Interspecies hybrids of deer - a ruminant resource for gene mapping and quantitative trait studies

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ABSTRACT

Deer are unique among New Zealand's farmed ruminants in the existence of hybridising species and subspecies which exhibit major differences in physiology and production traits. We used protein electrophoresis to examine genetic differences between two farmed deer species with fertile hybrids: red deer (*Cervus elaphus*) and Père David's deer (*Elaphurus davidianus*) (in both species 2n=68). Fixed differences between the species were found for 19 proteins. A further 3 proteins were polymorphic in red deer but not in Père David's deer. Using the present techniques, segregation of 17 of the protein differences between the species could be confirmed in a backcross pedigree to red deer. The large number of genetic markers segregating in backcross hybrids make them an excellent resource for gene linkage mapping among genetic markers and for experiments which examine the relationship between genetic markers and quantitative traits.

Keywords interspecies hybrid, genetic linkage, quantitative traits, Père David's deer, red deer, electrophoresis, genetic distance.

INTRODUCTION

The relationship between molecular genetic variation and genetic variation in traits, upon which natural or artificial selection may act, remains unresolved in most species. In farmed animals, an understanding of the molecular genetic variation underlying economically important quantitative traits such as fibre, meat, or milk production could provide genetic markers valuable in selective breeding (Geldermann, 1975; Lande and Thompson, 1990). Recent advances in molecular genetics provide a highly systematic approach to finding such markers (Lander and Botstein, 1989). The approach contrasts with many previous studies which have examined the relationship between individual markers and economically important traits (Lewontin, 1991). Instead of examining markers one by one, a large number of genetically mapped polymorphic markers are examined in the same pedigree allowing the segregation pattern of each chromosome segment to be deduced. The analysis then tests the relationship between the inheritance of particular chromosome segments and the quantitative traits of interest (Lander and Botstein, 1989).

While theoretically it may be possible to perform this analysis in any pedigree, in practice, there is a lack of sufficiently informative polymorphic markers within most species, and as a result, a lack of information on the order of markers along the chromosome and their distance from one another. However, interspecies hybrids do not have these limitations, and studies in both plants and animals have found sufficient genetic variation in hybrid pedigrees to generate detailed linkage maps which describe the chromosomal relationships of markers and to conduct linkage studies with quantitative traits. The essential feature of these hybrids is that they have high degree of divergence in molecular markers but are fertile and can be used to produce large numbers of backcross or intercross (F2) offspring in which markers and traits segregate.

In laboratory mice, an interspecies hybrid between *Mus spretus* and *Mus musculus domesticus* has played a fundamental role in genetic linkage mapping studies which have revealed a high degree of similarity between the genetic linkage maps of mice and humans (Erickson, 1989). The mouse hybrids have great potential for further fundamental genetic studies, which include linkage studies with complex quantitative traits (Avner *et al.*, 1988). In agricultural plants, interspecies hybrid experiments examining the relationship between markers and complex traits have been attempted. The seminal example is that of Paterson *et al.*, (1988) who used a wide interspecies cross in the tomato to identify marked chromosome segments which explained a large proportion of the variation in some quantitative traits. For example, in backcross hybrids, the segregation of 4 chromosome segments explained 44% of the variation in soluble solids, an economically important trait.

In traditionally farmed ruminants such as sheep and cattle, crossing between closely related domestic breeds is common, but interspecies hybridisation is rare. In contrast, the New Zealand deer farming industry has used hybridisation of species and subspecies widely in order to introduce desirable traits into local herds of red deer (predominantly *Cervus elaphus scoticus*). Red deer have been hybridised extensively with larger subspecies (eg: *C.e.hippelaphus*, from Europe and *C.e.nelsoni* from North America) as a means of increasing growth rate, mature weight and antler weight. Hybrids between red deer and other species have also been bred, including sika deer (*C. nippon manchuricus*) hybrids for grazing in forestry plantations, rusa (*C. timorensis*) hybrids for tropical environments (Queensland, Australia) and Père David's deer (*Elaphurus davidianus*) hybrids as a means of advancing the calving season of red deer.

