Article Author:
Article Title: Fertility in a F1 male hybrid of white-tailed deer (Odocoileus virginianus) X mule deer (O. hemionus).
Pages: 111-117
Fertility in an $F_1$ male hybrid of white-tailed deer ($\textit{Odocoileus virginianus}$) × mule deer ($\textit{O. hemionus}$)

J. N. Derr$^1$*, D. W. Hale$^2$, D. L. Ellsworth$^1$ and J. W. Bickham$^1$

$^1$Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, USA 77843; $^2$Department of Biology, Texas A&M University, College Station, TX, USA 77843

Summary. The fecundity of an $F_1$ male hybrid deer, from a cross between a male $\textit{Odocoileus virginianus}$ (white-tailed deer) and a female $\textit{O. hemionus}$ (mule deer), was assessed by cytotogenetic and flow cytometric techniques. Analysis of chromosome morphology, nucleolus organizer expression, meiotic chromosome pairing, sperm production, and nuclear gene inheritance revealed no genetic anomalies that could potentially impair normal fertility. These observations are discussed in relation to recent reports of hybridization between natural populations of these two species.

Keywords: deer; hybridization; synaptonemal complex; flow cytometry; fertility

Introduction

Hybridization between morphologically distinct and fully differentiated mammalian species under captive regimens is not uncommon. Most examples of interspecific hybridization are in domesticated or zoo-propagated animals in the families Bovidae, Cervidae, Equidae and Suidae, although hybridization has been reported between members of many other mammalian taxa (Gray, 1972). Isolation mechanisms before and after mating usually limit interspecific interactions and most mammalian hybrids are partially or completely sterile, but some hybridization experiments have resulted in fertile hybrid offspring (e.g. horse and donkey, Rong et al., 1985; Ryder et al., 1985). Nevertheless, documented observations of natural hybridization between large mammal species are rare and the offspring are usually sterile (Geist, 1981).

The two endemic species of New World deer, $\textit{Odocoileus virginianus}$ (white-tailed deer) and $\textit{O. hemionus}$ (mule deer), occur in sympatry across much of western Canada and the western USA. Where they co-occur, white-tailed and mule deer are usually separated by ecological preferences and habitat affiliation (Baker, 1984). Allozymic studies indicate potential hybridization between the two species in Montana (Cronin et al., 1988), Washington (Gavin & May, 1988) and Texas (Stubblefield et al., 1986; Ballinger, 1987; Derr, 1991). The two species share mitochondrial DNA (mtDNA) haplotypes in areas of the south-western USA (Carr et al., 1986; Ballinger, 1987; Derr, 1990). However, hybridization is not evident in all areas of sympatry and, in most cases where hybridization does occur, $F_1$ hybrids are rare. No hybrid swarm or clearly defined hybrid zones have been identified. The factors that prevent or limit interspecific hybridization are unknown.

This study assessed the fecundity of an $F_1$ white-tailed × mule deer hybrid to determine whether any structural genetic differences exist that would cause fertility problems associated with hybridization.

*Present address: Department of Animal Sciences, Molecular and Cell Biology, Texas A&M University, College Station, TX, USA 77843.
†Present address: The Jackson Laboratory, Bar Harbor, ME, USA 04609.
Materials and Methods

Animal. The individual examined was the F₁ offspring of a cross between a male white-tailed deer (O. v. texana) from central Texas and a female mule deer (O. h. crooki) from the Davis Mountains of west Texas. Both parents were long-term research animals at the Texas Agriculture Experiment Station, Uvalde Research Center, Texas A&M University. Hybrid parentage was assured in that the dam was the only mule deer at the facility. In autumn 1987, the 2-5-year-old hybrid deer was anaesthetized and biopsies were taken for analyses.

