A New Gene Mapping Resource: Interspecies Hybrids Between Père David's Deer (*Elaphurus davidianus*) and Red Deer (*Cervus elaphus*)

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ABSTRACT

Three male F_1 hybrids between Père David's deer and red deer were mated to red deer to produce 143 backcross calves. The pedigrees are a rare example of a fertile hybrid between evolutionarily divergent species. We examined the use of these families for genetic mapping of evolutionarily conserved (Type I) loci by testing for genetic linkage between five species-specific protein variants and 12 conserved DNA probes. Two probes were homologous, and the remainder syntenic, to the protein coding loci in cattle or humans. Using six restriction enzymes, each DNA probe detected one or more restriction fragments specific to Père David's deer. Linkage analyses among the species-specific variants placed the loci into four linkage groups within which linkage between adjacent loci and gene order was supported by a LOD > 3. The linkage groups were (*HPX, HBB*)-*FSHB-ACP2, LDHA-CD5-IGF2, BMP3*-(*GC, ALB*)-(*KIT, PDGFRA*) and *LDLR-C3-FGF1*. Southern and protein analysis of *LDHA* and *ALB* provided identical segregation data. These linkage groups were consistent with the cattle gene map and provide new information for comparing the gene maps of ruminants, humans and mice. The deer hybrids are an important new resource that can contribute to the comparative analysis of the mammalian genome.

IN the 1970s, cytological analyses demonstrated that karyotypic banding patterns could be used to align chromosomes and chromosome segments between genera, families and, in some cases, orders of mammals (SUMNER 1990). These data have been extended using gene maps that compare the chromosomal location of homologous loci among species (O'BRIEN *et al.* 1985, 1988) and such comparisons are most advanced in the extensively mapped genomes of mice and humans (O'BRIEN 1991; NADEAU *et al.* 1993). There is considerable interest in refining and extending the comparison of gene maps to a wider variety of species in an attempt to unify the genetic analysis of mammals and to provide insights into mammalian genome organization and evolution (EDWARDS 1991; FARR and GOODFELLOW 1992; O'BRIEN *et al.* 1993).

Genetic linkage mapping is currently the most productive technique in the refinement of gene maps of a wide variety of mammals, such as humans (WEISSENBACH *et al.* 1992), mice (DIETRICH *et al.* 1992; COPELAND *et al.* 1993), rats (SERIKAWA *et al.* 1992), cattle (BARENDSE *et al.* 1994; BISHOP *et al.* 1994), sheep (CRAWFORD *et al.* 1994) and pigs (ELLIGREN *et al.* 1994). Linkage maps provide the ability to rapidly determine the location and order of polymorphic genes and the potential to locate genes responsible for variation in phenotypic traits. However, with the exception of the mouse map, very few of the markers on linkage maps are suitable for cross referencing and alignment of the various mammalian genome maps. Typically linkage maps are composed of highly polymorphic microsatellites (WEBER and MAY 1989), which are usually anonymous and, with a few notable exceptions (STALLINGS *et al.* 1991), not widely conserved among mammals. O'BRIEN *et al.* (1993) has formalized this distinction of marker types, defining the evolutionarily conserved markers as "Type I" markers and the highly polymorphic but anonymous markers as "Type II" markers.

Only the mouse has a detailed linkage map of Type I markers and the key to the production of this map has been the use of interspecies hybrid backcross mapping pedigrees (COPELAND and JENKINS 1991). The principle example is an interspecies hybrid between *Mus spretus* and *M. musculus* (the laboratory strain C57BL/6J). These and other mouse interspecies hybrids continue to be the method of choice for mapping expressed genes and refining the genetic map of the mouse (COPELAND *et al.* 1993). The important feature of these interspecies hybrids is that the genetic divergence between the species is such that species specific variants can be rapidly identified with virtually any DNA gene probe. Each marker with species specific variants is heterozygous in the F_1 hybrid and thus fully informative in backcross panels.

