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# Age determination, reproduction, and mortality of the Gray fox (Urocyon cinereoargenteus) in Maryland, U.S.A.<sup>1</sup>

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#### Abstract

Four techniques for determining age were investigated for 143 gray foxes (Urocyon cinereoargenteus) collected in Maryland, 1976-1979: 1. counts of cementum layers in the teeth, 2. epiphyseal closure of the humerus, 3. eye lens weights and 4. baculum length and weight. Age distribution analyses indicated a balanced age structure. The juvenile segment of the population exceeded 50 % in each of the three seasons studied. The overall sex ratio for Maryland gray foxes was 123.4 males per 100 females. Mean testis weights and spermatogenic activity suggested that adult male gray foxes become fertile sooner than juveniles entering their first mating season. The onset of estrus in female gray foxes from Maryland appeared to occur in early February. Mean litter size, estimated from placental scar counts, was 4.42 (range 3-5) pups per year. The prenatal mortality rate was estimated to be 39 % and the implantation rate of ova was 88 %. The proportion of barren females was 45 %.

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## Introduction

Historically there has been a great deal of interest in foxes in North America and Europe. The red fox (*Vulpes vulpes*) has received considerable attention from wildlife researchers, which has seemingly resulted from conflicting views regarding its intrinsic value. Some view the red fox as a disease-spreading predator, while others view it as a valuable furbearer, and a great sporting animal.

The gray fox (Urocyon cinereoargenteus), on the other hand, is of less coursing value and until recently of much lower fur value than the red fox. Its role as a predator has been considered to be less significant. Nevertheless, the gray fox has almost always been included in red fox management policies as these species are sympatric throughout much of North America and capture techniques are essentially the same for both species.

During the past decade, the North American furbearer harvest has escalated in response to increasing consumer demand and higher pelt prices (SAMUEL and NELSON 1982). The increased demand on the gray fox population has resulted in the need for a more comprehensive understanding of the ecology of this species and the effects of the present harvesting regimes.

## Study area

This study was conducted in the Appalachian and Piedmont Plateau Provinces of western Maryland (Fig. 1). These two provinces are described by MILLER (1967) and BRUSH et al. (1977) as follows:

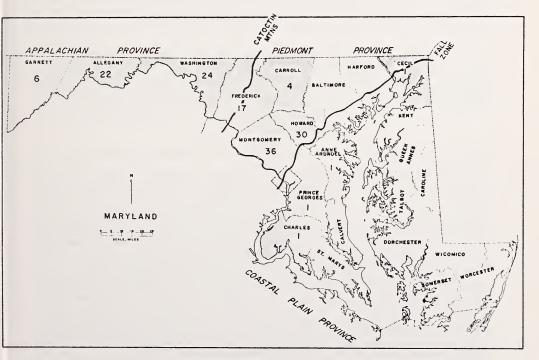


Fig. 1. Study area from which 143 gray foxes were collected 1976–1979 in western Maryland. Numbers in the shaded counties denote the number of gray foxes collected from that county

# The Appalachian Province (Region 1)

The Appalachian Province is the most western province and extends from the West Virginia border to the Catoctin Mountains in central Frederick County. The topography varies from a broad highland plateau in the west to wide valleys besected by long ridges in the east. Elevations decrease from a maximum of 1,024 m in the western highlands to 457 m in the eastern ridge and valley section. Temperatures average from 8.9 °C to 10.6 °C annually and from 120 to 150 days a year a below freezing (0 °C). Precipitation averages 109 cm annually.

Gray foxes collected in Garrett, Allegany and Washington Counties were categorized as Region 1.

## The Piedmont Plateau Province (Region 2)

The central province of Maryland, the Piedmont Plateau, extends from central Frederick County east to the Fall Zone. The topography ranges from ridges and valleys in the west to hilly county with well developed floodplains in the east. Elevations range from 335 m in the west to 91.4 m near the Fall Zone in the east. Temperatures average from 11.1 °C to 12.8 °C annually with a mean of 110 days per year below freezing (0 °C). Precipitation averages 104 cm annually.

