Chapter 1.

1. Introduction

The occurrence of naturally generated hybrids between Père David's (*Elaphurus davidianus*, 2n=68) and red (*Cervus elaphus*, 2n=68) deer raises several interesting questions since they are currently classified as separate genera (Harrington 1985; Groves and Grubb 1987). While the Cervidae (deer) family is notable for its exceptionally high capacity for hybridisation between the various species and subspecies (Fennessy and Dratch 1984; Harrington 1985; Fennessy 1992) this hybrid is between two very genetically distinct species which taxonomists have consistently classified as belonging to separate genera.

Prior to the introduction of Père David's deer to New Zealand in 1982 there were two accounts of naturally produced hybrids with red deer; both were female and were fertile. Natural hybridisation between a red stag and a Père David's deer hind resulted in the birth of a female F_1 in January 1978 at Woburn Abbey, England (Jones *et al.* 1983). This female subsequently produced a backcross calf with a typically Père David appearance. The other report of a naturally generated F_1 hybrid was a female born at Jardin des Plantes, Paris in August 1900; she was also fertile and produced several offspring. Subsequently, hybrid males and females have been generated using artificial breeding techniques (Asher *et al.* 1988; Fennessy and Mackintosh 1992). Haldane's law (Haldane 1922) states "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex". Thus, based on Haldane's observations and the fact that Père David and red deer are in fact different genera (and as such an even wider than interspecies hybrids) suggests that if it was possible to generate hybrids they would most likely be infertile. However this is not the case as both F_1 males and females are fertile thus posing some questions about either the evolutionary process, evolutionary mechanisms involved in controlling fertility and/or the classification system.

Red deer are a common species naturally distributed through Europe, North America and Asia (Whitehead 1993). In contrast, Père David's deer originate from China and are considered an endangered and highly inbred species (Jones et al. 1983). Significantly, the two species are very different in their appearance and biology the most notable being the antler, foot and tail morphology (Wemmer 1983), seasonality and mature size (Loudon et al. 1989), disease resistance (Orr and Mackintosh 1988) and behaviour (Altmann and Scheel 1980). The Père David's deer is a long day breeder, with conceptions occurring during summer and calving taking place around 283 days later (Weinmer et al. 1989) during the early spring/summer. In contrast, red deer are short day breeders, conceiving in autumn and giving birth around 234 days later (Fennessy et al. 1991a).

The knowledge of these hybrids generated considerable interest in the New Zealand deer farming industry, particularly in light of the vast seasonal and genetic differences between these species. The New Zealand deer farming industry has been particularly proactive in securing a diverse array of deer genetic resources, including North American wapiti or elk, European and Mesopotamian fallow deer and various Cervus elaphus subspecies. Thus the opportunity to explore the genetic potential of Père David's deer was eagerly adopted in light of the markedly earlier seasonality of this species compared to farmed New Zealand red deer.

Mouse interspecies hybrids have been particularly useful in refining and clearly defining linkage groups and orders of markers on linkage groups, there is also potential to detect quantitative trait loci (QTL) (Avner et al. 1988; Copeland and Jenkins 1991). Based on the large genetic and phenotypic differences between the mouse species and the subsequent ease of determining linkage suggested that the differences between the two species of deer would likely also provide a powerful experimental design for the detection of QTL in deer. Consequently restriction fragment length variants (RFLV) have been used to build a linkage map of deer using backcross ¼ Père David / ¾ red deer hybrid (Tate et al. 1995a). Such a linkage map thus provides the opportunity to rapidly determine the location and order of newly identified genes and offers the potential to locate genes or regions of chromosomes responsible for phenotypic variation. In addition to these developments in the molecular field, a significant contribution to the statistical analysis of such data (Lander and Botstein 1989) provided a major impetus to the success of quantitative trait loci detection. This mathematical technique recently developed

(from human linkage analysis) uses maximum likelihood to test for associations between quantitative trait expression and genome regions (Lander and Botstein 1989).

With respect to red deer and Père David's deer, estimates of the genetic distance (Nei 1972) between these species are 0.35 from 22 protein loci (Emerson and Tate 1993) and 0.48 from 45 protein loci (Tate *et al.* 1992). These genetic distances are large by comparison with those between the mouse species (extensively used for mouse genome research) *M. musculus* and *M. spretus* of 0.46 (Bonhomme *et al.* 1984). The vast differences in genetic constitution as is illustrated by the Nei values and the large differences in phenotypically expressed traits which can be quantified make these hybrids a unique resource for genetic studies. The interspecific hybrid between these species is of considerable interest as detailed studies may shed light on the genetic control of traits such as seasonality, gestation length, birthweight, body growth, disease resistance and other production traits.

One of the first and benchmark studies to use backcross progeny and the maximum likelihood technique as a means of defining sections of chromosomes likely to be responsible for quantitative traits in food producing species was in tomatoes (Paterson *et al.* 1988). In this study, domestic tomato (*Lycopersicon esculentum*) and wild South American green-fruited tomatoes (*L. chmielewskii*) were hybridised. These species show considerable genetic differences including fruit mass and concentration of soluble solids were hybridised. They were then used to map at least six QTL (quantitative trait loci) controlling fruit mass, four QTL for the concentration of soluble solids and five QTL for fruit pH, all traits of major economic importance in the tomato processing industry. Subsequently several experiments using backcrosses and similar analyses have been used to detect QTL for growth rate, fatness and length of small intestine in pigs (Andersson *et al.* 1994), live weights and carcass quality traits in cattle (Lewin *et al.* 1990) and milk characteristics and yield in dairy cattle (Georges *et al.* 1995).

While the insights to be gained from biological analyses of pure species and their hybrids may be many and diverse, they should also be considered in the light of the evolutionary processes which contributed to the present form. In this respect it appears Père David's and red deer have taken quite different evolutionary paths as is reflected by, among other things their seasonality. Deer partly express their seasonality through their annual breeding patterns where

both sexes are synchronised by environmental cues (usually mostly daylength) so that males are in hard antler for the breeding season. Evolutionary strategies such as these develop slowly and in response to environmental and other cues and may provide significant insights into the biology of living species and in particular for these deer species. Therefore in this thesis the evolutionary processes which have moulded these two deer species are discussed and intensive studies on the genetic control of seasonality, gestation length, birthweight and body growth as well as comparisons with red deer for production characteristics.

In this thesis the word genotype is used in two contexts as is common in the animal breeding literature. Firstly it can mean the genetic constitution of a hybrid animal in terms of the proportion of its genes which originate from different pure grandparental species and secondly it can also mean the genetic (allelic) constitution at a specific position on the genome (genetic marker). Specific meaning is clear in text as they are sufficiently different to avoid ambiguity.

This thesis was compiled so that chapters are stand alone and entities in their own right and as such there is some repetition between chapters where materials and methods and/or common data have been used.

Chapter 2.