Mitotic analysis. Scrotal biopsy served as the source for primary cultures for the mitotic analyses. Sterile tissue samples were obtained and fibroblast cultures were established in Ham's F-10 medium supplemented with 10% fetal calf serum and 1% penicillin and streptomycin. Fibroblasts were grown at 37°C and sampled using a modified version of the trypsin and karyotyping techniques of Baker et al. (1982). Cell division was arrested with 0.2 µg colchicine/ml for 45 min before sampling and the hypotonic treatment (0.075M KCl) was extended to 40 min at 37°C. Cells were fixed 3 times with acetic acid:methanol (1:3), dropped onto wet slides and allowed to air dry. Metaphase configurations were stained with Giemsa (1% in 0.01M phosphate buffer, pH 7.0) and examined with a Leitz Orthoplan light microscope. Silver staining for nucleolar organizer regions (NORs) followed the procedures described by Gold & Ellison (1983).

Analysis of synaptosomal complex of zygotene and pachytene nuclei. For the meiotic analyses, ~3 g of seminiferous tubules were collected from one testis of the anaesthetized animal. A portion of the testicular material was prepared using surface spreading (Moses, 1977a) and silver nitrate staining (Howell & Black, 1980). Preparations were mounted on copper grids (100 mesh) and examined using a Zeiss EM10C transmission electron microscope at 60 kV. Zygotene and pachytene nuclei were photographed at magnifications between ×1000 and ×2000.

Diakinesis, metaphase I and metaphase II analyses. A second portion of the testicular material was prepared using a modification of the technique described by Elder & Pathak (1980). Cytological staining was accomplished with Giemsa as for the mitotic preparations.

Flow cytometry analysis of testicular tissues. Testicular material (0.5 g) was prepared for examination by flow cytometry. The tissue was reduced to a monolayer suspension in a 1:1 fixative solution of Hanks' balanced salt solution and absolute ethanol. After filtration with a 37-µm nylon mesh screen, the sample was centrifuged at 1500 g for 10 min and resuspended in two volumes of stain (0.5 mg 4,6-diamidino-2-phenylindole (DAPI) in Tris·HCl buffer, pH 8.4). The cell suspension was stained for 24 h at 4°C. Nuclear fluorescence was estimated using a Leitz MPV flow cytometer equipped with a 100-W mercury-vapour bulb. Samples were analysed while suspended in the staining solution. Nuclear fluorescence was recorded through a pulse-height analyser, transferred to an Apple II computer and plotted using a program written by M. J. Smolen. At least 500 cells were counted in the median channel of the 1C peak (corresponding to haploid cells) for each of five replicates.

Protein electrophoresis. Whole blood was collected from the hybrid deer and both parents. Immediately after collection, samples were centrifuged at 3000 g for 15 min to separate the plasma from the red blood cells, which were frozen separately at −80°C. For protein electrophoresis, plasma was diluted 1:50 with distilled water and fractionated in starch gels (Clayton & Tretiak, 1972). Proteins were visualized with Coomassie brilliant blue.

Results

The mitotic karyotype of the hybrid was identical to that of white-tailed and mule deer described by Hsu & Benirschke (1967). The diploid number (2n) was 70, with 66 acrocentric autosomes, two submetacentric autosomes, a small bi-armed Y chromosome and a large bi-armed X chromosome. The NORs in both species occur on the distal portion of the two largest autosomal pairs (J. N. Derr, unpublished observation). Although NOR dominance has been reported in other hybrids (Ward & Cole, 1986). NOR expression in all 25 cells examined from the hybrid was identical to that in each parental species (Fig. 1).

The pairing of chromosomes at meiosis in the hybrid was analysed in a total of 88 zygotene and pachytene nuclei prepared by surface-spreadings. Silver staining revealed synaptonemal complexes similar to those described in other mammalian species (e.g. Moses, 1977a; Greenbaum et al., 1986; Dollin et al., 1989). During zygonema, autosomal synapsis was usually initiated at one set of telomeres and proceeded unidirectionally towards the opposite end of the forming bivalents. Pairing of the autosomes was completed by pachynema, each bivalent being represented by a fully formed synaptonemal complex. Autosomal pairing appeared completely normal (Fig. 2a), with no evidence of pairing failures or other synaptic anomalies. Two densely stained nucleoli, associated with the two largest synaptonemal complexes (Fig. 2b). Analyses of subsequent metaphase I and metaphase II cells were identical. These preparations also revealed no
A male white-tailed deer (O. v. texanus) in the mountains of west Texas. Both parents were at the Uvalde Research Center, Texas A&M. Hybrid deer at the facility. In autumn 1987, the analyses.