Gray foxes from all of Frederick, Carroll, Montgomery, Howard, Harford, and Cecil Counties

were categorized in Region 2. Three additional foxes, collected from counties just east of the Piedmont

Plateau Province, were also included in Region 2.

## Materials and methods

Gray fox carcasses were collected from November 1976 through January 1979. Carcasses were collected primarily from trappers. However, additional gray foxes were obtained via year-round trapping efforts by Appalachian Environmental Laboratory personnel. Victor number 2 and 11/2 coil spring traps (Woodstream Corporation) and standard fox trapping sets (dirt-hole and scent post) were used.

# Necropsy

After the eye lenses were removed, gray fox carcasses were frozen. Weights (g) and standard body measurements (mm) were recorded before dissection. The testes, epididymides, and ovaries were weighed to the nearest 0.001 g on a top-loading Mettler Balance (Model P-163). The adrenal glands and the male and female reproductive organs were preserved in Bouin's fluid. The skull, humerus, and baculum from each animal were also collected and cleaned by boiling for later examination.

## Sex and age determination

The sex of gray foxes was determined by examination of the external genitalia and internally by the

presence of ovaries and uteri.

The age of foxes was determined by a variety of methods. A count of the annular rings in the cementum layer of teeth has been successfully used to determine the age of many canid species (GRUE and Jensen 1973; Monson et al. 1973; Linhart and Knowlton 1967; Nelson and Chapman 1982). One lower canine tooth was removed from each skull and completely decalcified in Decal (Decal Corporation, Pomona, N. Y.). Histological sections, 18 thick, were made on a freezing microtome (International Cryostat Model CTL) at -20 °C. These sections were mounted on slides, stained with Harris' haematoxylin, and examined under a light microscope at 40X. Animals without annulations were considered juveniles and it is assumed that one annular ring was layed down each year thereafter. Ages, as determined from cementum annuli, were used to assign gray foxes to year classes.

Because cementum layers are more dependent upon seasonal (GRUE 1976) rather than age-specific factors, an estimate of the reliability of this technique was made by comparing the number of cementum annuli with the age as determined by an age-specific technique, such as epiphyseal closure. Juvenile animals having open or only partially ossified humeral epiphyses were selected for this comparison. Sullivan and Haugen (1956) reported that the distal epiphyses of the ulna and radius in gray foxes close between 8 and 9 months humerus closed about one week later than the distal epiphyses of the ulna and radius in red foxes. Thus, it is reasonable to assume that Maryland gray foxes

with incompletely ossified humeral epiphyses are less than one year of age.

Eye lens techniques, as described by LORD (1961), FRIEND (1967) and NELSON and CHAPMAN (1982) were also investigated. Whole eye balls were collected from freshly killed gray foxes and

preserved in 10 % buffered (CaCO<sub>3</sub>) formalin for a minimum of 3 months. Lenses were then removed from the eyes and dried at 80 °C in a drying oven for 72 hours or until no additional weight loss was noted. The dried lenses were weighed on a FPE Precision Balance to the nearest 0.1 mg.

Gray fox bacula were also collected for purposes of age determination (Pertides 1950). Lengths of bacula were recorded to the nearest 0.1 mm. They were then oven dried at 80 °C for 72 hours and

weighed on a top-loading Mettler Balance to the nearest milligram.

## Life table

A life table similar to those presented by STORM et al. (1976) and MACPHERSON (1969) was constructed following the guidelines prescribed by CAUGHLEY (1966). A stable age distribution and an exponential

growth rate of 0 (a stationary population) must be assumed in this procedure.

Survivorship (1x) is the number of individuals in an initial cohort of 1,000 animals that survive to age × (CAUGHLEY 1966). Survivorship was calculated by dividing the adjusted frequency of females from each year class by the sum of the adjusted frequencies from the preceding year classes times 1,000. The number dying during the interval between 2 year classes (dx) was determined as the difference between the 1x values of the 2 year classes (CAUGHLEY 1966). The mortality rates (qx) for each year class were calculated by dividing the dx value by the corresponding 1x value for each year class. Multiplied by 1,000, qx is the number of females that died before age x + 1 out of an initial 1,000 that were alive at age x (CAUGHLEY 1966).

