Chapter 7.

7. Comparative patterns of growth in live weight, antler and testes in red deer (*Cervus elaphus*) and their hybrids with Père David's deer (*Elaphurus davidianus*).

7.1 Abstract

The patterns of growth in red and their hybrids with Père David's deer (¼ Père David / ¾ red deer) were compared from birth to 15 months of age. Hybrids had significantly ($P<0.01$) higher live weight than reds from around six months of age in late May to their peak live weight at around 16 months in mid March. Hybrid males were also significantly heavier than reds at birth (9.37 vs 8.85kg, $P<0.05$) and at weaning (52.3 vs 48.8kg, $P<0.01$) approximately three months later. There were no significant differences in growth rates between hybrids and reds during the winter period of late May to late August and the early autumn period of mid January to mid March. For all other growth periods including birth to weaning, weaning to early winter and spring to peak summer hybrids grew significantly faster than reds in both sexes. Antler growth cycles indicated hybrid males initiated their pedicle growth significantly later (29 Aug vs 16 Aug, $P<0.001$) and at significantly higher body weight (77.2kg vs 68.4, $P<0.001$) than reds. In addition to this, hybrids cleaned their antlers earlier (14 Feb vs 24 Feb, $P<0.001$) and cast their hard antler buttons earlier (6 Oct vs 25 Oct, $P<0.001$) indicating an earlier pattern of seasonal response.

Keywords: growth, seasonality, Père David's deer, red deer, hybrid vigour

7.2 Introduction

Temperate species of deer, such as red deer (*Cervus elaphus*, red) and fallow deer (*Dama dama*), are seasonal breeders and unlike Père David's deer (*Elaphurus davidianus*, PD) are short day breeders. That is both the male and female reproductive systems are entrained by the decreasing daylength in the autumn (Guinness *et al.* 1971; Lincoln 1971b) whereas PD are

long day breeders (Curlewis *et al.* 1988). Red deer calves are born following a gestation of around 234 days in the early summer (November - December) (Fennessy *et al.* 1991b). However Père David's deer are long day breeders with the stags rutting (i.e. breeding) in December with hinds calving around October (Southern Hemisphere) following a gestation of around 283 days (Wemmer *et al.* 1989). The antler cycles are similarly synchronised so that in all species the stag is in hard antler for the rut. Similarly, using progesterone profiles the difference between PD and red females in the onset of the breeding season (first oestrous cycle) was estimated at 90 days (Loudon *et al.* 1989) while there were no differences in the length of the breeding season. However Père David's and red deer hybridise producing fertile hybrids (Asher *et al.* 1988; Fennessy and Mackintosh 1992). Therefore the seasonality of the interspecies hybrid is of particular interest. We have used fertile F_1 hybrid sires (from artificial insemination of red hinds with PD semen) to generate the quarter backcross (¼PD / ¾R) and conduct subsequent comparisons with red deer for body growth and seasonality traits (antlers and testes) in males.

7.3 Materials and Methods

Hybrid generation. Hybrid generation began with the production of F_1 hybrids using Père David semen in red females (Asher *et al.* 1988; Fennessy and Mackintosh 1992) and specialised techniques developed for deer (Fennessy *et al.* 1990, 1991a; Asher *et al.* 1993; Fennessy *et al.* 1994). Subsequently, backcross progeny were generated using these F_1 hybrids in both artificial insemination ((PDxR)xR)) and multiple ovulation and embryo transfer programs (MOET) (Rx(PDxR)) using the techniques described previously (Tate *et al.* 1997).

Management. At weaning all animals were vaccinated against clostridial diseases (Ryvac clostridial vaccine, Young's Animal Health (NZ) Ltd, Upper Hutt, NZ), yersiniosis (Yersiniavax, AgVax Developments Ltd, Upper Hutt, NZ) and treated with copper (Bayer copper capsules, Bayer NZ Ltd, Auckland), and selenium (selenium selenate) and an anthelmintic (Cydectin, Cyanamid NZ Ltd, Auckland) at recommended rates. Four weeks later they received booster injections for protection against clostridial diseases and yersiniosis. At the same time and thereafter in May, June, July, September and January they were further treated with Moxidectin.

Measurements. Live weights were recorded at birth, weaning (c. 3 months of age) and thereafter at least once per month (every second week through spring period) until 15 to 16 months of age (16m). Antler growth parameters were recorded at two weekly intervals from the first sign of pedicle development through till the antlers were cleaned of velvet (due to rising testosterone) in the autumn (February-March). The date of pedicle initiation was defined as the date on which pedicles reached a height of 1.5 cm above the skull (medial aspect of the pedicle) (Meikle *et al.* 1992). Casting date was recorded in the spring as the mean date on which the hard antler buttons were cast off. The testes diameters were measured at six and 16 months of age while males were anaesthetised.

Statistical analysis. All data were analysed using REML analysis (SAS 1989b). Live weights and live weight gains were modelled fitting genotype, sex, birth day, year, sire within genotype and a sex by year interaction. Dam live weight was also included in birth weight and weaning weight / growth rate to weaning analyses while calf fate was also included in the former analysis. Antler parameters were modelled fitting genotype, birth day, year and sire within genotype while weight at pedicle initiation date was also included in the analysis of pedicle initiation date. Change in mean testes diameter was modelled fitting genotype, birth day, year, and sire within genotype while testes diameters at 6m and 15m also included live weights at the same age. All traits were tested for homogeneity of variance between genotypes prior to REML analysis.

For birth weight, 14m, 16m weights, 14 to 16m live weight gain, date of pedicle initiation and 6m testes diameter hybrids had significantly greater standard deviations than reds. For 14m and 16m live weights the range in weights increased substantially with increasing standard deviation in a manner which suggested a log transformation would be appropriate. For these traits the data were log transformed prior to REML analysis however this made no difference to either the significance levels or the least square means for the genotype groups. For birth weight, pedicle initiation, 6m testes diameter and live weight gain between 14 and 16m the examination of the residual plots did not suggest a log transformation was appropriate and was therefore not carried out. The results presented for all these traits are for the non transformed data.

