Inherited protein variation and parentage testing in farmed red deer

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ABSTRACT

Inherited protein variation in the blood of domestic species can provide the means to resolve cases of uncertain parentage. In red deer, protein variation has been described from natural populations in Europe, although the inheritance of this variation has not been verified using family data. This paper examines protein variation in the blood of New Zealand farmed red deer and tests the inheritance of each protein variant using family data from 46 matings.

The 2 most variable proteins reported in European deer, transferrin and isocitrate dehydrogenase, were also variable in New Zealand farmed deer. The inheritance of variation in each of these proteins was consistent with co-dominant Mendelian inheritance at a single locus. In addition variation was found in plasma proteins in the postalbumins and the gamma-globulins. Variation in the postalbumins was consistent with co-dominant Mendelian inheritance at a single locus while variation in the gamma-globulins did not appear to be inherited.

The inherited protein variants identified in red deer provide a blood test which detected 70% of the possible mismatches between parents and calves in the sample of 46 matings.

Keywords protein variation; parentage; red deer.

INTRODUCTION

Correct parentage information is a pre-requisite for rapid progress in the genetic improvement of a species for farm production. In farmed red deer there are problems in determining both paternity and maternity. In red deer paternity is usually assigned by extrapolation from calf birth date and mean gestation length. Individual variation in gestation length means that even when stags are mated consecutively to a group of hinds there is, for each stag replacement, a period of about 12 d during calving in which the sire of calves is unknown. Maternity problems result from cross-suckling and adoption of calves at calving. Red deer behind fences appear to be more prone to cross-suckling and adoption than in the wild. Furthermore, disturbance such as tagging and observing calves to determine parentage may be an important factor causing cross-suckling and adoption (Cowie *et al.*, 1985).

In other valuable domestic species such as horses and cattle, biochemical tests are available to ensure correct pedigree information. Inherited protein variation found in the blood forms an important part of these parentage tests. Parentage problems can be solved using inherited protein variation because there is a simple relationship between the blood type of parents and their offspring. If the blood type of the offspring is not related to that of the suspected parents this parentage must be incorrect. This situation, termed exclusion provides a way to solve parentage problems by excluding all but 1 of the possible sets of parents.

Protein variation reported in the blood of European red deer includes variation in transferrin (McDougal and Lowe, 1968) and haemoglobin (Herzog, 1986) and the proteins, Pa, alpha-2 and Sd-2 (Kravchenko and Kravchenko, 1971; Bergmann, 1976). Among blood proteins only transferrin has been widely surveyed in European deer populations where it is highly polymorphic in most populations (McDougal and Lowe, 1968; Bergmann, 1976; Wegge, 1978; Gyllensten *et al.*, 1980; 1983). Tissue enzymes have also been surveyed in European populations and polymorphisms have been found at Ap-2, GPI-1, IDH-2, MDH-2, ME-1, MPI, PGM-1, PGM-2, SOD-1 and an esterase locus (Baccus *et al.*, 1983; Gyllensten *et al.*, 1983; Dratch and Gyllensten, 1985). Of these enzymes IDH-1 (Isocitrate dehydrogenase-1) is the most polymorphic across all the populations surveyed.

The inheritance of the protein variation described in red deer has not been tested as family data are generally unavailable in natural populations. Parentage testing in farmed deer requires a set of protein variants which are both known to be inherited and are readily detectable in blood. This study examined protein variation in New Zealand farmed deer, including the 2 most variable proteins reported from European deer (transferrin and isocitrate dehydrogenase), and tested the inheritance of this variation. Finally it examined the power of a parentage test using all the inherited protein variation characterised in deer blood.

METHODS

Forty-six matings of red deer were chosen for the reliability of parentage information based on calving

date, mating records, and calving records. Blood was taken from the jugular vein of each sire, dam and calf(s) using a 15ml heparinised evacuated tube and 20 guage needle. The blood was spun for 15min at 2,500 rpm and 4°C. Plasma, red blood cells and white blood cells were removed from the centrifuged blood using a Pasteur pipette and placed in seperate tubes. All samples were held at -80°C until use. Alkaline horizontal polyacrylamide gel electrophoresis was used to characterise deer plasma proteins. The technique followed that of Gahne *et al.* (1977) with the following modifications: main gell 11% acrylamide, gel buffer adjusted to pH 7.9, gels loaded with 1mm x 4mm x 4mm inserts placed on the gel for 40min at 60mA. After electrophoresis gels were silver stained using the protocol of Shaklee and Keenan (1986). Isocitrate dehydrogenase variation was resolved using the technique of Dratch and Gyllensten (1985) except that white blood cells were substituted for tissue homogenates.