Of these species, the Père David's deer is considered by taxonomists to be the most distantly related to red deer (Groves and Grubb, 1987). Although they have the same chromosome number (2n=68), Père David's deer have numerous features which distinguish them morphologically from members of genus *Cervus* (Wemmer, 1983). Père David's deer x red deer hybrids are fertile (Asher *et al.*, 1988; Fennessy *et al.*, 1991) and so have the potential to be useful in genetic linkage experiments but there is no information on the molecular genetic divergence between them. This paper examines the molecular genetic differences between Père David's deer and red deer using electrophoresis of

blood proteins and examines the segregation of some of the genetic markers which distinguish the species in the progeny of an F1 Père David's x red deer male backcrossed to red deer. We discuss the potential of this hybrid resource for linkage mapping in ruminants, and for experiments to identify the chromosome segments associated with quantitative traits.

METHODS

The animals sampled in this study were: 5 Père David's deer, 1 F1 Père David's deer x red hybrid male and 16, 1/4 Père David (backcross) progeny of this male and their red deer dams. The farmed red deer, from Invermay Agricultural Centre, were predominantly *C.e.scoticus* from the Otago population, New Zealand.

Blood samples were taken from the jugular vein of each animal using an evacuated tube containing sodium heparin with an 18 gauge needle. The blood samples were spun in a bench centrifuge (5,000g for 10min.) and separate samples of plasma, white blood cells (WBC), and red blood cells (RBC) removed. The WBC and RBC were washed twice in four times their volume of 0.9M NaCl. Samples were either frozen at -80°C or used immediately. Initial experiments used a wide range of methods. Proteins which stained clearly in these experiments (Table 1) were selected for an electrophoretic comparison of the five Père David's deer and five of the red deer.

The electrophoretic methods used for this comparison differed depending on the how the protein was stained. Proteins identified by immunological or general protein staining were compared using both polyacrylamide gel electrophoresis (PAGE) following the method of Gahne *et al.*, (1977) with the modifications of Tate *et al.*, (1992) and isoelectric focusing (IEF) on native and urea gels using an LKB Bromma Ultraphor and the manufacturers' recipes. Isoelectric focusing experiments initially examined a wide pH range (3-10) to determine the isoelectric point of a protein, and then examined a narrower pH range around the isoelectric point of the protein (eg. 2.5-5.0, 5.0-8.0, 6.7-7.7). Plasma proteins were examined on each of these systems both in their native state, and after treatment with an equal volume of a solution of neuraminidase from *Clostridium perfringens* (approx. 5 U/ml) at 37°C overnight. Antibody staining involved passive transfer blotting onto 0.45 micron nitrocellulose and staining as described by Tate *et al.*, (1992). All the primary antibodies used were polyclonal rabbit antibodies to specific human plasma proteins (obtained from Dakkopatts, Denmark).

Enzymes detected by specific activity staining were analysed by starch gel electrophoresis (SAGE) using the methods described by Selander *et al.*, (1971) and cellulose acetate (CA) electrophoresis following the methods of Hebert and Beaton (1989). The buffers used for SAGE were, LiOH (Selander *et al.*, (1971) buffer#2) and AC (N-(3-aminopropyl)-morpholine citrate, Clayton and Tretiak, 1972) and the buffers used for CA were TG (Hebert and Beaton, 1989) and AC (as above). All red blood cell proteins were analysed by SAGE and CA, in each case using both buffers. In addition, for some proteins the pH and buffer system were modified to improve resolution (Table 1); e.g., the diaphorase enzymes used the TC buffer described by Cepica and Stratil (1978). For white cells, only the CA techniques described above were used for the majority of enzymes (Table 1) as only small amounts of sample were available. Activity stains for specific enzymes followed Harris and Hopkinson (1976) for SAGE and Hebert and Beaton (1989) for CA.

The techniques which provided the best resolution for each protein are listed in Table 1. Proteins which showed a difference between Père David's deer and red deer samples were analysed, using the techniques in Table 1, in samples from the F1 sire, backcross progeny and the remainder of the red deer dams.

