Development and applications of a genetic linkage map of deer

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Summary

The rapid progress toward a primary genetic map of deer is reviewed and interspecies hybrid backcross pedigrees between Père David's deer (Elaphurus davidianus) and red deer (Cervus elaphus) described which have made this progress possible. Gene probes or primer pairs with good homology to deer can be mapped by analysis of DNA from the interspecies hybrid pedigrees. This DNA is now available as an international gene mapping resource. The interspecies hybrids have been used to chromosomally locate and order 150 gene markers into 29 linkage groups. Two applications of this gene map to (1) systematically search for key genes which determine the differences in morphology, physiology and biology of deer species and (2) compare and integrate genetic studies of deer with those of more intensively studied mammals, such as mice, humans and cattle, are discussed. It is argued that in the future a deer genetic map and the interspecies hybrid pedigrees will become basic tools in the study of deer genetics and will give new insights into evolution of natural populations and the production and breeding of farmed deer.

Introduction

New molecular tools are providing a new understanding of how genes act to produce the characteristic morphology, physiology and behaviour of animals (Davies & Tilghman, 1991; Wilkins, 1993). Remarkably, the same technology allows the alignment of the chromosomes of diverse mammals so that a gene responsible for a particular phenotypic trait in one mammal can be rapidly identified in other mammals (O'Brien *et al.*, 1993). In deer, these techniques offer new ways to examine fundamental questions in deer biology and genetics and offer the ability to integrate this information with knowledge from other mammalian species. We anticipate such studies will provide innovative approaches in cervid veterinary diagnostics, the genetic improvement of captive deer and the management of wild and endangered populations.

Two technologies play a central role in these advances: genetic markers and genetic maps. Variation in "marker" genes is used to trace the evolution of genes in phylogenies and populations, and the segregation of genes in pedigrees. Genetic marker techniques, such as protein polymorphism, have been used widely in the study of the evolution and biology of deer (Dratch & Pemberton, 1992) and other papers in the current volume describe the application of powerful new marker technologies in deer (Cronin, 1996; Pemberton, 1996).

Genetic maps describe the location and order of genes and genetic markers on the chromosomes of an animal. Just as geographic maps allowed explorers to systematically search for and record new continents and trade routes, genetic maps provide the ability to systematically search the mammalian chromosomes for a particular gene and accurately record that gene's location in the genome. Humans, mice and a growing number of domestic animals have

detailed and rapidly advancing genetic maps (O'Brien, 1994). This paper reports progress on the first substantial genetic map of a wild species (deer), and discusses the application of this technology which has the potential to revolutionise genetic studies in both captive and naturally evolving populations.

Development of a deer genetic map

Linkage mapping and interspecies hybrids

There are numerous techniques for genetic mapping in mammals and some new techniques have potential for mapping of novel species such as deer (Walter *et al.*, 1994). Currently the technique giving the highest resolution and most rapid development of genome maps is genetic linkage mapping (Schmitt and Goodfellow, 1994). Surprisingly, deer offer some of the best resources for linkage mapping of any mammal (Tate *et al.*, 1995).

Linkage mapping analyses the segregation of polymorphic markers in families; markers which are located close together on the chromosomes tend to co-segregate during meiosis while markers which are distant, or on different chromosomes, segregate independently (Fig. 1 and see below). Linkage analysis requires markers which show variation or polymorphism within a particular linkage pedigree and so linkage maps primarily consist of highly polymorphic but anonymous DNA markers, such as microsatellites (Bishop *et al.*, 1994; Crawford *et al.*, 1994; Schmitt & Goodfellow, 1994). Unfortunately, these highly polymorphic markers are not usually conserved among mammals so hundreds of new markers are usually needed for each new species being studied (O'Brien, 1991; O'Brien *et al.*, 1993).

The rare cases where different species hybridise and produce fertile offspring provide an exception to this situation and are a remarkable resource for linkage mapping (Avner *et al.*, 1988). The essential feature of these hybrids is that the parental species are as widely diverged as possible, so it is easy to identify variation, even in evolutionarily conserved markers, but the cross must also produce fertile hybrids so that extended pedigrees can be produced for linkage analysis. In the mouse, hybrids between *Mus spretus* and laboratory strains have been pivotal to the very rapid development of mouse genetic maps which are now at the highest resolution in any mammal (Copeland *et al.*, 1993). However, a wide evolutionary genetic divergence between two species and the ability to produce fertile hybrids are usually mutually exclusive and attempts to produce similar hybrids for linkage mapping in other mammalian orders have either been unsuccessful (Hill & Broad, 1991) or are at an early stage (O'Brien, 1991).

