitol, 10mg NAD, 15mg NBT, 15mg MTT and 2mg PMS in 50ml 0.2 tris-hydrochloric acid buffer, pH 8.0. Incubate 30 minutes to 1 hour.

APPENDIX 2

ELECTROPHORETIC BUFFER SYSTEMS

1. Tris-hydrochloric acid. Electrode: 0.30 M borate, pH 8.2 (=18.55g boric acid and 2.40g sodium hydroxide dissolved and diluted to 1 liter with deionized water). Gel: 0.01 M tris-hydrochloric acid, pH 8.5 (=1.21g tris diluted to 1 liter; pH adjusted with concentrated hydrochloric acid). Potential: 250 v for 2 hours.
2. Lithium hydroxide. Electrode: Stock solution A. Gel: 1:9 mixture of stock solutions A and B. Stock solution A: 0.03 M lithium hydroxide, 0.19 M boric acid, pH 8.1 (=1.20g monohydrate lithium hydroxide and 11.89g boric acid diluted to 1 liter). Stock solution B: 0.05 M tris -0.008 M citric acid, pH 8.4 (=6.2g tris and 1.6g monohydrate citric acid diluted to 1 liter). Potential: 300-350 v until buffer line reaches anodal sponge (ca. 4 hours).

3. Discontinuous tris-citrate (Poulik). Electrode: 0.30 M borate, pH 8.2 (=18.55g boric acid and 2.40g sodium hydroxide diluted to 1 liter). Add 10mg NADP to cathodal electrode tray. Gel: 0.076 M citric acid, pH 8.7 (=9.21g tris and 1.05g monohydrate citric acid diluted to 1 liter). Add 10mg NADP to gel solution before aspirating. Potential: 250-300 v until buffer line reaches anodal sponge (ca. 3½ hours).
4. Continuous tris-citrate. Electrode: 0.687 M tris -0.157 M citric acid, pH 8.0 (=83.2g tris and 33.0 monohydrate citric acid diluted to 1 liter; pH adjusted with 1.0 M sodium hydroxide). Gel: 1:29 dilution of electrode buffer. Potential: 130 v for 4½ hours.
5. Tris-maleate. Electrode: 0.10 M tris -0.10 M maleic acid, 0.01 M EDTA -0.01 M magnesium chloride, pH 7.4 (=12.1g tris, 11.6g maleic acid, 3.72g disodium salt of EDTA, and 2.03g magnesium chloride (hexahydrate) diluted to 1 liter; pH adjusted with 2.0 M sodium hydroxide). Add 10mg NADP to cathodal electrode tray. Gel: Same as electrode buffer. Add 10mg NADP to gel solution before aspirating. Potential: 100 v for 5 hours.

NUTRIENT CONTENT AND YIELD OF BURNED OR MOWED JAPANESE HONEYSUCKLE

by
JOHN J. STRANSKY
Southern Forest Experiment Station, Forest Service, USDA
Nacogdoches, Texas 75961[1]
JAMES N. HALE
Florida Department of Pollution Control
Tallahassee, Florida 32301[2]
LOWELL K. HALLS
Southern Forest Experiment Station, Forest Service, USDA
Nacogdoches, Texas 75961

ABSTRACT

Burning reduced the dense growth between 3-year-old Japanese honeysuckle plants and prevented the resprouting of runners. Mowing removed the dense accumulation of vines, but the severed runners resprouted to create a uniformly dense carpet. Crude protein of foliage was highest on the burned plots, but neither calcium nor phosphorus were significantly affected by the treatments.
Key words: Lonicera japonica, protein, phosphorus, calcium.

INTRODUCTION

Japanese honeysuckle *(Lonicera japonica)* produces an abundance of palatable and nutritious leaf-browse, which is available during the critical late fall and winter months when other nutritious browse is scarce (Segelquist *et al.* 1971, Craft and Haygood 1972). Some southern states rely extensively on honeysuckle for wildlife habitat management. Since little is known about managing honeysuckle on game food plots, this study was conducted to determine how yields and nutrient content are influenced by late winter burning or mowing.

METHODS

The study took place on an abandoned field at the Stephen F. Austin Experimental Forest near Nacogdoches, Texas. The area had not been cultivated, grazed, or burned since the mid-1950's. Soils were of the Kalmia and related Ruston series; they were moderately well-drained and well-drained upland fine sandy loams.

In February 1970 the area was plowed, disked, and planted with rooted honeysuckle cuttings spaced at 10 x 10 feet. One week later, 270 lbs. per acre of ammonium nitrate was spread over the area.

---

[1] In cooperation with the School of Forestry, Stephen F. Austin State University.
[2] At the time of the study, Hale was a graduate student at Stephen F. Austin State University.