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Hybridization of Père David's deer (*Elaphurus davidianus*) and red deer (*Cervus elaphus*) by artificial insemination

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(With 2 plates in the text)

In three separate trials, 83 mature red deer hinds had their oestrous cycles synchronized early in the 1986 breeding season by the removal, after 12 or 14 days, of either intravaginal silastic devices (CIDR, Controlled Internal Drug Release) containing 1·0 g progesterone ($n=69$) or polyurethane sponges (Chronogest) containing 45 mg fluorogesterone acetate ($n=14$). Immediately following CIDR/sponge removal each hind was given an i.m. injection of 250 i.u. PMSG (pregnant mare serum gonadotrophin). Hinds received intrauterine inseminations, via laparoscopy ($n=63$) or laparotomy ($n=20$), of either Père David's deer (PD) semen ($n=77$ hinds; 18–46×10^6 live sperm per inseminate) or red deer semen ($n=6$ hinds; 40×10^6 live sperm per inseminate) 54–56 h after CIDR/sponge removal and PMSG administration. Six hinds inseminated with PD semen produced hybrid calves (two females and four males) following gestations of between 262 and 274 days. Five of the six hinds artificially inseminated with red deer semen (Trial 1) calved to that insemination following gestations of 223–239 days. Most remaining hinds produced red deer calves following return services by fertile red stags. Five calves were verified as hybrids by electrophoresis. Hybrid calves exhibited physical characteristics common to both species but superficially tended more closely to resemble PD calves.

Contents

Introduction

The systematic position of the Père David's deer (*Elaphurus davidianus*) within the Cervidae has remained a subject of controversy since the species was discovered in China in the 19th century.

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Elaphurus has been accepted as a distinct genus that is allied to *Cervus* (Whitehead, 1972). However, there is anecdotal but reliable evidence of fertile red deer (*Cervus elaphus*) × Père David's deer hybrids occasionally occurring within some sympatric populations (Gray, 1972; Jones & Manton, 1983), indicating an even closer genetic affiliation than is presently recognized.

Red deer form the basis of the deer farming industry in New Zealand and there is presently considerable interest in hybridizing this species with other closely related taxa to obtain breeds more suited to particular environments or production requirements. Since 1984, 77 Père David's (PD) deer have been imported into New Zealand by various research and farmer groups with the intentions of establishing viable breeding populations within the southern hemisphere and of producing hybrids with red deer.

This paper describes some of the first attempts to produce F_1 PD × red deer hybrids by artificial insemination.

Materials and methods

Semen collection and processing

Single ejaculates (Table I) were collected from 2 mature PD stags (January/February 1986) and a mature red stag (July 1985) by electro-ejaculation following anaesthetization with an i.m. injection of 0·4 mg xylazine hydrochloride (Rompun; Bayer New Zealand Ltd.) and 0·008 ml Fentaz (Ethnor Pty Ltd., North Ryde, Australia; 10 mg/ml fentanyl citrate and 80 mg/ml azaperone) per kg liveweight for PD stags or 2·0 mg xylazine hydrochloride (Rompun) per kg liveweight for the red deer stag. The ejaculates were collected in pre-warmed glass vials and immediately assessed for volume, spermatozoa density and motility (Table I). The ejaculates were extended in 2·9% sodium citrate–20% egg yolk diluent (after Krzywinski & Jaczewski, 1978) to concentrations of 160–168 × 10^6 (PD) or 200 × 10^6 (red deer) spermatozoa per ml and loaded into either 0·25 (PD) or 0·5 (red deer) ml straws. The semen was then frozen in N_2 vapour to −125 °C in a programmable freezer (6 °C per minute reduction) and later transferred to liquid N_2 until required for insemination. A single straw from each processed ejaculate was thawed several weeks later and assessed for post thaw motility (Table I).