Père David's deer x red deer hybrids : a new linkage mapping resource.

The Cervinae are notable for the degree of hybridisation between the various species and subspecies (Gray, 1972). Of these, the cross between Père David's deer and red deer is among the widest and one of the few confirmed cases where members of different taxonomic genera produce fertile male and female hybrids (Van Gelder, 1977). These species share the same chromosome number and similar chromosome banding pattern (Wang, 1988), but protein marker studies confirm the wide genetic divergence between these species which was expected from taxonomy (Emerson & Tate, 1993; Tate *et al.*, 1992). In total 17 of the 43 putative protein loci examined had a different type in Père David's deer and red deer. Comparison of these results with similar studies in the mouse (Bonhomme *et al.*, 1984) showed the Père David hybrids had a similar level of divergence to the *Mus spretus* hybrids and a much wider divergence than any other cross currently available for genetic mapping (Tate *et al.*, 1992).

The wide divergence between Père David's deer and red deer makes it possible to use conserved, DNA gene probes for linkage mapping (Table 1). Approximately 60% of human gene (cDNA) probes hybridised well to deer DNA using our methods for Southern analyses (Tate *et al.*, 1995) while 80% of probes from Artiodactyl species hybridise well to deer DNA (Table 1). Of the probes that "work" in deer, over 90% show a difference between Père David's deer and red deer and so can be ordered to form a map (see

Table 1. Summary of genetic markers tested in Père David's deer and red deer

Marker type	Total number of markers tested	Number of markers which "work" in deer	Number of markers which distinguish Père David's deer and red deer
Protein polymorphism[1]	43	43	17
RFLV, human cDNA[2]	84	54	49
RFLV, artiodactyl cDNA[2]	108	88	80
Sheep microsatellites[3]	102	19	18

1 from Tate *et al.* (1992).

2 RFLV - restriction fragment length variant, the exact methods are described by Tate *et al.* (1995).

3 The microsatellite methods and primers used are described by Crawford *et al.* (1994).

below). In most cases these differences were found using restriction fragment length variation and a standard screen of only six restriction enzymes (Tate *et al.*, 1995). The use of gene probes from other species has allowed the rapid construction of the deer genetic map, firstly, because there are a very large number of probes available and, secondly, because the map location of these probes in other species, such as mouse and human, provides clues as to their likely arrangement in deer (see below).

In addition to the conserved markers over 100 sheep microsatellite primers have been tested in the deer pedigrees. Only a few were conserved between sheep and deer and about 20% amplified a microsatellite in deer (Table 3). However, of these, all but one could be mapped in hybrid pedigrees and linked with the known genes. These results suggest that it will be possible to use the interspecies hybrid pedigrees to map virtually all polymorphic microsatellites isolated from other members of the Cervinae.

Production of Père David x red deer hybrid pedigrees

During the history of Père David's deer in captivity, hybridisation with red deer has been noted several times and in at least two previous cases these hybrids have produced offspring (Jones *et al.*, 1983). More recently Père David's deer were introduced to New Zealand as a conservation measure and because of the potential to introduce desirable traits, particularly variation in seasonality, into farmed red deer (Asher *et al.*, 1988). Unfortunately the pure species have not thrived in New Zealand because of susceptibility to malignant catarrhal fever (MCF) (Orr & Mackintosh, 1988). There is now only one herd of 17 pure Père David's deer left in New Zealand.