Table 7.1 Mean birth dates (±SD days) for hybrids (¼ Père David / ¾ red) and red deer.

	Year of birth	Hybrid (±SD)	n	Red (±SD)	n
Male	1991	28 Nov ± 12.4	31	3 Dec ± 7.5	23
	1993	19 Nov ± 9.0	39	26 Nov ± 9.1	15
	1994	18 Nov ± 8.6	51	22 Nov ± 12.0	21
	1995	22 Nov ± 6.3	49	28 Nov ± 10.3	17
	Total		170		76
Female	1991	30 Nov ± 16.3	18	9 Dec ± 5.1	20
	1993	19 Nov ± 7.2	40	19 Nov ± 4.9	15
	1994	18 Nov ± 8.2	28	28 Nov ± 12.1	19
	1995	19 Nov ± 5.4	36	23 Nov ± 5.0	16
	Total		122		70

7.4 Results

Live weights and growth. The differences between genotypes in mean birth dates (Table 7.1) were small and the mean dates overlapped in all years. Mean birth dates over four years are presented for hybrids despite the significant segregation observed in this trait (Goosen *et al.* 1997a).

Live weights were recorded at similar dates over the four years and subsequently adjusted to a common date using covariance procedures. Live weight gains were calculated for six periods to coincide with the seasonal pattern of live weight gain defined in red deer (Fennessy *et al.*

Table 7.2 Adjusted[1] live weights (kg) at five points on the growth curve up to 16 months of age for hybrids (¼ Père David / ¾ red) and red deer.

	Birth	Weaning (26 Feb)	Early winter (28 May)	Early spring (26 Aug)	Early summer (19 Nov)	Mid summer (14 Jan)	Peak autumn (12 Mar)
Male							
Hybrid	9.37 *[3]	52.3 **	66.8 ***	75.2 ***	99.7 ***	118.0 ***	122.9 ***
Red	8.85	48.8	61.4	69.2	89.9	104.7	108.4
Female							
Hybrid	8.74 NS	47.2 NS	60.3 **	64.6 **	82.7 ***	93.7 ***	99.7 ***
Red	8.28	45.0	55.5	59.5	74.3	84.7	89.2
SED[2] within sex between genotypes	0.239	1.26	1.52	1.63	2.03	2.28	2.21
SED between sexes	0.207 *	0.71 ***	0.94 ***	0.99 ***	1.26 ***	1.57 ***	1.60 ***

[1] adjusted for environmental effects and sire
[2] standard error of difference
[3] * P<0.05; ** P<0.01; *** P<0.001

Table 7.3 Adjusted[1] live weight gains (g/d) for the four stages of growth up to 16 months of age for hybrids (¼ Père David / ¾ red) and red deer.

	Birth - weaning (± 90days)	Autumn (92 days)	Winter (90 days)	Spring (85 days)	Summer (56 days)	Early Autumn (57 days)
Male						
Hybrid	460 ***[3]	155 *	92 NS	286 ***	329 ***	75 NS
Red	408	141	87	241	267	61
Female						
Hybrid	409 **	138 ***	45 NS	212 ***	195 ***	113 NS
Red	373	115	43	173	163	83
SED[2] within sex between genotypes	13.0	6.5	5.3	8.7	10.1	17.9
SED between sexes	7.5 ***	6.1 ***	4.2 ***	6.2 ***	8.0 **	10.6 ***

[1] adjusted for environmental effects and sire
[2] standard error of difference
[3] * P<0.05; ** P<0.01; *** P<0.001

1981, 1991c) namely birth to weaning (approximately 90 days) to start of winter (Autumn, 92 days), to start of spring (Winter, 90 days) with the spring to autumn (198 days) period being further split into three periods of 85, 56 and 57 days in order to allow investigation of the rapid spring growth rate and the late summer/autumn surge in the pattern of live weight gain prior to the rut (Fennessy *et al.* 1991c).

Adjusted live weights and live weight gains are presented in Tables 7.2 and 7.3. Hybrid males had significantly greater body weights at all seven defined points from birth through to 16m (8-13%). Hybrid male growth rates were higher than their red contemporaries by 13, 10, 6, 19, 23 and 21% for the six growth intervals (all except winter and early autumn were significant, Table 7.3). Hybrid females averaged 8% heavier at the seven points through the growth period although the differences were not significant at birth and weaning. Hybrid female growth rates were superior to their red counterparts by 10, 21, 4, 23, 20 and 23% for the six growth intervals (again differences for the winter and early autumn periods were not significant).

Pubertal and seasonality traits. Pubertal and seasonality parameters for males are presented in Table 7.4. Pedicle initiation is a pubertal trait (Meikle *et al.* 1992). Hybrids initiated their pedicles 14 days later (P<0.001) and at around 13% higher body weight (77.2 kg on 29 August vs 68.4 kg on 16 August, P<0.001) than reds. Antler cleaning is a seasonal

Table 7.4 Adjusted[1] antler and testes seasonality parameters for hybrids (¼ Père David / ¾ red) and red deer.

	Pedicle initiation		Date of		Testes diameter (cm) at	
	Date	Live weight (kg)	Antler cleaning	Antler casting[2]	6 months	15 months
Hybrid	29 Aug **[3]	77.2 ***	14 Feb ***	6 Oct ***	1.2	2.9
Red	16 Aug	68.4	24 Feb	25 Oct	1.4 ***	3.2 ***
SED	4.9	1.45	2.1	2.8	0.39	0.73

[1] adjusted for environmental effects and sire
[2] casting as 2 year olds
[3] * P<0.05; ** P<0.01; *** P<0.001

phenomenon associated with rising testosterone prior to the rut. Mean adjusted cleaning dates for hybrids and reds were 14 February and 24 February respectively being 10 days earlier in the hybrids. Antler casting is also a seasonal phenomenon occurring due to the decline in testosterone in the early spring. Hybrids cast their hard antler buttons on average 19 days earlier (6 October) than their red counterparts (25 October). Testes growth is a phenomenon caused by two effects; namely pubertal and seasonal influences. Mean testes diameters of red males were significantly larger than hybrids at 6m and 16m (1.4 vs 1.2 cm, P<0.001 and 3.2 vs 2.9 cm, P<0.001). The calculated increase in testes diameter over the 250 day period between 6 and 16m was not significantly different between reds and hybrids ($7.0x10^{-3}$ vs $6.8x10^{-3}$ cm, SED=$0.29x10^{-3}$).

