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ScienceDirect
Forensic Science International: Genetics 2 (2008) 190–197
FSI GENETICS
www.elsevier.com/locate/fsig

DNA typing in populations of mule deer for forensic use in the Province of Alberta

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Received 31 December 2007; accepted 25 January 2008

Abstract

The present study involves the development of forensic DNA typing tests and databases for mule deer in the Province of Alberta. Two multiplex PCR reactions interrogating 10 loci were used to analyze samples from three populations of mule deer. Additionally, an amelogenin based sex-typing marker was used to determine the gender of samples. Results show that the tests and databases are appropriate for use in forensic applications. Additionally, the results indicate that there is little population structure in mule deer in Alberta and that no changes to management of this game species are suggested.

Keywords: Forensic science; Wildlife; Alberta; Mule deer; DNA typing; Microsatellite; *Odocoileus hemionus*; Poaching; Polymerase chain reaction; Sika amelogenin; BM4107; T7; Rt30; Rt7; Rt5; BM1225; OheN; BM4208; OheQ

The mule deer (*Odocoileus hemionus)* is a highly prized North American big game animal that is hunted both as a trophy and also for its meat. Its range covers the Western portion of North America [1].

In Alberta, illegal taking of mule deer is an ethical and public safety concern. However, no estimates of how many deer are taken illegally have been made. In other areas in North America, radiocollar studies performed on deer indicate that the number of illegally taken deer is between 9 and 61% of the legal harvest [2–4]. These studies indicate that illegally taken deer represent a significant source of deer mortality, especially in areas that experience considerable hunting pressure. Protection of big game through enforcement activities presents some significant challenges. These difficulties stem from the fact that wildlife officers are typically responsible for patrolling large areas of undeveloped land. When deer are illegally taken, it usually takes place in remote areas where it is unlikely that there will be any witnesses who are not party to the offence. These circumstances make it quite difficult to detect these offences and make it even more difficult to convict individuals who commit them. However, the advent of forensic DNA typing has made it possible for officers to collect evidence from illegal kill sites and link it back to biological material that is associated with the individual who committed the offence.

In the present study, forensic DNA-typing tests and databases have been developed to protect mule deer in the Province of Alberta and surrounding territory. The goal of this project is to provide the court with acceptable evidence to assist in the prosecution of individuals who violate statutes designed to protect wildlife. DNA-based evidence has been used in the prosecution of human crime for over 20 years [5]. Currently, the analysis of microsatellite DNA via the polymerase chain reaction is the most commonly used forensic technique. This technology has been available to human forensics for over a decade [6,7]. DNA analysis has also been used in a forensic capacity in deer [8–10]. However, a majority of the published work on microsatellite analysis in deer has been aimed at answering questions regarding population structure and parentage [11–13]. The present study is the first in which DNA markers and mule deer populations are selected specifically to be used in a forensic capacity to protect mule deer in a defined geographic area. In addition to their use in a

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1872-4973/$ – see front matter
doi:10.1016/j.fsigen.2008.01.003

forensic capacity, data collected in this study may also be of interest to individuals who study and/or manage mule deer.

1. Methods

1.1. Location of deer populations

Three mule deer populations were used in this study to serve as forensic databases. The locations of these databases were selected so that they covered a majority of the mule deer range within the province and so that the number of samples available was adequate to constitute a forensic database. These databases included the following areas: (1) an area mostly to the south of Lethbridge, Alberta (STHMLD), (2) an area surrounding Lloydminster, Alberta (LYDMLD), and (3) an area surrounding Fort St. John, British Columbia (FSJMLD). The database samples were collected within a 100-km radius of the sites indicated in Fig. 1. Although some mule deer can be found north of the FSJMLD database, there are relatively few deer in the extreme northern part of the Province (unpublished data from government deer counts). This low density of animals and limited accessibility has resulted in too few samples being available for an adequately sized database to represent the northern limit of the species in Alberta.