Artificial insemination

Trial 1: 27 mature (>4 years old) red deer hinds located on the Ruakura Animal Research Station (37°46′S, 175°20′E) had their 1985 born calves weaned on 10 March 1986. They were randomly divided into 2

TABLE I

Characteristics of semen collected from PD and red deer stags and processed for frozen storage in straws

Ejaculate number	Species	Collection date	Ejaculate volume (ml)	Sperm density (× 10^6/ml)	Sperm motility (%)	Sperm concentration extended (× 10^6/ml)	Straw volume (ml)	Sperm stored per straw (× 10^6)	Post-thaw motility (%)	Live sperm per straw (× 10^6)
1	PD	27.2.86	3·0	950	95	160	0·25	40	45	18
2	PD	23.1.86	8·0	420	90	168	0·25	42	55	23
3	red	10.7.85	1·2	1450	100	200	0·50	100	40	40

insemination groups of 15 (Group 1) and 12 (Group 2) hinds, respectively. On 1 April (Group 1) or 2 April (Group 2) each hind received 2 intravaginal silastic devices containing a total of 1·0 g progesterone (CIDR-type S; NZ Dairy Board, Hamilton, NZ). At CIDR removal 12 days later (13 and 14 April), each hind received an i.m. injection of 250 i.u. PMSG (Folligon; Intervet, Arturmon, Australia). Thereafter, each group was run with a vasectomized stag until the time of artificial insemination. The hinds were laparoscopically inseminated into both uterine horns with the contents of a single thawed straw of either Ejaculate 1 (PD; $n=15$), Ejaculate 2 (PD; $n=6$) or Ejaculate 3 (red deer; $n=6$) between 54 and 56 h from CIDR removal/PMSG administration. For insemination, the hinds were anaesthetized with an i.m. injection of 1·0 mg xylazine hydrochloride (Rompun) and 2·0 mg ketamine hydrochloride (Ketalar; Parke-Davis Pty Ltd., USA) per kg liveweight. Following insemination, external puncture wounds were treated with topical antibiotic powder (Aureomycin; Cyanamid NZ Ltd., Auckland) and 10 ml of a long-acting antibiotic (Propen LA, Glaxo NZ Ltd., Auckland) was administered by i.m. injection. Anaesthesia was reversed with an i.v. injection of 0·25 mg yohimbine hydrochloride (Recervyl; Aspiring Animal Services, NZ) per kg liveweight and on recovery the hinds returned to pasture. The total insemination procedure, from administration of anaesthetics to complete reversal, lasted approximately 20 min.

On day 12 (Group 2) or 13 (Group 1) from insemination (i.e. 28 April), all hinds were grouped together and run with a single crayon-harnessed fertile red deer stag until 30 June. Twice daily observations were conducted to detect crayon mating marks that would indicate return services (i.e. failure to conceive to the artificial insemination).

Trial 2: 20 mature (> 4 years old) red deer hinds located near Kaukapakapa, North Auckland (36°32′S, 174°27′E) had their 1985 born calves weaned on 10 March 1986. The oestrous synchronization technique was identical to that of Trial 1 except for a 14-day CIDR insertion period between 22 March and 5 April and the absence of a vasectomized stag following CIDR removal. The hinds were each inseminated with the contents of 2 thawed straws (1 straw per uterine horn) of either Ejaculate 1 (PD; $n=14$) or Ejaculate 2 (PD; $n=6$) between 54 and 56 h from CIDR removal/PMSG administration. They were anaesthetized with an i.m. injection of 1·0 mg xylazine hydrochloride (Rompun) and 0·02 ml Fentaz per kg liveweight. Laparotomies were performed following left flank incisions and the semen was injected directly into each uterine horn. Anaesthesia was later reversed with an i.v. injection of 0·25 mg yohimbine hydrochloride (Recervyl) and 0·04 mg naloxone hydrochloride (Narcan; Du Pont Pharmaceuticals, Auckland, NZ) per kg liveweight following the i.m. administration of 10 ml long-acting antibiotic (Propen LA; Glaxo New Zealand Ltd., Auckland) and on recovery the hinds were returned to pasture.

On 1 May (24 days after artificial insemination) until 30 June the hinds were run with a fertile red deer stag.