7.5 Discussion

Infusion of Père David genes into red deer increased live weight overall from birth to 16 months of age with hybrids attaining around 13% higher live weights than reds. Pure Père David's have a greater mature live weight than reds (Loudon *et al.* 1989; Whitehead 1993) and this is reflected in the higher growth rate of the hybrids, although heterosis could not be calculated as pure PD were not run with the hybrid and red genotypes. Calculations from the current data and assuming no heterosis infer that the PD would be expected to be around 52% (i.e. 4x13%) heavier than reds. In fact the difference between these pure species reported by Loudon *et al.* (1989) was 70%. This suggests the backcross hybrids in the current study performed below expectation which may provide evidence for interesting genetic effects. Considering the large genetic divergence between PD and red deer (Tate *et al.* 1995b) and the highly inbred nature of the PD deer (Bedford 1950, 1951; Glover 1980) the assumption of no heterosis effects in their hybrids may be somewhat conservative. If infusion of PD gene/s

increased live weight then it may have been due to either heterosis or a PD gene effect *per se* however a negative effect i.e. where PD gene/s decreased live weight, was indicative of interesting genetic effect.

Red deer had excellent growth rates and exceeded target live weights which attract price premiums (83kg for females and 105kg for males in early March) reaching 89 and 108kg respectively (Fennessy and Milligan 1987). The weaning weights, live weight gains and 16m weights of red deer are superior to other red deer performance recorded in New Zealand (Fennessy and Milligan 1987; Suttie *et al.* 1987; Moore *et al.* 1988). The date and weight at pedicle initiation in reds in this study compares well with previous studies at Invermay (e.g. pedicle initiation on 24 August at 66.1 kg for animals born on 2 December; Meikle *et. al* (1992)). The observed 16m testes diameters were similar to previously published data at the same age (Suttie *et al.* 1992) and also coincide with peak testes development described in wild deer (Lincoln 1971b; Mitchell *et al.* 1976).

The findings in this study for testes growth and regression are similar to other studies. For example, the magnitude of change in testes size in adult fallow buck has been shown to increase from a seasonal trough of 22g to a seasonal peak of 133 g indicating a six fold increase in paired testes weight during the annual cycle (Chaplin and White 1972). This study indicated backcross hybrids increased 2.4 fold in testes diameter between 6 and 15m just prior to the rut.

Based on the significantly larger standard deviations for 14 and 16m live weight in hybrids than reds and the residual plots, a log transformation seemed appropriate. The significance levels and least squares means remained almost identical after transformation which indicated that the differences between genotypes were real. Also it has been shown that there is evidence for quantitative trait loci for these two traits (see Chapter 9) thus making the transformation inappropriate. For live weight gain between 14 and 16m and using the residual plots transformation did not seem appropriate but when seven obvious outliers were excluded the genotype standard deviations were no longer significantly different. For birth weight a log transformation seemed inappropriate and there is also evidence to indicate a quantitative trait locus for this trait (see Chapter 8) which is a distortion that a log transformation is unable to remedy.

Table 7.5 Comparative data for mean (±SD) casting dates (n) for 3 F_1 stags (Père David x red), 2 and 3 year old hybrids (¼ Père David / ¾ red) and red deer. Expectations are based on additivity.

Age (years)	Genotype			
	Red	F_1 (PDxR)	Backcross hybrid	
			Expected	Observed
2	24-Oct ± 9.7 (47)	5 Sept ± 13.9 (3)	30 Sept	5-Oct ± 11.4 (83)
3	9-Oct ± 4.5 (10)	29 Jul ± 12.6 (3)	4 Sept	10-Sept ±14.3 (16)

Père David's deer have a unique antler form which is markedly different from red deer as the antlers appear reversed. Probably the most accurate indicator of seasonality in males is casting date. Adult PD cast their antlers in June/July and clean their hard antlers in late October/November (Fennessy and Mackintosh 1992). In comparison, the mean dates for four year old reds were September 15 (± 17.8 days SD) and February 3 (± 6.9 days) (Fennessy *et al.* 1992). Thus the difference in seasonality between the grand-parental species for the antler cycle is in the order of three months. The F_1 hybrid antler data are few due to the difficulties associated with generating these animals but the data recorded at Invermay are presented in Table 7.5. Females of the two species also show differences in reproductive parameters similar to these with pubertal and adult PD hinds exhibiting a significant 90 day earlier onset of oestrus than reds (Loudon *et al.* 1989).

From this it is apparent that there is large variation in casting dates and that the date of casting for both F_1 and hybrids is intermediate between the grand-parental species and roughly in proportion to their genetic makeup; older animals also tend to cast earlier. The casting dates for the backcross hybrids are about midway between those of their parents (reds vs F_1; Table 7.6). In the yearling animals, hybrids had a more condensed antler growing season with later pedicle initiation and earlier antler cleaning than reds. The large standard deviations in hybrid antler traits are indicative of the extreme differences in the grand-parental species for these seasonal parameters and the fact that genetic recombination events occur in these hybrids.

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Chapter 8.

8. Genetic analysis of gestation length and live weights to weaning in Père David's (*Elaphurus davidianus*) x red deer (*Cervus elaphus*) interspecies hybrids.

