A Forensics Program for Identification of Fish and Wildlife Species

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Abstract: As a part of a cooperative effort between the Fisheries and Law Enforcement Division of the Texas Parks and Wildlife Department, a comprehensive program for forensic identification of fish and wildlife species has been implemented. Results of comparisons of blood stains from various wildlife species indicate that isoelectric focusing of blood produces genetic "fingerprints" that are characteristic for individual species. Results of 3 representative cases are reported and the program enactment is outlined.

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Species identification of evidentiary material has been a difficult task ever since the first game warden or conservation officer pinned on a badge and stopped the first night hunter. Methods for determining the species of origin of samples of blood or pieces of animal flesh have been investigated for many years with varying degrees of success. The use of precipitin tests (Boyden 1926, Brohn and Korschgen 1950), serologic methods (Whitehead et al. 1974), and immunodiffusion/immunoelectrophoretic methods (Oates and Weigel 1976) proved to be very useful in species identification.

More recently, protein separatory techniques such as starch-gel electrophoresis (Morgan et al. 1976) and isoelectric focusing (Oates et al. 1979) have been employed to evaluate natural genetic variation of fish and wildlife populations. These techniques, particularly isoelectric focusing, can be used to separate and characterize proteins with extraordinary resolution (Vesterberg and Svensson 1966). The utility of isoelectric focusing and its remarkable level of resolution makes this technique a prime candidate for use in forensic identification of samples of blood and

tissue. Preliminary studies (Bunch et al. 1976, Oates et al. 1979, Lawton and Sutton 1982) indicate that isoelectric focusing of blood and muscle tissue provides protein banding patterns which can be used to successfully identify species of origin of these samples.

The law enforcement potential for such a technique is both obvious and enormous. In some situations, the only evidence of a suspected game-law violation may be (for example) a drop of blood, a piece of tissue, or a fish fillet. The ability to determine the species of origin of such evidentiary samples could allow prosecution of a suspected offender when, in the past, such offenders might not even be cited. In addition, such a capability would likely serve as a significant deterrent to some types of game law violations.

In a cooperative effort between the Fisheries and Law Enforcement Divisions of the Texas Parks and Wildlife Department (TPWD), we are using isoelectric focusing of tissue and blood for development of a comprehensive library of genetic "fingerprints" of important fish and wildlife species and in provision of scientific evidence for prosecution of game law violations. The objectives of this paper are three-fold: (1) to describe the techniques and equipment we are currently using for species identification of blood samples, (2) to report the results of 3 cases representing use of this technique, and (3) to outline the program we have set up for collection of reference samples of fish and wildlife species.

We would like to acknowledge Bill Rutledge, Chester Burdett, and a multitude of state game wardens for their support of this project. Most of the samples used in baseline data determinations for this study were provided by Calvin Turner, Law Enforcement Division, and Donald Chumley, Fisheries Division, TPWD.

Methods

Sample Preparation

Blood samples of several different game and domesticated species (Fig. 1) were collected, placed in individual weigh-boats, and allowed to dry to completion at room temperature. Samples were then stored in plastic vials at 1° C until analyzed by isoelectric focusing. Blood samples were rehydrated with deionized water. Approximately 2 μl of solution was placed in a weigh-boat and again allowed to air dry. Dried bloodspots were then rehydrated with 2 μl of 0.1 M dithiothrietol (Cleland's reagent) immediately prior to electrofocusing.

Gel Preparation and Running Conditions

Gels were prepared with acrylamide and N,N-methylenebisacrylamide such that a final composition of 7.5% T (acrylamide in the gel) and 3% C (crosslinker) was achieved. The gels also contained a pH 7–8 carrier ampholyte (3% wt/vol). The resultant gels were 0.25 mm thick and were formed using the "flap" technique described by Radola (1980). Gels were run on a flatbed electrofocusing unit (LKB–Produkter) with temperature held at 10° C for the entire focusing period.

The anolyte consisted of 0.5 M acetic acid while the catholyte solution used was 0.5 M sodium hydroxide. Initial power was set at 3.5 watts with a voltage limitation of 1,700 volts. Current was adjusted so that the starting voltage was 150 volts. Gels were prefocused for 1 hour prior to application of the samples.

Samples were applied using a mask with 1.0 mm × 10.0 mm wells. The mask was placed 2.5 cm from the anode and 2 μl of sample was placed in each well. The application mask was removed after all the samples entered the gel, usually within 30 minutes. Samples were focused until the voltage reached the 1,700 volt limit. This required approximately 6 hours of total run time.

Gels were fixed for 5 minutes in 200 ml of 20% trichloroacetic acid and then washed for 5 minutes in 200 ml of destaining solution (35% ethanol and 10% acetic acid in water). Gels were stained with 300 ml of 0.5% Brilliant Blue–R in destaining solution. Gels were destained with several changes of destaining solution and then allowed to air dry.