In spite of this problem with Père David's deer, the production of hybrids has been successful, using the breeding potential of a few Père David's deer males. In 1986, 83 red deer hinds were inseminated and produced 5 hybrid calves (Asher *et al.*, 1988), while subsequent insemination of about 180 red deer hinds between 1987 and 1989 produced an additional 4 living calves (G.W. Asher pers. comm.). In addition, a further 9 calves were produced from 166 inseminations between 1988 and 1991 (Fennessy & Mackintosh, 1992) and 7 additional hybrids are known to exist on commercial farms. Currently of these 25 F1 hybrids, 10 females and 5 males are known to be alive, with 11 of these being produced from the semen of one pure Père David's deer stag. In contrast to the low fertility of the first cross, the fertility of the F1 hybrid appears comparable, or slightly lower than red deer (P. F. Fennessy,

unpublished data). In matings with red deer, male and female F1 hybrids at Invermay Agricultural Centre, New Zealand have produced a total of 182 and 19 backcross calves respectively (Fennessy and Mackintosh, 1992). At least 80 additional backcross calves have been produced on other farms (Otway, 1993).

Gene mapping in the interspecies hybrid pedigrees

For each marker in which Père David's deer and red deer differ, the "Père David's deer" type is heterozygous in the F1 sire and, either present or absent in the backcross (depending on whether the chromosome segment on which it resides is inherited from the Père David's deer or not). In the backcross, markers from the same chromosome segment will tend to occur together, whereas markers distant from one another segregate independently because of recombination, and independent assortment of chromosomes during meiosis in the F1 hybrid (Fig. 1).

The mapping pedigrees presently used consist of 123 backcross animals sired by three F1 hybrids mated to red deer (Fig. 1). One of the few drawbacks of these pedigrees, sired by a male hybrid, is that the X-chromosome cannot be mapped. The technical specifications of the pedigrees are that they will provide linkage to 33 cM and resolution of order within 4 cM (Tate *et al.*, 1995). The genome of mammals is about 1500 - 3000 cM long so between 100 and 200 well-spaced markers in these pedigrees should provide good coverage of the 33 autosomes in these deer and a primary linkage map of deer. Currently over 150 markers have been examined in these pedigrees and preliminary analysis indicates that the markers fall into 29 chromosomal groups with a further 4 markers unlinked to any other marker (M. L. Tate and H. C. Mathias, unpublished data). We estimate that this map covers at least two thirds of the deer genome. Published data from 5 chromosomal linkage groups mapped in these pedigrees (Tate *et al.*, 1994; Tate *et al.*, 1995) have given no evidence of recombination suppression or segregation distortion occasionally seen in the mouse interspecies hybrid pedigrees.

Applications of a deer gene map

Genetic analysis of traits

The relationship between molecular genetic variation and variation in phenotypic traits, upon which natural or artificial selection may act, remains unresolved in most species, including deer. In a few cases, tantalising associations between traits and particular protein variants have been described in deer (Chesser *et al.*, 1982; Roed, 1987; Smith *et al.*, 1993). Studies by Pemberton *et al.* (1988; 1991) provide the most compelling evidence that some protein variation is selectively maintained but, even if this is generally true, it seems clear that currently known protein variants explain, at best, a small proportion of the variation in only a few traits. Usually it is assumed that the genetic markers are selectively "neutral" and therefore not directly related to traits of biological or evolutionary significance.

The deer gene map provides a powerful and systematic approach to identification of the genes which underlie trait variation in deer. This "trait linkage analysis" tests the relationship between the inheritance of each marked chromosome segment and variation in the trait or traits of interest (Lander & Botstein, 1989). The key requirements of trait linkage analyses are, firstly, large pedigrees (e.g. 200 progeny) where there is measurable phenotypic variation in the trait of interest and, secondly, a map of markers relevant to these pedigrees. While these techniques have been primarily applied to traits known to be affected by single genes, both the theory and a growing number of examples show the utility of these approaches to identification of multiple genes underlying quantitative variation (Avner, 1994).

The same features, that make the Père David's deer pedigrees useful for linkage mapping, also make them useful for the genetic analysis of these complex traits (Lander & Botstein, 1989). Père David's deer are distinct in numerous traits: these include behaviour (e.g. vocalisations, neonatal behaviour and gait, Altman & Scheel, (1980)), morphology (e.g. hoof, tail, rump and antler shape, Wemmer & Wemmer, (1983)) and physiology (e.g. gestation length, seasonality, Loudon *et al.*, (1989)) and disease resistance