The meiotic analyses, sterile tissue was prepared for the mitotic analyses. Sterile tissue medium supplemented with 10% foetal at 37°C and sampled using a modified Bullet. Cell division was arrested with 0.2 µg Mitotic media [3µM-KCl] was extended to 40 min at 37°C. Slides and allowed to air dry. Metaphase (pH 7.0) and examined with a Leitz (NORS) followed the procedures as described

The testicular material was prepared using a saline solution of Hanks' balanced salts and centrifuged at 1500 g for 10 min and a cytometer, pH 8.0). The nucleolar organizer regions (arrows) and the X and Y chromosomes; × 2000.

Fig. 1. Chromosomes of the hybrid deer at metaphase showing the distribution of nucleoli organizer regions (arrows) and the X and Y chromosomes; × 2000.

with the two largest synaptonemal complexes, were visible in many of the pachytene nuclei (Fig. 2b). Analyses of subsequent stages of meiosis revealed examples of typical diakinesis and metaphase I and metaphase II cells. Thus, no pairing or disjunction anomalies were observed. These preparations also revealed many fully formed and apparently normal spermatozoa.
The meiotic analyses. Sterile tissue of the testicular material was prepared using a modified Zygote buffer, pH 7.0 (Black, 1980). Preparations were mounted on electron microscope at 60 kV. Zygote and ×2000.

The testicular material was prepared using a classical staining was accomplished with a solution of Hanks' balanced salts and was centrifuged at 1500 g for 10 min and DAPI in Tris-HCl buffer, pH 8.4). The cells were then stained using a Leitz MPV flow cytometer and suspended in the staining solution. The DNA content was determined using an Apple II computer and plotted against the stimulation index (SI).

We observed meiotic behavior and both parents. Immediately after the spermatozoa from the red blood cells, which were stained with ethidium bromide in a solution of distilled water and fractionated with a suction

In the hybrid white-tailed and mule deer described by with 66 acrocentric autosomes, two largest autosomal pairs of J. N. N. has been reported in other hybrids from the hybrid was identical to that

The karyotype was analysed in a total of 88 zygote and 88 metaphase cells. Revealed synaptonemal complexes (Fig. 2b). Analyses of subsequent stages of meiosis revealed examples of typical diakinesis and metaphase I and metaphase II cells. Thus, no pairing or disjunction anomalies were observed. These preparations also revealed many fully formed and apparently normal spermatozoa.

Fig. 1. Chromosomes of the hybrid deer at metaphase showing the distribution of nucleolus organizer regions (arrows) and the X and Y chromosomes; ×2000.
Fig. 2. Electron micrographs of silver-stained pachytene configurations from the hybrid deer: (a) completely paired autosomal synaptonemal complexes of normal morphology (× 5170), (b) densely stained nucleoli corresponding to the two bivalents with nucleolus organizer regions (× 2570) and (c) heteromorphic X and Y chromosomes (× 3960) exhibiting a short region of synaptonemal complex formation (arrow). The unpaired telomeres of the sex chromosomes (arrowheads) are often associated end-to-end during pachynema.

As in other mammalian species (Moses, 1977b; Hale & Greenbaum, 1986; Schmid et al., 1987; Dollin et al., 1989), pairing of the X and Y chromosomes was asynchronous with that of the autosomes in zygotene and pachytene nuclei in the hybrid. The sex chromosomes initiated synopsis end-to-end during late zygonema or early pachynema and achieved maximum pairing by midpachynema. The axes of the heteromorphic X–Y bivalent were easily identified, as they were darkly stained and unpaired along most of their length (Fig. 2c). The sex bivalent exhibited a short pairing segment involving ~50% of the length of the Y chromosome; most of the length of the X chromosome remained unpaired throughout pachynema. The unpaired telomeres of the X and Y chromosomes were associated in many of the pachytene nuclei examined.