RESULTS

The five Père David's deer were monomorphic for all the proteins examined while red deer showed intra-specific variation in C3, DIA1, IDH, GC, PLG, ME, TF. Interspecies differences were found between red deer and Père David's deer in a total of 19 of the 43 loci examined (Table 2). Seventeen proteins distinguished all five red deer from the Père David's deer, while for three (DIA1, IDH, PLG) the Père David's deer were identical to one of the protein types present in red deer. The differences between the species were all in the electrophoretic mobility of protein bands with the exception of LDHA and HEX. For both these proteins, Père David's deer samples stained strongly while red deer samples did not stain. In addition to these differences, two protein bands were observed in plasma which showed a migration difference between Père David's deer and red deer. These were not included in Table 2 because it was uncertain how many unidentified protein bands were compared which showed no difference between the species. The first unidentified protein (Unid1) was in the post-albumin region of PAGE gels and although originally detected on an 11% gel it was best resolved using a 15% gel. The second protein (Unid2) was a sharply resolved set of bands with an isoelectric point between pH 4 and 5 on a pH 3-10 isoelectric focusing gel where both the gel and sample contained 6M urea.

The proportion of loci showing differences between Père David's deer and red deer varied between the tissues examined. In plasma 10 out of 12 loci examined were variable while comparable proportions for white blood cells and red blood cells were 8/16 and 2/15 (Table 2). These differences may be at least partially explained by the use of a greater range of techniques, and more sophisticated techniques in plasma proteins, and some red blood cell proteins compared with white cells proteins (Table 1). Based on the similarities and differences between the 5 Père David's deer and 5 red deer (Table 2) for the 43 proteins examined (Table 1) we calculated a Nei (1972) standard genetic distance (D) of 0.48 between the two species.

The proteins CAT, ORM, and ME distinguished all the red deer from the Père David's deer with an intermediate type in the F1 hybrid but the difference between the F1 hybrid and red deer type could not be scored reliably. IDH and DIA1, which showed a frequency difference between red and Père David's deer were not heterozygous in the F1 hybrid and so did not segregate in this backcross pedigree.

Table 3 lists the frequency of animals with a "Père David" protein type (termed 'P') in 16 backcross hybrids and the allele frequency of this type in their dams for 17 of the proteins which showed a difference between Père David's deer and red deer. Given that the protein differences are the result of mutations in a single gene locus coding for the protein in question, we would expect the F1 sire to be heterozygous with one red deer type (allele) and one Père David type (allele) for each protein. Therefore, we would expect that for fixed protein differences, one half of the backcross progeny would inherit a Père David allele from their F1 sire and the other half a red deer allele, in which case, the latter would have a type identical to a red deer for that particular protein. For the proteins examined in Table 3, in no case did the frequency of 'P' progeny differ significantly from the expected

TABLE 1 Proteins compared between C. elaphus and E. davidianus and the analytical method used.