8.1 Abstract

Père David's deer (PD) have a 49 day longer gestation than red deer (283.4 ± 6.1 (SD) and 234.4 ± 3.4 days). In F1 hybrids (PD sire), male progeny had a longer gestation than females (268.8 ± 6.4, n=10 and 262.0 ± 4.5, n=10, P<0.01), both greater than the mid-parent mean of 259 days. The male and female progeny of F1 sires and red dams had a gestation length of 248.8 ± 5.5 days (n=275). Mixture distribution analysis suggested two normal distributions with a difference in means of 7 days. In the reverse hybrid (embryo transfer progeny of F1 dams), the gestation length of 242.5 ± 3.3 days (n=19) was significantly shorter than the backcross hybrid progeny of F1 stags. The control of gestation length in this interspecies hybrid is clearly very complex although we have found evidence of four quantitative trait loci (QTL) of which three are on the same linkage group (16) with the other being on linkage group 18. These QTL (LOD scores > 3.0) accounted for around 3 days and between 7 to 7.9% of the phenotypic variance in gestation length in backcrosses. We also provide good evidence (LOD=2.80) for the existence of a QTL for birth weight. In backcross hybrids the size of this effect was 0.60 kg and the mean birth weight was 9.3 ± 1.39 (SD) with the QTL explaining 5.3% of the phenotypic variance.

Keywords: Père David's deer; red deer; hybrid; gestation length; birth weight; weaning weight; quantitative trait loci; QTL

8.2 Introduction

Père David's deer (*Elaphurus davidianus*, PD) were originally imported to New Zealand for several reasons, the major one being the possibility of hybridisation with red deer (*Cervus elaphus*, R) to advance the time of calving in farmed deer species. PD are long day breeders typically mating in December and calving around 9 months later. In contrast red deer are short day breeders mating in March/April and calving after a gestation of 234 days which is 49 days shorter than PD. Two reports of hybrids between these species, namely a fertile female born around the turn of the century and another in 1978 of unknown fertility status (Beck and Wemmer 1983) stimulated interest in generating hybrids. This resulted in a number of importations of PD and several attempts at natural and artificial hybridisation (Asher *et al.* 1988; Fennessy and Mackintosh 1992).

In the light of these successful hybridisations we have generated interspecies hybrids between Père David's and red deer as a tool in the search for QTL in deer. This parallels a considerable world-wide effort in the quest to find quantitative trait loci (QTL) in a wide variety of farmed species, including dairy cattle (Georges *et al.* 1994), sheep (Crawford *et al.* 1997) and pigs (Andersson *et al.* 1994). A significant feature of this deer hybrid is that the genetic divergence between the parental species is so large that almost all DNA polymorphisms identified are species-specific, thus providing a very powerful gene mapping resource (Tate *et al.* 1995b). In addition, the species also show large differences in morphology and production traits. We have previously presented preliminary evidence for QTL for gestation length (Goosen *et al.* 1997a). This paper summarises the results of these studies with special emphasis on gestation length, birth weight, weaning weight and pre-weaning gain in this unique deer hybrid.

8.3 Materials and Methods

Hybrid generation and phenotypes. All F_1 hybrids were generated by artificial insemination (AI) of R hinds with PD semen. Backcross hybrids (¼ PD / ¾ R) were generated over a period of four years using three methods, namely artificial insemination (AI), multiple ovulation and embryo transfer (MOET) and synchronised natural mating. Artificial insemination was carried out using R hinds with F_1 hybrid (PDxR) semen, multiple ovulation and embryo transfer (MOET) using F_1 hybrid hinds (PDxR) as donors (R sire) and R hinds as recipients and synchronised natural mating using an F_1 hybrid stag over R hinds. Semen from six F_1 stags was

used in a total of 841 laparoscopic intrauterine inseminations (Fennessy *et al.* 1991a; Asher *et al.* 1993). Semen was collected on the day of AI by electro-ejaculation (Asher *et al.* 1993) and hinds were each inseminated with 3 to 30 million live sperm. For MOET, 34 embryos obtained from the synchronised natural matings of five superovulated F_1 hinds were transferred to R recipients (Fennessy *et al.* 1994). For synchronised natural mating, hinds were synchronised using the same techniques as for AI (Fennessy *et al.* 1991a). More details of the methods used have previously been provided (Tate *et al.* 1997).

Hinds in the three mating programs were examined by rectal ultrasonography (Wilson and Bingham 1990), 32 to 42 days after insemination or transfer, to assess pregnancy status. During the calving season hinds were monitored daily, new-born calves tagged and birth weight, sex and dam recorded. A total of 339 backcross progeny were generated of which 275 were by artificial insemination with the remainder being produced by MOET (19) and synchronised natural mating (45). Of the 275 AI progeny only the 240 live at birth were used in ANOVA and interval mapping analyses. For hinds conceiving to AI, conception was taken as the AI date, while for hinds conceiving to ET, it was taken as 72 hours after withdrawal of progesterone treatment. Each cohort of backcross hybrid animals was raised on pasture with a comparison group of at least 30 red deer.

Genotyping. The hybrid status of all backcross hybrids was confirmed by DNA typing and that of F_1 hybrids by DNA and/or protein testing (Tate *et al.* 1995b). The segregation of up to 250 genetic markers was analysed in the backcross herd including restriction fragment length variants (RFLV), protein variants and microsatellites. Numbers of animals scored at each locus are given in Appendix 5. The term "variant" has been used for the fixed differences observed between the species whereas the word "polymorphism" has been reserved for variation within a species. The linkage relationships of the markers were analysed using MAPMAKER/EXP using the Kosambi mapping function for all autosomal chromosomes (Tate *et al.* 1995b; Tate 1997). An animal is described as "informative" if genotype data at a particular locus can be used to identify the parental origin of gametes (i.e. which allele was inherited from the sire and which from the dam). Informativeness is a prerequisite for linkage analysis, which examines the inheritance of gametes at each marker locus. The species specific variants used (Tate 1997) were close to fully informative and on average were 96% informative in backcross offspring in the mapping panels thus providing a powerful resource for QTL detection.