In cases of suspected infertility, flow cytometry of testicular tissues allows for the measurement of reduction in spermatogenesis (Clausen & Abyholm, 1980; Pfitzer et al., 1982). Because DNA content in thousands of cells can be analysed rapidly at all stages of the cell cycle, this method provides a powerful tool in the detection of aneuploidy and other DNA anomalies (Lovett et al., 1980; Kent et al., 1988). Haploid sperm cells (1C peak) from the hybrid deer represented the largest cell population from the testicular tissue (Fig. 3). The half-height coefficient of variation from the 1C peak was 7.17. Such variation is reasonable considering the 1C peak is made up of 2 cell populations, one corresponding to X- and the other to Y-bearing spermatozoa (Evenson & Melamed, 1983). The second peak (2C) is composed of diploid somatic cells (i.e. spermatogonia, Sertoli cells, Leydig cells, interstitial tissue and nucleated blood cells) and spermatocytes with reduced DNA content after the first meiotic division. The third peak (4C) represents cells undergoing DNA synthesis. The histogram presented in Fig. 3 represents the DNA content of ~20,000 cells of which more than half constitute the 1C haploid sperm peak. Cell populations with aberrant DNA contents were not evident. These results are similar to those reported for other mammalian species (Meistrich et al., 1978; McLean-Grogan et al., 1981; Pfitzer et al., 1982; Evenson, 1989) and indicate a high production of normal haploid cells.

The electrophoretic mobility of the plasma protein albumin was examined in both parents as well as the hybrid deer. White-tailed deer and mule deer typically differ by 3% in the mobility of this protein (Derr, 1990). In deer and other mammals, albumin is a monomeric protein. The electrophoretic behaviour of a made and mule dam to be homozygote. These result for this protein.

Haldane's Rule (Haldane, 1922) predicts that crosstypes of two species, that sex linkage in the heterozygous condition, will result from the hybrid deer after examining production and inheritance of the number of cases of intermediate morphology in the western USA and California. Morphological characters distinguish antlers, length of the scrotum, and colour of the tail in the hybrid deer and ≤42 mm long in which all the hind legs, with the body length, and most key characters appear.

Two other studies explored...
Fig. 2. Electron micrographs of silver-stained pachytene configurations from the hybrid deer: (a) completely paired autosomal synaptonemal complexes of normal morphology (× 5170), (b) densely stained nucleoli corresponding to the two bivalents with nucleolus organizer regions (× 2570) and (c) heteromorphic X and Y chromosomes (× 3960) exhibiting a short region of synaptonemal complex formation (arrow). The unpaired telomeres of the sex chromosomes (arrowheads) are often associated end-to-end during pachynema.

As in other mammalian species (Moses, 1977b; Hale & Greenbaum, 1986; Schmid et al., 1987; Dollin et al., 1989), pairing of the X and Y chromosomes was asynchronous with that of the autosomes in zygote and pachytene nuclei in the hybrid. The sex chromosomes initiated synapsis end-to-end during late zygote or early pachynema and achieved maximum pairing by midpachynema. The axes of the heteromorphic X-Y bivalent were easily identified, as they were darkly stained and unpaired along most of their length (Fig. 2c). The sex bivalent exhibited a short pairing segment involving ~50% of the length of the Y chromosome; most of the length of the X chromosome remained unpaired throughout pachynema. The unpaired telomeres of the X and Y chromosomes were associated in many of the pachytene nuclei examined.

In cases of suspected infertility, flow cytometry of testicular tissues allows for the measurement of reduction in spermatogenesis (Clausen & Abyholm, 1980; Pfetzer et al., 1982). Because DNA content in thousands of cells can be analyzed rapidly at all stages of the cell cycle, this method provides a powerful tool in the detection of aneuploidy and other DNA anomalies (Lovett et al., 1980; Kent et al., 1988). Haploid sperm cells (1C peak) from the hybrid deer represented the largest cell population from the testicular tissue (Fig. 3). The half-height coefficient of variation from the 1C peak was 7.17. Such variation is reasonable considering the 1C peak is made up of 2 cell populations, one corresponding to X- and the other to Y-bearing spermatozoa (Evenson & Melamed, 1983). The second peak (2C) is composed of diploid somatic cells (i.e. spermatogonia, Sertoli cells, Leydig cells, interstitial tissue and nucleated blood cells) and spermatocytes with reduced DNA content after the first meiotic division. The third peak (4C) represents cells undergoing DNA synthesis. The histogram presented in Fig. 3 represents the DNA content of ~20,000 cells of which more than half constitute the 1C haploid sperm peak. Cell populations with aberrant DNA contents were not evident. These results are similar to those reported for other mammalian species (Meistrich et al., 1978; McLean-Grogon et al., 1981; Pfetzer et al., 1982; Evenson, 1989) and indicate a high production of normal haploid cells.

