A marker for red deer — wapiti hybrids

P.A. DRATCH

Invermay Agricultural Centre
Ministry of Agriculture and Fisheries, Mosgiel

ABSTRACT

Red deer comprise over 80% of New Zealand farmed deer. The larger body and antler weights and faster growth rate of wapiti, originating in North America, have encouraged many deer farmers to hybridise these animals with red deer.

This study sought a biochemical marker in blood which could discriminate red deer, wapiti and F_1 hybrids. Blood samples were taken from over 200 red deer, North American wapiti, and F_1 hybrids. Haemolysed red blood cells were treated with potassium cyanide and subjected to isoelectric focusing (pH range 5 to 8). Starch gel electrophoresis was performed on untreated samples which were scored for both haemoglobin and superoxide dismutase.

Haemoglobin from North American wapiti had a higher isoelectric point than that of red deer. Haemoglobin of all F_1 hybrids tested showed a double banding pattern representing both parental types. All F_1 hybrids were also heterozygous for superoxide dismutase, though this marker was not absolute. It correctly discriminated 95% of the animals tested.

This blood test could be used to verify hybridisation in live deer sales, identify hybrids among captured deer, and select for wapiti if those traits are desired.

Keywords Wapiti; red deer; haemoglobin; superoxide dismutase; isoelectric point; hybrid marker.

INTRODUCTION

Red deer, which comprise over 80% of New Zealand's farmed deer, hybridise with both wapiti (Banwell, 1966: Murie, 1967) and sika deer (Lowe and Gardiner, 1975; Harrington, 1979). Interbreeding of red deer and wapiti has recently been used as a method of rapid genetic progress in body weight, velvet antler cut and growth rate (Moore, 1984). Verifying hybridisation can be difficult, however, particularly in immature animals. This study sought a protein marker which differs in red deer and wapiti so that controlled F_1 crosses could be objectively identified from a blood sample. Such a test is the first step in identifying hybrids when the parentage is unknown.

MATERIALS AND METHODS

Whole blood was collected in 10ml heparinised vacuum tubes from 159 red deer raised at Invermay and on commercial deer farms, 27 North American wapiti imported to New Zealand within the last 5 years, and 38 F_1 hybrids from red deer hinds sired by North American wapiti bulls. Samples were refrigerated until processed; they were then centrifuged (3000 RPM/15 minutes/4° C) and the plasma removed to plastic 5ml tubes. The remaining red blood cells were then washed with 0.88% sterile saline by repeated dilution and centrifugation. After washing, saline was removed and red cells were lysed with an equal volume of distilled water. Three lysate samples from each animal were put in plastic tubes and stored with the plasma sample at -75° C.

Red cells lysates were subjected to isoelectric focusing using an LKB Ultraphor. Prior to focusing, 1 ml lysate was further diluted with 2.5ml 1% potassium cyanide. Samples were then applied to 0.5mm agarose ampholine gels (pH range 5 to 8) by saturating Whatman #3 filter paper wicks. The anode electrode solution was 0.01M sulphuric acid and the cathode was 0.5M sodium hydroxide. Gels were focused at a maximum of 15 watts and cooled from beneath at 15° C. Sample wicks were removed after 10 minutes (900v/21mA) and focusing was complete after 30 minutes (1300v/13 mA). Gels were fixed in a dilute solution of ethanol-glacial acetic acid. Haemoglobins showed discrete red bands which could be scored without staining.

Samples from the same animals were also analysed by starch gel electrophoresis. Gels were 14% hydrolysed potato starch (Electrostarch Lot #392) dissolved in a citric acid buffer. (Electrode buffer 0.04M citric acid titrated to pH 6.0 with aminopropyl morpholine; gel buffer 1:19 dilution of electrode buffer, final gel pH 6.1). Filter paper wicks were saturated in untreated lysates from each animal and inserted into the gels midway between cathodal and anodal ends. Sample wicks were left in for 10 minutes (230v/30mA). The voltage was then increased (320v/50mA) for the remainder of the 3 hour run.