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Conceptually, this approach could be used to efficiently generate linkage maps of Type I loci from a wide variety of mammalian genera. Parallels have been drawn between the mouse hybrids and crosses created for genetic mapping in domestic animals such as the *Bos taurus* × *B. indicus* hybrid (ROBERTS 1990) and crosses among divergent strains of domestic pig *Sus scrofa* (ARCHBALD *et al.* 1991). Although these crosses introduce some additional polymorphism into mapping panels, linkage maps of Type I markers comparable with those produced with *M. spretus* hybrids (eg: BUCHBERG *et al.* 1989) have not been forthcoming suggesting these large mammal hybrids do not share the wide divergence of the mouse hybrids. Breeding of wider hybrids for genetic linkage mapping has been attempted in sheep (sheep × goat hybrids; HILL and BROAD 1991) and cats (Asian leopard × domestic cat; LYONS and O'BRIEN 1994) but few backcross animals have been produced. In marsupials, a subspecific backcross between tammar wallabies is being evaluated for linkage mapping (MCKENZEE *et al.* 1993).

The interspecies hybrid we have developed is between the Père David's deer or Milu, *Elaphurus davidianus,* and red deer, *Cervus elaphus.* The deer species show high level of genetic divergence, similar to that between *M. spretus* and *M. musculus* (BONHOMME *et al.* 1984). Estimates of the genetic distance (NEI 1972) between the deer species are 0.35 from 22 protein loci (EMERSON and TATE 1993) and 0.46 from 45 protein loci (TATE *et al.* 1992). Red deer are the common large deer of Europe and closely related subspecies and species are naturally distributed throughout Europe, Asia and North America (WHITEHEAD 1972). In contrast, Père David's deer, originally from China, are one of the world's rarest deer species (JONES *et al.* 1983). The two species have very large differences in their appearance and biology, the most notable being the antler, foot and tail morphology (WEMMER 1983), seasonality (LOUDON *et al.* 1989), disease resistance (ORR and MACKINTOSH 1988) and behavior (ALTMANN and SCHEEL 1980). However, both species have the same number of chromosomes and very similar karyotypes (WANG 1988) and fertile hybrids have occasionally been produced (BEDFORD 1951). Recently, in New Zealand, Père David's deer males that were in excess to the breeding requirements of the Père David population have been successfully crossed to red deer females using artificial insemination (ASHER *et al.* 1988).

In this study, we report on the fertility of the F_1 hybrid males and the production of large backcross families. The aim of this study was to evaluate these pedigrees as a resource for linkage mapping Type I markers and comparing the chromosomal arrangement of loci in deer with other species. The experimental approach was to use restriction fragment length variants (RFLVs) detected by conserved coding sequences to build linkage groups around five protein variants known to be segregating in the backcross herd (TATE *et al.* 1992; EMERSON and TATE 1993). We have used the term "variant" for the fixed differences observed between the species and reserved the word "polymorphism" for variation within a species. The five protein variants were assumed to reflect variation in the coding gene loci, namely, *C3* (*complement component 3*), *GC* (*vitamin D binding protein*), *ALB* (*albumin*), *LDHA* (*lactate dehydrogenase A*) and *HPX* (*hemopexin*). Loci were chosen for RFLV investigations on the basis of homology or conserved synteny with these protein loci in the human and cattle gene maps (Table 1). Three of the proteins, (*HPX, LDHA* and *C3*) are close to proposed evolutionary break points between human and cattle chromosomes (O'BRIEN *et al.* 1993) so we selected gene probes flanking these breakpoints (Table 1).

MATERIALS AND METHODS

Pedigrees and sampling: Three F_1 stags, produced from artificial insemination of red deer hinds with Père David's deer semen (ASHER *et al.* 1988; FENNESSY *et al.* 1991), were mated to red deer from 1989 to 1991 on three New Zealand deer farms. During this period, the three stags naturally mated with 91, 21 and 32 red deer, respectively, and semen from the latter two stags was used to artificially inseminate 31 and 83 red deer, respectively, using methods described by FENNESSY *et al.* (1991). All animals were maintained outdoors on pasture. Blood samples (50–100 ml) were taken from all the available parents and progeny of these matings. The parents of the sires had either died soon after mating or were not available for sampling. However, reference samples were available from five Père David's deer from the grandsires' herd.