RESULTS

Protein Variation

Clearly scoreable variation in plasma proteins occured in 3 regions on polyacrylamide gels, the gamma-globulins, beta-2-globulins, and alpha-1-globulins.

Gamma-globulins: Variation in this region consisted of 2 band positions, a slower (S) and a faster (F) migrating band. Individual deer had either the S band, F band, both S and F bands or no bands (Fig. 1). In 46 red deer matings no relationship was found in the banding pattern of parents and offspring. For example, in 1 case both parents contained a band which was absent in the calf. In 3 other cases, calves had the band while it was not present in either parent. In 5 animals repeat samples showed different phenotypes.

Beta-2-globulins (transferrin): Protein banding in this region was typical of transferrin; primary band staining intensity was second only to albumin and each primary band was associated with anodally migrating sub-bands. Variation consisted of 2 primary band positions termed A (more cathodal) and B (more anodal) in accordance with the 2 most common alleles found in European deer by Bergmann (1976). Individual deer had either the A band or the B band or both bands (Fig. 1). In the 46 red deer matings the inheritance of this variation agreed exactly with the predictions of a simple Mendelian model of 2 co-dominantly inherited alleles segregating at a single locus (Table 1).

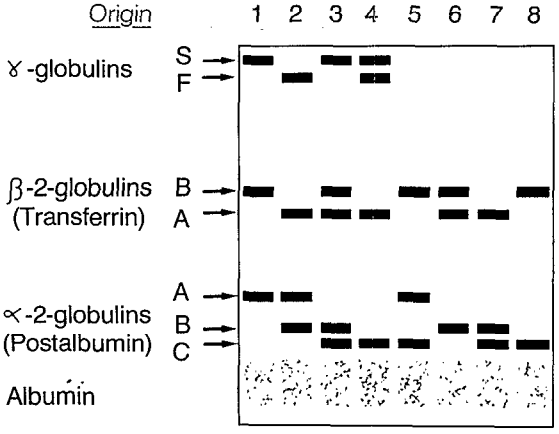

FIG. 1 Diagram of variation in the gamma-globulins, transferrin and postalbumin of 8 red deer plasma samples compared by alkaline acrylamide electrophoresis and silver staining.

TABLE 1 The inheritance of variation in transferrin for 46 matings compared to the expectations of co-dominant inheritance of 2 alleles at a single locus.

Parental types	No. of calves		Calf type AA	AB	BB
AA x *AA*	10	Expected	10	0	0
		Observed	10	0	0
AA x *BB*	12	Expected	0	12	0
		Observed	0	12	0
AA x *AB*	24	Expected	12	12	0
		Observed	12	12	0
AB x *AB*	2	Expected	0.5	1	0.5
		Observed	0	2	0

Alpha-1-globulins (postalbumin): Three protein types termed A, B and C from cathode to anode were observed in this region. Their relative migration as a proportion of the most anodal type (C) was 1.00, 0.98, 0.90. Each animal had either 1 or a combination of 2 of these protein types (Fig. 1). In 46 red deer matings, inheritance of this variation followed a simple Mendelian model of 3 co-dominantly inherited alleles segregating at a single locus (Table 2). In the absence of other data characterising this protein in deer it is termed *postalbumin.*

Isocitrate dehydrogenase variation: Two band positions were present termed slow (S) and fast (F) in order of anodal mobility. Individual deer contained either the S or the F or both S and F bands. In deer with both the S and F band an intermediate band was also present. Dratch and Gyllensten (1985) reported similar variation in tissue isocitrate dehydrogenase which is typical of inherited variation in a dimeric protein. Isocitrate dehydrogenase could not be scored in 4 deer due to poor preparation of white cells. In 42 red deer matings the inheritance of this variation followed a simple Mendelian model of 2 co-dominantly inherited alleles segregating at a single locus (Table 3).

Application of Inherited Protein Polymorphism to Parentage Testing.

The value of the inherited variation found in New Zealand farmed red deer for transferrin, isocitrate dehydrogenase and postalbumin as a parentage test was calculated empirically by assuming no knowledge of the parentage of the test group of 46 matings. Each of the calves were tested for parentage against each of the 46 parental pairs. The number of parental combinations shown by the blood test to be

TABLE 2 The inheritance of variation in postalbumin for 46 matings compared to the expectations of co-dominant inheritance of 3 alleles at a single locus.