2. Literature review

"Nothing in nature makes sense except in the light of evolution"

(Dobzhansky 1973)

Speciation denotes the genetic changes whereby new species come into existence and it also recognises that these processes are a distinct part of the general course of evolution. Speciation is quite clearly the result of the combined action and interaction of many processes including geographical, climatic, biological and phyletic (presumed evolutionary descent) (White 1978). Consequently any model which relies on a single process to the exclusion of others to infer speciation is undoubtedly simplistic in structure and prone to error. Species have been defined as "... groups of actually or potentially interbreeding natural populations which are reproductively isolated from other groups" (Mayr 1963) and as "... systems of populations within which the gene exchange between systems is limited or prevented by a reproductive isolating mechanism or perhaps a combination of several such mechanisms" (Dobzhansky et al. 1975). There are two significant points worth noting with regard to these definitions; firstly both definitions exclude species status based purely on geographical distribution and secondly neither definition allows that reproductive compatibility (even the production of fertile offspring) is sufficient to establish species designation. Thus distinctiveness at several levels including differences at the ecological, physiological, karyotypic and molecular levels as well as reproductive isolation mechanisms and the selection for or against hybrids should be used in defining different species.

2.1 Evolution and speciation within the Cervidae

Evolutionary divergence can arise from three processes

i) phylogenetic change in the course of geological time (anagensis),

ii) speciation or cladogenesis where a single genetic lineage splits into two more lineages and

iii) sub-speciation or the local adaptation of populations to their localised environments and activities (Dobzhansky et al. 1975).

Other bases have also been used, such as biological, morphological, presence or absence of hybrids, reproductive isolation and morphological discontinuity. Thus robust studies on speciation should aim to include as many sources of information from a variety of disciplines in attempting to define speciation. Generally there is no single basis for any systematic arrangements but instead each method should be considered against postulated relationships between taxa. As with other species classification systems, several methods (including comparative anatomy and morphology, the primary method of classification for many phylogenetic trees) have been used for deer.

Anatomy. The Cervidae, or deer family, are of the order Artiodactyla (even toed hoofed ungulates) and as such are representatives of one of the most successful orders of large mammals. Deer can be traced back to the Miocene, 7 to 26 million years ago, lying between the Tragulina (cheviotains) and the Giraffidae (giraffe and okapi). They can be distinguished from other ungulates by the following features:

i) they are true ruminants (Pecora)

ii) they retain rudiments of the first two phalanges of the lateral digits

iii) their molars are brachydont

iv) they have a cotyledonary placenta and

v) they have fenestrated lachrymal bullae (Brooke 1878; Young 1962)

Four broad classification systems have been proposed over the last 120 years, namely those of Brooke (1878), Harrington (1985), Groves and Grubb (1987) and Whitehead (1993). They are all very similar and follow the Telemetacarpi / Plesiometacarpi split of Brooke (1878) which

Table 2.1 The two major sub family groups within the Cervidae (after Harrington 1985).

Cervinae (Plesiometacarpi)	Odocoileinae (Telemetacarpi)
	Section A
Muntiacus	Moschus
Elaphodus	Section B
Dama	Hydropotes
Axis	Capreolus
Elaphurus (Père David's deer)	Alces
Cervus (including red deer - *Cervus elaphus*)	Section C
	Rangifer
	Odocoileus
	Blastocerus
	Ozotocerus
	Hippocamelus
	Mazama
	Pudu

divided deer into two families based on the regression of the lateral metacarpals. Considering the 25 to 30 million year evolutionary history of the Cervidae it is not surprising that many evolutionary lines have disappeared. This increases the difficulty in assembling or compiling the phylogenetic relationships of the living deer species (Harrington 1985). Within the Telemetacarpi, the 11 members are split into three sections based on skull morphology and metatarsal gland positions (Table 2.1) (Brooke 1878). The Telemetacarpi include two genera of antlerless deer, namely Moschus and Hydropotes. The isolation of the musk deer in this classification structure appears to be related to their close relationship with the cheviotains which may be close to the ancestry of all ruminants (Young 1962).

An extensive array of morphological and anatomical features as well as antler structure and karyotype have been used in determining the phylogenetic relationships among living Cervidae (Groves and Grubb 1987). They propose three subfamilies within the Cervidae namely, Hydropotinae, Odocoileinae and Cervinae. Hydropotes include Chinese water deer which are unique in that they are antlerless. The Odocoileinae subfamily includes three sub groups namely Capreolini, Alcini and Odocoileini which are also referred to as the New World deer. The Odocoileinae and Hydropotinae subfamilies combine to comprise the equivalent of the Telemetacarpi while the Muntiacini and Cervini combine to comprise the Plesiometacarpi described elsewhere (Harrington 1985). This highlights some of the slight variations in the levels of classification within the family Cervidae. There is also another variation which includes six sub families within Cervidae and lists 16 genera which consist of about 41 species

and 196 sub-species (Whitehead 1993) although the previous classification systems seem more robust based on all the evidence available.

Ecology and physiology. Life history strategies (r vs K strategists) (MacArthur and Wilson 1967; Pianka 1970) have also played a role in the classification within the Cervidae. The two basic life history strategies differ in that r-strategists aim to maximise their reproductive capacity and the potential growth rate of the population whereas K-strategists maximise their competitive ability and the stability of the population. Species tend towards one or other extreme in response to their habitat. Unstable habitats favour the r-strategy while stable habitats favour the K-strategy (Harper *et al.* 1961). The Telemetacarpi are mostly r-strategists while the Plesiometacarpi tend to be K-strategists. The r-strategists are clearly identifiable from their high reproductive potential with all major genera having multiple births and/or early sexual maturation. They are also predominantly browser or concentrate (low fibre food) feeders in contrast to K-strategists which are generally mixed grazers / browsers (high fibre food eater) and can feed on roughage (Hofmann 1985). K-strategists have a lower reproductive potential reaching maturity at two to three years and normally have singleton progeny. The Plesiometacarpi tend to have higher levels of social contact and appear more responsive to social modification than deer of the r-strategy Telemetacarpi (Moore 1984).

Based on the definition of species, one of the classical means of determining phylogenetic relationships is the ability for hybridisdation between species and the subsequent fertility of the offspring. The degree of reproductive isolation between species within the Telemetacarpi is clearly greater than that between species within Plesiometacarpi; for example, interbreeding between the Siberian roe deer (*Capreolus capreolus pygargus*) and the European roe deer (*C. c. caprelous*) results in sterile male hybrids but fertile female hybrids (Stubbe and Bruholz 1979; cited by Groves and Grubb 1987) while white-tailed deer (*Odocoileus virginianus*) have only limited capacity for hybridisation with black tailed deer (*O. hemionus*) (Cowan 1962; Gray 1971). In contrast, within the Plesiometacarpi, the European red deer (*Cervus elaphus*) and Japanese sika deer (*Cervus nippon*) hybridise relatively easily with both sexes being fertile (Harrington 1979c) while the New Zealand (Moore and Littlejohn 1989; Pearse 1992) and Chinese (Rongqian 1996) deer industries utilise hybridisation at the subspecies level between wapiti (often known as elk) and red deer. Hybrids between sambar (*Cervus* (Rusa) *unicolor*) and rusa (*Cervus* (Rusa) *timorensis*) deer have also been reported (van Mourik *et al.* 1985). In

addition, natural intergeneric hybridisation between Père David's (*Elaphurus davidianus*) and red (*Cervus elaphus*) deer has been reported (Gray 1971; Jones *et al.* 1983) although the wider hybrid between red and sambar deer (*Cervus unicolor*) has been produced, fertility following artificial insemination was very low with evidence of early foetal mortality and for non-viability of neonates (Muir *et al.* 1997).