Protein	Tissue method	Electrophoretic method	Detection
6 phosphogluconic acid (6PGD)	RBC	SAGE, AC, pH6.0	activity
Acid phosphatase (ACP)	RBC	SAGE, AC, pH6.0	activity
Adenosine deaminase (ADA)	WBC	CA, TG, pH 8.6	activity
Adenylate kinase (ADK)	WBC	CA, TG, pH 8.6	activity
Alpha-1-antitrypsin (AAT)	P	PAGE, 11%	antibody
Alpha-1-antichymotrypsin (AACT)	P	PAGE, 11%	antibody
Albumin (ALB)	P	IEF, urea pH 3-10	protein
Antithrombin III (AT3)	P	PAGE, 11%	activity
B-galactosidase (GAL)	WBC	CA, TC, pH 6.0	activity
C3 Complement (C3)	P	PAGE, 11%	antibody
Carbonic anhydrase 1 (CA1)	RBC	PAGE, 9%	protein
Carbonic anhydrase 2 (CA2)	RBC	PAGE, 9%	activity
Catalase (CAT)	RBC	SAGE, LiOH, pH 8.1	activity
Diaphorase 1 (DIA1)	RBC	SAGE, TC, pH 7.2	activity
Diaphorase 2 (DIA2)	RBC	SAGE, TC, pH 7.2	activity
Esterase1 (EST1)	WBC	CA, AC, pH 5.3	activity
Esterase2 (EST2)	WBC	CA, AC, pH 5.3	activity
Fumarate hydratase (FH)	WBC	CA, TG, pH 8.6	activity
Glucose-6-phosphate dehydrogenase	WBC	CA, TG, pH 8.6	activity
Glucose phosphate isomerase (GPI)	RBC	SAGE, LiOH, pH 8.1	activity
Glutamate oxaloacetate transaminase (GOT1)	WBC	CA, TG, pH 8.6	activity
Glutamate oxaloacetate transaminase (GOT2)	WBC	CA, TG, pH 8.6	activity
Haemoglobin alpha chain (HBA)	RBC	IEF pH 6.7-7.7	protein
Haemoglobin beta chain (HBB)	RBC	IEF pH 6.7-7.7	protein
Haemopexin (HPX)	P	PAGE, 11%, Neu.	antibody
Hexokinase (HEX)	RBC	SAGE, AC, pH 6.0	activity
Isocitrate dehydrogenase (IDH)	WBC	SAGE, LiOH, pH 6.0	activity
Lactate dehydrogenase (LDHA)	RBC	SAGE, AC, pH 6.0	activity
Lactate dehydrogenase (LDHB)	WBC	CA, TG, pH 8.6	activity
Leucine aminopeptidase (LAP)	P	SAGE, LiOH, pH 8.1	activity
Malate dehydrogenase (MDH)	RBC	SAGE, AC, pH 6.0	activity
Malic enzyme (ME)	RBC	SAGE, LiOH, pH 8.1	activity
Mannose phosphate isomerase (MPI)	WBC	SAGE, AC, pH 6.0	activity
Nucleoside phosphorylase (NP)	RBC	CA, TG, pH 8.6	activity
Orosomucoid (ORM)	P	IEF, urea, pH 2.5-5, N	antibody
Phosphoglucomutase 1 (PGM1)	WBC	SAGE, AC, pH 6.0	activity
Phosphoglucomutase 2 (PGM2)	WBC	SAGE, AC, pH 6.0	activity
Plasminogen (PLG)	P	PAGE, 11%	antibody
Post-transferrin (PTF)	P	PAGE, 11%	protein
Pyruvate kinase (PK)	WBC	CA, AC, pH 5.3	activity
Superoxide dismutase (SOD)	RBC	SAGE, AC, pH 6.0	activity
Transferrin (TF)	P	PAGE, 11%	protein
Vitamin D binding protein (GC)	P	PAGE, 11%	antibody

P - plasma, RBC - red blood cells, WBC - white blood cells, PAGE - polyacrylamide gel electrophoresis (gel concentration %), IEF - isoelectric focusing (pH range), SAGE -starch gel electrophoresis, CA - cellulose acetate gel electrophoresis, LiOH, AC, TC, or TG -buffer sytstem as explained in the text and pH of buffer system, Neu - samples treated with neuraminidase, Urea - samples and gel contained specified molarity of urea.

TABLE 2 Proteins found to distinguish Père David's deer (n=5) and red deer (n=5) using the electrophoretic methods listed in Table 1. For loci in bold typeface all Père David's deer were distinct from all red deer. For other loci the frequency of the Père David's deer type in the red deer is given in parenthesis.

	No. of protein loci examined	No. of protein loci distinguishing the species.	Loci
Plasma	12	10	AAT, AACT, ALB, C3, HPX, ORM, PLG (0.5), PTF, TF, GC
Red Blood Cells	16	8	CA1, CAT, DIA2 (0.6), HBA, HEX, LDHA, ME, NP
White Blood Cells	15	2	ADA, IDH (0.8)

proportion of 0.5. With two exceptions, the proteins listed in Table 3 were codominantly inherited and were clearly heterozygous for the 'Père David' allele in the F1 hybrid male and approximately one half of the backcross offspring. For HEX and LDHA, the F1 male showed a moderate staining intensity, whereas the 16 red deer dams showed an absence of staining, while the backcross offspring showed either moderate staining (P) or did not stain. For all proteins except PLG and ADA, all of the red deer dams of the backcross hybrids had types which were clearly distinct from the F1 hybrid. In PLG and ADA one and

four of the mothers respectively had types which could not be distinguished from the F1 hybrid type and so their progeny were not informative and were not included in the segregation analysis. In the case of PLG this was due to polymorphism in red deer, while for ADA it was uncertain whether this was due to sample deterioration or polymorphism not observed in the sample of 5 red deer.