1.2. DNA processing

Tissue samples were collected from hunter kills, found dead animals and animals that were culled as part of a disease prevention program. Samples were extracted using a Kingfisher ML purification system (Thermo Electron Corp., Waltham, MA, USA) and Kingfisher, genomic DNA purification kit (Thermo Electron Corp., Waltham, MA, USA). These kits have been discontinued but can be replaced by the MagExtractor, Nucleic acid purification kit (Toyobo Co. Ltd, Osaka, Japan). The DNA in each sample was quantified using a Biophotometer (Eppindorf, Hamburg, Germany). DNA samples were diluted, to a concentration of 5 ng/μl, in water, which was filtered, autoclaved and deionized. Different DNA extraction techniques were not compared in this study; however, the methods used in this study have been shown to be effective in extracting DNA from biological material commonly encountered in casework [14].

Fig. 1. An outline map of three Provinces in Western Canada (British Columbia, Alberta and Saskatchewan). This map also shows the locations of the mule deer populations studied, Fort St. John (FSJMLD), Southern Alberta (STHMLD) and Lloydminster (LYDMLD).

Primers for 10 microsatellite markers were utilized (Table 1). These markers were amplified in two multiplexed PCR reactions. Multiplex 1 consists of 1× concentration of GeneAmp® PCR Buffer II (Roche Molecular Systems Inc., Almeda, CA, USA), 2.1 mM $MgCl_2$ (Roche Molecular Systems Inc., Almeda, CA, USA), 20 mM GeneAmp® dNTP mix with dTTP (Roche Molecular Systems Inc., Almeda, CA, USA), 3 U AmpliTaq Gold® (Roche Molecular Systems Inc., Almeda, CA, USA), 0.10 μM BM4107 primers, 0.20 μM T7 primers, 0.20 μM Ovir A primers, 0.20 μM Rt30 primers, 0.16 μM Rt7 primers, 5 ng DNA template and enough filtered–autoclaved–deionized water to make a 25-μl reaction volume. Multiplex 2 consists of GeneAmp® PCR Buffer II (Roche Molecular Systems Inc., Almeda, CA, USA), 1.8 mM $MgCl_2$ (Roche Molecular Systems Inc., Almeda, CA, USA), 20 mM GeneAmp® dNTP mix with dTTP (Roche Molecular Systems Inc., Almeda, CA, USA), 3 U AmpliTaq Gold® (Roche Molecular Systems Inc., Almeda, CA, USA), 0.08 μM Rt5 primers, 0.40 μM BM1225 primers, 0.20 μM OheN primers, 0.80 μM BM4208 primers, 0.80 μM OheQ primers, 10 ng DNA template and enough filtered–autoclaved–deionized water to make a 25-μl reaction volume. Primers for Rt7 and OheQ were produced by Applied Biosystems, Foster City, CA, USA. All other primers were produced by Integrated DNA Technologies, Coralville, IA, USA. Both multiplexes use the same amplification conditions. The amplification conditions are as follows: (1) (hot start) 95 °C for 4 min, (2) (denaturation) 94 °C for 30 s, (3) (annealing) 54 °C for 30 s, (4) (extension) 72 °C for 60 s, (5) steps 1 through 4 repeated 30 times, (6) (final extension) 60 °C for 45 min. Sika deer amelogenin primers were not included in the multiplex reactions. This locus is amplified alone when required under the following conditions: 1× concentration of GeneAmp® PCR Buffer II (Roche Molecular Systems Inc., Almeda, CA, USA), 1.3 mM $MgCl_2$ (Roche Molecular Systems Inc., Almeda, CA, USA), 20 mM GeneAmp® dNTP mix with dTTP (Roche Molecular Systems Inc., Almeda, CA, USA), 1 U AmpliTaq Gold® (Roche Molecular Systems Inc., Almeda, CA, USA), 0.20 μM sika amelogenin primers, 10 ng DNA template and enough filtered–autoclaved–deionized water to make a 25-μl reaction volume. The amplification conditions are as follows: (1) (hot start) 95 °C for 4 min, (2) (denaturation) 94 °C for 30 s, (3) (annealing) 57 °C for 30 s, (4) (extension) 72 °C for 60 s, (5) steps 1 through 4 repeated 30 times, (6) (final extension) 60 °C for 45 min. Amplified DNA was stored at −20 °C. The amplifications were performed on an MJ Research PTC-200 DNA Engine® (MJ Research Waltham, MA, USA).

The amplified DNA fragments were diluted (60 times dilution) in Hi-Di™ formamide (Applied Biosystems, Foster City, CA, USA) and a 400 times dilution of GenScan® 400HD [ROX] size standard was added to each sample (Applied Biosystems, Foster City, CA, USA). This mixture was