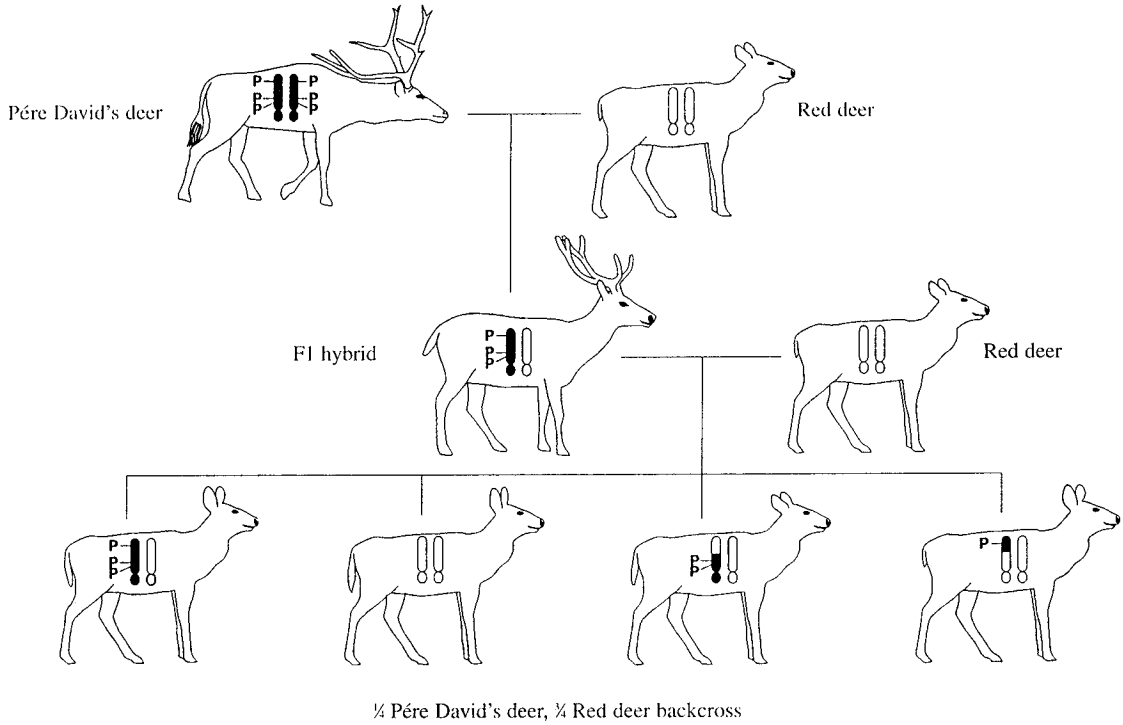


Figure 1. Segregation of chromosomes and markers in **Père David's deer x red deer pedigrees.** The lines indicate the familial relationships of the animals; in each case the progeny are below the parents. Each animal has 34 pairs of chromosomes and an example of one of these chromosome pairs is shown. Chromosomes and chromosome segments inherited from the Père David's deer are black while those from red deer are clear. The locations of three genetic markers with Père David's deer specific types 'P' is indicated on the Père David's deer chromosomes. The segregation of markers and chromosomes shown is described in the text.

Waldrup KA, Mackintosh CG (1993b): A difference in abomasal pH between Canadian wapiti affected with chronic illthrift and unaffected deer. *NZ Vet J. 41*: 142 - 143.

Waldrup KA, Mackintosh CG, Labes RE, Rhodes AP (1993c): The use of Proftril boluses in weaner red deer hinds (*Cervus elaphus*).
Proceedings Deer Branch NZVA Course No 10 : 151 - 161.

Table 1: The original trial design with 3 treatments across three subspecies of deer and 6 animals per group.

	Parasitised *Control*	Parasitised *Alb treated*	Non-parasitised *Alb treated*
Red	6	6	6
F1	6	6	6
Elk	6	6	6

Table 2: The modified trial design with the elimination of the elk control group and a summary of pretrial animal losses.

	Parasitised *Control*	Parasitised *Alb treated*	Non-parasitised *Alb treated*
Red	6	5	6
F1	6	5	6
Elk	0	8	5
Animal losses:		Red deer	# leg
		F1	# leg
		Para-Elk	lungworm dis
			7, 8, 15 and 16
		Non-para Elk	MCF

Table 3: Total worm counts for all the trial animals together with group arithmetic and geometric means and the standard error of the geometric means.