Parental types	No. of calves		Calf type AA	AB	AC	BB	BC	CC
AB x *AA*	10	Expected	5	5	0	0	0	0
		Observed	8	2	0	0	0	0
AB x *AB*	9	Expected	2.2	4.5	0	2.2	0	0
		Observed	2	6	0	0	0	0
AB x *AC*	3	Expected	0.75	0.75	0.75	0	0.75	0
		Observed	1	0	1	0	1	0
AB x *BB*	2	Expected	0	1	0	1	0	0
		Observed	0	1	0	1	0	0
AB x *BC*	1	Expected	0	0	1	0	1	0
		Observed	0	0	0	1	0	0
AB x *CC*	2	Expected	0	0	1	0	1	0
		Observed	0	0	1	0	1	0
BB x *BB*	4	Expected	0	0	0	4	0	0
		Observed	0	0	0	4	0	0
BB x *CC*	1	Expected	0	0	0	0	1	0
		Observed	0	0	0	0	1	0
BB x *BC*	9	Expected	0	0	0	4.5	4.5	0
		Observed	0	0	0	3	6	0
BC x *BC*	3	Expected	0	0	0	0.75	1.5	0.75
		Observed	0	0	0	2	0	1
BC x *CC*	2	Expected	0	0	0	0	1	1
		Observed	0	0	0	0	1	1

incorrect were recorded for each calf. In this theoretical problem only if all 45 incorrect parents were identified would it be possible to identify the correct parents. For 1 calf, 43 of the 45 incorrect parents were excluded by protein type leaving only 3 potential sets of parents. For the rest of the calves a lower number of parental combinations were excluded by protein type. On average 32 of the 45 incorrect parents or 70% of parent-calf mismatches were identified by protein type.

DISCUSSION

The variation found in transferrin and isocitrate dehydrogenase in New Zealand farmed red deer is consistent with that already described in European populations and is not unexpected as New Zealand red deer populations were established by introduction from Europe (Donne, 1924). Variation in the alpha-globulin region was noted by Kravchenko and Kravchenko (1971) and Bergmann (1976) but has not been fully described and has not been surveyed in European populations. Post-albumin was the most polymorphic of the proteins examined in this study, making it particularly valuable in parentage testing. Also the degree of variation found in New Zealand deer suggests this protein will be valuable as a genetic marker in European populations. Variation in the gamma-globulin region in this study was not inherited, and is therefore not useful in parentage testing or protein differentiaiton. Non inherited protein variation can be related to a variety of environmental or physiological factors (Hopkinson and Harris, 1971). The similarity of some aspects of variation in the gamma-globulins to genetic variation in other proteins highlights the importance of obtaining family data before inheritance is assumed.

In combination, the proteins transferrin, isocitrate dehydrogenase and postalbumin provide the means to test for parentage in deer. In the test group of 46 red deer matings, 70% of incorrect parentage assignments were detected using the test. A test of this power will not identify all parents with offspring in a large calving herd, but the test does provide a means of estimating the rate of cross-suckling and adoption at calving. In comparison to other methods of estimating cross-suckling levels such as detailed behavioural observations (Alexander *et al.*, 1983), blood testing requires less effort and can be applied without special management or disturbance at calving. Furthermore, estimates of cross-suckling and adoption can be gained for herds under a wide variety of management conditions. This factor may help identify management procedures to reduce parentage problems in herds used for genetic selection or sire referencing.

A blood test which detects 70% of parentage misidentifications provides the means to solve parentage problems involving only a few animals. A typical problem is when 2 stags have been used sequentially over a group of hinds and because of variation in gestation length of hinds either stag could be the sire of a calf. This problem will be solved by blood typing if the incorrectly assigned sire can be detected. Assuming the level of protein variation in other farmed deer is similar to that in the test matings the incorrect sire will be detected by the present test about 70% of the time. Further inherited variants

TABLE 3 The inheritance of variation in isocitrate dehydrogenase for 42 matings compared to the expectations of co-dominant inheritance of 2 alleles at a single locus.

Parental types	No. of calves		Calf type FF	FS	SS
FF x *FF*	1	Expected	1	0	0
		Observed	1	0	0
FF x *FS*	13	Expected	6.5	6.5	0
		Observed	7	6	0
FF x *SS*	6	Expected	0	6	0
		Observed	0	6	0
FS x *FS*	4	Expected	1	2	1
		Observed	2	2	0
SS x *FS*	9	Expected	0	4.5	4.5
		Observed	0	4	5
SS x *SS*	4	Expected	0	0	12
		Observed	0	0	12

found either using the proteins or DNA of farmed deer would increase the power of this test.

In addition to their immediate application in parentage testing transferrin, isocitrate dehydrogenase and postalbumin variants have application as genetic markers in studies of population differentiation within red deer. Furthermore these proteins form a base of single gene markers useful in studying the relationship between genetic variation at the molecular level and variation in agricultural productive characters in red deer.

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