Trial 3: 36 mature (> 3 years old) red deer hinds, located near Balfour, Southland (45°50′S, 168°35′E), had their 1985-born calves weaned on 20 March 1986. They were randomly allocated to 2 treatment groups of 10 (Group 1) and 26 (Group 2) hinds each. Oestrous synchronization methods were identical to those of Trial 1 for 22 of the hinds, with CIDR removal/PMSG administration occurring on either 15 April ($n=7$; Group 1) or 13 May ($n=15$; Group 2). The remaining 14 hinds received single intravaginal polyurethane sponges containing 45 mg fluorogesterone acetate (Chronogest 45; Intervet, Angers, France) for 14 days, with sponge removal and PMSG (250 i.u.) administration occurring on either 15 April ($n=3$; Group 1) or 13 May ($n=11$; Group 2). All hinds were laparoscopically inseminated with a single straw of either Ejaculate 1 (PD; $n=6$) or Ejaculate 2 (PD: $n=2$) as for Trial 1 but using anaesthetic procedures of Trial 2.

All hinds were run with fertile red deer stags from approximately 20 days after artificial insemination.

Calving records

During the subsequent calving period in December and January, the artificially inseminated hinds in each trial were monitored daily and new-born calves were tagged, weighed (except Trial 3), sexed and identified to dam (after Asher & Adam, 1985).

PLATE I. F_1 hybrid calves (two weeks old) on the Ruakura Animal Research Station (Trial 1).

Validation of hybridization

All live potential hybrid calves (i.e. born to hinds not observed to return to service in Trial 1 or outwardly resembling Père David deer in Trial 2 and 3) were blood sampled (5 ml heparinized Vacutainer) by jugular venepuncture (20-gauge needle) at time of tagging (Trial 1) or weaning at 3 months of age (Trials 2 and 3). Plasma was removed following centrifugation (1000 G for 25 min) and stored at -20 °C until required for electrophoretic analysis of transferrin genotype.

Plasma samples (10 μl aliquots) were subjected to horizontal polyacrylamide gel electrophoresis (PAGE) using the alkaline buffer system described by Gahne, Juneju & Grolmus (1977), as unique alleles for albumin and transferrin which discriminated PD and red deer had been previously resolved by this method (P. Dratch, unpubl. data).

Results

Conceptions to insemination

Trial 1: Two of the 21 hinds artificially inseminated with PD semen gave birth to singleton F1 hybrid calves (one male and one female) following gestations of 262 and 267 days, respectively. Thirteen of the remaining 19 hinds inseminated with PD semen had been observed to return to service between 17 and 21 days later (mean cycle length $\pm$ S.D.; $18{\cdot}1 \pm 1{\cdot}4$ days). All produced singleton red deer calves (eight males and five females) following gestations of between 228 and 239 days (mean gestation length $\pm$ S.D.; $234{\cdot}5 \pm 2{\cdot}9$ days). The remaining six hinds produced singleton red deer calves (four males and two females) at intervals of between 247 and 265 days from artificial insemination, indicating undetected return services.

