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Phenotyping Phosphoglucose Isomerase in West Coast Cervids for Species Identification and Individualization

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ABSTRACT: Phosphoglucose isomerase (PGI) from 111 Columbia blacktail deer, 65 mule deer, 21 Sitka blacktail deer, 100 moose, 46 elk, 75 caribou, 46 reindeer, and 2 whitetail deer was examined using cellulose acetate electrophoresis. Mule deer and Columbia blacktail deer exhibited a biallelic polymorphism at the PGI-1 locus. Allele frequencies were 0.18 and 0.82 for Columbia blacktail deer and 0.70 and 0.30 for mule deer. No PGI variation was present in the other cervids examined, except for a single caribou variant. PGI phenotyping can be of value in individualizing meat from mule and blacktail deer for wildlife law enforcement. Comparison of PGI mobilities can also be used to differentiate between several of the monomorphic species, and can differentiate between beef and all the cervids except elk.

KEYWORDS: criminalistics, phosphoglucose isomerase, big game animals, deer, elk, moose, caribou, reindeer, beef, enzymes, isozymes, phenotyping, electrophoresis

The individualization of deer blood or meat is of primary importance to the criminalist attempting to determine if a game violation involves more than one deer, or trying to compare a bloodstain or portion of meat to a given deer kill.

Previous serologic techniques for the identification of cervid tissues have centered about using immunochemical methods for identification of the family level and then applying electrophoresis to identify the species [*1,2*]. Individualization within a big game species is not a common forensic science technique [*3*].

A survey of the literature revealed limited information on enzyme polymorphisms on blacktail and mule deer. Enzyme screening of other cervids have found variation present at low levels, with the greatest average heterozygosity present in whitetail deer [*4*].

The methods employed in these previous studies were starch gel electrophoresis, which requires an overnight run [*5*] and is sometimes difficult to interpret because of background staining. The use of cellulose acetate as a support medium for electrophoresis of human bloodstains has been well documented [*6*]. This medium allows for a small sample size and permanent preservation of the stained results. The phosphoglucose isomerase (PGI) isozymes of up to 20 samples can be separated in 30 to 40 min using this technique.

This study describes the polymorphism of glucose 6-phosphate isomerase (PGI: EC 5.3.1.9)

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in Columbia blacktail deer (*Odocoileus hemionus columbianus*) and mule deer (*Odocileus hemionus hemionus*), and the relative mobilities of PGI from Sitka blacktail deer (*Odocoileus hemionus sitkensis*), whitetail deer (*Odocoileus virginianus*), Roosevelt elk (*Cervus elaphus roosevelti*), Rocky mountain elk (*Cervus elaphus nelsoni*), moose (*Alces alces*), reindeer (*Rangifer tarandus*), and barren ground caribou (*Rangifer tarandus granti*) as determined by cellulose acetate electrophoresis. Proteins from muscle, whole blood, serum, and bloodstains on paper and cloth were examined.

Materials and Methods

Bloodstains from Columbia blacktail deer, mule deer, whitetail deer, and elk were randomly collected from carcasses by game officers of the Oregon State Police. Whole blood and muscle were also submitted to the laboratory when possible. These specimens were obtained incident to contact with successful hunters in the field or upon removal of road-killed animals. Meat, blood, and serum samples from Sitka blacktail deer, moose, elk, reindeer, and caribou were collected by Alaska Department of Fish and Game biologists from chemically immobilized animals or by Alaska Division of Fish and Wildlife Protection Officers contacting hunters in the field. Bovine blood samples were collected from local slaughter houses. Samples from both sexes were collected. All specimens were frozen at −20°C. The specimen age varied from one week to one year.

Meat samples were homogenized in the membrane buffer or water, for a final dilution of 1:50. The bloodstains were also dissolved in the membrane buffer or water. The dilution was dependent on the size and concentration of the stain, 1:1 to 1:3 being the average. Those meat and blood extracts were centrifuged (12 000 × *g*) for 3 min before electrophoresis. The use of sulfhydryl reagents does not alter isoenzyme patterns or improve the results on old stains.