Animal No.	Group	*Ostertagia* - type				*Trichs*	Lungworms	
		Adult	*L5*	*LL4*	*EL4*	*Adult*	*Adult*	*Immature*
R1	Red	2900	0	2300	12500	0	10	29
R2	Control	4700	300	300	9400	0	34	122
R3		4900	1200	1000	8400	0	1030	197
R4		2600	400	400	4400	0	42	6
R5		8400	3100	2800	41800	0	173	1060
R6		3100	500	1800	15400	200	1	54
Mean		4433	917	1433	15317	33	215	245
Geom mean		4063	246	1053	11848	1	42	80
SE		0.9934	1.0816	0.8000	0.7551		0.6641	0.4151
R 8	Red	500	0	100	500	0	53	25
R 9	Treated	0	0	0	0	0	76	32
R 10		2800	900	600	1300	0	48	64
R 11		0	500	0	0	0	17	10
R 12		300	500	900	200	0	14	68
Mean		720	380	320	400	0	42	40
Geom mean		52	46	34	41	0	34	32
SE		1.0224	1.1848	0.8764	0.8272		0.7275	0.4548
BY31	F1	8500	700	2600	13100	0	41	114
BY32	Control	1800	600	700	4200	0	1	22
BY33		16200	300	2100	10700	0	32	87
BY34		13100	1200	4400	43500	200	10	181
BY35		9100	900	3600	10000	0	27	278
BY36		4500	300	1200	3800	0	1433	152
Mean		8867	667	2433	14217	33	257	139
Geom mean		7114	589	2038	9996	2.4	32	109
SE		0.9334	1.0816	0.8000	0.7551		0.6641	0.4151
BY 38	F1	400	0	0	100	0	18	101
BY 39	Treated	1800	500	100	500	0	15	56
BY 40		700	0	0	100	0	179	254
BY 41		900	100	0	1700	0	9	62
BY 42		1100	600	100	600	0	12	79
Mean		980	240	40	540	0	47	110
Geom mean		870	31	5.4	350	0	23	94
SE		1.0224	1.1848	0.8764	0.8272		0.7275	0.4548
P 61	Elk	0	100	0	100	0	1373	101
P 63	Treated	0	100	0	100	0	216	502
P 65		100	0	0	100	0	97	0
P 66		1200	100	0	200	0	553	399
P 67		0	0	0	200	0	45	68
P 68		300	200	0	200	0	1385	303
P 70		0	0	0	0	0	142	351
P 71		300	100	100	400	0	76	305
Mean		238	75	13	163	0	486	254
Geom mean		17	19	1.7	86	0	239	223
SE		0.8083	0.9367	0.6928	0.6539		0.5751	0.3595

SE = standard error of geometric mean

Table 4: Estimated efficacies (with 95 % confidence interval) for albendazole against deer parasites in the three subspecies of deer.

Group (n)	*Ostertagia*-type				*Trichostr.*	Lungworm	
	Adult	*L5*	*LL4*	*EL4*	*Adult*	*Adult*	*Immature*
Red deer (6)	98.7 (99.9-77.4)	81 (99.3-0)	96.7 (99.7-61.5)	99.6 (99.9-96.4)	100 (ND)	17.3 (89-0)	58.9 (88-0)
F1 (6)	87.8 (99.3-0)	94.7 (99.8-0)	99.7 (99.9-96.4)	96.5 (99.7-64.8)	100 (ND)	28.1 (90-0)	13.9 (75-0)
Elk(8)*	99.7 (99.9-96.8)	96.7 (99.8-37)	99.9 (99.9-99.2)	99.1 (99.9-93.2)	100 (ND)	[0]	[0] (34-0)

L5 - fifth stage larvae
LL4 - late fourth stage larvae
EL4 - early fourth stage larvae
Trichostr. - *Trichostrongylus* spp

Fig 1:Group mean plasma concentrations of albendazole sulphoxide in parasitised and non-parasitised red, F1 and elk deer over the 48 hour period after treatment.