Immunology. When taxa within the Cervidae are cross immunised, taxon-specific antibodies are produced which provide a measure of the structural difference or similarity between these groups and related taxa. Japanese sika, red, wapiti and their hybrids as well as Père David's deer have been examined in this way using quantitative immuno-electrophoresis. This illustrated that while there was considerable genetic variation, relationships between taxa were identifiable. This method also detected both intergeneric and interspecific hybrid deer through three generations of backcrossing to parental forms (Harrington 1979b; cited by Harrington 1993).

Karyotype. The contrast in reproductive isolation within the Telemaetacarpi compared with the Plesiometacarpi is also supported by their karyotypes and chromosome fundamental number (FN). Most Telemetacarpi have a FN greater than 70 with slight differences in the number of microchromosomes between closely related species. In contrast all species within the Plesiometacarpi have FN of ≤ 70 and no microchromosomes (Table 2.2). As a general rule species with the same diploid chromosome number (2n) and the same FN should be able to hybridise and produce offspring (Short 1985). In general if the FN is the same but the 2n is different, chromosomal rearrangement has occurred which is normally the result of Robertsonian translocations during which two acrocentric chromosomes fuse. Within the Plesiometacarpi, vast differences in diploid chromosome numbers between Indian muntjac (*Muntiacus muntiacus*, 2n = 6 and 7) and Chinese muntjac (*M reevesii*, 2n = 46) deer was not sufficient to produce complete reproductive isolation (Shi *et al.* 1980; Shi and Pathak 1981). While the muntjac have a FN of 46, most other genera in the Cervinae have a FN of 70. These unique features imply a long independent evolutionary history of muntjac (Dobzhansky *et al.* 1975) compared to the Cervini group and add further justification for this split (Groves and Grubb 1987).

Table 2.2 Division within the family Cervidae based on anatomical, karyotypic, biographical, physiological and ecological barriers (after Harrington 1993).

Cervinae (Plesiometacarpi)	Odocoileinae (Telemetacarpi)
Anatomical	
greater regression of lateral metacarpals	less regression of lateral metacarpals
Ecological / Physiological	
K-strategist	r-strategist
roughage feeders	concentrate feeders (mainly browse and herbs)
uniparous	multiparous
breeding age at 2 or more years	breeding age in first year
relatively long lived	relatively short lived
highly social	solitary or poorly social
mainly palaerctic distribution	mainly neoarctic distribution
Karyotype	
FN ≤ 70	FN > 70
no microchromosomes	microchromosomes
frequent Robertsonian translocations	few Robertsonian translocations
Genetic compatibility	
hybridise freely	little hybridisation
genetically continuous	genetically fragmented

Molecular genetics. Using mitochondrial DNA sequences as a quantitative measure of genetic relatedness, a Cervini / Muntiacini clade (single genetic lineage) has also been supported and this excludes the Odocoileini (Miyamoto *et al.* 1990).

An electrophoretic analysis between Scottish red deer and North American elk or wapiti indicated differences (D=0.025) of a magnitude less than between European and North American Moose (D=0.060) (Dratch and Gyllensten 1985). These data thus support wapiti and red being sub-species within the *Cervus* genera. Also, using protein electrophoresis, the genus *Cervus* was split into two distinct groups with red, wapiti and sika in one clade and sambar and rusa in another, suggesting that genera within the Cervinae are not monophyletic (Emerson and Tate 1993). This split also reflects a geographical split between temperate and tropical species.

A RAPD (Random Amplified Polymorphic DNA) analysis on five species within the Cervidae (Comincini *et al.* 1996) also supports the morphological and palaeontological studies. This RAPD analysis showed a low degree of similarity between *C. capreolus* and *O. h. hemionus* although they belong to the same subfamily which supports other studies (Brooke 1878; Groves and Grubb 1987). In addition this analysis also suggested that *C. elaphus* and *Dama*

dama appear more closely related than either of these two with *M. muntiacus* (Indian muntjac) which also supports the anaomical studies. These data suggest the best split would be two groups namely, Capreolini and Odocoleini. A mitochondrial DNA analysis revealed no variation in moose (*Alces alces*) and little in elk / wapiti (*Cervus elaphus*) while caribou (*Rangifer tarandus*), white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) showed considerable variation (Cronin 1992).

Speciation. Speciation has been described as a three stage process through time which collectively concludes with sufficient differences in the form of geographical, reproductive and other processes to confer speciation (Lewontin 1974). Thus a new species is created in three consecutive stages according to Lewontin's theory of geographic speciation as follows.

Stage I: geographic isolation causes severe restriction of gene flow between populations. The resulting accumulation of genetic differences between geographically separated populations initiates their reproductive isolation. Therefore, genes controlling the reproductive isolation in the first stage are causally involved in speciation.

Stage II: the reproductive isolation is reinforced after the isolated populations come into contact again.

Stage III: this is characterised by a continuous accumulation of genetic differences between separated species.

These genetic differences are consequences of speciation and some can manifest themselves as sterility genes. Allopatric speciation refers to species which inhabit different areas, niches or have different distributions and this does not necessarily require different geographic distribution. It seems likely that the time components in allopatric and sympatric exposure as well as the degree of genetic exchange in this process would play a significant role in speciation.

In the sub families Cervinae and Odocoileinae, antlers (which form an integral part in the phylogenetic classification of deer) are usually large and complex structures, consisting of a long, multibranched distal element attached to a shorter nondeciduous pedicle or base. In contrast the antlers of Muntiacinae are relatively small with a short simple distal region connected to a long pedicle. This latter expression is considered more primitive because it is

found in the earliest fossil antlered deer, the Dicrocerini, from the early and middle Miocene of Eurasia (Goss 1983). Previously the similarities in antler structure lead to the assignment of both Dicrocerines and the living Muntiacines into one group (Miyamoto *et al.* 1990). This has subsequently been shown to be unlikely, based on paleontological and mitochondrial DNA phylogeny suggesting these two groups are not monophyletic (Miyamoto *et al.* 1990). The split between Cervini and Muntiacini has been estimated at 6 to 8 million years before present (MYBP) (Han 1985; cited by Miyamoto *et al.* 1990). By adopting a date of 6-8 MYBP for the Cervine / Muntiacini split, the time of origin for extant antlered deer may be estimated from the relatively equal amounts of change leading to Cervus, Muntiacus and Odocoileus. Using a simple clock calculation for rate of change of the genome (with supporting paleontological evidence) infers that the Odocoileines diverged from other antlered deer between 9.3 and 12.4 MYBP (Goodman 1986). This also agrees with a late Miocene origin for the subfamily, as hypothesised from paleontological data (Vislobokova 1980).