Protein variation: Five proteins known to distinguish red deer and Père David's deer were scored in the pedigrees using previously described methods (TATE *et al.* 1992; EMERSON and TATE 1993). Briefly, LDHA was examined by native starch gel electrophoresis of red blood cell lysates followed by histochemical staining. GC, C3 and HPX were analyzed by native polyacrylamide gel electrophoresis of plasma followed by western analysis using antibodies specific to the human form of these proteins (Dako, Carpenteria, CA). ALB was analyzed by isoelectric focusing at pH 3–10 with 6 M urea followed by general protein staining.

Selection of DNA probes: Large numbers of cDNA probes from a variety of mammalian sources had been previously screened for hybridization with red deer DNA on "zooblots," which included *Eco*R1-restricted DNA from human, sheep, goat, cow, red deer, pig and possum (*Trichurus vulpecula;* J. M. PENTY and H. C. MATHIAS, unpublished data). From this information, we selected 12 mammalian probes which showed good homology with red deer DNA (Table 1). The probes were selected from analysis of published comparative gene maps of human, mouse, and cattle (see Introduction). They were not preselected on the basis of DNA polymorphisms.

Restriction fragment length variation: DNA was extracted using the salt method, blotting and hybridization protocols described by MONTGOMERY and SISE (1990) with the exception that probes were labeled using a "megaprime" random prime labeling system (Amersham, UK). Probes were screened for variation on filters, which contained DNA from

TABLE 1

Loci examined in the deer interspecies pedigrees; comparative map locations, methods, restriction enzymes and DNA probes used

Locus name	Symbol	Chromosomal Location			Deer species specific variants[e]	Clone used in RFLV		
		Human[b]	Mouse[c]	Cattle[d]		Name (species)	Genbank account	Reference
Bone morphogenetic protein 3	*BMP3*	4p14-q21	5.50	(6)	RFLV:*Hin*dIII, ***Msp*I**, *Pvu*II	BMP3-315 (human)	M22491	WOZNEY *et al.* (1988)
Albumin	*ALB*[a]	4q11-q13	5.46	6	Protein and RFLV: *Hin*dIII, ***Msp*I**	SSA1 (ovine)	X17055	BROWN *et al.* (1989)
Vitamin D binding protein	*GC*	4q12-q13	5 syn	6	Protein variation only			
Hardy-Zuckerman 4 feline sarcoma viral oncogene	*KIT*[a]	4p11-q22	5.37	6	RFLV: ***Pst*I**, *Pvu*II	hckit-171 (human)	M16592	YARDEN *et al.* (1987)
Insulin-like growth factor II	*IGF2*[a]	11p15.5	7.74	25	RFLV: ***Pvu*II**	B5 (bovine)	X53553	BROWN *et al.* (1990)
Hemoglobin, beta	*HBB*[a]	11p15.5	7.49	15	RFLV: ***Msp*I**, TaqI	G4EC3HA3 (caprine)		TOWNES *et al.* (1984)
Hemopexin	*HPX*	11p15.5-15.4	—	—	Protein variation only			
Lactate dehydrogenase A	*LDHA*[a]	11p15.1-p14	7.23	25	Protein and RFLV: *Msp*I, *Pvu*II, ***Taq*I**	LDH12 (bovine)	D90143	ISHIGURO *et al.* (1990)
Follicle stimulating hormone, beta polypeptide	*FSHB*[a]	11p13	2.42	15	RFLV: *Hin*dIII, *Bgl*II, ***Msp*I**, ***Taq*I**	bovFSH31 (bovine)	M14853	MAURER and BECK (1986)
Acid phosphatase 2, lysosomal	*ACP2*[a]	11p11	2	15	RFLV: ***Hin*dIII**	CT29-8 (human)	X53061	POHLMANN *et al.* (1988)
CD5 antigen	*CD5*[a]	11q13	19.08	(25)	RFLV: ***Bgl*II**, *Taq*I	BCD5 (bovine)	X12548	YU *et al.* (1990)
Low-density lipoprotein receptor	*LDLR*[a]	19p13.3	9.04	7	RFLV: ***Hin*dIII**, *Pvu*II	LDLR-1 (bovine)	M11341	HOBBS *et al.* (1985)
Complement component 3	*C3*	19p13.3-p13.2	17.28	(7)	Protein variation only			
Fibroblast growth factor 1 (acidic)	*FGF1*[a]	5q31.3-q33.2	18.18	7	RFLV: *Bgl*II, ***Msp*I**, *Taq*I	JC-3-5 (human)	M13361[f]	JAYE *et al.* (1986)