TABLE 3 Occurrence of 'Père David' alleles in backcross hybrid progeny and their red deer dams.

	Frequency of 'Père David' allele in 16 red deer dams	No of informative progeny	Proportion of progeny with a 'Père David' allele
AAT	0.000	16	0.43
AACT	0.000	16	0.43
ADA	0.125	12	0.75
ALB	0.000	16	0.50
C3	0.000	16	0.56
CA1	0.000	16	0.37
HBA	0.000	16	0.56
HEX	0.000	16	0.43
HPX	0.000	16	0.50
LDHA	0.000	16	0.62
NP	0.000	16	0.62
PLG	0.680	15	0.40
PTF	0.000	16	0.50
TF	0.000	16	0.37
GC	0.000	16	0.50
Unid 1	0.000	16	0.31
Unid 2	0.000	16	0.56

DISCUSSION

The large number of protein markers found to distinguish Père David's deer and red deer in this study and evidence that these markers segregate in backcross offspring suggest that deer hybrids may provide an excellent resource for linkage studies. Electrophoretic protein differences between species have been widely used as measure of genetic and evolutionary divergence (Avise, 1974) which enables comparison of our results with studies in other species. In a similar study to our own, Bonhomme *et al.*, (1984) examined the differences between mouse species in 42 proteins (22 of which were in common with this study). They found 10 fixed differences between *Mus spretus* and *Mus musculus*, and 9 loci with a strong frequency difference (>0.2), and calculated a genetic distance (D) of 0.46 between the species. In subsequent studies of these mouse hybrids, DNA variation informative for linkage studies has been found using both standard techniques and novel, rapid techniques which are only possible with such a wide cross (Siracusa *et al.*, 1991).

In the ruminants the only interspecies hybrid which is presently being used for linkage mapping is that between domestic cattle (*Bos taurus*) and the zebu (*Bos indicus*) (Roberts, 1990). However, in comparison with the mouse hybrids, there appear to be few proteins, if any, which distinguish the species. Baker and Manwell (1980) compiled data from nearly 1000 papers on protein variation in cattle and tabulated 10 proteins from blood and milk which distinguished zebu and other cattle breeds. These gene frequency data identified zebu and breeds originating from zebu x domestic cattle hybrids as the most diverged 'breeds' from domestic cattle but found no fixed differences between the species (Manwell and Baker, 1980).

These comparisons support the conclusion that the deer hybrids will be valuable for genetic linkage studies. They suggest that the molecular divergence between the deer species is at least comparable to the mouse interspecies hybrids used very successfully for gene linkage mapping and is much greater than the divergence between cattle hybrids currently being used for linkage mapping in the ruminants. A disadvantage of using deer hybrids for linkage studies is lack of molecular genetic and quantitative trait studies in these species. However these disadvantages may not be great as DNA probes from other ruminants (eg: sheep and cattle) hybridise well with deer DNA (D.F. Hill, pers. comm.), and at least on research farms there are few practical constraints to measuring most quantitative traits in deer.

The potential value of this deer hybrid resource is twofold. Firstly, it is an efficient means of estimating the distance between genetic markers and their order along the chromosomes (genetic linkage mapping). These data are expected to have application in all ruminants, and would provide important comparative data on the arrangement of genes in ruminants and other species under intense genetic investigation, principally, mice and humans. The second area where the deer hybrid resource will be of value is in conducting linkage analyses with quantitative traits. There are a large number of traits which distinguish red deer and Père David's deer. Genetic analysis of some traits, such as the differences in the seasonality of reproduction (Wemmer *et al.*, 1989), antler morphology (Wemmer, 1983), gestation length (Fennessy *et al.*, 1991) and disease resistance (Orr and Macintosh, 1988) have direct application to New Zealand deer breeding, while a genetic analysis of other distinctive differences between the species such as tail length and foot morphology (Wemmer, 1983) are of less direct application. However, there is already some evidence that associations found between chromosome regions and quantitative traits in one interspecies hybrid are also present in other crosses (Paterson *et al.*, 1991). If this is generally true, then, as with genetic linkage mapping studies, the results of studies examining linkage between chromosome segments and quantitative traits using Père David's deer hybrids could have more general significance.

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