Distribution. The Cervidae are indigenous to the moist and cooler regions of the world including Eurasia, North Africa and the Americas. The two major groups within the Cervidae have allopatric ranges. The natural range of the Telematacarpi is through The Americas and the north of the Russian continent (Kurten 1972). For the Plesiometecarpi their natural range spans a narrow band from Indonesia through South East Asia including Japan and southern China through to Europe and Britain and includes a small part of Morocco. The relatively recent arrival of wapiti in the neoarctic (during the Pleistocene) probably explains why they are an exception to this. It has been suggested that several outliers including Rangifer, Capreolus and Alces survived in the palearctic as remnant specialist lines within the epicentre of Cervid evolution. (Young 1962). Significantly, deer are absent from both the arid Ethiopian and Australian regions as with many other groups of holarctic mammals and birds (Kurten 1969). This complements the continental drift theory and may explain the discontinuous distribution patterns exhibited by both plants and animals.

Temperate deer within the *Cervus* genera express a vast range in live weights as is indicated in Figure 2.1. There appears to be a distinct cline starting with Japanese sika deer (50 kg) and radiating west the *Cervus* species tend to a larger genetic size, reaching a peak body weight in Europe with *C.e.hippelaphus* (270-300 kg) then declining again to *C.e.scoticus* (200 kg) in Britain. Similarly there is another cline starting in Japan and radiating east, *Cervus* species tend
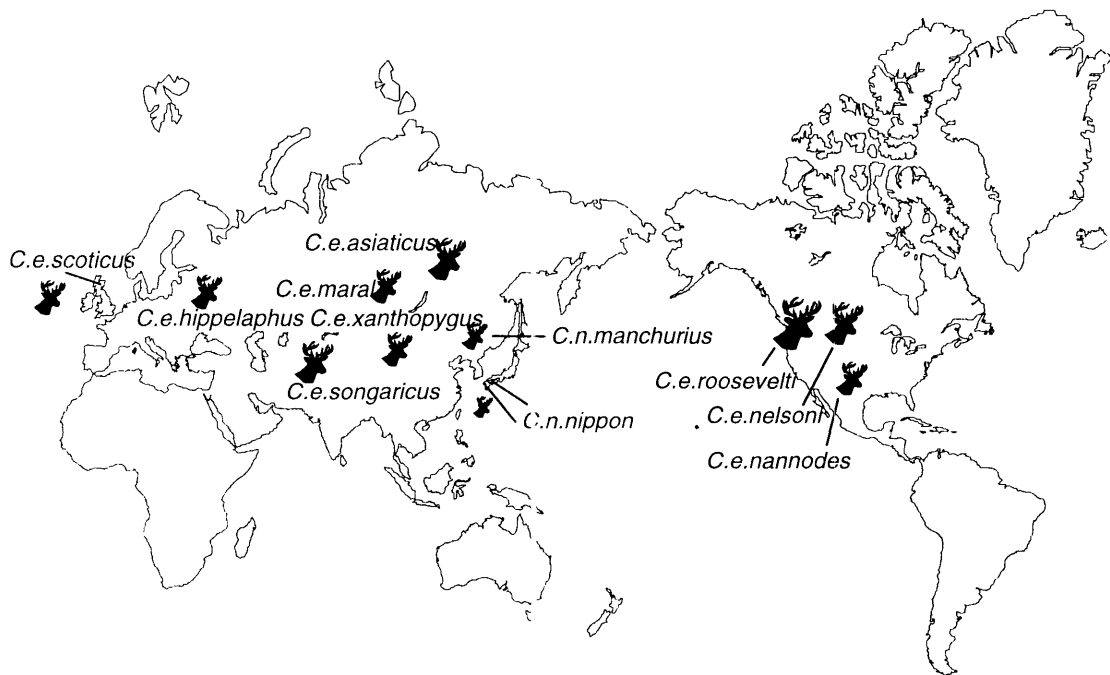

Figure 2.1 Natural distribution for several Cervus genera and species (the approximate relative live weights are indicated by the size of the head).

Typical live weights for adult stags: C.*nippon nippon* (Sika) 50 kg Japan; C.*nippon manchurius* (Sika) 70 - 100 kg Manchuria, Korea; C.*e.scoticus* 200 kg Scotland; C.*e.hippelaphus* 270 - 300 kg Europe; C.*e.xanthopygus* 300 kg China, Russia; C.*e.nannodes* (Tule Elk) 180 - 230 kg California, C.*e.maral* 300 kg Russia; C.*e.songaricus* (Wapiti) 380 kg Tien Shan Mountains, China; C.*e.asiaticus* 400 kg Russia; C.*e.nelsoni* (Wapiti) 400 kg Western North America except coast; C.*e.roosevelti* (Wapiti) 500 kg Western North America. Source: Whitehead (1993) and Fennessy (pers. comm).

to a large genetic size through China and Russia with peak body weights in C.*e.roosevelti* (500 kg) thereafter declining again to a body weight of around 200 - 300 kg in C.*e.nannodes*.

Hybridisation. While there is little ambiguity about the phylogenetic relationships within the Telemetacarpi there are some difficulties associated with classification within the Plesiometecarpi especially within the genus Cervus. Most species within the genus Cervus do not appear to be reproductively isolated, suggesting there may be grounds for sub species status rather than species. For the red / wapiti relationship this is supported by the fact that they hybridise (producing fertile offspring) (Caughley 1971), and also partly supported by an electrophoresis study (Dratch and Gyllensten 1985). Extensive studies have shown a high degree of hybridisation between red and sika (C. nippon) deer (Harrington 1979a; Putman and Hunt 1993) and also that there may be some adaptive advantage over the Japanese sika form in

some areas. The similarity of these hybrids with the Asiatic sika suggest the non Japanese sika might have in fact been derived from hybrids with other species (Lowe and Gardiner 1975). Sika and wapiti have hybridised in the Soviet Far East but the ecological context of this hybridisation is not fully known (Groves and Grubb 1987). There have been reported hybrids between the chital species, *Axis axis* and *A. porcinus*, which were fertile as well as between *A. axis* and *Dama dama* suggesting genetic relationships between these are similar to those within *Cervus* (Gray 1971). There are also the fertile hybrids between the tropical species sambar (*Cervus unicolor*) and rusa (*Cervus timorensis*) deer (van Mourik *et al.* 1985).

The high degree of hybridisation within the Cervini is probably best illustrated by the generation of fertile offspring between Père David's (*Elaphurus davidianus*) and red (*Cervus elaphus*) deer (Gray 1971; Jones *et al.* 1983) despite their current classification as separate genera (Groves and Grubb 1987). There has also been a report of interspecies hybridisation between sambar (*Cervus unicolor*, 2n=56) and red (*C. elaphus*, 2n=68) deer using artificial insemination in which the hybrid had a 2n of 62 (Muir *et al.* 1997). Insemination of 400 red deer hinds with sambar semen resulted in 31 pregnancies at day 40 and the birth of four calves while insemination of 10 sambar hinds with red semen resulted in five pregnancies at day 40, none of which went to term. A natural hybrid between sika (*Cervus nippon*, 2n=68) and axis

Table 2.3 Interspecies hybridisation among the Cervini: comparisons of pregnancy rate (day 40), birth rate and perinatal death rate (after Tate *et al.* 1997).