Samples were electrophoresed on either Beckman Microzone or Sartorious Sartophor equipment using Sartorious 12200BB 70/145 membranes. The buffers and reaction mixtures were those described by Grunbaum [*6*] and Wolfe [*7*]: a Tris (hydroxymethyl) amino methane-borate stock tank buffer (109.03 g of Tris, 30.92 g of boric acid, and 7.44 g of ethylenediamenetetraacetate (EDTA) were dissolved to 1 L distilled water) was adjusted to pH 8.7. A 1:20 dilution in distilled water was used for the membrane buffer. A 1:7 dilution of stock was used for the working tank buffer. Specimens were usually electrophoresed at 400 V for 40 min. Double row 20-sample applications were run at 300 V for 30 min. The minimum sample size was 0.5 μL. The reaction mixture consisted of 13 mg of fructose-6-phosphate, 3 mg of nicotinamide-adenine dinucleotide phosphate (NADP), 12.5 U of glucose-6-phosphate dehydrogenase, 3 mg of 3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyl tetrazalium bromide (MTT), and 3 mg of phenazine methosulfate (PMS) in 5 mL of 0.06*M* Tris, pH 8.0 buffer. This was added to 0.25-g Noble agar liquified in 10-mL buffer.

Results and Discussion

PGI exhibits a definite polymorphism in only two of the eight cervid species/subspecies examined (Table 1). This polymorphism in Columbia blacktail and mule deer appears to be a biallelic polymorphism at the PGI-1 locus, with three phenotypes present in both subspecies (Fig. 1). Both homozygous phenotypes appear as a three-banded pattern, with the most cathodal band of each phenotype being the most intense. The heterozygous phenotype appears as a six-banded pattern with the intermediate bands being the most intense. Such an isozyme pattern would be expected from a dimeric enzyme coded for with two loci, one fixed for a single allele and one polymorphic with two alleles. A similar pattern was found in Scandanavian moose by Rymen et al [*8*], in which those moose exhibited variation at the anodal locus PGI-2; however, no moose homozygous for the rare allele were reported in Ryman's study.

Multiple secondary anodal banding was observed in many samples in the present study,

TABLE 1—*Listing of PGI screening results for eight members of the Cervidae.*

Species	Locale	Number	Allele Frequencies, PGI-1
Mule deer	Oregon	65	0.70, 0.30
Columbia blacktail deer	Oregon	111	0.18, 0.82
Sitka blacktail deer	Alaska	21	monomorphic
Whitetail deer	Oregon	2	monomorphic
Elk	Oregon	45	monomorphic
Elk	Alaska	2	monomorphic
Moose	Alaska	100	monomorphic
Caribou	Alaska	75	monomorphic[a]
Reindeer	Alaska	46	monomorphic

[a]One variant was found; the small sample size precludes classifying caribou as polymorphic.

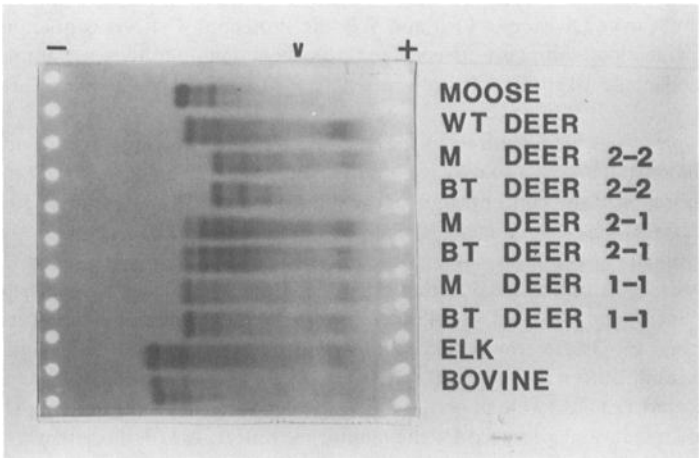

FIG. 1—*Cellulose acetate electrophoretogram of blood samples from moose, whitetail* (WT) *deer, Columbia blacktail* (BT), *mule* (M) *deer, elk, and beef* (bovine) *stained for PGI. Deer PGI pattern identifications are listed along the right side. All deer samples were dried blood extracts. The ten samples were applied at the arrow. (Photo by Peckles).*