[a] Comparative loci from the list compiled by O'BRIEN *et al.* (1993).
[b] Data from the Human Genome Database (GDB), March 1994.
[c] Data from HILLYARD *et al.* (1993) and NADEAU *et al.* (1993).
[d] Data from WOMACK *et al.* (1993) and MEZZELANI *et al.* (1994), bracketed assignments were made by interpolation from the comparative maps presented by O'BRIEN *et al.* (1993).
[e] Details of screening procedure are given in the text; the enzyme(s) in bold type were used to genotype family samples.
[f] The sequence is from independently isolated clone of the same gene.

two or three Père David's deer, an F_1 sire, and three red deer each cut with six restriction endonucleases (*Bgl*II, *Hin*dIII, *Msp*I, *Pst*I, *Pvu*II, *Taq*I). RFLVs were then examined on family filters that contained DNA from all progeny, sires and dams digested with one of the six endonucleases identified from the screening filters.

Linkage analysis: All experimental results were independently scored by two people and any equivocal results excluded from the analyses. Where a polymorphism was identified in red deer, alleles were named alphabetically in order of decreasing fragment length whereas the Père David allele was designated '*P*' irrespective of the size. The full genotypic data was used to check pedigree information. For linkage analysis, genotypic data in the backcross were simplified to a single score indicating the presence (in heterozygous form) or absence of the Père David allele. Data from the three sires were combined and analysed using the computer package MAPMAKER version 3.0 (LANDER *et al.* 1987) with data entered in the backcross format. Two-point linkage analysis was used to identify linkage between loci, and thus construct linkage groups (LOD > 3). The relative likelihoods of all possible orders of loci in each linkage group were then compared and a maximum likelihood map for the most likely gene order was calculated using the MAP function of MAPMAKER. The error detection option was used initially with the probability for error set at 1% for each locus. Potentially incorrect genotypes were checked and in two cases a mistaken scoring was corrected. The analysis was then repeated without using error detection.

RESULTS

Pedigrees: The three F_1 Père David × red deer hybrids stags produced 58, 34, 51 living progeny respec-

tively in the three years of matings, 43 of these were from artificial inseminations and the remainder from natural mating. In total, 123 of these backcross animals were available for sampling. The dams of 79 of these calves were sampled, while the dams of the remaining 44 calves were either not individually identified in pedigree records or could not be located for sampling. No twin calves were recorded but nine of the red deer dams had hybrid calves in two different years.

Molecular markers: Each of the DNA probes screened for fragment length variation identified differences between Père David's deer and red deer with at least one of the six restriction enzymes used (Table 1). For all but the *ACP2* (*lysosomal acid phosphatase*) and *IGF2* (*insulin-like growth factor II*) probes, two or more restriction enzymes identified an RFLV. For each probe the restriction enzyme producing the clearest RFLV difference between Père David's deer and red deer with the lowest number of additional bands was chosen for further study. The restriction enzyme(s) used are indicated in boldface type in Table 1.