## Reproduction

Uteri and ovaries were examined for visible signs of pregnancy to determine the onset and duration of the gray fox breeding season. The testes of males were also examined for the presence of sperm (Sullivan 1956; Layne 1958; Wood 1958). Sperm was categorized as absent (0), present (1), or

abundant (2) for both the testes and epididymides.

The mean litter size was estimated by counting the number of placental scars on the uteri (LAYNE and McKeon 1956; Wood 1958). Ovulation rates were determined by examining the ovaries for the presence of corpora lutea. Preserved ovaries were sectioned on a rotary microtome (American Optical Model 820). Serial sections, 10 thick, were mounted on slides and stained with Delafield's haematoxylin and eosin.

## Statistical methods

Data transformations and linear regressions, similar to those prescribed by Dudzinski and Mykytowycz (1961), were used to analyze eye lens weights and baculum lengths and weights. Where applicable, t-tests for unequal subsamples were used (Mendenhall and Ott 1976). Statistical tests were considered significant when P < 0.05 and highly significant when P < 0.01.

## Results

# Age determination

Eye lens weights

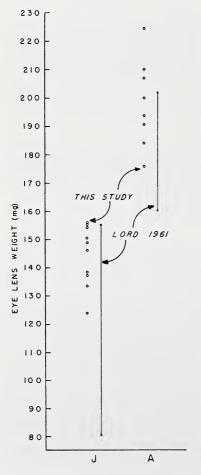
The mean dried weights of eye lenses of 19 gray foxes from western Maryland were categorized by age in months (Table 1). Since no animals of known age were available, it was necessary to assume a common birth date. It was presumed all foxes were born in April. Ages were estimated by counting the number of months from the month of collection back to April and then adding 12 months for every cementum annuli. These data suggest that lens weights increased with age; however, fluctuations in weight are apparent after 15 months.

Lens weights were separated into categories for juveniles less than one year old (5 to 7 months) and adults greater than one year old (15 to 79 months) (Fig. 2). These data indicate that lens weight can be used to separate juveniles (< 156.6 mg) from adults (> 176.6 mg) (P < 0.05, t = 9.037, df = 17). Similarly, the lens weights of 74 gray foxes from Florida (LORD 1961a) also effectively separated juveniles (< 155.3 mg) from adults (> 160.3 mg).

Table 1

Mean dry eye lens weights (mg) of gray foxes collected in Maryland, 1976–1979

Estimated age (months)	N	Mean lens weight (mg)	Range
5	1	124.0	
6	4	142.8	133.4-151.5
7	6	149.9	138.4-156.6
15	1	191.0	
16	1	176.6	
31	1	184.3	
37	1	210.2	
42	1	200.2	
51	1	194.0	
<i>7</i> 5	1	224.9	
79	1	207.3	



Two problems noted by Dudzinski and Mykytowycz (1961) when dealing with organ weights in relation to age were the significant differences in organ growth rates between young and older animals and the lesser amount of organ variability (in absolute) in young animals than for older ones. They found that by transforming the lens weight values to  $Y = \log_{10}$  (lens weight), and the age to X = 1/(age + constant), a linear model and homogenity of variance would result, solving both problems.

Gray fox eye lens data from this study were transformed to  $Y = \log_{10}$  (lens weight) and X = 2/(age - 0.75). The constant, -0.75, was determined by trial and error to give linearity (r = -0.958). These data (Fig. 3) are represented by the equation

$$Y = 2.329 - 0.951x$$

For comparison, the mean weights from LORD (1961a) (Fig. 3) were similarly transformed (Y =  $\log_{10}$  [lens weight] and X = 1/[age + 1.05], r + -0.992) and are represented by

$$Y = 2.352 - 1.828x$$

Eye lens weights increased with age in gray foxes from both Maryland and Florida. However, the significant difference between the slopes of these two equations (P < 0.05, t = 8.289, df = 28) suggest that the eye lens growth rates were greater for Florida gray foxes than for those from Maryland.