however, it did not appear to have a genetic basis. Over 80% of the reindeer in our study exhibited additional anodal bands, and no pattern consistent with an anodal rare allele homozygote was found. Multiple secondary anodal banding in a deer PGI Type 1-1 could be confused with a Type 2-1 if the criteria that the inner bands must be the most intense were not applied. Although the moose, caribou, reindeer, whitetail deer, elk, and Sitka blacktail deer samples examined in this study were monomorphic, the relative mobilities of PGI can still be of use for species identification (Figs. 1 and 2). Elk and beef can be readily differentiated from the rest of the cervids, and Sitka blacktail deer (monomorphic for PGI Type 2-2) can be distinguished from Alaska moose and caribou. These relative mobilities are identical to what Baccus et al [*9*] reported in their study, except they observed no variation in mule deer PGI. This discrepancy was probably due to their small sample size (N 2 for mule deer).

Our reporting of whitetail deer PGI as being monomorphic, although based on small sample size, is supported by the large number of whitetail deer examined by Baccus (N = 753). Although no confirmatory family studies were performed to prove the PGI variation is actually

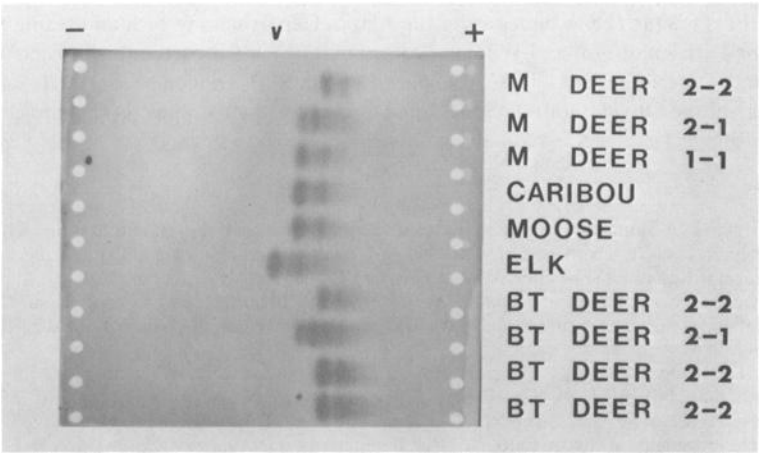

FIG. 2—*Cellulose acetate electrophoretogram of meat samples from mule deer* (M), *caribou, moose, elk, and blacktail* (BT) *deer. Some deer samples were frozen for up to three years. (Photo by Peckles).*

genetic, the observed frequencies closely match the Hardy-Weinberg expectations ($P > 0.90$). The banding patterns are also similar to what Vanderberg and Stone [*10*] observed in Rhesus monkeys, in which the three phenotypes were determined by two autosomal codominant loci PGI-1 and PGI-2.

It is interesting that the most common PGI phenotype in Columbia blacktail deer is Type 2-2 where the most common phenotype for mule deer is Type 1-1 (Table 2). Since both subspecies of deer can interbreed, it is fortunate that the rarer phenotypes of each subspecies were also found in areas of Oregon where the complimentary species is not found. In the high Cascade Mountain areas where blacktail and mule deer are known to interbreed, samples from bucks only were used. The subspecies was determined by the antler fork pattern.

Identification of the PGI patterns in game enforcement is useful for quickly differentiating between certain closely related members of the deer family for species identification. It also provides a useful "blood type" for mule deer and Columbia blacktail deer in situations involving multiple deer carcasses.

TABLE 2—*Phenotype and gene frequencies for PGI in Columbia blacktail deer and mule deer. Expected frequencies calculated assuming Hardy-Wienberg Equilibrium.*

	Phenotype				Gene Frequency	
Population Sample	1	2-1	2	Total	PGI^1	PGI^2
Blacktail deer						
Number observed	3	31	77	111	...	...
Incidence observed	0.027	0.279	0.693	...	0.178	0.822
Incidence expected	0.032	0.292	0.676	...	...	...
Mule deer						
Number observed	31	29	5	65	...	...
Incidence observed	0.477	0.446	0.077	...	0.700	0.300
Incidence expected	0.490	0.420	0.090	...	...	...

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