Analysis. Gestation length was modelled fitting sire, year and sex, while birth weight included sire, year, sex and breeding method with dam live weight at conception fitted as a covariate (there was no relationship between gestation length and birth weight). Similarly weaning weight and pre-weaning gain were modelled including sire, birth day, year, sex and a sex by year interaction with dam live weight at conception fitted as a covariate (Moore *et al.* 1988). Genetic analysis of backcross progeny for gestation length, birth weight, weaning weight and pre-weaning gain included the use of three techniques namely, mixture distribution analysis (see Appendix 4), which did not use marker information, ANOVA and multiple marker interval mapping analyses, which used markers to test for associations between chromosome sections and/or individual genetic markers and trait expression. Backcrosses generated by MOET were not used in the analysis for the detection of QTL because of the small number of animals generated.

Mixture distribution analysis used a maximum likelihood technique to test the null hypothesis of one normally distributed population against the alternative hypothesis of two equal sized normally distributed populations with different means in the backcross hybrid population (Wuliji *et al.* 1993). This analysis did not utilise marker information and as such was an alternative test of the datum. The same coefficient of variation as for red deer was used for this test. Using residual degrees of freedom (df) representative of the traits in Table 8.3 a power analysis of this technique revealed that with 246 df this test had power of 78% and 96% of detecting two sub-populations if their means differed by 1 and 1.2 standard deviation units respectively while with 278 df 1 and 1.2 standard deviation units conveyed 83% and 98% power respectively.

Linkage analyses were carried out testing across sires and investigated differences in backcross individuals which inherited either PD or R alleles. The ANOVA approach used the regression relationship between individual markers and adjusted phenotype to test for point associations between genetic markers and traits (Soller *et al.* 1976). Given the large number of single point tests conducted across the genome it was important to determine the true or trait-wise 5% significance threshold. Using a technique which simulated a normally distributed population for the 240 individuals and, using 1000 iterations, the trait-wise 5% and 1% thresholds were estimated as probabilities of P=4.48x10-4 and P=7.44x10-5.

In addition, the more accurate interval mapping maximum likelihood technique was used to test for QTL (Lander and Botstein 1989) using MAPMAKER/QTL. For the multiple marker interval mapping analyses we used the more stringent significance threshold from the ANOVA simulation to determine the appropriate significance threshold (Knott *et al.* 1996). The genome-wide simulated thresholds given above were equivalent to LOD scores of 2.70 and 3.50 (Champoux *et al.* 1995) and were used to test for significance. All LOD scores greater than 1.90 are indicative of "suggestive linkage" and have therefore been reported (Lander and Kruglyak 1995).

8.4 Results

The gestation lengths for the parental species and various hybrids are presented in Table 8.1. The mean gestation length for the 20 F_1 singleton progeny of PD stags and R hinds was 265.4 days, 31 days longer than the R×R mean and 6 days longer than the expected mid parent mean of 259 days, although there was a significant difference between males (268.8 ± 6.4, n=10) and females (262.0 ± 4.5, n=10, SED ± 2.4 $P<0.01$).

The large standard deviation in gestation length in this backcross hybrid population relative to red deer (5.5 vs 3.4) suggested potential segregation in this interspecies population and further analyses were conducted to test this hypothesis. The 240 AI observations from the (PDxR) sire backcross were initially analysed using a simple general linear model (SAS 1989a) which revealed significant sire and year effects. Males had a longer gestation length than females but the difference (0.7 days) was not significant. Figure 8.1 illustrates the distributions for the three populations adjusted for sire, year and sex effects.

Table 8.1 Gestation lengths for Père David's deer, red deer and their hybrids

Species	n	Mean ± SD	Expected[1]	Difference[2]
Red (R)	86	234.4 ± 3.4	-	-
Père David's (PD)	21	283.4 ± 6.1	-	-
(PD x R) female	10	262.0 ± 4.5	258	+ 4
(PD x R) male	10	268.8 ± 6.4	259	+ 10
(R x (PD x R))	19	242.5 ± 3.3	247	- 5
((PD x R) x R)	275	248.8 ± 5.5	247	+ 2

[1] This assumes normal additive genetic variation and thus the expected gestation length is the mid parent mean, or in the case of the ¼PD / ¾R is the weighted mean of the grand-parental values ((283.4 - 234.4) /4) and adjusted for sex differences. The maternal effect is assumed to be constant as both backcrosses were carried to term by red mothers.
[2] Difference between observed and expected gestation lengths.

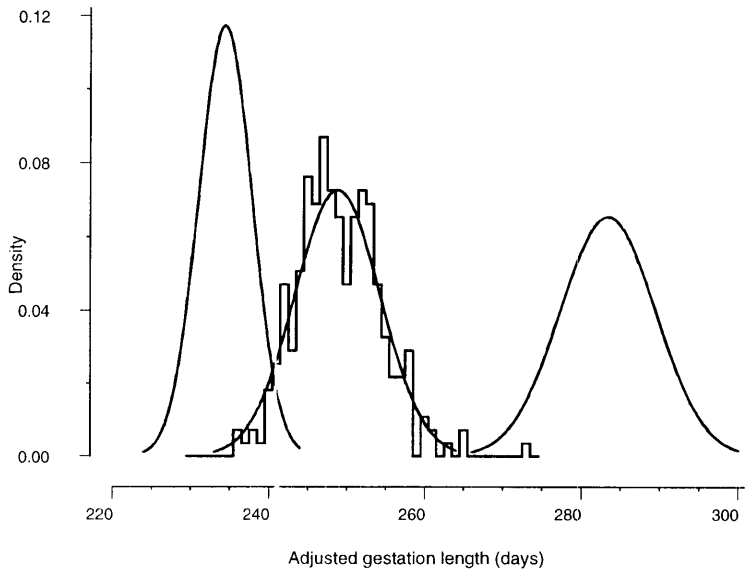

Figure 8.1 Probability density distributions for gestation length. Distributions from left are: red deer (Fennessy *et al.* 1991a), ((PDxR)xR) both as a histogram and a normal approximation after adjustment for sire, year and sex effects, and Père David's deer (Wemmer *et al.* 1989).