Fig. 2. Eye lens weights (mg) for gray fox juveniles (J) and adults (A) from Maryland, 1976–1979, and from LORD (1961a)

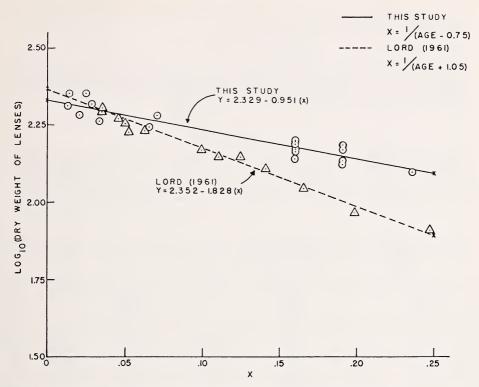


Fig. 3. Dry weights (mg) of gray fox eye lenses (log<sub>10</sub>) and the reciprocal of age (months and constant) for Maryland gray foxes collected in 1976–1979 compared to LORD (1961a)

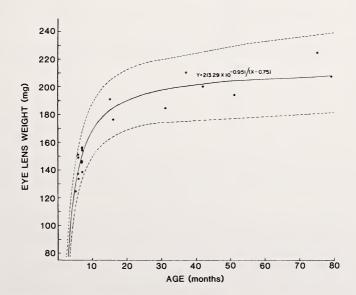


Fig. 4. Dry lens weights (mg) plotted with age (months), growth curve  $Y = 213.29 \times 10^{-0.951/(\times -0.75)}$ , and 95 % confidence limits for predicted lens weights, for Maryland gray foxes collected 1976–1979

## R. A. Wigal and J. A. Chapman

Eye lens weights from the 19 Maryland gray foxes and their corresponding ages in months were used to calculate the growth curve,

$$Y = 213.3 \times 10^{-0.951/(x - 0.75)}$$

and the 95% confidence limits for predicted lens weights (Fig. 4). By reading the confidence limits horizontally, it is readily apparent that the eye lens technique for determining age becomes much less reliable as age increases.

#### Bacula

Bacula were collected from 44 gray foxes from western Maryland. The mean baculum lengths and weights were assigned ages in the same manner as for eye lens weights (Table 2). While it is apparent that both the baculum lengths and weights generally increased with age, a great deal of variability existed both within and between age groups.

Table 2 Mean baculum lengths (cm) and weights (mg) in relation to age (months) for 44 male gray foxes collected in Maryland, 1976-1979

Age (months)	N	Mean baculum length (cm)	Range	Mean baculum weight (mg)	Range
5	2	4.75	4.60-4.90	134	117–151
6	6	4.86	4.16-5.71	177	97-265
7	10	5.03	4.37-5.62	216	153-312
8	8	5.16	4.58-5.57	254	172-318
10	1	5.06		291	
16	1	5.52		307	
19	1	5.48		392	
20	1	5.24		229	
30	1	5.63		382	
32	1	5.58		345	
34	1	5.73		414	
37	1	5.26		389	
45	1	5.59		402	
55	1	6.27		450	
67	1	5.10		247	
68	1	5.73		393	
81	2	5.67	5.52-5.82	473	453-493
90	1	5.49		589	
91	2	5.48	5.29-5.67	472	428-516

The lengths of the 44 gray fox bacula and their ages (in months) were transformed to  $Y = log_{10}$  (baculum length) and X = 1/(age - 1.5) to give linearity (r = -0.579) (Fig. 5). The growth curve,  $Y = 5.65 \times 10^{-0.287/(x-1.5)}$ 

and 95 % confidence limits for predicted baculum length were also calculated. By reading the confidence limits horizontally, it is apparent that baculum length is not a satisfactory technique for determining age in gray foxes.

The weights and corresponding ages of the 44 gray fox bacula were also used to calculate the growth curve, and 95 % confidence limits. These data were also transformed [Y =  $\log_{10}$ (baculum weight) and X = 1/(age - 1.5)] to give linearity (r = -0.832) and the growth curve is from the equation  $Y = 448 \times 10^{-1.826/(x-1.5)}$ 