Results

Species Identification

Initial results of isoelectric focusing of several game and non-game species indicate that different species of animals have very different banding patterns (Fig. 1). While some variation within species is present, these banding patterns tend to be quite recognizable with little overlap in the isoelectric region characteristic of each species.

These results are also quite reproducible when Cleland's reagent is used as part of the processing procedure. The utility of the procedure lies in the fact that dried blood samples are identifiable for as long as 5 years after drying (Harvey, unpubl.

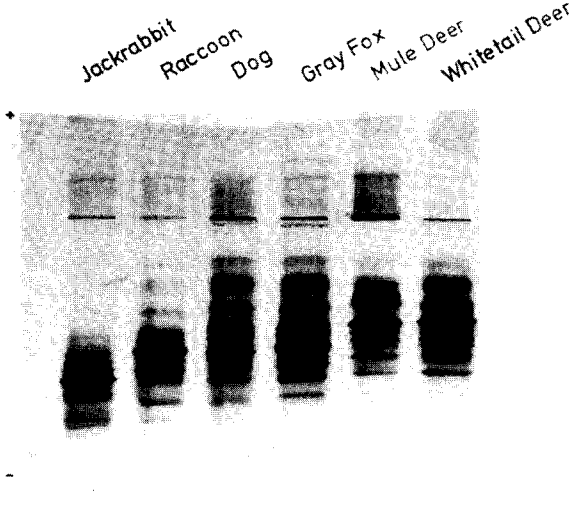

Figure 1. Comparison of blood stains of 6 different species. Proteins were separated by isoelectric focusing and illustrate differences in species specific blood proteins.

data) and the procedure can be done on samples of dried blood that are as small as 1 mm in diameter.

Case Results

Case 1

As a result of a call to Texas' Operation Game Thief program, a state game warden was dispatched to the home of a suspected poacher. The suspect admitted to having killed a feral Spanish goat which, in this case, did not constitute a game-law violation. The warden found traces of fresh blood in the bed of the suspect's pickup truck and submitted these for analysis.

Comparison of the samples collected by the warden to samples of Spanish goat and white-tailed deer indicated that the blood stains were indeed not those of goat, but rather those of a white-tailed deer (Fig. 2). When presented with this evidence, the suspect admitted the game-law violation and subsequently paid the levied fine.

Case 2

During a routine license check a Texas state game warden noticed blood on the clothing of 2 hunters stopped for the check. The suspects claimed to have killed a javelina and had already processed the animal. The warden subsequently noticed hair in the bed of the pickup being driven by the suspects and believed the hair was atypical of javelina.

Samples of blood extracted from the clothing of the suspects was compared with that of javelina and several other species. The banding patterns of these samples were consistent with those of white-tailed deer and were distinctly different from those of javelina (Fig. 3). The suspects subsequently pleaded guilty to a game-law violation and paid the levied fine.

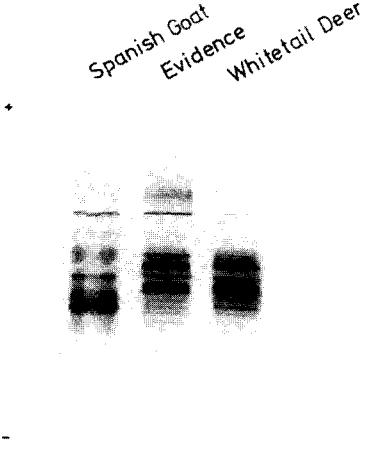

Figure 2. Comparison of banding patterns of blood stains from Spanish goat and white-tailed deer to those of samples collected as evidence. Note the similarity of white-tailed deer to evidence and dissimilarity of Spanish goat.

Case 3

While investigating a report of illegal "night hunting" a state game warden came upon several "varmint hunters." The hunters claimed that they had not taken any animals during the hunt, yet both the clothing of one hunter and the pickup bed of the hunters' vehicle were noticeably blood stained. The driver of the pickup stated that the blood stains came from a dog which had been accidentally struck with the vehicle and later disposed of.

Samples from both the clothing of the suspect and the pickup bed were submitted for analysis. Comparison of the blood samples and several game species indicated that the samples in question were not those of a game species and were,

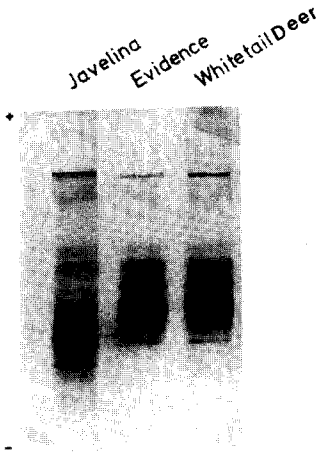

Figure 3. Comparison of banding patterns of javelina and white-tailed deer blood stains to that of samples submitted as evidence. Note the similarity of the white-tailed deer pattern to that of evidence and the lack of similarity to that of javelina.

