THE HYBRID IDENTIFICATION TEST - RESEARCH AND COMMERCIAL SERVICES

M.L. Tate, R.T. Rummel and P.A. Dratch, Invermay Agricultural Centre

INTRODUCTION

Inherited protein variation in the blood of deer forms the basis of tests for hybrid identification and parentage which are currently available to the deer industry (Dratch 1987, Tate and Dratch 1987). Similar techniques are used in parentage testing and hybrid identification but the concepts used in the application and interpretation of these services are quite different (Dratch and Fennessy 1983). The hybrid identification test uses concepts which will be new to many veterinarians and animal breeding consultants. Unlike parentage testing which is available in other valuable domestic species (for example horses, Braend 1973) the hybrid identification test has no parallels in other animal industries.

This paper is intended as a reference for veterinarians who have clients with a need for the hybrid identification services. It reviews the methods and research results which form the basis of testing and then discusses the interpretation and application of these services in the deer industry. Current policies regarding the interpretation and dissemination of results are outlined.

RESEARCH

Methods

The Invermay Genetic Markers Laboratory uses protein gel electrophoresis to separate and compare blood proteins. The same technique plays an important role in parentage testing in horses (Braend 1973) and cattle (Gahne et al 1977).

By comparing protein molecules from different animals, electrophoresis provides a means of studying variation in the structural (protein coding) part of an animal's DNA. Examining structural genes in this way is

considerably cheaper than examining the DNA directly. The relatively low cost of protein techniques is an important factor in the viability of routine testing in agricultural animals.

The technique of electrophoresis involves loading proteins onto a gel with a pore size similar to the size of the proteins to be analysed. The pH of the gel is adjusted so that the proteins have a net charge and an electric current is applied causing the proteins to migrate. The migration rate of a protein depends on its charge and also its size and shape as the movement of larger or arkwardly shaped proteins tend to be retarded by the gel pore size.

The requirements for testing are two 10ml heparinised (green top) vacutainers per animal. Two tubes provide insurance against breakage in transit. However if large numbers of animals are to be bled one 15ml vacutainer is sufficient. Samples should be chilled but not frozen.

Genetic Variation in Red Deer

With few exceptions the genetic differences between farmed deer stem directly from the variation present in natural populations of deer. This contrasts with other agricultural animals such as sheep and cattle where the genetic differences between breeds are largely due to a long history of controlled animal breeding. In natural populations the genetic differences between individuals are generally least within one local population. These differences increase between members of more distant populations and members of different subspecies and species (Ayala 1975).

This hierarchy of genetic variation is reflected in the genetic variation detected by protein electrophoresis (Avise 1975) and this technique has been widely used to study the relationships of red deer populations in Europe (Gyllensten et al 1983). Although populations of European deer can be differentiated by electrophoresis the level of the genetic differences between populations is so small that an animal can only rarely be assigned to a population or subspecies by its protein type (Gyllentsten et al 1983). Populations of red deer in New Zealand show electrophoretic variation in the same proteins which are highly variable in European deer (Tate and

Dratch 1987). This is to be expected as New Zealand red deer were established by introduction from Europe. On New Zealand deer farms within species variation is valuable in parentage testing (Tate and Dratch 1987).

Genetic Variation Between Deer Species and Subspecies

The genetic differences between red deer populations in Europe are small compared to the genetic differences between populations of red deer and cervids from other continents such as the North American elk and the the Japanese Sika (Dratch and Gyllensten 1983, Harrington 1985). The electrophoretic differences in blood plasma and red cell proteins between red deer, elk, sika deer and Pere David's deer have been examined by the Invermay Genetic Markers Laboratory. Table 1 lists the proteins in which these species and subspecies have large frequency differences in electrophoretic type.

TABLE 1: Electrophoretic Types of Four Deer Species

	RED CELL PROTEINS		PLASMA PROTEINS			
	n	Hb	SOD	Trf	Ptf	Alb [1]
Red deer	>400	AA	SS(SF,FF)[2]	AA,AB(BB)	22	SS
Elk	>200	BB	FF(SF,SS)	BB(AB,AA)	11	SS
Sika	20	AA	FF(SF,SS)	BB(AB,AA)	11	SS
Pere David	35	AA	SS	EE	*	FF

[1] Hb - Hemoglobin, SOD - Superoxide dismutase, Trf - Transferrin, Ptf - Post-transferrin, Alb - Albumin.

[2] Rare types (<10% of animals) are indicated in parenthesis.

* - not detected in this species.