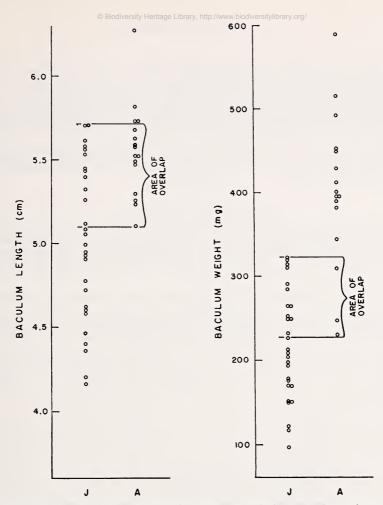


Fig. 5. Baculum lengths (cm) and weights (mg) for juvenile (J) and adult (A) male gray foxes collected in Maryland 1976-1979

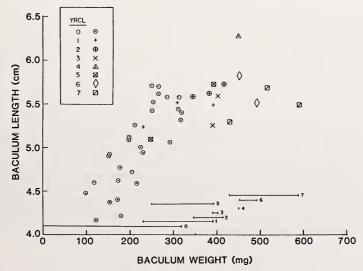


Fig. 6. Baculum length (cm) plotted against baculum weight (mg) and the ranges of baculum weight by age (year classes) for Maryland gray fox males collected 1976–1979

While these data suggest that baculum weight may be more reliable than length for determining age, the divergence of the 95% confidence limits again suggest that this technique is not suitable for separating Maryland gray foxes into age groups by month.

Baculum lengths (cm) and baculum weights (mg) were separated into juvenile and adult categories (Fig. 5). Unlike the complete separation noted for eye lens weights, there was a considerable area of overlap between juveniles and adults for both baculum length and weight.

Baculum weight generally increased with length, however, the range of weights for a given length was variable (Fig. 6). This variability is illustrated by the poor array of weight ranges for the 8 year classes.

# Epiphyseal closure of the humerus

The degree of epiphyseal closure was recorded for each of the 143 gray foxes examined. Because most carcasses were obtained from furtrappers, no animals with open (un-ossified) epiphyses appeared in the sample until September (Table 3). Juvenile foxes with open

Table 3

Date of closure of the humeral epiphysis for 85 Maryland gray foxes (less than 18 months old) from Maryland, 1976–1979

Year	Month	N	Open percent	N	Closing percent	N	Closed percent
First year							
	April						
	May June						
	July						
	August	2	400				
	September October	3 17	100 94.4	1	5.6		
	November	26	96.3	1	3.7		
	December	12	40.0	18	60.0		
	January February			4 1	100 100		
	March			1	100		
Second year							
,	April						
	May						
	June July					1	100
	August					1	100
	September						

epiphyses appeared in the sample through December, beyond which time no additional unossified epiphyses were noted. Partially-ossified (closing) epiphyses first appeared on October 6. Additional closing epiphyses were observed through early February. While no first year animals appeared in the sample from early February through late July, these data suggest that the humeral epiphysis has at least begun to close by February. Thus, it appears that the proximal epiphysis of the humerus may be reliably utilized to separate juveniles from older age groups through December. The remaining 58 animals, older than 1 year of age, all had completely ossified epiphyses.

## Cementum annuli

The number of cementum annuli were counted for 143 gray foxes from Maryland. A total of 82 juvenile Maryland gray foxes with less than complete epiphyseal ossification were examined for the presence of cementum annuli. One juvenile had canine teeth with annulations. A fox collected on January 9, showed partial epiphyseal closure and also possessed one cementum annulus. This translates into 1.2 % error rate for placement into the juvenile or 0 year class.

## Mortality

# Age distribution

Cementum annuli data from only those foxes collected October to January were used for age distribution analyses because they were collected during relatively short periods of time and accounted for 90 % of the animals collected (Table 4). The size of the juvenile

Table 4

Age distribution of gray foxes collected in Maryland from October through January, 1976–1979

Year		5–1977	1977	′–1978	1978	-1979
class	N	Percent	N	Percent	N	Percen
0	11	55	38	58	30	68
1	0	0	8	12	3	7
2	2	10	7	11	1	2
3	1	5	2	3	4	9
4	2	10	5	8	3	7
5	2	10	0	0	2	5
6	2	10	0	0	1	2
7	0	0	4	6	0	0
8	0	0	1	2	0	0
Total	20	100	65	100	44	100

cohorts exceeded 50 % in each of the 3 years of the study. The oldest gray fox collected was in its ninth year.