Deer Species			Number					
Sire	Dam	Breeding method	Insemina tions or matings	Hinds pregnant[a]	Calves born	Pregnan cies lost (%)	Calves born dead (%)	Reference
Red	Red	AI	140	102	99	2.9	-	1)
Red	Red	AI	-	167	164	2.4	-	1)
F₁ Père David's x red	Red	AI	841	321	301	2.4	18	2)
F₁ Père David's x red	Red	Natural	155	56	53	6.5	9	2)
Wapiti	Red	AI	59	39	36	7.1[c]	-	1)
Red	F₁ (PDxR)	MOET[b]	52	34	25	30	24	2)
Père David's	Red	AI	116	15	9	40	11	3)
Sambar	Red	AI	400	31	4	87	75	4)
Red	Sambar	AI	10	5	0	100	-	4)

[a] Pregnancy diagnosis by ultrasound at 32-42 days.
[b] Red deer recipient dams (multiple ovulation and embryo transfer).
[c] Includes one set of twins.
1) (Fennessy *et al.* 1991a)
2) (Tate *et al.* 1997)
3) (Fennessy and Mackintosh 1992)
4) (Muir *et al.* 1997)

(*Axis axis*, 2n=66) deer has been reported from Tennessee, USA although the fertility of this female (2n=67) has yet to be assessed (Asher *et al.* 1997). Pregnancy, birth and perinatal mortality rates for several species within the Cervini (Table 2.3) illustrate where losses occur and the extent of these in attempts to generate various deer hybrids.

Conclusions. The evolutionary divergence within the Cervidae family is a reflection of multiple environmental and other processes which have, through time, tended to direct or guide the two subfamilies in different evolutionary directions. These differences manifest themselves through the variation in anatomical, immunological, physiological and genetic differences which are subsequently used in the determination of the phylogenetic relationships within and between taxa. While it seems reasonable that the Cervidae may be monophyletic, the very distinct division into the Telemetacarpi and Plesiometacarpi provided by the various sources above suggest that a polyphyletic origin of the family is worth considering. Groves and Grubb (1987) consider the Plesiometecarpi, but not the Telemetacarpi deer are monophyletic which seems reasonable given the vast differences within the latter.

Assuming Odocoileines diverged from other antlered deer between 9.3 and 12.4 MYBP and the split between Cervini and Muntiacini occurred 6 to 8 MYBP (Han 1985; cited by Miyamoto *et al.* 1990) then within the Cervini, divergent genera have developed more recently than the Cervini / Muntiacini split. This also agrees with a late Miocene origin for the subfamily, as hypothesised from paleontological data (Vislobokova 1980). This recent and rapid evolution within the Cervini may explain why such a large degree of hybridisation occurs between the various species.

2.2 Mammalian hybrids and gene mapping

Mouse crosses. Traditionally the mouse has been used as the species of choice for genetic studies because of its short gestation period, large litter size, the availability of inbred strains and the ability to perform controlled matings. The mouse has served as an important model in the study of human genetic diseases such as birth defects, cancer, diabetes and reproductive anomalies to mention a few. This combined with new marker technologies, which allow mapping of DNA polymorphisms, has allowed the rapid development of genetic maps for mouse, humans and several other species. The objectives of these maps are firstly to provide a tool for genetic analysis and manipulation, including mapping of biologically interesting traits

as well as quantitative traits, and secondly to develop a physical map which lays down the order of sequences on the genome.

Laboratory strains and inbred strains of t1e mouse (*Mus musculus*) have low allelic variation which limits their use in terms of generating informative crosses and determining the order of genes on the genome. The development of new crosses has played a dramatic role in the explosion of mouse gene mapping as they overcome low allelic variation because of their large genetic divergence (Copeland *et al.* 1993). Interspecific crosses between laboratory strains and distantly related species are unique in that they exploit the inherent genetic diversity between these two taxa. This results in polymorphic genes or DNA sequences and facilitates mapping of multiple genes simultaneously, thus allowing easier identification and gene order placement. One of the most genetically distant mouse species that is capable of interbreeding with laboratory species is *M. spretus*. In the hybrids, males are sterile but females are fertile thus providing the resource for backcrossing and gene mapping (Avner *et al.* 1988). There are also various other species of mice which differ significantly from *M. musculus* (Bonhomme and Guenet 1989) using Nei's genetic distance (Nei 1972) as a quantitative measure. Using these techniques a comprehensive and detailed genetic map has been built up from around the 1970's. The current (October 1997) estimates indicate that there are around are 20,000 marker loci and 6,000 genes mapped on the mouse genome (Mouse Genome Database) reflecting a very rapid increase in recent years.

Sterility in these mouse hybrids follows Haldane's rule which states "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex" (in this case XY or males). The mechanism/s which control this are as yet unclear but studies in mice suggest there are specific DNA regions which contribute to this sterility phenotype (Forejt 1996).

Applications of the map. The genetic divergence of man and mice lineages is apparent in terms of the different organisation of their respective genomes. While there are remarkable homologies between the two genomes, the organisation of the genomes is different, with rearrangements of the 'founder' mammalian chromosome through a multitude of mechanisms including chromosome translocations, inversions, insertions and other complex rearrangements. Despite this there are also large segments of conserved DNA which are

consistent, not only across these species but in others as well. These conserved segments allow comparisons between various species where the conserved segments can act as reference points to assist in the transfer of linkage information from more densely mapped species (e.g. mouse, human) to less densely mapped species (e.g. pig, cow, deer). Another significant application of this comparative mapping involves the analysis of complex traits. Susceptibility to many human diseases is often controlled by several genes rather than one specific DNA segment and the identification of these genes is often easier in mice than in humans. Once candidate genes have been isolated, their homologue in humans can be studied for associations between disease and DNA sequence. There are also numerous other applications of genome maps which are covered in greater detail elsewhere (Copeland *et al.* 1993).

Breeding for genetic linkage mapping has been attempted using sheep x goat hybrids where attempts to produce offspring from a single female were unsuccessful (Hill and Broad 1991) and domestic x Asian Leopard cat hybrids (Lyons and O'Brien 1994). Not surprisingly the male F_1 hybrid cat is sterile while the female has low fecundity but is fertile (O'Brien *et al.* 1997). Several cattle hybrids have also been produced in recent years including the very common *Bos taurus* x *B. indicus* (Roberts 1990) and *B. taurus* x *B. gaurus* (Bongso and Hilmi 1988; Riggs *et al.* 1997). Both these hybrids, but especially the latter, have been used to assist in linkage map construction. In marsupials, a sub-specific cross between tammar wallabies is being evaluated for linkage mapping (McKenzie *et al.* 1993). American bison (*Bison bison*) and domestic cattle have long been known to hybridise (Makobo *et al.* 1981) and have since been in utilised in commercial operations.

