PARENTAGE TESTING IN FARMED RED DEER

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INTRODUCTION

Modern animal breeding methods need accurate pedigree information to select genetically superior individuals. In the New Zealand deer industry, accurate parentage data is virtually essential when selling high priced breeding stock.

Biochemical parentage testing provides a means of checking pedigree records and solving cases of unknown parentage. This paper outlines the present status of parentage testing in red deer using protein polymorphisms and reports the first case of DNA parentage testing in New Zealand deer. The results of protein parentage testing on 199 pedigrees are summarised and the rate of pedigree misidentification is calculated in 8 breeding groups.

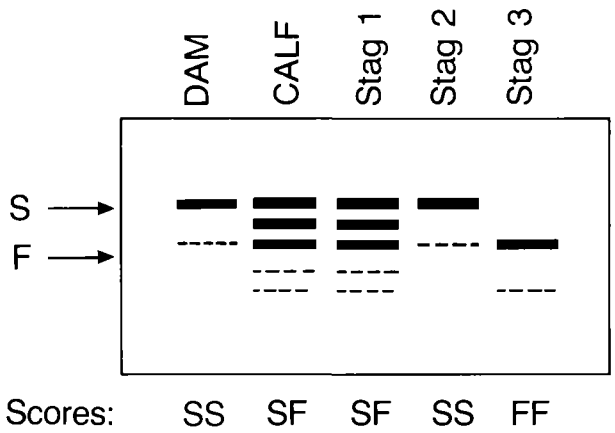
PROTEIN PARENTAGE TESTING

Most protein variation in animals is inherited in a simple and very predictable fashion. Each animal inherits one protein type (called an allele) from its sire and one protein from its dam. Parentage can be excluded when a calf contains alleles which are not compatible with its parents.

Variation in the red blood cell enzyme, glucose phosphate isomerase (GPI) provides an example of how protein parentage testing works in red deer. GPI has two alleles (called 'S' and 'F') which by combination provide three possible GPI blood types in deer - SS, SF and FF. Figure 1 shows the GPI types of 5 animals involved in a case of unknown paternity. The calf typed SF and the dam SS, the sire therefore must have at least one 'F'. This means the SF and FF stags would qualify for parentage while the SS stag is excluded.

In the Genetic Markers Laboratory at Invermay we presently use 8 protein systems for parentage testing in deer (Table 1). The power of protein parentage testing is summarised by calculating the probability that a misidentification will be detected by the test. One measure of this is the probability of exclusion (PE) (Jamieson 1966). The PE for each protein system and for all proteins combined was calculated from the frequency of types in 450 unrelated New Zealand farmed red deer (Table 1). The total probability of exclusion was 0.81, with most of the exclusions likely to come from two of the proteins, GC and PLG. An additional estimate of the probability of detecting a parentage error was calculated within several herds where a high proportion of pedigrees had been tested. This estimate used a computer program to check the

Figure 1: Parentage exclusion provided by protein variation in red deer GPI. The calf could have been sired by any of the three stags. The GPI blood type of each animal was scored from the starch gel electrophoretic patterns of GPI shown in the diagram. Stag 2 was excluded from parentage because it did not have the 'F' allele needed to explain the calf genotype. Stag 1 and 3 have an 'F' allele and so qualify as potential sires.



Parentage exclusion

CALF SF DAM SS ∴ Sire must have 'F'

Stag 1 SF ✓ qualifies
Stag 2 SS × excluded
Stag 3 FF ✓ qualifies

parentage of each calf against all the possible parental pairs and then calculate the proportion of parents and calves which were incompatible. Using the seven protein systems available at the time of testing the probability of detecting an error in a herd ranged from 58% to 83% (Table 2). These results show the dependence of parentage testing on the level of genetic variation. The power of the test is reduced in herds with a low level of genetic variation which may arise through line breeding or breeding from a very small genetic base. Conversely out bred herds including stock from different red deer strains are likely to have a high probability of exclusion. Research is continuing to identify further parentage markers which will increase the power of protein parentage testing still further.

Table 1: Frequency of alleles for 8 protein systems in 450 farmed red deer and the calculated probability of exclusion

Proteins		Frequency of types in 450 farmed red deer					Probability of exclusion (PE) *
		A	B	C	D	E	
Glucose phosphate isomerase	(GPI)	0 807	0.193				0.13
Isocitrate dehydrogenase	(IDH)	0 516	0.484				0.19
Mannose phosphate isomerase	(MPI)	0 990	0.010				0.01
Phosphoglucomutase	(PGM)	0.049	0 946	0.005			0.05
Plasminogen	(PLG)	0 005	0.452	0.153	0.165	0.225	0.45
Superoxide dismutase	(SOD)	0.936	0.064				0.06
Transferrin	(TRF)	0 588	0.412				0.18
Vitamin D binding protein	(GC)	0 153	0.418	0 429			0 43
		Probability of exclusion over all 8 loci					0.81

* PE was calculated according to Jamieson (1969) and is the probability of detecting an error when one parent is known.

DNA PARENTAGE TESTING

The DNA parentage test used specific probes developed in the MAFTechnology Molecular Biology Unit (Crawford and Buchanan 1990). The probes identify many different regions of the genome so that each individual animal has its own unique 'bar code' or pattern. Approximately half the bands of an individual are inherited from its sire and half from its dam. In humans the probability of exclusion for DNA fingerprinting is estimated to be greater than 99% (Jeffreys et al. 1985). This new technology provides a powerful new parentage test in deer.