# Life table

The data from the 1977–1978 season were utilized for the construction of a life table. This life table was based on less than 50 ages at death and may, therefore, be subject to bias (CAUGHLEY 1966).

The observed frequencies for each year class were adjusted (r = 0.951) by the equation

$$\log Y = 3.24 - 1.09x + 0.19X^2 - 0.01x^3$$

where Y is the log of the predicted frequency and x is the age. Because the number of juveniles in the sample is not representative of the number of juveniles born, but rather of the number of juveniles from 6 to 9 months of age in the population, the age categories are  $0.5, 1.5, \ldots 8.5$ .

Estimates of survivorship (1x) and the number of deaths (dx) were calculated from the adjusted frequencies for each year class and applied to an initial cohort of 1,000 (at 0.5 years of age). These data represent the number of gray fox vixens from the initial cohort that have survived (1x) to age x and died (dx) between age x and x + 1 (Table 5). Approximately 80 % of the gray fox vixens alive at 6 months of age die by the age of 2.5 years and while less than 10 % lived beyond 4.5 years of age, 8 % lived for 7 or more years.

Table 5

Life table for female gray foxes collected from October 1977 to January 1978 in Maryland

Year class	Frequency	Adjested frequency	1000 1x	1000 dx	1000 qx
0.5	18	15.51	1000	526	526
1.5	6	7.35	474	275	580
2.5	4	4.56	199	75	377
3.5	0	3.41	124	32	258
4.5	4	2.84	92	20	217
5.5	0	2.44	72	17	236
6.5	0	1.99	55	18	327
7.5	1	1.42	37	16	432
8.5	1	0.82	21		

Assuming the population was stationary, the mean rate of mortality (qx) for all age groups (CAUGHLEY 1966) was:

 $\overline{q}x = \frac{1}{1x} = 0.48.$ 

Of primary interest, however, were the differences in mortality rates (qx) estimated for each year class interval (Table 5). The mortality rates were greatest for juveniles and yearlings and lowest for females in their fifth year. Beyond year class 5.5, mortality rates increased steadily with age suggesting that the effects of senectitude become greater as old age approaches.

#### Sex ratios

The sex ratio for the combined sample of Maryland gray foxes, collected from 1976–1979, was about 123 males per 100 females. However, the difference was not statistically different from 1:1.

## Reproduction

# Male reproductive cycle

Testes and epididymides were collected from 35 males. Mean testes weights for adults steadily increased from October through February (Fig. 7). A similar increase was also observed for juveniles from September through January although these weights were generally below those of adults.

Spermatogenic activity, determined from testicular and epididymal smears, was first noted in the testis in September and in the epididymis in October (Table 6). The percentage of testes with sperm increased rapidly from October to January and all males examined in January and February (n = 4) had testes with sperm (Table 6, Fig. 8). The percentage of epididymides with sperm increased from September to February with a slight decrease in January (Table 6, Fig. 8).

## Female reproduction

All females collected in January and February, were in at least the early phases of proestrum (Table 7). Two adults, collected in late January and early February, had large tertiary follicles located near the surface of their ovaries indicating they were in late proestrum.

Estrus in female Maryland gray foxes appears to begin in early February. This corresponds to the maximum testes weights of males.

Uteri from 52 female gray foxes were examined for the presence of placental scars.

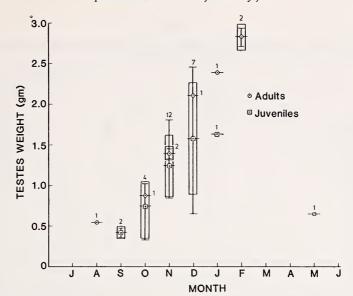


Fig. 7. Testes weight (gm) by month for juvenile and adult male gray foxes collected in Maryland 1976–1979. Horizontal lines, vertical lines and vertical bars represent the means, ranges and one standard deviation on each side of the means, respectively. Numbers indicate sample size