In their natural range these deer species and subspecies are separated by continental boundaries. However the introduction of the species to New Zealand about the turn of the century has led to hybridisation in the wild between red and sika deer in the central North Island and between elk and

red deer in Fiordland (Challies 1983). More recently elk and red deer have been hybridised under farm conditions (Moore 1987) and red deer and Pere Davids deer have been hybridised using artificial insemination (Asher et al. 1987)

The blood types of hybrids bred under farm conditions confirm that the protein differences detected between these species are simply inherited. The 45 elk red deer F1 hybrids analysed to date type HB - AB, SOD - SF, TRF - AB and rarely (BB), Ptf - 12 at the four marker proteins distinguishing these species. In each protein one gene from each parental type is expressed in the hybrid offspring. Similarly each of the 5 Pere David's deer red deer hybrids produced by artificial insemination contain one red and one Pere David gene at transferrin (Trf) and albumin (Alb), the two plasma proteins distinguishing the species.

Animals captured in the wild from the Fiordland area show a wide variety of types at the four marker proteins including a large proportion of animals with combinations of genes from both elk and red deer (Dratch 1987a). Similarly a limited sample of red sika hybrids from Taupo show a combination of red and sika genes in the types at SOD, Trf, and Ptf (P.A. Dratch and M.L. Tate, unpublished data).

In addition to the markers discussed above we have markers which distinguish European and Mesopotamian fallow and also Mollucan rusa, Javan rusa and red deer.

COMMERCIAL SERVICES

The discovery of inherited protein variants which distinguish deer species provides the means to clearly identify as hybrids animals which contain a combination of protein types from different species. Hybrid identification using these inherited protein variants has been available to commercial deer farmers from mid 1987 (Dratch 1987).

The test for elk red hybridisation has found wide application in the New Zealand deer industry and overseas with over 2500 samples received to date. For testing to be of greatest value to the commercial deer breeder the

capabilities of the test must be clearly understood and it must be applied correctly.

In addition to the explanatory letter accompanying results the Genetic Markers Laboratory provides free consultancy to any veterinarian or farmer submitting samples. However all correspondence with farmers is also sent to the veterinarian involved except in cases where the farmer specifically requests complete confidentiality of results. All results should be treated as confidential between the veterinarian, the farmer and the Invermay laboratory.

The Capabilities of the Elk Red Hybrid Identification Test

When all elk contain one inherited protein variant and all red deer a different inherited variant any animal with a combination of elk and red types must be a hybrid.

At two protein markers hemoglobin (Hb) and post-transferrin (Ptf) we have typed over 400 known pure red deer from a range of populations in New Zealand and over 200 known pure elk from populations in Canada. All the red deer score Hb-AA, Ptf-22 and all the elk score Ptf-11 Hb-BB. These standards show that the chance of pure animals showing a combination of elk and red types at these markers is extremely low - less than 0.0025 for red deer and less than 0.005 for elk.

In test results, hemoglobin and post-transferrin are referred to as primary markers. Animals with a combination of elk and red deer types at these primary markers are identified as hybrids.

The other markers used in the hybrid identification test, superoxide dismutase and transferrin are referred to as secondary markers. This is because, at these markers, a small proportion of pure red deer and elk show the types typical of the other species (Table 1).

Each of the four protein markers used in the elk red hybridisation test are inherited in a simple and predictable way. In any mating, for each protein marker, one type (which geneticists call an 'allele') is inherited from

each parent. Thus, in a cross between a pure elk and a pure red deer all the offspring (F1 hybrids) are clearly identified as hybrids by the test because at each marker they inherit one 'elk' allele and one 'red' allele (Table 2).

TABLE 2: Inheritance of protien markers in a elk red cross

ANIMALS		RED DEER	X	ELK	------>	F1 HYBRID
MARKERS	Hb	AA	X	BB	------>	AB
	SOD	SS	X	FF	------>	SF
	Ptf	22	X	11	------>	12
	Trf	AA	X	BB	------>	AB

After the F1 generation, in a hybrid mating one or both of the parents will contain a combination of elk and red deer genes. In this situation it is possible that, at any one protein marker two 'elk' alleles or two 'red' alleles will be inherited resulting in a type identical to that in the pure animals. Table 3 shows the inheritance of the two primary markers in a backcross mating of an F1 hybrid over a red deer. In this example 75% of the progeny would be identified as hybrids by the primary markers.

TABLE 3: Inheritance of protien markers in backcross

ANIMALS		RED DEER	X	F1 HYBRID	----->	1/4 ELK PROGENY			
						25%	25%	25%	25%
PRIMARY	Hb	AA	X	AB	------>	AB	AB	AA	AA
MARKERS	Ptf	22	X	12	------>	12	22	12	22
							---		--
							|		|
Identified as hybrid at primary markers							75%		|
Indistinguishable from red deer at primary markers									25%

The advantage of having both primary and secondary protein markers is that the animals not detected by the primary markers are likely to be picked up by

the secondary markers. In test results animals with no hybridisation detected at the primary markers but a combination of elk and red deer genes in the secondary markers are identified as 'suspect' if the probability of a false positive is less than 5% or to put it another way the probability the animal is a hybrid is greater than 95%.