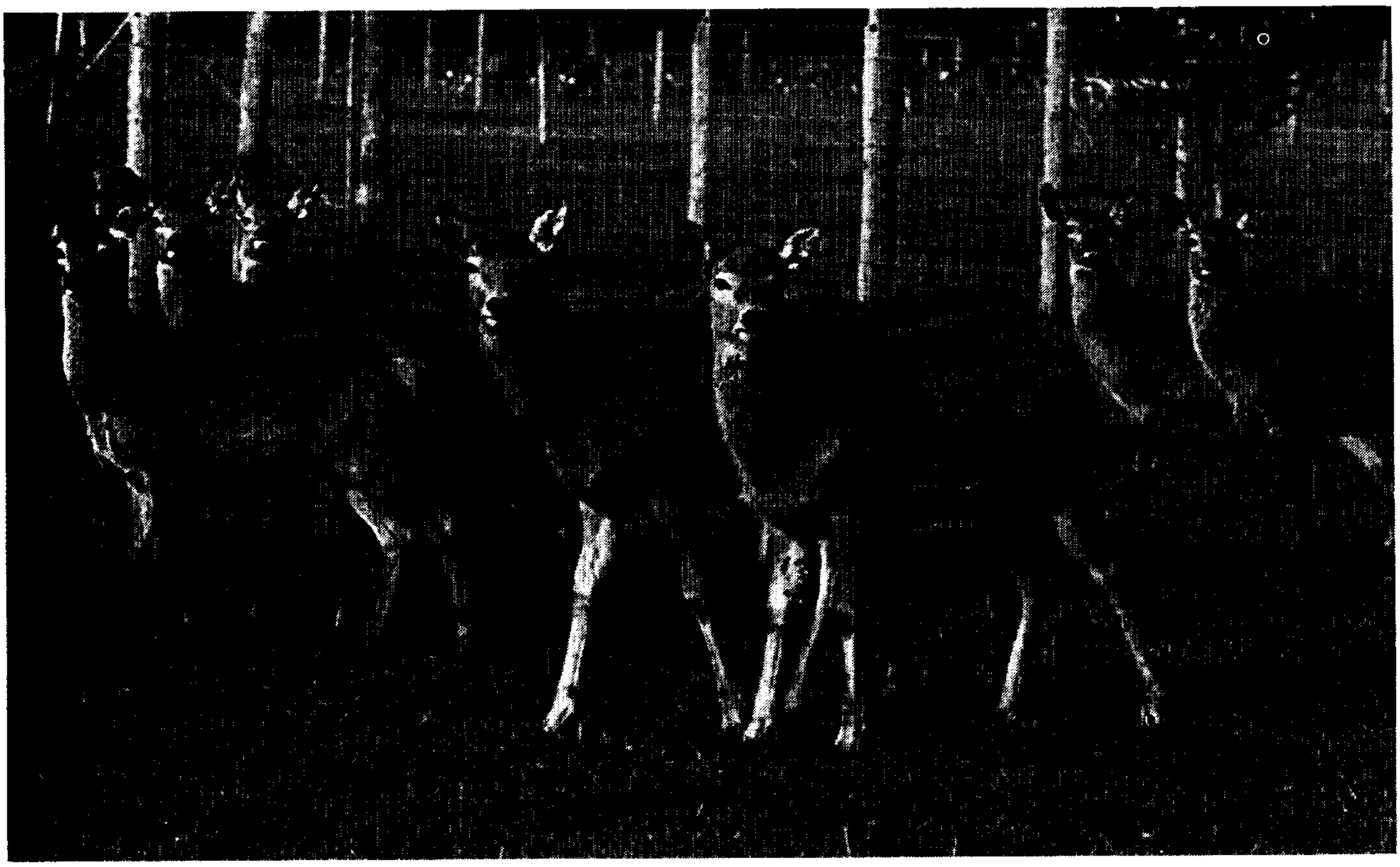

PLATE II. The same hybrids (foreground) at five months of age, weighing 70·8 kg (male) and 64·2 kg (female), with $5\frac{1}{2}$–6 month old red deer in the background (45–55 kg).

Five of the six hinds artificially inseminated with red deer semen gave birth to singleton ($n=4$) or twin ($n=1$) calves (one male and five females) following gestations of between 223 and 239 days (mean gestation length $\pm$ S.D.; $232{\cdot}6 \pm 6{\cdot}4$ days), indicating conception to that insemination. The remaining hind was observed to return to service 21 days after insemination and calved (one female) 236 days after that mating.

Trial 2: One of the 20 hinds in this trial gave birth to a singleton F1 hybrid male calf following a gestation of 274 days. All the remaining 19 hinds produced red deer calves between 260 and 280 days from artificial insemination.

Trial 3: Three of the 36 hinds gave birth to single F_1 hybrid calves (two males and one female) following gestations of 264, 265 and 271 days, respectively. One of the male calves died at birth. Most of the remaining hinds gave birth to singleton red deer calves >250 days after artificial insemination. Three hinds failed to calve.

Hybrid phenotype

The six F_1 hybrid calves were biochemically and visually distinct from red deer calves. Of 11 live calves (including some randomly selected red deer calves) blood sampled for electrophoretic analysis, only five that outwardly appeared to be hybrids exhibited the albumin and transferrin alleles that characterize Père David's deer. All five were heterozygous at these two loci, also having typical red deer alleles. The remaining calves were homozygous for albumin and exhibited the

intraspecific variation previously described for red deer transferrin (Bergman, 1976). Another hybrid calf that died at birth (Trial 3) could not be confirmed as a hybrid by the electrophoretic technique.