When family samples were analyzed the validity of the species differences identified in screening was strongly supported for all protein and DNA markers. The red deer dams were monomorphic for 10 of the markers but showed polymorphism in the remaining seven loci, namely the protein loci *ALB, C3* and *GC* and the RFLVs detected with *FGF1, FSHB, LDHA* and *LDLR*. In each case, the polymorphism within red deer was clearly distinct from the Père David variant. No genetic variation was detected in the Père David's deer. For all markers, the F_1 sires showed a heterozygous type with one allele being the unique Père David allele and the other, an allele found in red deer. In each case, the backcross calves were either heterozygous for the Père David allele or had only the red deer alleles. The frequency of heterozygotes in the calves for the Père David allele did not differ significantly from the expected proportion of 0.5 for any marker. The protein variants in GC, C3, ALB and HPX were inherited codominantly in the backcross. LDHA had a band of activity present in Père David's deer that was not present in red deer. Hence whether an individual was homozygous or heterozygous for the $LDHA^P$ allele could only be distinguished on the intensity of staining.

Linkage analysis: Linkage was examined by testing for cosegregation between species-specific markers. The *HPX* and *LDH* protein variants, which required fresh blood samples, were genotyped in the 31 progeny of only one sire. The remainder of the loci were tested in all 123 available progeny and unequivocal genotypic data was obtained for either 123 or, in eight loci, 122 progeny. Table 2 shows the significantly linked pairs of loci from pair-wise tests of all combinations of the 17 markers tested. The two point linkage data identified four groups of linked loci (Table 2), namely, *ACP2, FSHB, HBB* and *HPX; CD5, IGF2* and *LDHA; ALB, BMP3, GC, KIT, PDGFRA;* and *C3, FGF1* and *LDLR*. Among loci from different linkage groups the highest LOD score was 1.1 giving little evidence of any association between the groups. In two cases, *ALB* and *LDHA*, the same locus was genotyped using both protein variation and RFLVs. In these comparisons, no recombination was detected between the protein variants and RFLVs (Table 2) providing confirmation of the identity of the locus and the segregation pattern in the backcross pedigrees. For subsequent analyses, data from the RFLVs were used as these had no missing data.

TABLE 2

Linkage groups (LOD > 3) identified by two-point analysis

Loci	No. recombinants/no. informative meosis.	rf	LOD
Linkage Group 1			
HPX[a]-*HBB*	0/30	0.00	9.03
HPX[a]-*FSHB*	3/31	0.10	5.05
HBB-FSHB	8/122	0.07	23.9
HBB-ACP2	35/121	0.29	4.82
FSHB-ACP2	28/122	0.22	8.72
Linkage Group 2			
LDHA[a]-*LDHA*	0/31	0.00	9.33
LDHA-CD5	24/122	0.20	10.5
CD5-IGF2	19/122	0.16	13.8
Linkage Group 3			
ALB[a]-*ALB*	0/122	0.00	36.7
ALB-GC	0/123	0.00	37.0
ALB-BMP3	13/122	0.11	18.8
ALB-KIT	6/123	0.05	26.6
ALB-PDGFRA	6/123	0.05	26.6
GC-BMP3	13/122	0.11	18.8
GC-KIT	6/123	0.05	26.6
GC-PDGFRA	6/123	0.05	26.6
BMP3-KIT	19/122	0.16	13.8
BMP3-PDGFRA	19/122	0.16	13.8
KIT-PDGFRA	0/123	0.00	37.0
Linkage Group 4			
C3[a]-*LDLR*	7/121	0.06	24.8
C3[a]-*FGF1*	25/121	0.21	9.65
LDLR-FGF1	31/121	0.26	6.52

[a] Loci genotyped using protein variation.

Comparison of the likelihood of all possible orders of loci within each linkage group gave strong support for the following orders: (*HPX, HBB*)-*FSHB-ACP2, LDHA-CD5-IGF2, BMP3*-(*GC, ALB*)-(*KIT, PDGFRA*) and *LDLR-C3-FGF1*. Markers within brackets showed no recombinants and therefore could not be ordered. The next most likely gene orders had relative LOD scores of −3.8, −8.0, −4.9 and −3.6 for groups 1, 2, 3 and 4, respectively. The maximum likelihood map produced for each of these gene orders is shown in Figure 1. Typically each backcross individual was informative for