After electophoresis, gels were sliced into 1.5mm slabs. Haemoglobin bands were scored on one slab, and later checked for agreement with isoelectric focusing results. A second slab was put in a formazan dye bath and incubated at 38° C in the dark. When subjected to light after 15 minutes the slab stained purple, and unstained bands of the protein superoxide dismutase (SOD) could be scored.

RESULTS

The isoelectric focusing gels revealed 2 primary regions of haemoglobin activity (Fig. 1). Red deer bands focused more anodally (region A) than North American wapiti (region B). All F_1 hybrids had 2 haemoglobin bands, 1 in each region. After this difference between the 3 groups was found in 8 animals, subsequent samples were scored AA, AB, or,BB (Table 1) without prior knowledge of their parentage.

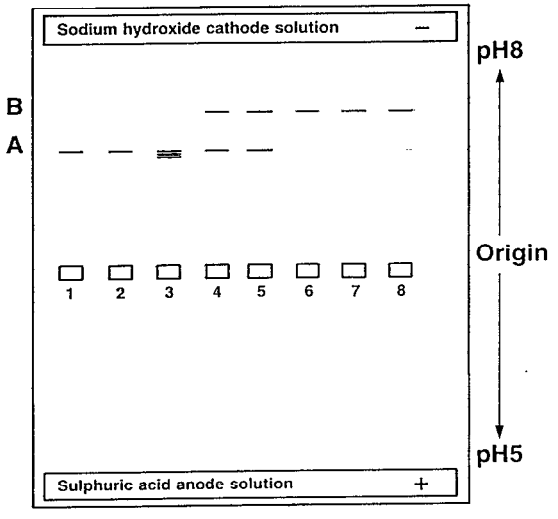

FIG. 1 Diagram of an agarose gel at the completion of isoelectric focusing. Lanes 1, 2 and 3 are red deer, 4 and 5 are F_1 hybrids and 6, 7 and 8 are North American wapiti. Lane 3 illustrates red deer region A sub-banding. From left to right, samples are scored AA, AA, AA, AB, AB, BB, BB, BB for haemoglobin.

TABLE 1 Genotype numbers observed for the 3 deer groups analysed by isoelectric focusing at the haemoglobin locus.

	n	AA	AB	BB
Red deer	159	159	0	0
F_1 hybrids	38	0	38	0
North American wapiti	27	0	0	27

Subsequent isoelectric focusing over narrower pH ranges (pH 6 to 8 and pH 6.7 to 7.7) suggested variation among red deer in region A banding. This variation, which requires further work to determine if it is inherited, did not prevent differentiation of red deer from wapiti or hybrids.

After the haemoglobin marker was found through isoelectric focusing, the experiment was repeated using starch gel electrophoresis. Haemoglobins of all deer types migrated anodally in the citric acid buffer. They again showed 2 different regions of activity in red deer and wapiti, and both regions of activity in hybrids. Though resolution was poorer than by isoelectric focusing, all but 2 samples were scorable and all genotypes matched the focusing results.

After formazan staining, superoxide dismutase (SOD) bands appeared to the cathode side of the origin. Homozygotes showed single bands in either of 2 regions, as with haemoglobin. Heterozygotes were 3-banded, however, with a bright band between the homozygous regions (Fig. 2). Depending on their migration distances from the origin, samples were scored homozygous slow (SS), homozygous fast (FF) or heterozygous slow-fast (SF). In 4 animals which were scored for haemoglobin, SOD genotypes could not be resolved. Complete superoxide dismutase results are listed in Table 2.