Birth weights of three of the hybrid calves (11·3 and 12·2 kg, respectively, for the male and female calves from Trial 1; 13·0 kg for the male calf from Trial 2) were heavier than for the 26 red deer calves from Trial 1 (mean ± S.D.; 8·5 ± 1·3 kg; range: 5·5–10·5 kg). Other physical and behavioural characteristics of the hybrids closely resembled Père David's deer (Plates I and II). In comparison to red deer calves, the pelage at birth was light in colour, well spotted, fine and with well interspersed guard hairs. Tails were considerably longer than those of red deer and were invariably held horizontal when the calves trotted behind their dams. Apart from the ears, which resembled those of red deer, the facial features were distinctly like Père David's deer; in particular the straight, elongated muzzle and the flattened dorsal surface between the eyes and nose.

Discussion

There are a number of anecdotal reports of hybridization between red deer and Père David's deer in zoo and park situations and the hybrids appear to be fertile (Jones & Manton, 1983). The present trials have shown conclusively that such hybridization is possible, although the fertility of the present hybrids has yet to be assessed. However, there is little doubt that hybridization is a rare event in situations where sympatric populations of the two species exist. Attempts to produce F_1 hybrids in New Zealand by joining PD stags with red deer hinds have been completely unsuccessful as the two species remained completely segregated and there is little seasonal overlap in breeding activity. There are clearly strong behavioural and seasonal barriers between the two species and artificial insemination of red deer hinds with PD semen appeared the only practical alternative to produce hybrids.

The high conception rate in red deer hinds following intrauterine insemination with red deer semen in Trial 1 (i.e. 5/6; 83%) demonstrates the suitability of oestrous synchronization and insemination techniques. The low apparent conception rate in hinds following insemination with PD semen in Trial 1 (2/21; 9·5%), using identical techniques, and similar results in Trial 2 (1/20; 5%) and Trial 3 (3/36; 8·3%) may be due to either generally lower numbers of live spermatozoa per inseminate (i.e. $18–46 \times 10^6$ vs. 40×10^6) and/or cross species fertilisation/conception failure. The fact that most hinds inseminated with PD semen in Trial 1 were observed to return to service 17–21 days later, an interval that corresponds to a natural oestrous cycle length (Guinness, Lincoln & Short, 1971; Krzywinski & Jaczewski, 1978), suggest that the lower calving rate to artificial insemination was not mainly due to later embryonic mortality, as this would tend to lengthen the interval between insemination and return oestrus.

Gestation lengths of 19 hinds bearing red deer calves to artificial insemination or observed return services in Trial 1 (mean ± S.D.; 234·1 ± 3·9 days) are in agreement with other published data on red deer (Krzywinski & Jaczewski, 1978; Kelly, McNatty, Moore, Ross & Gibb, 1982). The considerably longer gestation lengths of the six red deer hinds bearing hybrid calves (mean ± S.D.; 267·2 ± 4·5 days) were intermediate between those of red deer and PD deer (282 days; Wemmer, 1983), indicating that foetal genotype influenced gestation length.

What does the PD × red deer hybrid have to offer? Besides demonstrating a close genetic and taxonomic affiliation between the two species, the F1 hybrid, should it prove fertile, may be of considerable value to the deer farming industry. Red deer, the predominant species farmed in New

Zealand and Britain, are highly seasonal breeders, conceiving in autumn and calving in summer. Under intensive pastoral grazing regimens, the high energy demands of summer lactation are poorly aligned with peak spring pasture production and quality that occur several months earlier. On the other hand, Père David deer conceive in summer and, despite a longer gestation, calve 4–6 weeks earlier than red deer. Obtaining F1 hybrids, therefore, may be the first step in producing a breed with a more flexible reproductive seasonality.

Secondly, Père David's deer are highly susceptible to Malignant Catarrhal Fever (MCF), a viral disease which has decimated the population recently imported into New Zealand (Orr & Mackintosh, In press) and has resulted in numerous deaths in North America (Heuschele, Fletcher, Oosterhuis, Janssen & Robinson, 1984). As the world population of this species is extremely small (< 2000 individuals; Jones & Manton, 1983) it is vulnerable to extinction as a result of a variety of viral epidemics, including MCF. The incorporation of Père David genes into hybrids of possible lower MCF susceptibility may provide some safeguard that the Père David-like genotype will not become extinct; albeit a less desirable and purely alternative strategy to ensuring the survival of the purebred genotype.

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