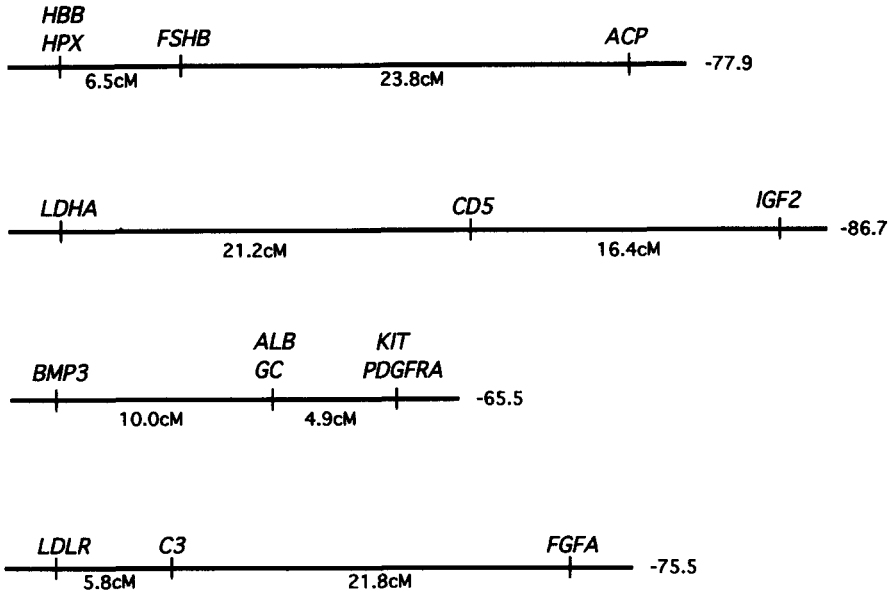

FIGURE 1.—Graphical representation of the maximum likelihood map for each linkage group derived from the MAPMAKER analysis. The map shows the chromosomal order and distance between loci in Kosambi centi-Morgans (cM). The absolute likelihood of the map is given on the right.

all markers so the location of recombination events in each individual could be placed. Figure 2 summarizes all the genotypic data for each linkage group giving the number of animals with no recombination across a linkage group (□ or ■) or with a recombination event in a particular marker interval in a linkage group (a transition from ■ to □). This presentation of the data confirms the stated gene order, which was the only order in which no double recombination events occurred.

DISCUSSION

The value of interspecies hybrid backcrosses for linkage mapping has been convincingly demonstrated in the mouse, a "map rich" species where interspecies backcrosses play an on-going role in the construction of a genetic map (COPELAND *et al.* 1993). Similar genetic mapping resources are currently lacking in other mamalian orders and, compared with the mouse, there are few alternative resources for mapping and ordering "comparative" or Type I markers. We have identified a wide interspecies hybrid in deer, a ruminant species with no previous genetic mapping information, and successfully used it to build linkage groups and order evolutionarily conserved loci. We evaluate the deer interspecies hybrids as a gene mapping resource and discuss their use in a comparative approach to gene mapping and genetic analysis of traits in deer and other mammals.

Genetic divergence and fertility: The key feature of interspecies hybrid pedigrees is that they contain useful genetic variation in coding regions that are typically conserved within a species. The expectation, from protein data, of a wide molecular genetic divergence between Père David's deer and red deer (TATE *et al.* 1992)

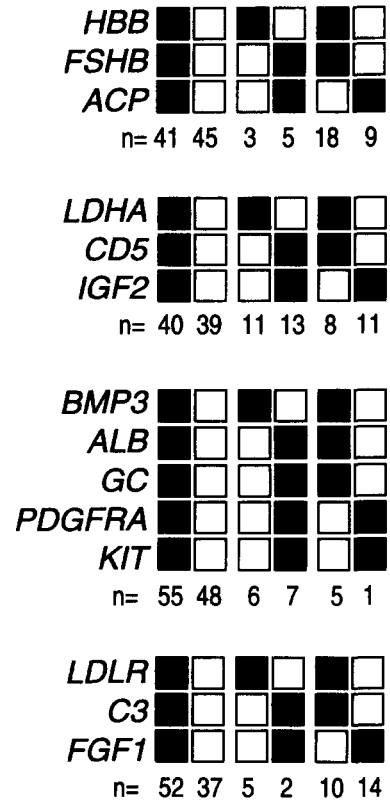

FIGURE 2.—Summary of the results of the linkage analysis in the Père David's deer × red deer backcross families. Each column represents the chromosome inherited from the F_1 Père David × red deer sire in the backcross progeny. The order of markers along the chromosome derived from our linkage analysis is shown on the left. The presence of Père David alleles (■) and red deer alleles (□) are indicated. The number of each type of chromosome observed in the linkage analysis is listed below. Seven chromosomes with missing data points are excluded.