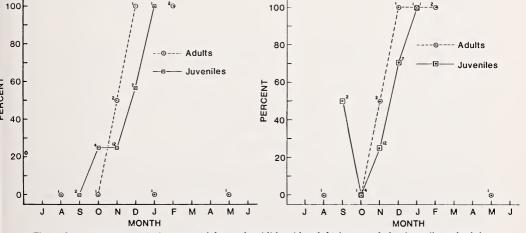


Fig. 8. Sperm presence (%) in testes (right) and epididymides (left) by month for juvenile and adult male gray foxes from Maryland 1976–1979

Thirty of these foxes were young-of-the-year females, not yet experiencing their first mating season, and did not possess uteri with placental scars. The mean number of placental scars per female, from those 1-year-old or older that possessed at least 1 scar (n = 12), was 4.42 (range 3-5). The remaining 10 adult females possessed uteri without scars.

Three adult females collected in July had ovaries containing corpora lutea (mean = 5.67, range 4-7). One had 7 corpora albicantia visible in the ovaries. However, it was not lactating, nor did it possess placental scars, indicating reproductive failure. The other 2 females had placental scars and were lactating.

Table 6

Presence of sperm (percent) in gray fox testes and epididymes from Maryland, 1976–1979

		Percentage	e with sperm
Month	Number	Testes	Epididymis
July			
August	1	0	0
September	2	50	0
October	5	0	20
November	14	28	28
December	8	62	62
January	2	100	50
February	2	100	100
March			
April			
May	1	0	0
June			
Total	35		

Table 7

Status of ovarian follicle development and estrous cycle phases for female gray foxes collected in January and February from Maryland, 1976–1979

Specimen number	Date	Age <sup>1</sup>	Follicle <sup>2</sup> status	Estrous³ phase
102	1/6	J	S	E
19	1/9	Ĭ	S	E
56	1/25	Å	T	L
28	1/26	I	S	E
18	2/1	Ĭ	S	E
23	2/4	Å	T	L
67	2/12	Α	S	E

 $<sup>^{1}</sup>$  Juveniles (J) and Adults (A).  $^{2}$  State of follicular development:  $S = \text{small secondary follicles in ovarian medulla; } T = \text{large tertiary follicles near medulla surface.} - <math>^{3}$  Estrous cycle phase: E = early proestrum; L = late proestrum.

Ovulation rates were also estimated from the number of corpora albicantia. Pairs of ovaries from 23 female gray foxes, at least 1-year old, were examined for the structures. Corpora albicantia were observed in 15 females and the mean was 4.6 (range 3–7). Five of the remaining females showed no sign of corpora albicantia and the other 3 animals (one each from December, January and February) had regressing corpora albicantia.

Differences in the total number of ovarian corpora and the number of placental scars from 19 female gray foxes, were used to estimate prenatal mortality (Table 8). Ovarian corpora totaled 74 (mean = 3.9) and the number of placental scars equaled 45 (mean = 2.4) for an estimated prenatal mortality rate of about 39 %.

Ten of the females, which showed signs of successfully producing litters, had a total of 51 ovarian corpora and 45 placental scars. From these females, an implantation rate of 88 %, a mean ovulation rate of 5.1 ova per female, and a mean litter size of 4.5 per female, were determined.

Ten of 22 (45 %) females over 1-year of age were thought to have been barren during the last reproductive season. Five of the barren females apparently failed to ovulate while the other 5 ovulated, but were either not fertilized, or the fertilized blastocysts failed to implant.

Table 8

Number of corpora lutea, corpora albicantia and placental scars for 22 female gray foxes from Maryland, 1976–1979

Specimen number	Month	Total corpora lutea	Total corpora albicantia	Total placental scars	Comments
24	July	7	6	0	B,1,2
25	July	6	Ō	5	F,6(1)
81	July	4	Ō	4	F,4
44	October	0	7	4	F,6(3)
46	October	0	6	4	F,6(2)
136	October	0	0	0	B,3
35	November	0	6	5	F,6(1)
108	November	0	5	4	F,6(1)
123	November	0	3	5	F,5,7
124	November	0	3	0	B,1,2
7	December	0	4	0	B,1,2
11	December	0	4	5	F,5,7
37	December	0	0	0	B,3,7
93	December	0	5	5	F,4
95	December	0	0	0	B,3,7
112	December	0	5	0	B,1,2
141	December	0	$O^a$	3	F,7
142	December	0	5	4	F,6(1)
143	December	0	4	0	B,1,2
56	January	0	1 <sup>a</sup>	0	B,1,2,3
23	February	0	1 <sup>a</sup>	5	F,5,7
67	February	0	0	0	B,3