Figure 2 shows the first parentage problem in farmed red deer solved by DNA fingerprinting. The problem was a case of unknown paternity. A Yugoslavian stag calf due to be sold could have been sired by one of two imported Yugoslavian stags and the vendor wanted to provide accurate pedigree information with the sale. The two possible sires were closely related and protein testing could not solve the problem. We DNA tested the two sires, the dam and the calf. Comparison of the calf and dam DNA banding patterns identified bands in the calf which were not present in the dam these therefore must have been inherited from the sire. The results clearly identified stag 1 as the sire because it contains all the unaccounted for bands while stag 2 does not (Figure 2).

THE ACCURACY OF FARM PEDIGREE RECORDS

Protein parentage testing was used to assess the accuracy of farm pedigree records from 8 breeding groups, totalling 199 pedigrees. The groups tested came from South Island research herds and commercial deer breeders. Either three or seven of the parentage test loci were used to check the pedigree records depending on the number parentage test loci defined at the time of testing. Of the 199 pedigrees tested 24 (12%) were identified as incorrect. However the actual rate of parentage misidentification in the herds is higher as the tests used do not detect all parentage errors. The actual rate of mismatching was estimated by dividing the observed error rate by the probability of detecting an error (Table 2). A second method of estimation using maximum likelihood methods (McCoubrey et al. 1983) gave similar results and only the more straight forward calculation is presented here.

Table 2 . Detection of pedigree errors in 8 groups of red deer hinds using protein parentage testing.

Deer Group Farm		Hinds	Mating	No loci	PE[1]	No. in group	Detected pedigree Errors Sire	Dam	Total	Actual percentage pedigree errors [2]
A	1	First calving	Natural	7	0 682	23	2	1	4	25%
A	2	Mixed age	Natural	7	0.580	26	0	0	0	None detected
B	3	First calving	Natural	3	0 442	28	0	1	4	32%
B	4	Mixed age	AI	7	0 757	30	0	1	3	13%
C	5	Mixed age	ET	7	0.756	28	0	0	1	5%
C	6	Mixed age	Natural	7	0 833	13	0	0	0	None detected
D	7	Mixed age	Natural	3	0.462	27	0	0	0	None detected
E	8	First calving and mixed age	Natural and AI	7	0 810	24	2	6	12	61%

1. PE was calculated by checking each calf against all the possible parents.

2 The actual number of pedigree errors was estimated by dividing the detected rate by the probability of exclusion.

The estimated rate of pedigree misidentification in individual groups varied from nil to 61% (Table 2). The three groups including first calving hinds had the three highest error rates of 25%, 32% and 61%. The six groups of mixed age hinds tested had error rates ranging from nil to 13%. Some of the parentage errors detected could be identified as specifically due to sire error or dam error. Identifiable dam errors occurred in the four data sets with the highest error rates. Identifiable sire errors occurred in the two data sets with the highest overall errors.

DISCUSSION

The development of protein parentage testing and DNA testing in deer provides the ability to detect pedigree misidentifications and solve parentage problems in deer. The two techniques are complementary. Protein parentage testing will not detect every parentage error but the testing is cheap and rapid compared to present DNA techniques, in addition protein test results can be easily recorded for later reference. By comparison DNA testing is likely to detect almost all pedigree errors but it is a more expensive new technique which needs to be trialed more widely in deer. In addition DNA tests require all the samples for a particular problem to be run together, at this stage there is no way

of storing the results for later reference. Instead the DNA itself must be stored and retested with each pedigree problem. For important sire stags this may mean the same sample will be rerun several times.

Some of the deer pedigrees tested show a very high level of errors. Parentage errors of greater than 10% identified in half of the groups tested could seriously compromise any breeding program involving the deer (Gelderman et al. 1986). While in the sale of elite breeding stock, especially where embryo transfer has been used, a single misidentification may be of concern if detected by the buyer. In general the records tested in this study are likely to be more accurate than average as the farmers involved were willing to be tested and had some choice of animals they tested. Overall, therefore the results suggest pedigree records in many farmed red deer herds are not sufficiently accurate for their intended use. Although they do show that it is possible to obtain accurate records at least in older hinds.

The rates of mismatching observed is too high and consistent to be accounted for by record keeping errors. More parentage testing needs to be done to confirm the exact reasons for the high rate of pedigree errors observed. However a high level of cross suckling and adoption among farmed red deer would explain the parentage errors. The hypothesis is consistent with first calvers having a higher rate of misidentification than more experienced mothers. The stag errors identified in the two farms with the highest errors may also result from a wrongly identified dam, as in these groups, the progeny of several stags were calved together. In all cases the calves were not matched until just prior to weaning which means adoption would not be detected and cross suckling may not be detected unless repeated observations are made. Matching at birth is difficult in red deer as they are very prone to mismothering when disturbed, in addition hinds tend to 'hide' their calves during the first few days postpartum making matching difficult (Kelly and Drew 1986). These features contrast with sheep and cattle where tagging and matching at birth are more feasible.

The combination of protein testing and a fully developed DNA test provides the tools for a deer farmer or veterinarian to detect and solve virtually any case of uncertain parentage. If parentage records are important to the breeding program or credibility of a farmer in live sales the use of protein parentage testing to check a proportion of records is a prudent option. This can be followed by further testing to solve the parentage problems and/or modification of mating and calving management to obtain more accurate records.

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Figure 2 : DNA parentage testing in red deer. The calf's band pattern is inherited from its parents. The calf could have inherited the bands shown in black in the diagram from its mother. The remaining bands (hatched) did not occur in the dam and so must have come from the sire. Only stag 1 has the all bands required to qualify for paternity.