The electrophoretic mobility of the plasma protein albumin was examined in both parents as well as the hybrid deer. White-tailed deer and mule deer typically differ by 3% in the mobility of this protein (Derr, 1990). In deer and other mammals, albumin is a monomeric protein. The electrophoretic behaviour of albumin and other proteins in the hybrid deer was similar to that of the albino and mule deer dam. This suggests that the hybrid deer is not a heterozygote.

Fig. 3. DNA flow cytometry corresponds to haploid spermatogenesis undergoing DNA synthesis.

Haldane's Rule (Haldane, 1922) predicts that the most fit hybrid will be the progeny of two species, that sex is determined by the observation that many genes are transmitted from the heterozygous condition. The hybrid deer after examination of the production and inheritance of normal morphology. Nevertheless,
Hybrid deer fertility

![DNA flow cytometry of testicular material from the hybrid deer. The 1C peak corresponds to haploid spermatozoa, the 2C peak to diploid cells and the 4C peak to cells undergoing DNA synthesis.](image)

Electrophoretic behaviour of albumin from these three animals revealed the white-tailed deer sire and mule deer dam to be homozygous, 100/100 and 103/103, respectively. The hybrid was a 103/100 heterozygote. These results are consistent with Mendelian inheritance of the nuclear genes that code for this protein.

Discussion

Haldane’s Rule (Haldane, 1922) states that when one sex is absent, rare, or sterile among the F1 progeny of two species, that sex is the heterogamic sex. Support for this assumption is based on the observation that many genes have deleterious effects when in the hemizygous condition, but not in the heterozygous condition. Results presented in this report indicate no genetic anomalies in the hybrid deer after examination of mitotic chromosomes, synaptonemal complexes, sperm cell production and inheritance of nuclear genes.

Deer with intermediate morphological characteristics have been reported in areas of sympatry in the western USA and Canada (Cowan, 1962; Krammer, 1973; Wishart, 1980). Several key morphological characters distinguish white-tailed deer from mule deer: the shape and configuration of antlers, length of the sub-basal snag on the antlers, ear length, size of the metatarsal glands on the hind legs, colour of the dorsal tail hair and depth of the lachrymal fossa (Baker, 1984). Generally, the most reliable character is the length of the metatarsal gland, ≥70 mm long in mule deer and ≤42 mm long in white-tailed deer. In the hybrid, the metatarsal gland was 52 mm long and most key characters appeared intermediate between those of the parents.

Two other studies explored various aspects of viability and hybrid deer fertility. Nichols & Murrey (1973) examined 34 F1, 27 BC1 and 8 F2 hybrid offspring, of which 12.5% of the hybrids and 12.0% of a control population of pure C-bred parents were infertile. Fertility was assessed simply by the demonstrated ability to produce offspring. In a separate study, Wishart et al. (1988) demonstrated fertility in 10 female hybrid deer: 7 were F1s and the remaining three were BC1 crosses. All 10 produced offspring as yearlings, but in 2 F1 male hybrids, representing both reciprocal crosses, one male hybrid (mule deer sire × white-tailed dam) was sterile with no spermatozoa and the other (white-tailed sire × mule deer dam) was considered subfertile, based on the observation of a high number of nuclear defects in mature spermatozoa. A third male hybrid, a BC1 (3/4 to white-tailed deer) was potentially fertile with a high production of spermatozoa of normal morphology. Nevertheless, none of these 3 male hybrids sired offspring.
What factors limit hybridization between these two species in natural populations? Our data do not support the contention that hybrid sterility limits interspecific hybridization. Studies documenting the exchange of genetic markers (Carr et al., 1986; Ballinger, 1987; Derr, 1990) suggest that hybridization between deer species may be strongly correlated with environmental factors. Krämer (1973) proposed that differences in social behaviour and scent may account for the separation between these two species. Nevertheless, mtDNA analyses indicate that white-tailed and mule deer have hybridized in the past, resulting in the replacement of mule deer mtDNA by white-tailed deer mtDNA in some areas of the south-western USA (Carr et al., 1986; Ballinger, 1987; Derr 1990). Although hybridization in most areas of sympatry is rare, historical interactions have clearly influenced the genetic architecture of both species.