was confirmed by the RFLV data. Père David's deer and red deer showed fixed allelic differences with each of the 12 coding sequence gene probes chosen for study. The loci were not preselected on the basis of polymorphism and so we expect that virtually any single-copy probe that hybridizes to deer DNA will identify a difference between Père David's deer and red deer. Zooblot data including >200 cDNA probes (J. M. PENTY and H. C. MATHIAS, unpublished data) show a very large number of useful probes are available for deer mapping. In these data, 80% of ovine and bovine cDNA probes hybridized strongly to deer DNA at moderate stringency, whereas under similar conditions 60% of porcine and human probes hybridized. The recent history of Père David's deer may contribute to the number of fixed allelic differences between the species. Père David's deer have undergone repeated founder effects and a bottleneck of small population size possibly lasting >1000 yr (JONES *et al.* 1983).

A wide evolutionary genetic divergence between two species and the ability to produce fertile hybrids are usually mutually exclusive. In *M. spretus* hybrids, which show a similar level of genetic divergence to the deer hybrids (BONHOMME *et al.* 1984), only females, the homogametic sex, are fertile (COPELAND *et al.* 1993). In contrast, both male and female F_1 hybrids are fertile in the Père David's deer × red deer (FENNESSY and MACKINTOSH 1992). Female deer, typically have only one offspring per year, so the fertility of male hybrids is essential to the rapid production of large numbers of backcross hybrids for linkage analysis. We found no evidence of infertility in the three F_1 males used in this study as their calving results were within the normal range expected for red deer mated under similar conditions. We are currently producing a further 200 fully recorded backcross animals and, in addition, hybrids are being produced on commercial deer farms because they offer the potential of introducing desirable Père David's deer traits into New Zealand's farmed deer herd, which includes >1,000,000 red deer and wapiti (*C. elaphus* ssp.). We know of no other large mammal where such large numbers of a wide hybrid have been produced.

Genetic segregation and linkage analysis: In the present deer backcross, only male meioses are examined so segregation among *X*-chromosome markers cannot be tested. For linkage analysis of autosomal markers with species-specific alleles, we see no important differences between the half-sib deer and the full-sib mouse interspecies backcrosses. One practical aspect of using half-sib families is that there are a relatively large number of parental animals. DODDS *et al.* (1993) have shown that in many instances little or no extra linkage information is gained from genotyping the dams of large paternal half-sib families. In the present analysis, we typed all available parents ($n = 70$) and assumed that the parents not available for typing showed the absolute species-specificity of alleles found in the genotyped animals. This assumption was essential to determine both the phase of linkage and the segregation of sire alleles in some progeny and introduces a slight possibility, first, that the *P* (Père David) allele in one F_1 hybrid was actually inherited from its red deer dam and, second, that a backcross calf whose mother was not available for genotyping inherited a *P* allele from its dam and a *red deer* allele from its Père David sire. Clearly this is very unlikely as 70 red deer and five Père David's deer showed fixed differences. We calculated that, even given an unlikely distribution of gene frequency, the probability of the first possibility was <1 in 3000 per locus and the second possibility <1 in 150 per locus, per calf. Low frequency errors, such as these, would be likely to be detected by analysis of double recombinants and other procedures used to identify genotyping errors in the data set (LINCOLN and LANDER 1992) but no anomalous results were identified in these analysis. In future pedigrees, we suggest it will not be necessary to routinely genotype the red deer dam of each backcross but it would be preferable to have the dams available for genotyping if an unlikely distribution of genotypes is detected.