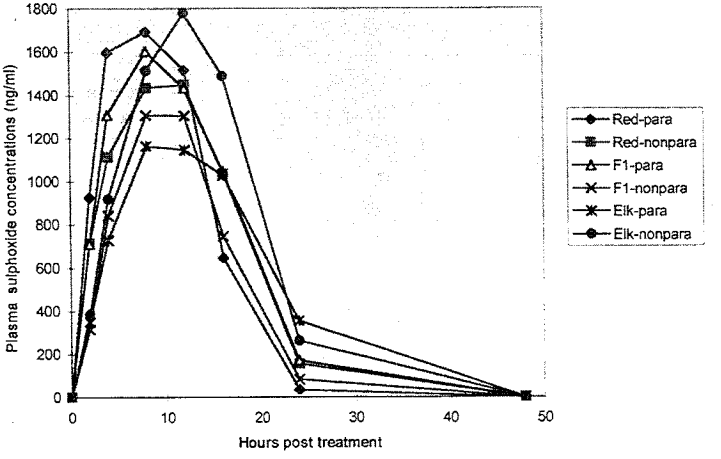


Fig 2: Group mean plasma concentrations of albendazole sulphone in parasitised and non-parasitised red, F1 and elk deer over the 48 hour period after treatment.

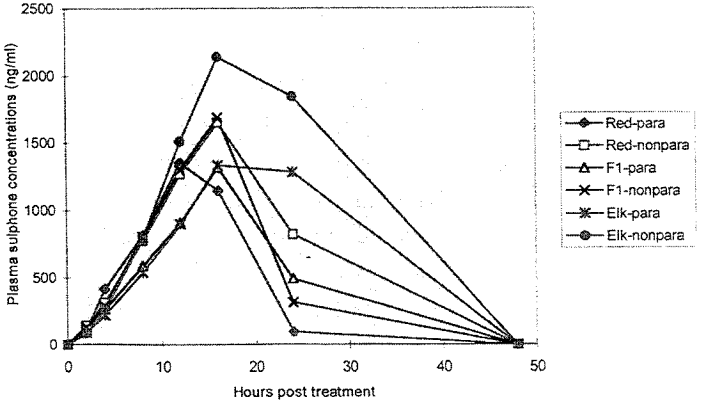


Fig 3: Group mean plasma concentrations of albendazole sulphoxide in non-parasitised red, F1 and elk deer

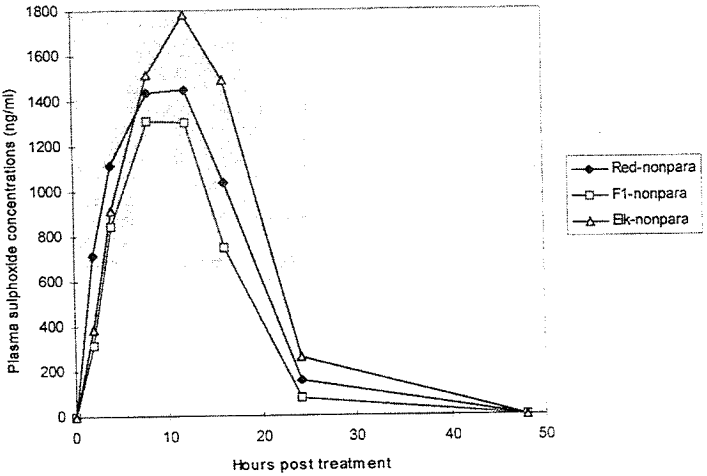
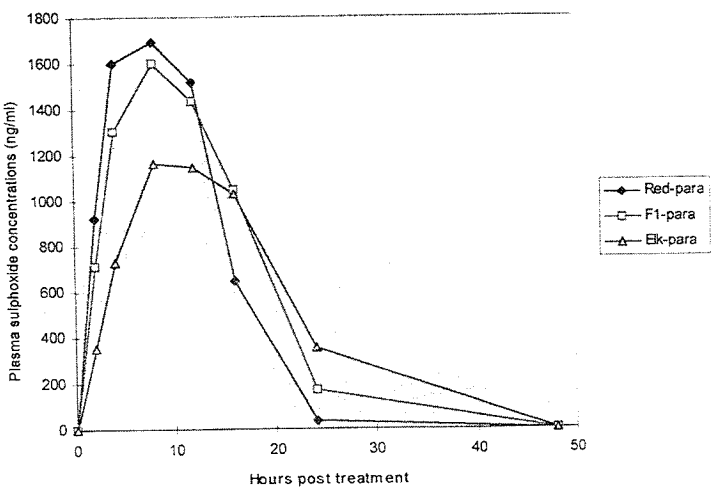


Fig 4: Group mean plasma concentrations of albendazole sulphoxide in parasitised red, F1 and elk deer.