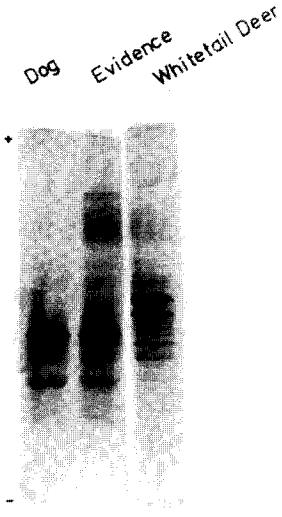

Figure 4. Comparison of banding patterns from blood stains of domestic dog and white-tailed deer to those of samples submitted as evidence. The banding pattern of the evidence is similar to that of dog and not white-tailed deer.

most likely, those of some type of dog (Fig. 4). The suspect later admitted to having shot his neighbor's troublesome German shepherd.

These cases represent just 3 of many that the TPWD Game Wardens are confronted with each year. Each of these would have been virtually impossible to investigate further without the utilization of the technology we have begun to employ. However, in many cases, violators will not plead guilty to charges and the case will eventually go to court. In these situations, a strong body of background genetic information concerning the characteristics of fish and wildlife species must be in place to support the validity of the technique to allow judges and juries sufficient information to evaluate the evidence, and, most importantly, to protect the accused. In order to secure this information, the Law Enforcement and Fisheries Divisions of TPWD have put into motion a comprehensive plan for development of a genetics library for fish and wildlife species.

Program Implementation

The TPWD Law Enforcement Division field staff is divided into 10 administrative regions. Each region is headed by a regional director. Under this program, it is the responsibility of these regional directors to compile a listing of those fish and wildlife resources which are considered under the classifications of game species, protected species, endangered species, and certain unprotected species. This listing will also include certain domestic or exotic species that may be pertinent. Representative samples of human blood will also be submitted and analyzed.

From each region, 10 samples of blood, meat, and hair or feathers (when pertinent) will be submitted for analysis. Reference samples, like evidence, will be submitted using a strict chain-of-custody providing a background sample total of 100 from each of these species. This will allow for comparison of genetic variation between regions of the state and will provide necessary background information for use in litigated cases.

After samples have been analyzed by isoelectric focusing or other separatory techniques (i.e. electrophoresis, 2 dimensional separation), the resulting genetic "fingerprints" will be analyzed by scanning with a laser-scanning densitometer. This information will then be loaded onto a computer disc and stored for future reference.

After this information is in place, evidentiary samples can be analyzed through computer comparison of the reference library and the sample in question. The computer comparison will then give a best "fit" of the evidence to those samples in the library. The result is an objective determination of the species of origin for evidentiary samples. This will minimize any bias that might be associated with a strictly visual interpretation of genetic data and will present a much stronger basis for the utilization of such evidence in court.

Discussion

The implementation and execution of this program will have numerous positive effects. First, the program will allow state wardens to pursue many cases that were essentially impossible to even consider in the past. Those cases which involve only the intuition of the warden and a few spots of blood can now be successfully investigated.

We believe that the program will act as a significant deterrent to many types of game-law violations. We are actively spreading word of this program through various media sources in order to publicize both the program itself and the success we have enjoyed to this point. This program may not slow the "hard core" violator, but it is likely that it will significantly impact illegal hunting and fishing through discouragement of a percentage of illegal activity.

This program will also serve as another mechanism for protection of the resources themselves. Each time that an animal is spared as a result of this program, the overall resource is enhanced. The effect is the same as if another fawn is produced, another turkey hatched, another largemouth bass spawned.

Last, but certainly not least, this will serve as a powerful addition to those tools available to the game warden. The results of tests can be reported in a matter of hours and provide hard scientific evidence. This should make the warden's task a little easier, more fruitful, and more rewarding. It could conceivably result in fewer cases, less time spent in investigation of some cases, and in a higher success rate in prosecution of offenders.

We are fully cognizant of the enormity of the task before us in the successful enactment of this program. It could well take 5 years or more to develop a workable catalog of the genetic resources of the state of Texas. However, the technology is available for such a comprehensive program and the benefits will far outweigh the costs in the long term.

The TPWD has been given the privilege of both the guardianship and stewardship of the fish and wildlife resources of the state. Innovation is the key to meeting and managing those demands placed upon our wildlife resources. With this in mind, we are certain that this program will play a major role in the continued protection and enhancement of the living natural resources of Texas.

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