Mixture distribution analysis. Gestation length, birth weight, weaning weight and pre-weaning gain were analysed for evidence of segregation (Wuliji *et al.* 1993). The results for all four traits rejected the null hypothesis of one normally distributed population in favour of the alternative hypothesis of two normally distributed populations with means $\bar{x}_1$ and $\bar{x}_2$ as is evident from the chi-squared probabilities in Table 8.2.

ANOVA. Based on the above results genetic linkage between DNA markers and individual traits was investigated. An ANOVA approach was used to assess the relationship of individual markers to gestation length, birth and weaning weights and pre-weaning gains respectively.

Table 8.2 Mixture distribution analysis of gestation length, birth and weaning weight and pre-weaning gain for evidence of quantitative trait loci in the Père David's x red deer backcross hybrids (¼PD / ¾R).

Trait	Mean ± SD (n)	Effect	$\bar{x}_1$	$\bar{x}_2$	P
Gestation length (days)	248.8 ± 5.5 (275)	7.2	245.4	252.6	$<2.18\times10^{-8}$ ***
Birth weight (kg)	9.3 ± 1.39 (289)	1.5	8.48	10.02	2.18×10^{-8} ***
Weaning weight (kg)	51.0 ± 4.64 (271)	3.9	49.0	52.9	1.72×10^{-3} ***
Pre-weaning gain (g/d)	439 ± 45 (263)	34	422	456	5.17×10^{-3} **

* P<0.05; ** P<0.01; *** P<0.001

Table 8.3 ANOVA of gestation length, birth and weaning weight and pre-weaning gain traits for quantitative trait loci in the Père David's x red deer backcross hybrids (¼PD / ¾R).

Trait	Mean ± SD (n)	df	Effect	Linkage group	Marker (Deer map #)	P
Gestation length (days)	248.8 ± 5.5 (275)	266	2.7	16	75	0.006 **
			2.7	16	127	0.007 **
			2.6	16	149	0.020 *
			2.6	16	12	0.023 *
			2.7	16	236	0.028 *
			2.3	18	74	0.045 *
Birth weight (kg)	9.3 ± 1.39 (289)	278	0.56	4	112	0.09 NS
Weaning weight (kg)	51.0 ± 4.64 (271)	254	-	-	-	-
Pre-weaning gain (g/d)	439 ± 45 (263)	246	17	19	7	0.25 NS

* P<0.05; ** P<0.01; *** P<0.001

Using this method, backcrosses with PD alleles at several markers had significantly longer gestation lengths (Table 8.3). The original probabilities from this analysis were converted to trait-wise probabilities, which account for multiple tests conducted across the genome, and are presented in Table 8.3 to indicate the true magnitude of significance for these tests.

Backcrosses with the PD allele at marker #112 had significantly higher birth weights (9.63 vs 9.02 kg, P<0.05) compared to those without the PD allele and this accounted for 5.6% of the phenotypic variance in birth weight. Neither weaning weight nor pre-weaning gain produced significant results using the ANOVA (Table 8.3).

Multiple marker interval mapping analysis. This analysis detected several significant QTL for gestation length. Four of the five results for gestation length were significant (P<0.05) with three on linkage group 16 and one on linkage group 18 (Figure 8.2 and Table 8.4).

Interval mapping analysis detected significant linkage for a birth weight QTL on linkage group 4 close to marker #112. The position of the highest LOD score was at marker #41, 1.2 cM from marker #112. The effect size was 0.60 kg and explained 5.3% of the variance in birth weight. For this trait there was evidence of allele variation between sires with progeny of one sire used in 1990 being lighter than progeny of all other sires. When this sire's progeny were excluded from the analysis the maximum LOD score was 2.80, and when his progeny were included the maximum LOD score was 2.12 and still suggestive of linkage. The LOD of 2.00 ("suggestive linkage") for pre-weaning gain mapped to an interval of 25 cM on linkage group

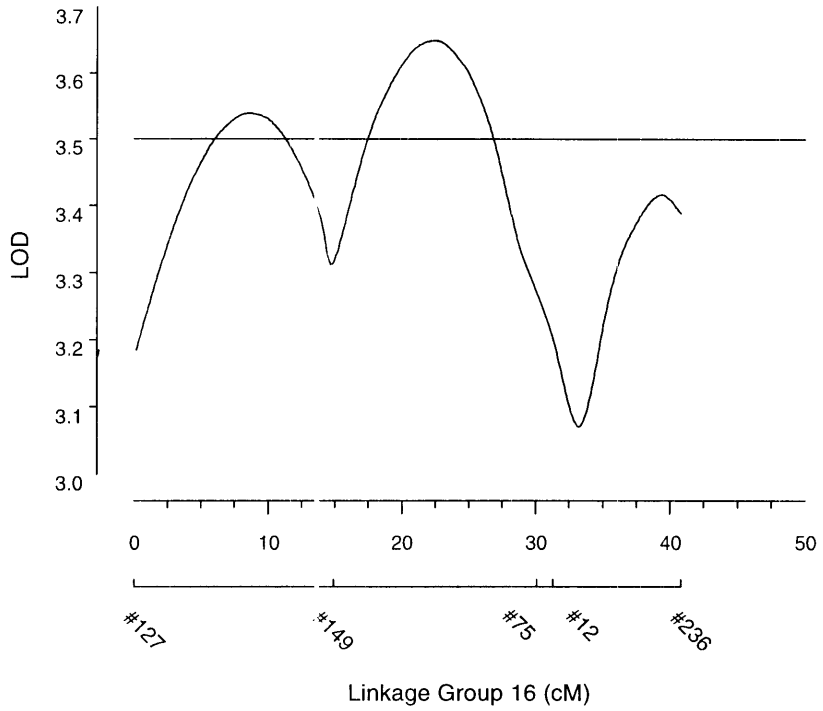

Figure 8.2 Maximum likelihood surface for gestation length on linkage group 16 with 1% threshold at LOD = 3.5.