Horse / donkey interspecies hybrids. One mammalian interspecies hybrid, the mule (the hybrid of the horse (*Equus caballus*, 2n=64) x donkey (*E. asinus*, 2n=62) (Benirschke 1967) has been known and used for thousands of years by humans. However the advent of new genetic technologies and the ability to produce other hybrids (often using artificial breeding techniques) has opened up a vast area with many potential revelations about genetic control of complex traits. Like the genus *Cervus*, the genus *Equus* is remarkable in its ability for generating viable interspecies hybrids. These hybrids are unique and an intriguing model for the study of interactions between mother and foetus in the maintenance of equine pregnancy (Allen *et al.* 1987).

The mule (2n=63), the result of the mating of a domestic jack donkey (*E. asinus*) to a mare horse (*E. caballus*) and the reciprocal cross, the hinny (2n=63) are by far the most common equine hybrids. Horse and donkey gestation lengths are 335 and 385 days respectively but exhibit large variation; for example, the normal range for horses should be considered as 315 to 360 days (W.R. Allen pers. comm). Both the mule and hinny have gestation lengths close to the mid parent mean of around 360 days (W.R. Allen pers. comm). Transfer of donkey embryos into the horse resulted in 80% abortion between days 80 and 100 of gestation in conjunction with a vigorous immune response against the foetus (Allen *et al.* 1987) with only one quarter of the remaining pregnancies going to term; with horse-like gestation lengths of around 330-350 days. In contrast horse embryos transferred into donkeys go to term with highly variable donkey-like gestation lengths of around 365 days (W.R. Allen pers. comm). Using embryo transfer it has been shown mules (2n=63) can carry both horse and donkey foals and domestic mares can carry Przewalski's horse or Grant's zebra (*E. burchelli*, 2n=44) embryos while the horse can carry a donkey embryo and *vice versa* (Allen and Short 1997).

The infertility of both mules and hinnies has also received some attention. An incompatibility between paternal and maternal sets of chromosomes leading to a block during meiosis is thought to lead to the subsequent lack of spermatozoa production in the testes of males. The same chromosomal incompatibility was subsequently shown to lead to partial meiotic arrest in female mules and hinnies resulting in severely depleted stocks of oocytes at birth in these hybrids (Taylor and Short 1973). Despite the obvious incompatibility in chromosome number and poor fertility in the mule and hinny hybrids there have in fact been a few reports of these hybrids producing offspring (Allen and Short 1997).

The vast numbers of hybrids generated by the matings of jack donkey with mare horse across the world indicate this interspecies mating is fertile. In fact the interspecies mating of horse mare to jack donkey has greater conception rates than intraspecies matings between either of the parental species (Allen and Short 1997). The reciprocal hybrid, the hinny, appears much more difficult to generate because conception rates to this mating are very low (14%) whether by natural mating or insemination (Allen and Short 1997). In addition the estrous display of the jenny donkey appears less arousing to the horse stallion than *vice versa* (W.R. Allen pers comm).

It appears that a vast array of hybrid offspring can be generated with almost any two equine species. These include combinations of a) domestic donkey with the Somali, African and Asiatic wild asses, b) Przewalski's and domestic horse, c) Burchell's, Grevyi's and mountain zebra as well as a variety of others (Allen and Short 1997).

Pig intercrosses and hybrids. Various crosses among pig taxa (Archibald *et al.* 1991) have been studied. European and Chinese domestic pigs are assumed to originate from the European wild pig (*Sus scrofa scrofa*) and the Asiatic wild pig (*S. s. vittatus*) respectively and are considered distant subspecies. European wild and domestic (*S. s. domesticus*) pigs which are evolutionarily closely related have only evolved from their wild ancestor in the last 10 000 years. Using these powerful genetic linkage maps, quantitative trait loci (QTL) for production type traits have been successfully isolated in various pig hybrids (Andersson *et al.* 1994; Geldermann *et al.* 1996). These include the isolation of QTL for backfat thickness, an important trait in commercial pig production systems (Andersson *et al.* 1994). The Chinese and European pig interbreed with no observed reduction in fertility (Andersson 1997) and show vast differences in productive performance (Archibald 1994).

Sheep / goat hybrids. There are reports of the natural occurrence of sheep (*Ovis aries*, 2n = 54) x goat (*Capra hircus*, 2n = 60) hybrds (Berry 1938; Bunch *et al.* 1976; Pinheiro *et al.* 1989; Stewart-Scott *et al.* 1990). Failure of goat pregnancies in ewes or chimeras appeared to be due to species-specific maternal antibody response whereas in contrast a maternal cytotoxic antibody response to species specific antigen(s) may have contributed to the failure of hybrid or ovine pregnancy in does (MacLaren *et al.* 1992). Female sheep x goat hybrids (2n = 57) produced offspring (2n = 56) when mated to sheep, indicating these hybrid females are fertile while male hybrids appeared unable to fertilise females even though sperm production was apparently normal (Bratanov *et al.* 1972; Bratanov *et al.* 1980).

There is evidence to suggest that sheep, goats and the Barbary sheep or Aoudad (*Ammotragus lervia*, 2n = 58) have a common ancestor (Zeuner 1963) although the Barbary sheep may be more closely related to the goat than the sheep (Geist 1971). Natural matings between Barbary rams and nanny goats have been reported (Gray 1971; Bunch *et al.* 1977), whereas the crossing of Barbary rams and domestic sheep ewes has been unsuccessful (Gray 1971). When

embryos of Barbary ram x domestic ewe were transferred to nannies (4 embryos) and ewes (4 embryos) none survived. In contrast, when embryos of Barbary ram x nanny goat were transferred to ewes (4 embryos) none survived but of those transferred to nannies (4 embryos), resulted in one male hybrid offspring (2n = 59) (Moore *et al.* 1981). In addition, this hybrid male was also shown to produce excellent motility sperm with no evidence of gross abnormalities and resulted in fertilisation and pregnancy in does up to at least four weeks post embryo transfer (Moore *et al.* 1980).

Deer hybrids. Histological analysis of spermatogenesis and sperm phenotype as an indicator of fertility in three white-tailed deer (*O. virginianus dacotensis*) x mule deer (*O. hemionus hemionus*) hybrids revealed all animals were infertile but varied in their degree of testicular differentiation, spermatogenic activity and sperm production (Wishart *et al.* 1988). A unique experiment was conducted on the gaits of white-tailed deer, mule deer and their hybrids in which it is suggested there is a genetic basis to the way in which these deer run when alarmed (Lingle 1992). This indicated that white-tailed deer tend to gallop, mule deer tend to stot and hybrids tend to bound. The bound appears to be a slow and mechanically inefficient gait for deer suggesting that these hybrids may be ineffective in their response to predators providing a further intriguing barrier to hybridisation in situations where the species are sympatric.