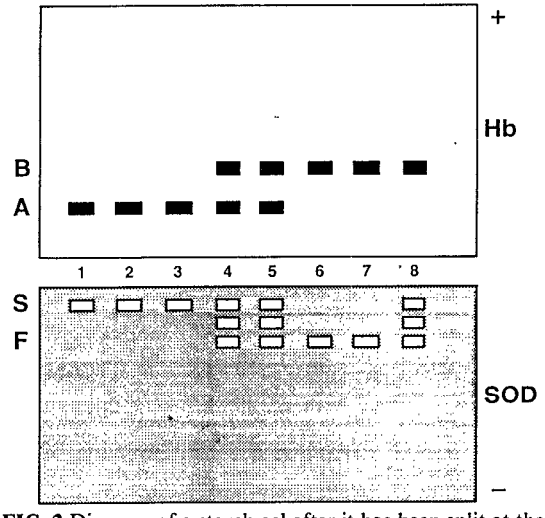

FIG. 2 Diagram of a starch gel after it has been split at the origin and the cathodal slab stained with formazan dye. Lanes 1, 2 and 3 are red deer, 4 and 5 are F_1 hybrids, and 6, 7 and 8 are North American wapiti. From left to right samples are scored for SOD: SS, SS, SS, SF, SF, FF, FF and SF. The heterozygous wapiti in lane 8 illustrates that SOD is not an absolute marker. The haemoglobin types are the same as in Fig. 1.

TABLE 2 Genotype numbers observed for the 3 deer groups analysed electrophoretically at the SOD locus.

		Superoxide dismutase		
	n	SS	SF	FF
Red deer	157	155	2	0
F_1 hybrids	37	0	37	0
North American wapiti	26	1	4	21

DISCUSSION

The recent trend among zoologists has been to consider red deer and wapiti one species (Bryant and Maser, 1982), particularly as interbreeding of intro-

duced animals in New Zealand has become widely known. Baccus *et al.* (1983), however, published evidence of fixed protein differences between these animals. Subjecting muscle homogenates to starch gel electrophoresis, they found red deer and wapiti had different alleles for albumin, esterase-4, malate dehydrogenase-1, mannose phosphate isomerase and superoxide dismutase.

Subsequent work with larger sample sizes has shown that for the latter 2 enzymes the differences are not absolute, though allele frequencies do differ in endemic red deer and wapiti populations (Dratch and Gyllensten, 1985). Nonetheless the Baccus study stimulated the search for protein differences in blood which could be used non-destructively by deer farmers and wildlife managers.

As haemoglobin is such an accessible protein, this marker might have been found earlier had the differences described not been obscured by several minor fractions of both higher and lower pH. Treatment with potassium cyanide, as used by Andersson *et al.* (1985) to unravel rabbit haemoglobins, eliminated these minor bands and revealed 2 codominant alleles at a single locus.

Two haemoglobin chains have been reported in many mammals (Garrick and Garrick, 1983) and this could explain the apparent variation in the red deer region A. If inheritance studies prove this variation is genetic it will add another useful marker for red deer blood-typing. Attributing the differences between red deer and wapiti to a particular Hb chain requires further work, using reversed-phase chromatography.

The 3-banded heterozygotes found when deer samples were stained for superoxide dismutase are characteristic of a dimeric protein. (Dimers such as SOD have 2 polypeptide chains in their quaternary structure which interact in heterozygotes to form a third intermediate band). Three-banded SOD heterozygotes have also been described in dogs (Baur and Schoor, 1969) and humans (Beckman *et al.*, 1973).

Although all of the F_1 hybrids analysed in this study were heterozygous for SOD, the protein is not an absolute marker discriminating red deer and wapiti. Five wapiti tested had the allele which predominates in red deer (1 was homozygous slow), and 2 red deer had the allele which predominates in wapiti.

CONCLUSION

Red deer, wapiti and F_1 hybrids can readily be differentiated by high resolution isoelectric focusing of treated haemoglobin samples. This marker has found its first use in eliminating a hybrid animal from research on red deer growth. As the animal was heterozygous for haemoglobin (Hb AB) it must have had hybrid genes and thus could skew red deer growth results.

Should the marker come into general use in the deer industry, well preserved blood samples can be typed less expensively using starch gel electrophoresis. The electrophoretic method showed a second marker, superoxide dismutase, which correctly classified 95% of the deer tested.

These biochemical markers, with others yet to be discovered, should prove valuable as interbreeding of red deer and wapiti becomes more common, particularly beyond the first cross. When the genetic background of the parents is unclear, blood-typing can help predict valued characteristics in the progeny.

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