19 between markers #209 and #7. The effect was -16 g/d and it explained 3.6% of the phenotypic variance in pre-weaning gain and was of similar size and on the same linkage group as the non significant effect noted for the ANOVA.

Table 8.4 Multiple marker interval mapping maximum likelihood analysis of gestation length, birth and weaning weight and pre-weaning gain traits for quantitative trait loci in Père David's x red backcross hybrids (¼PD / ¾R).

Trait	Mean ± SD (n)	Allele subsitution[1]			Marker interval		
		LOD	Effect	Variance explained[2]	Linkage group	Markers (Deer map #)	Span (cM)
Gestation length (days)	248.8 ± 5.5 (275)	3.65 **	3.0	7.9	16	149 - 75	15.2
		3.53 **	3.0	7.5	16	127 - 149	14.9
		3.41 *	2.9	7.1	16	12 -236	9.5
		3.05 *	2.9	7.0	18	74 - 85	21.3
		1.94 NS	3.1	8.2	6	120 - 108	60.5
Birth weight (kg)	9.3 ± 1.39 (289)	2.80 *	0.60	5.3	4	41 - 230	3.2
		2.37	0.74	8.2	23	195 - 125	30.1
Weaning weigth (kg)	51.0 ± 4.64 (271)	-	-	-	-	-	-
Pre-weaning gain (g/d)	439 ± 45 (263)	2.00 NS	-16	3.6	19	209 - 7	24.5

[1] Effect of substitution of red deer allele with a Père David's deer allele.
[2] Percentage of variance explained by QTL σ2% = ((effect/2)2/σ2)
* P<0.05; ** P<0.01; *** P<0.001

Table 8.5 Haplotype analysis of gestation length.

Linkage group 16 haplotype	n	Gestation length ± SD (days)	Effect (days)[1]
Red	64	247.7 ± 4.97	
Père David	64	251.8 ± 5.72	4.1
Linkage group 16 and 18 haplotype			
Red 16 / Red 18	30	247.1 ± 4.37	
Red 16 / PD 18	31	247.5 ± 4.50	0.4
PD 16 / Red 18	29	250.1 ± 4.52	3.0
PD 16 / PD 18	30	252.3 ± 5.51	5.2

[1] Difference from red haplotype.

Haplotype analysis. Based on the consistent support for several QTL affecting gestation length on linkage group 16 a further analysis was conducted to determine if there was a haplotype effect influencing gestation length. For the four markers segregating on linkage group 16 (#127, #149, #75, #236) backcrosses were divided into those which were heterozygous PD/R and those which had all red (R/R) alleles. Markers #75 and #12 were only 1.2 cM apart and this basis marker 12 was eliminated from the haplotype analysis. The difference in gestation lengths between these two groups was 4.1 days which supports the other analyses in suggesting there are several important components which control gestation length on linkage group 16 (Table 8.5). Given the evidence for a QTL for gestation length on linkage group 18 a further haplotype was investigated. For the marker segregating on linkage group 18 (#74) backcrosses were divided into those which were heterozygous PD/R and those which had all red alleles. The difference in gestation lengths between PD/R and R/R was 5.2 days. The virtually equal numbers of individuals in each of the haplotypes in Table 8.5 support the genome map constructed in MAPMAKER in that there is no evidence of segregation distortion either within linkage group 16 or between linkage groups 16 and 18. In addition there was no evidence for differences in gestation length between males and females within the two linkage group haplotypes.

8.5 Discussion

PD have a gestation length significantly longer than any other deer species except roe deer which exhibit embryonic diapause and a gestation of around 300 days. There is no evidence for embryonic diapause in Père David's deer (Brinklow and Loudon 1993) so it seems most unlikely that this contributes to the observed gestation lengths.

The 6.8 day difference in gestation length between F_1 male and female calves is very large compared with sex differences in other ruminant species. For example the differences in sheep of 1 - 2 days (Mali *et al.* 1985; Kassem *et al.* 1989), cattle of 1.4 - 2.2 days (Azzam and Nielsen 1987) and red deer of less than a day (Fennessy *et al.* 1991a) are all much smaller; the standard deviation are also large compared with that of 3.4 days in red deer (Fennessy *et al.* 1991a).

The high standard deviation in backcross progeny of F_1 hybrid stags (backcross 5.5 vs Red 3.4) suggested there may be some evidence for an interesting genetic effect or major gene. The mixture distribution analysis confirmed this, providing evidence for two segregating sub-populations with a difference in means of 7.2 days. This difference was not due to an obvious effect such as sex and must have been due to the PD influence. However the backcross progeny of F_1 hybrid hinds did not exhibit a similarly high variance despite eliminating the hybrid hind effect by using red hinds as recipients in a MOET program. In addition the mean gestation length from this cross (242.5 days) was significantly shorter (6 days) than for progeny from F_1 stags and more akin to one of the sub-populations from the mixture distribution analysis of the progeny of F_1 stags at 245.4 days.