(Mackintosh, 1992). Hybrids show measurable variation in some of these traits including growth rate and gestation length (Fennessy *et al.*, 1992; Fennessy & Mackintosh, 1992). Currently a large number of hybrids are being produced at Invermay Research Centre, New Zealand, with the aim of using the genetic map to analyse variation in these traits. At the very least we expect to be able to estimate the number of genes which determine the differences between Père David's deer and red deer and potentially identify genes which determine a significant portion of some traits.

The outcome of these studies is potentially very significant as this will be the first comprehensive genetic analysis of phenotypic traits in a cross between evolutionary diverged mammalian species. Comparable studies in domestic animals examine traits under artificial selection (Hetzel, 1993). These studies will provide new information on the process of speciation and evolutionary change and, on farms, provide the potential to use genetic markers in the selection of hybrid animals with desirable characteristics for farming.

Aligning the genomes of mammals

One of the fundamental findings of genetic mapping and karyotypic studies is that the genomes of mammals are very similar (O'Brien *et al.*, 1993). In deer, karyotypic studies of deer have identified putative relationships between the karyotypes of deer species and potential mechanisms, largely involving Robertsonian translocation, for the evolution of deer karyotypes (Herzog, 1987; Herzog & Harrington, 1991; Neitzel, 1987; Wang, 1988). These studies also identified the remarkable case of the Indian muntjac which has only six chromosomes in the female and seven in the male and this species continues to attract considerable attention in the study of comparative cytogenetics (Lee *et al.*, 1993; Lee *et al.*, 1994; Levy *et al.*, 1992). Chromosome banding studies suggest many deer chromosomes are very similar in banding pattern and therefore presumed to be homologous with other members of the Artiodactyls (Buckland & Evans, 1978). The concept that the chromosomes of diverse mammals may be very similar in structure is attractive because of the potential to unify genetic and chromosomal studies of different mammals.

Virtually every marker mapped in the Père David's deer x red deer hybrids has been mapped in other species so the deer gene maps provides the means to extend comparisons and test theories of chromosomal evolution by comparing the position of homologous loci in different species. Indeed the deer map may assist in comparative mapping of related domestic species, such as sheep and cattle, where wide interspecies hybrid pedigrees are not available (Tate *et al.*, 1995).

This alignment of maps is also of some practical significance as it allows the rapidly expanding information base on the function of particular mammalian chromosome to be used directly in studies of deer. Tate *et al.* (1994) provide an example of this by using comparative information to locate genetic markers, in deer, which are known to be adjacent to the mammalian major histocompatibility complex (MHC), a complex of genes that is likely to be responsible for the differences in disease resistance of different individuals (Lui *et al.*, 1993).

Mapping the future: international reference pedigrees

Our Père David's deer x red deer interspecies hybrid backcross has allowed rapid construction of a deer genetic map which defines the relationship of the deer genome to other species and allows a comprehensive genetic analysis of traits which distinguish Père David's deer and red deer. However the comparative markers used to construct the current map are not useful for genetic analysis of other deer crosses. To facilitate future mapping we intend to make DNA from these interspecies hybrid pedigrees internationally available. This will enable any new deer gene markers to be given a chromosomal location relative to all other markers tested in the pedigrees.

A map location is particularly important for new microsatellite markers which are highly polymorphic (and so useful for genetic analysis in almost any pedigree) but tend to be anonymous and species-specific (Pemberton, 1993). Mapping these markers to a chromosomal location relative to comparative markers will provide access to comparative information from

homologous chromosome segments in other mammals. In addition, once sufficient mapped polymorphic markers are available, it may possible to conduct, in any deer pedigree, a systematic genomic analysis, such as that described for the Père David's deer hybrids. We look forward to this new wave of studies in deer to provide a new understanding of the relationship between genes, traits and evolution.

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