Interspecies hybrids can show unusual patterns of inheritance that may affect linkage analysis. Segregation distortion has occasionally been reported in mouse interspecies hybrids (SIRACUSA *et al.* 1989) and recombination suppression, caused by small chromosomal inversions that have accumulated between diverged species, is also a concern (HAMMER *et al.* 1989). The linkage analysis in deer demonstrated stable inheritance and segregation of markers in the hybrid pedigrees, at least for the chromosome segments covered by the present markers. Also, recombination rates appeared to be compatible with map information from other species. Loci showing no recombination have been mapped very close to each other in other species. For example, *KIT* and *PDGFRA* show no recombination in interspecies hybrid mouse panels (KOSAK and STEPHENSON 1992) and *ALB* and *GC* are very closely linked in a wide variety of mammals (O'BRIEN 1993). We could not find any comparative linkage data for *HPX* and *HBB* but these loci do map to the same chromosome band in humans (Table 1).

Comparative gene maps: The basic tenet of comparative gene mapping is that the high degree of similarity between the gene maps of mammals can be used to unify many aspects of mammalian genetics. This has been demonstrated repeatedly in comparisons of mouse and human gene maps and in the use of mouse models for human disease (HILL and VAN HEYNINGIN 1992). The use of comparative maps within a mamma-

lian order such as the ruminants (to which cattle, sheep and deer belong) is likely to be even more robust. Strong similarities between the gene maps of cattle and deer are expected considering the close evolutionary relationships of the families Cervidae and Bovidae and the similarities in the G banding pattern of Père David's deer and cattle chromosomes (BUCKLAND and EVANS 1978). Our data are a good illustration of the use of the cattle map to construct linkage groups in deer. The loci examined fell into linkage groups which are identical to their syntenic grouping in cattle (Table 1). The five loci not mapped in cattle (*BMP3, C3, CD5, HPX* and *PDGFRA*) were placed into a group predicted from human-bovine comparative mapping data (Table 1). The deer linkage data, therefore, provide support for the gene assignments in cattle. With the exception of *FSHB* and *HBB*, the loci examined in this study are either not assigned in cattle or assigned by only one method in a single study (WOMACK *et al.* 1993). Furthermore even in this limited data set the new gene order information from the deer indicates additional rearrangement within a syntenic group that is conserved between human and ruminants because in the *HSA11p* loci *IGF2* and *LDHA* are separated by *CD5* which is from *HSA11q* (Figure 1). As these data illustrate, the development of gene maps in at least three ruminant species (deer, sheep and cattle) provides a "comparative strength" as inference among maps can be used to confirm map locations or identify incorrect assignments and predict the location of genes not mapped in a particular species. The deer hybrid pedigrees are currently the most informative ruminant resource for mapping and ordering evolutionary conserved (Type I) markers and their use may allow more rapid and detailed comparison of the ruminant genome with other mammalian orders. We believe these advantages of a comparative approach within the ruminants and among mammals in general far outweigh potential disadvantages involved in confusion over the inference of homology among species.

The primary goal of ruminant linkage mapping programs, including the deer, is to use maps to locate the loci underlying phenotypic variation in commercially important traits (*e.g.*, HETZEL 1993; MONTGOMERY *et al.* 1993). Although the use of diverse mapping resources and a comparative approach is of value in gene mapping, it will be even more valuable in identifying loci underlying variation in phenotypic traits. Successful genetic analysis of phenotypic traits, particularly complex traits, requires large pedigrees with clearly measurable variation. However, even in the most well characterized species or cross, only a few traits will be amenable to analysis. Père David's deer are distinct in behavioural traits (*e.g.*, vocalizations, neonatal behavior and gait), morphological traits (*e.g*, hoof, tail, rump and antler shape) and physiological traits (*eg.* gestation length, seasonality and disease resistance). Hybrids show measurable variation in some of these traits (FENNESSY and MACKINTOSH 1992). The same features that make the pedigrees useful for linkage mapping, also make them advantageous for the genetic analysis of these complex traits (LANDER and BOTSTEIN 1989).

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