The current classification has Père David's (*Elaphurus davidianus*, 2n=68) and red (*Cervus elaphus*, 2n=68) deer as separate genera (Groves and Grubb 1987). Given this these species would not be expected to hybridise, yet prior to their introduction to New Zealand there were two accounts of fertile female hybrids namely, one born in 1900 and another in 1978 (Jones *et al.* 1983). Subsequently numerous hybrids have been produced (Asher *et al.* 1988; Fennessy and Mackintosh 1992) albeit all by artificial breeding techniques. Estimates of the genetic distance (Nei 1972) between these species are 0.35 from 22 protein loci (Emerson and Tate 1993) and 0.48 from 45 protein loci (Tate *et al.* 1992). These genetic distances are comparable with those between *M. musculus* and *M. spretus* of 0.46 (Bonhomme *et al.* 1984). Red deer are a common species naturally distributed through Europe, North America and Asia (Whitehead 1993). In contrast, Père David's deer originate from China and are considered an endangered and highly inbred species (Jones *et al.* 1983). Significantly, the two species have large differences in their appearance and biology, the most notable being the antler, foot and

tail morphology (Wemmer 1983), seasonality and mature size (Loudon *et al.* 1989), disease resistance (Orr and Mackintosh 1988) and behaviour (Altmann and Scheel 1980).

There are large differences in both genetic and phenotypic characteristics between the Père David's and red deer. These are of a similar magnitude to the differences between the mouse species which have been used to generate interspecies hybrids and which have had a massive impact on map construction and QTL detection. These similarities may indicate that the interspecies hybrids between Père David's and red deer may be extremely useful in the search for QTL in deer.

Evolutionary divergence. Postulated divergence time is a prediction of the date at which taxa diverged based on a number of lines of evidence including phylogenetic hypotheses, comparisons of divergence estimates based on fossil records in sister taxa, information about geographic distribution and robustness of the fossil record in key areas. From other Artiodactyls the postulated divergence time for the Bovidae family is 20 MYBP while for the Cervidae family it is similar at 19 to 21 MYBP, during the early Miocene (Miyamoto *et al.* 1993). Within the Bovidae, the Bovinae subfamily (including buffalo and cattle) are estimated to have diverged some 7 MYBP in the early Pliocene while the *Bos* taxa are estimated to have diverged as recently as 3 to 4 MYBP (Miyamoto *et al.* 1993). There are also estimates for the divergence times between the various *Bos* species based on mitochondrial (Bradley *et al.* 1996) and microsatellite (MacHugh *et al.* 1997) information, but these vary considerably.

Based on current estimates the Cervinae subfamily appears to have diverged some 6 to 8 MYBP (Miyamoto *et al.* 1993) which indicates that the Muntiacini / Cervini split is more recent. It is well known that there is a high degree of hybridisation between the various cattle species and breeds within *Bos taurus* (domesticated cattle). Hybrids between *Bos gaurus* and *Bos taurus* also appear to be fertile (Bongso and Hilmi 1988) while the hybrids between domestic cattle and European bison (*Bison bonasus*) (Krasinska 1971) show signs of infertility in the F_1 males (Fedyk and Krasinska 1971). American bison x *Bos taurus* appear to generate hybrids successfully and have been produced on a commercial basis for several years.

The Asian leopard cat and domestic cat are estimated to have diverged 8 to 10 MYBP with the male F_1 offspring being sterile while the female is fertile (O'Brien *et al.* 1997). The tammar

wallaby (*Macropus eugenii*) populations from Western Australia and South Australia are estimated to have diverged 50,000 to 100,000 years ago (Oliver *et al.* 1979), show morphological (Poole *et al.* 1991) and genetic (van Oorschot and Cooper 1988) differences and have a Nei genetic distance of 0.16 (McKenzie and Cooper 1997). They produce infertile F_1 male offspring and there appears to be some evidence for evolved behavioural barriers to reproduction between these populations or simply reduced hybrid viability. The wild and domesticated pigs are estimated to be separated by a mere 10 000 years (Andersson 1997) but the genetic distance between these populations based on phenotypic characters are as large as found between species in nature yet in their hybrids both sexes are fertile.

Muntiacini and Cervini sub-families within the Cervidae are estimated to have differentiated some 6 to 8 MYBP (Han 1985; cited by Miyamoto *et al.* 1990), while the estimated time of the divergence of Père David's deer and reds would be less than this. The Nei genetic distance between Père David's and red deer has previously been estimated at between 0.35 and 0.48 (Tate *et al.* 1992; Emerson and Tate 1993) compared with 0.46 in the *M. musculus* and *M. spretus* mice species (Bonhomme *et al.* 1984). Thus while criteria such as hybrid fertility status, years apart and Nei genetic distance between taxa are useful, they are not definitive but merely a guide to the degree of genetic divergence between taxa. The rates of evolution for different taxa are likely to be influenced by many factors (White 1978) including the more obvious ones such as environment and generation interval.

The Equus family consists of nine living species of horses, asses and zebras and is considered to have a rapid rate of chromosomal evolution (Wijers *et al.* 1993). Also it is considered by some to have the most rapid rate of chromosomal evolution of any mammalian genus (Bush *et al.* 1977). There is a vast range in diploid number of species within the Equus family, from 32 in Hartmann's mountain zebra (*Equus zebra*) to 66 in Przewalski's horse which is interesting considering their rapid evolution from a common ancestor some 4 to 5 MYBP (Wichman *et al.* 1991). Even within three zebra species *E. Grevyi* (2n=46) , *E. burchelli* (2n=44), and *E. zebra*, the karyotype of *E. zebra* is markedly different from the other two (Ryder *et al.* 1978). These complex rearrangements appear to have occurred within 1 to 2 MYBP (Wichman *et al.* 1991) yet considerable hybridisation occurs not only with zebra species but also within the Equus family (Allen and Short 1997). There are 17 recorded interspecies hybrids with Equus (Wichman *et al.* 1991) and numerous accounts of successful embryo transfers between various

species (Allen and Short 1997) however, most interspecies hybrids are infertile. This suggests that the rapid rate of evolution in the wider sense (not simply chromosomal) within the Equus family may play a significant role in the above observations. While several of these interspecies hybrids have been generated with the assistance of artificial breeding techniques natural hybridisations within Equus do occur and thus it is tempting to speculate the rate of evolution in Equus has been so rapid that behavioural barriers to hybridisation have had insufficient evolutionary time to develop fully. In this respect ethological or behavioural barriers to random mating constitute the most important class of isolating mechanisms in animals (Mayr 1970). Parallels can be drawn with the Cervinae where karyotypes vary between 2n=6 (female) and 7 (male) in muntjac deer to 2n=68 in red deer, the estimated time of evolutionary divergence from Muntiacini is less than 6-8 MYBP and hybridisation within Cervini is widespread.