The mixture distribution analysis was partly supported by the ANOVA where effects of around 2.6 to 2.7 days of individual markers on linkage group 16 were detected. This was supported by the interval mapping maximum likelihood analysis where linkage groups 16 and 18 each accounted for around 3 days. Again this was supported by the haplotype analysis (Table 8.5) where approximately a quarter of the individuals with PD/R haplotype had a 4.1 day longer gestation length than R/R haplotypes. There was also no evidence of a sex effect within haplotype groups. Therefore in the backcross hybrids from F_1 stags gestation length appears to be controlled by a haplotype effect on linkage group 16 which accounts for 4.1 days and a QTL on linkage group 18 which accounts for a further 2.9 days. The additive effect of these two phenomena would have a similar influence on gestation length as suggested by the mixture distribution analysis (7.2 days). There was no evidence of segregation distortion in the population as linkage groups 16 and 18 appeared to be inherited independently. This was supported by the genome map constructed in MAPMAKER and the virtually equal numbers and proportions of individuals in each of the haplotypes in Table 8.5. Interval mapping is 5% more powerful than ANOVA for intervals of less than 20 cM and as the distance between

Table 8.6 Summary of key gestation lengths indicative of imprinting effect.

	Red	F_1	Backcross	PD
Male	234.6[1]	268.0	249.0[2]	283.4[4]
Female	234.1[1]	262.0		
Male	-	-	242.5[3]	-
Female	-	-		-

[1] Fennessy and Mackintosh (1992)
[2] Progeny of hybrid males
[3] Progeny of hybrid females
[4] Wemmer *et al.* (1989)

markers increases so does the power of interval mapping to about 30% when intervals are about 70 cM (Rebai *et al.* 1995). Also the deer genome map used here was 1240 cM long with an average spacing of 7.3 cM between markers which would indicate a small advantage of interval mapping over the ANOVA technique. An analysis capable of detecting multiple QTL simultaneously and interactions among them may provide further evidence to clarify these issues.

The differences in gestation lengths between backcross progeny of F_1 sires and F_1 dams (and the marker analyses which partly support the mixture distribution analysis) provide evidence for genomic imprinting in these hybrids where maternal and paternal chromosomes or chromosome sections are functionally non-equivalent (Latham 1996). Both the longer than expected gestation length in pregnancies sired by F_1 stags and the shorter than expected gestation length in pregnancies of F_1 hinds support the concept of imprinting (Table 8.6). Also the progeny of F_1 hinds did not show any evidence of segregation (in gestation lengths), providing further evidence that alleles inherited paternally and maternally appear to function differently. It has been proposed that there is a conflict between maternally and paternally inherited genes at the embryo stage in both mammals and angiosperms (Haig and Westoby 1989) where paternally inherited genes strive to attain maximum resources from the mother in an effort to be larger at birth, survive and reproduce. Simultaneously it is in the mothers interest to have large and successful offspring, although not at the expense of her potential future reproduction. This creates the conflict between paternally and maternally inherited genes. Thus a gene such as a placental growth factor which is expressed in embryos can influence the transfer of resources from mother to offspring. Therefore an imprinted or mutant variant of this gene has the potential to increase nutrient transfer thereby increasing foetal

growth or size which could well be associated with an increase in gestation length. However there is currently no evidence to suggest that any of the markers on linkage group 16 nor markers #74 and #85 on linkage group 18 are within or nearby known imprinted regions (Barlow 1995; Ledbetter and Engel 1995). All 15 known imprinted regions on the human and mouse genomes map to different chromosomes and syntenic groups compared with the regions identified on the deer genome which indicate evidence for genomic imprinting (Barlow 1995; Ledbetter and Engel 1995; Villar and Pedersen 1997). Unfortunately there are insufficient data on F_1 hinds carrying their own backcross calves to term. While MOET programs have been shown to have some unexpected effects on birthweight (Walker *et al.* 1996) there is no evidence of this in our data set although that does not preclude other potential side effects.

For birth weight the most significant markers from the ANOVA (#112) and multiple marker interval mapping techniques (#41) are both on linkage group 4 and only 1.2 cM (Kosambi) apart. The one LOD interval for this putative QTL is 26.4 cM and includes a total of 6 markers. The estimated size of the effect was 0.60 kg and the LOD of 2.80 provides evidence for a birth weight QTL. Both the mixture distribution and ANOVA analyses indicated a significant effect which must be due to PD alleles, although in the case of mixture distribution analysis, the predicted effect of the gene was 1.51 kg and much higher than the 0.64 kg from the ANOVA. There was also evidence for different PD alleles for this trait with the maximum LOD score varying depending on the sires included, with the progeny one sire in particular, being quite different to the rest. Thus, evaluation of genes in this region of the human genome may provide candidate genes for this effect.

Pre-weaning gain attained a reasonably sized LOD of 2.00 indicating that this region is certainly an area worthy of further investigation. Backcrosses with a PD allele at marker #112 on linkage group 4 had greater birth and weaning weights but animals with a PD allele at marker #7 on linkage group 19 had lower pre-weaning live weight gain than backcrosses without a PD allele. The mixture distribution analyses suggest there may be two segregating populations in the backcross hybrid population for weaning weight and pre-weaning gain. The trend for red type animals to have higher live weight gains pre-weaning was interesting and indicative of a negative heterosis effect which may well be a reflection of the extreme genetic divergence of the parental species. Pure PD have a larger mature size (Loudon *et al.* 1989)

than red deer, and hybrids between the two would be expected to express hybrid vigour and weights greater than the mid parent means and in proportion to their genotype.

The control of gestation length in the PD × R hybrids is clearly very complex. While there is intense international research effort to dissect the genetic basis of productive traits in farm animals e.g. (Andersson *et al.* 1994; Georges *et al.* 1994), we are not aware of any other evidence for QTL for gestation length. However a major gene for gestation length in cattle has been documented (Mead *et al.* 1949). Thus we have found an intriguing pattern of sex effects and segregation within populations in terms of gestation length. While direct effects of a major gene or genes for gestation length may account for the differences or a portion of them, other non-genetic (e.g. birth mother or MOET) or non-Mendelian genetic effects (imprinting) or sex effects (including X or Y chromosome effects) may also be involved. Investigation of the reverse hybrid (red deer male over PD female) and their backcrosses would be of value to elucidate the genetic control of gestation length. In general terms, interspecies hybrids such as these deer may well become an extremely useful tool in the quest to understand the mechanisms or control of complex genetic traits such as gestation length.

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