With test results which include a suspect animal a recommendation is made that the parents of the animal are typed. Identification of either parent as a hybrid at the primary markers confirms the suspect animal as a hybrid. No evidence of hybridisation at primary markers in the parents or a group of animals from the same source population is strong evidence that the animal is not a hybrid.

If the secondary markers are included in the backcross example (Table 3) a further 13.5% of animals would be identified as suspect. In this case typing of the parents or siblings would identify the suspect animals as hybrids giving an overall detection rate of 88.5%.

In practice a major use of the hybrid identification test is detecting hybrids with Fiordland wapiti. The degree of hybridisation in these animals is uncertain so the power of the test cannot be calculated directly. However typing of a large number of wild captured hybrid animals from the wapiti area in Fiordland indicates a high detection rate. In 216 wild captured Fiordland animals over 89% were identified as hybrids and a further 6% as suspect, 4% typed as pure elk and 1% as pure red. The progeny of this sample of animals if backcrossed over with red deer or elk would type over 70% hybrid and over 80% hybrid or suspect.

The important implication of the fact that a hybrid mating can produce progeny with a bloodtype identical to a pure animal is that for a single animal the hybrid identification test cannot be used as proof of breed purity. The strength of the hybrid identification test is the degree of certainty with which a high proportion of hybrids can be identified.

To use the hybrid identification test to examine breed purity the concept of typing groups or populations of animals of the same origin is important. Testing a group of greater than 25 animals from a single source provides a

powerful test which can detect the presence of up to 1/32 elk genes over 95% of the time. In addition typing a group of animals provides the means to distinguish classes of hybrids such as FI hybrids from F2 hybrids, or upgraded animals (Elk X Fiordland wapiti) from straight Fiordland animals.

APPLICATION OF TEST RESULTS

1. Verification of Hybridisation

In farmed deer hybridisation of deer species provides an opportunity to introduce genes for particular characters into a deer population For example by hybridising wapiti with red deer,and Mesopotamian fallow with European fallow deer it is possible to produce large carcasses at a younger age than with straight red or European fallow (Moore 1987, G.W. Asher pers comm.).

Bloodtyping provides an independent test by which the success of hybridisation programs can be measured. This is particularly relevant in the case of Mesopotamian European fallow hybrids where hybrid and straight European fallow fawns are morphologically very similar in their first year (Asher pers. comm.) and in distinguishing farm bred elk red F1 hybrids from hybrids of uncertain genetic composition captured in the wild.

2. Identification of Hybridisation in Animals of Uncertain Origin

The advantages of hybridisation in farmed deer are not without cost. Once fertile hybrids have been produced, maintainence of the pure populations becomes more difficult. For example in Alberta, Canada the importation of elk red hybrids for farming has been banned because of the danger of introducing red deer genes into natural populations of elk. Animals entering Alberta are tested for hybridisation by the Invermay Laboratory.

In New Zealand the presence of a large number of hybrid animals in the industry makes it increasingly difficult to maintain a herd of pure animals required to supply replacement stock for cross breeding