Given the ability for hybridisation and the fertility of both *Bos* and Equus hybrids, the fact that *Bos* diverged some 3 to 4 MYBP, while Equus diverged some 4 to 5 MYBP suggests that the divergence between Père David's and red deer is a more recent event although as with most predictions associated with the evolutionary process this should be interpreted with caution. Similarly the occurrence and degree of any genetic bottlenecks may have significant influences on the genetic makeup of populations and this certainly appears to be a distinguishing feature between Père David's and red deer as discussed in the next section.

2.3 Père David's deer (*Elaphurus davidianus*)

The history of the Père David's deer illustrates their inbred nature and contributes significantly to their use as a model species for hybridisation with red deer in the search for quantitative trait loci (QTL). This species originates from China, but evidence of its survival in the wild is scanty and incomplete and relies on the distribution of fossil remains for evidence of its habitat and geographical range. To date five species or sub-species of *Elaphurus* have been named from Pleistocene deposits in North China, Taiwan and Japan. It appears that *E. davidianus* was distributed widely in north-central China especially along the coast. The habitat was lowland plains with sparse trees and shrubs or swampy areas of riverine grasslands and reeds. Sub-fossil deposits suggest they were present in Hunan province during the Shang dynasty (1766 to 1122 BC) (Sowerby 1949) and became extinct in the wild around 220 AD (Keqing 1978). In any event the species would have become extinct had it not been for the specimens held at the Imperial Hunting Park of Nan-Hai-Tze, south of Beijing (Peking). They were "discovered"

there by French Basque missionary-explorer-naturalist Père Armand David in 1865, the first foreigner to see what lay inside the 72 kilometre walled park; the population was estimated at 120 animals (Chapman 1990). The park, established in the thirteenth century, was originally 160 square kilometres and once enclosed sika, deer, hares, and antelopes as well as Père David's deer (Chapman 1990).

Père Armand David managed to secure bones and hides from the park for the Paris Museum. The curiosity of zoologists and naturalists was aroused and between 1866 and 1869 several attempts were made to export live animals to London but these either died en route or within 12 months of arriving without leaving any progeny. During the next ten years other deer reached various zoos in Europe, but it was not until 1898, when a pair of young deer reached Woburn Abbey from a Paris zoo, that they began to breed outside China. Unfortunately by this stage tragedy had struck the Nan-Hai-Tze herd. In 1894 a river which flowed through the park had breached the wall in several places and the bulk of deer escaped only to be eaten by famine stricken peasantry. A small remnant population survived this tragedy and remained until about 1900. During the Boxer rebellion troops en route to the relief of the foreign legations in Peking broke into the park and the deer were either killed or captured, a few being shipped to Europe. A pair in Peking Zoo were the last Père David's deer in China and they died about 1921 (Dobson 1951). Thus the only specimens to reach Europe were selected from the original population of 120 from the Imperial Hunting Park. All evidence suggests that other populations of Père David's deer had died out in their native China.

About this time, the eleventh Duke of Bedford, realising the dire plight of this unique species, persuaded European zoos to let him have as many specimens as possible to release in his park at Woburn. Altogether, 18 Père David's deer were assembled at Woburn and by the outbreak of the first world war in 1914 the population had increased to 88 animals (Bedford 1951). However mainly because it was illegal to buy in extra winter feed, the population declined to about 50. Thus by 1918 in effect this was the total world population of this species, since it was all but extinct in its native China (one pair in Peking zoo), and there were none left in European zoos (Glover 1980). By the second world war, the population had grown to about 200 animals, all of which were closely related to the 18 survivors assembled at Woburn. In fact these 18 may have arisen from a mere seven breeding animals (Glover 1980). However, a summary of all published data (Appendix 1) suggests that the entire world population has

certainly descended from between 3 to 13 animals but it could not be determined exactly how many made up the nucleus due to poor or lost records as a result of the world wars (Flower 1883; Phillips 1925; Sowerby 1949; Bedford 1950; Bedford 1951; Dobson 1951; Glover 1980; Jones *et al.* 1983; Chapman 1990) (Appendix 1). In fact, evidence of an even narrower genetic bottleneck in the Père David has been suggested with all animals being the descendants of one stag from the original consignment of six from Paris (Sowerby 1949).

From the history of this species it becomes very clear that they have been subject to three genetic bottlenecks with the narrow genetic base established at Woburn in the early 1900's probably being the most severe of the three. The first of these bottlenecks was essentially the "random" sub-sample of 120 of the species selected from their native range in China. The gathering of all live animals at Woburn around the turn of the century was in essence a smaller sample of these original 120 animals from the Imperial Hunting Park. Of the 18 animals accumulated at Woburn it is well known that only 13 were reproductively active and that one stag in particular dominated the females thus narrowing the genetic base even further (Sowerby 1949). The increase in population size to 88 in 1914 and their subsequent decrease to about 50 animals was a further erosion of this unique genetic resource. As a consequence they are extremely homogenetic and exhibit a low degree of genetic variability. In some ways they are comparable to inbred strains of animals.

2.4 Red deer (*Cervus elaphus*)

Red deer have a much less colourful history than the Père David's deer. The original red deer in the Invermay herd descended from the Otago strain of Scottish red deer introduced into New Zealand in 1871 (Banwell 1968) although there have been recent introductions of other strains or subspecies. These animals have successfully lived in the wild and expanded their range since introduction. Deer were first captured from the wild on a large scale when deer farming was legalised in New Zealand around 1970 (Yerex 1982). Despite some major difficulties in the early days the deer industry has developed and expanded and is a well established and profitable export earner for the New Zealand economy (NZGIB 1997).

2.5 Hybridisation between Père David's and red deer

Père David's and red deer both have 34 pairs of chromosomes. Using blood protein eletrophoresis, 19 fixed differences at 43 loci were evident indicating large genetic differences

between the two species (Tate *et al.* 1992). In addition a further 3 proteins were polymorphic in red deer but not in Père David's deer. These differences in blood proteins produced a Nei genetic distance (D) of 0.48 (Tate *et al.* 1992). This compares favourably with a Nei genetic distance (D) of 0.46 for *M. musculus* and *M. spretus* (Bonhomme *et al.* 1984). The large number of genetic markers segregating in these interspecific deer backcross hybrids make them an excellent resource for linkage mapping among genetic markers and for experiments which examine the relationship between genetic markers and quantitative traits (Tate *et al.* 1992).

A mating program which takes advantage of the effects of chromosomal recombination can be used to explore the genetic differences between the parental species. At AgResearch Invermay technologies such as oestrous synchronisation, artificial insemination, embryo transfer and *in vitro* production of embryos have been used as means of producing the F_1 hybrids. These F_1 progeny were mated to red deer to produce backcross ¼ Père David's deer / ¾ red deer hybrids. The vast majority have been produced by using F_1 males over red females. Due to chromosomal recombination these progeny may exhibit morphological and production traits similar to either parental species or at any intermediary level.

This follows several experiments which using backcrosses or intercrosses and similar statistical analyses have isolated production QTL in pigs including growth rate, fatness and length of small intestine (Andersson *et al.* 1994), live weights and carcass quality traits in cattle (Lewin *et al.* 1990) and milk production in dairy cattle (Georges *et al.* 1995).