Attempts to cross the hybrid animal used in this report with female Texas white-tailed deer have proved unsuccessful. However, our observations do not reveal any structural genetic differences that could potentially reduce fertility in hybrids between white-tailed and mule deer. Maintenance of the genetic integrity of white-tailed and mule deer may best be explained by behavioural and ecological preferences that serve as semipermeable reproductive isolation mechanisms.

We thank L. H. Blankenship, T. Fillinger and G. Wampler for facilities and animal care. L. H. Blankenship, J. R. Gold, and I. F. Greenbaum provided laboratory space. B. G. Hanks and M. J. Smolen assisted with flow cytometry and photography. S. F. Lockwood, L. A. Reudas, and D. J. Schmidt provided helpful comments on earlier drafts of this paper. This research was funded through support from the Texas Agriculture Experiment Station Expanded Research Area (Program Development Funds 24464), the Caesar Kleberg Wildlife Foundation, the Department of Wildlife and Fisheries at Texas A&M University and a Tom Slikk Research Fellowship to J. N. Derr.

References


Cowan, I.M. (1962) Hybridization between the black-tailed deer and the white-tailed deer. J. Mammal. 43, 539–541.


in natural populations? Our data
and may be correlated with environmen
tial; and scent may account for the

The analyses indicate that white-tailed and

The female Texas white-tailed deer have

tailed and mule deer. Maintenance

for facilities and animal care. L. H.

in Montana J. Wildl. Mgmt 52, 320–328.

(1990) Genetic interactions between two species of American deer, Odocoileus virginianus and


(1991) Genetic interactions between white-
tailed mule deer in the southwestern United

M. S., Murray, J.D. & Gillies, C.B. (1989)

cellular complex analysis of hybrid cattle. I.

substaging and the normal full bloods.


staining of the behavior of silver-stained tria-
pachyteme cells of Sigmodon fulviventris

Muridae) heterozygous for centric fusion.

Cell Genet. 27, 31–38.

(1989) Flow cytometry evaluation of male

cell lines. In Flow Cytometry: Advanced Research

P. Press, Boca Raton, FL.


cell lines and abnormal cell types in human semen

biopsies by flow cytometry. J. Histochem.

31, 248–253.

& May, B. (1988) Taxonomic status and

identity of Columbian white-tailed deer. J.

52, 1–10. Flow cytometric analysis of spermen
cell lines in spermatocytes of the Chinese hamster (Cricetus griseus). I. Morphology of the autosomal


Moses, M.J. (1977b) Synaptonemal complex karyo-
typing in spermatocytes of the Chinese hamster (Cricetus griseus). II. Morphology of the XY pair in

spread preparations. Chromosoma 60, 127–137.

Nichols, R. & Murrey, J. (1973) Black-tailed deer–white-
tailed deer breeding study. Final report. P-R Project

W-46 (4-A, V-B). Game and Fish Commission, Nashvile, TN.


Ryder, O.A., Chemnick, I.C., Bowling, A.T. &

Benirschke, K. (1985) Male mule foal qualifies as the

offspring of a female mule and jack donkey. J. Hered.

76, 379–381.

Schmidt, M., Johannisson, R., Haaf, T. & Neitzel, H.

(1987) The chromosomes of Micromus minutus

(Rodentia, Murinae). II. Pairing pattern of X and Y


45, 121–131.


Hybridization of free-ranging white-tailed and mule
deer in Texas. J. Wildl. Mgmt 50, 688–690.


diploid and triploid parthenogenetic lizards of hybrid


Wishart, W.D., Hradka, F., Schmutz, S.M. & Flood, P.F.

(1988) Observations on spermatogenesis, sperm

phenotype, and fertility in white-tailed × mule deer


Wishart, W.D. (1980) Hybrids of white-tailed and mule

deer in Alberta. J. Mammal. 61, 714–716.