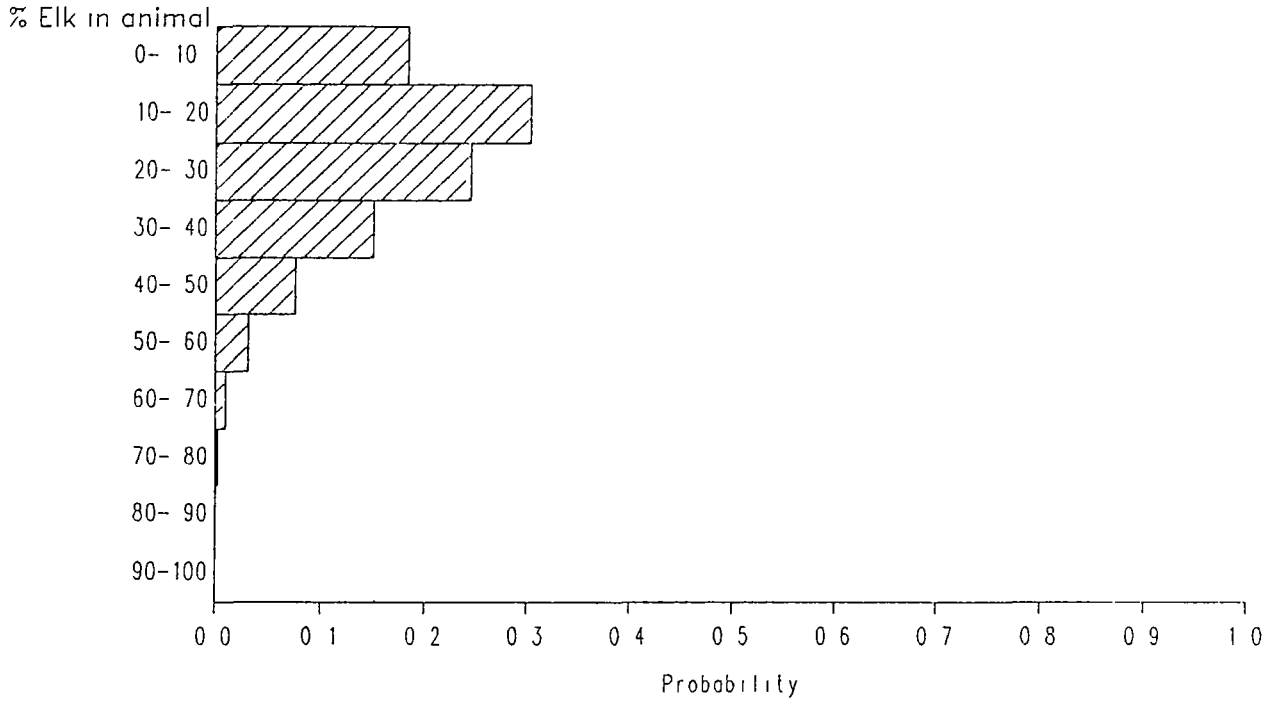
systems. For red deer breeders the use of the test to exclude hybrids from a herd helps ensure that performence gains made by a selection program within red deer are not merely a reflection of the influence of elk genes. The use of testing to exclude hybrids from pure herds of red deer and elk continues to provide the majority of samples tested in the Invermay laboratory. This is a valid use of the testing services. However it is important to note that the fact that an animal is not detected as a hybrid does not automatically mean the animal is of pure type (see 'Power of the test' above).

3. Hybrid Assessment

The hybrid identification test can be used to generate a probability distribution for the proportion of elk genes in a single hybrid of unknown origin. Figure 1 shows the probability distribution for an animal with one 'elk' allele at hemoglobin and 'red' alleles at the other markers. There is a 19% chance this animal is between 0 and 10% elk, a 30% chance this animal is between 10 and 20% elk and 25% chance the animal is between 20 and 30% elk (Figure 1). These estimates are additive to give a 74% chance that the animal is between 0 and 30% elk. Bloodtyping data is completely independent of other factors such as the appearance of an animal or its weight. We stress the importance of using as many factors as possible in estimating the proportion of elk in a single hybrid of unknown origin as estimates based on any one factor may be misleading.

Figure 1 Probability of animal with the following blood-type having a given % of Elk

HB=AB SOD=SS HPT=22 TRF=AA



SUMMARY

The hybrid identification service offered by the Invermay Genetic Markers Laboratory to the deer industry uses a method of protein separation called gel electrophoresis to identify protein variants which distinguish deer species. These protein variants provide the means to clearly identify as hybrids animals which contain a combination of protein types from different species.

The requirements for testing are two 10ml heparinised (green top) vacutainers per animal or if large numbers of animals are to be bled one 15ml vacutainer. Samples should be chilled but not frozen.

Detection of elk(wapiti) red deer hybrids is based on four single gene markers which distinguish elk and red deer. Hybrid animals or herds are clearly identified because they contain both elk and red deer blood markers.

Of the four blood markers used, two are primary and two are secondary markers. A combination of elk and red deer types in the primary markers indicates greater than 99.5% probability of hybridisation. Combinations of elk and red deer types in the secondary markers are not always certain evidence of hybridisation however they can provide grounds for a strong suspicion of hybridisation (>95% probability).

When results are sent out three types of animals are identified;

1. Hybrids --- animals with a combination of elk and red deer types at the primary markers.

2. Suspected Hybrids --- animals with a suspicious combination of elk and red deer types at secondary markers.

3. Animals in which no evidence of hybridisation was detected.

The inheritance of the blood markers is such that all F1 hybrids are clearly identified as hybrids by testing. However in subsequent generations it is possible that some hybrid progeny will inherit only the 'red' or 'elk' types from there parents and so be indistinguishable from a pure animal.

The important implication of the fact that a hybrid mating can produce progeny with a bloodtype identical to a pure animal is that for a single animal the hybrid identification test cannot be used as proof of breed purity. The strength of the hybrid identification test is the degree of certainty with which a high proportion of hybrids can be identified.

Over the last year hybrid identification test has found application in both the New Zealand deer industry and overseas. The main areas of application are in verification of hybridisation, identification of hybridisation in animals of uncertain origin and hybrid assessment.

The Invermay Genetic Markers Laboratory provides a consulting service to veterinarians and farmers who use the hybrid identification test. If large numbers of samples are likely to be taken it is worthwhile contacting the laboratory in advance.

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