Comments regarding fertility and pre-natal mortality are coded as follows: B = barren; F = fertile; 1 = failed to be fertilized; 2 = failed to implant; 3 = did not ovulate; 4 = corpora and placental scars equal; 5 = polyovular; 6 = ovular mortality (number of ova lost); 7 = corpora albicantia regressed. - a Corpora albicantia regressed.

## Discussion

## Age determination

The mean dried weights of eye lenses from Maryland gray foxes suggest that this technique is suitable for the separation of juveniles from adult animals. These results are similar to those reported by LORD (1961a) for lens weights from 74 Florida gray foxes up to 28 months of age. He found a 90 % agreement between lens weight and tooth wear techniques (WOOD 1958) for separating juveniles from adults and only 78 % agreement between the 2 techniques when estimating the year of birth for foxes up to 28 months of age. Apparently, however, much of the discrepancy between these techniques was with the tooth wear method as LORD (1961a) reported that of 21 cases of adult disagreement, tooth wear suggested that 20 of these animals were older than did the lens weights. Tooth wear was also found to be a less reliable method for determining age in gray foxes from Alabama and Georgia (NICHOLSON and HILL 1981). HARRIS (1978) found that tooth wear was a variable character in red foxes from England and subsequently felt that this method was unreliable for determining absolute age.

Bacula weights collected from 44 male gray foxes from Maryland were of less value as a criterion for age determination than eye lens weights. A small sample of gray fox bacula from Ohio was examined by Petrides (1950) from which he found no overlap of weights between juvenile and adult animals. The maximum juvenile weight was 330 mg and the

minimum adult weight was 420 mg. Bacula from the Maryland sample showed no such separation. The weights of 46 gray fox bacula from southern Georgia and northern Florida (WOOD 1958) also failed to separate juvenile from adult animals.

Examination of the epiphysis of the proximal humerus to determine the degree of ossification reliably separated juveniles from adult gray foxes through the end of December. Additional separation of some young-of-the-year gray foxes is possible, as evidenced by the presence of partially closed epiphyses through at least February; however, the overall reliability of the technique is thought to be considerably less at that time. Sullivan and Haugen (1956) reported similar results for both red and gray foxes from Alabama by taking X-rays of the distal epiphyses of the radius and ulna. Their results showed that both species of foxes could be reliably categorized as juveniles or adults through November and that some juveniles could be separated through December.

Counts of the annulations of the cementum layer of teeth have been recognized, in recent years, as a valuable technique for determining the absolute age of many mammalian species. Studies to determine the reliability of this technique have been conducted on several species of canids including the coyote (Canis latrans) (LINHART and KNOWLTON 1967; ALLEN and KOHN 1976); domestic dogs (Canis familiaris) (GRUE 1976); the arctic fox (Alopex lagopus) (GRUE and JENSEN 1976); and the red fox (MONSON et al. 1973; GRUE and JENSEN 1973; HARRIS 1978). In New York, the teeth of gray foxes, and other species, were histologically examined (STONE et al. 1975), but since the primary objective of this investigation was to compare the quality of several staining techniques, no inference to the reliability of the cementum annuli technique for this species was made.

A sample of animals of known age was utilized, in conjunction with the use of wild specimens, in most of the studies mentioned above. While a reference collection of knownaged animals would improve the validity of applying this technique to a population not previously investigated (GRUE and JENSEN 1973; HARRIS 1978), no gray foxes of known age were locally available. However, because the counting of cementum annuli has been demonstrated as a valid criterion for determining age, not only in canid species, but in a variety of animals (GRUE and JENSEN 1979), it appears that this method is equally applicable to gray foxes from Maryland. Histological sections from this study closely resembled photomicrographs of tooth sections from the red fox and arctic fox (GRUE and JENSEN 1973, 1976).

## Age distribution

The size and variability of the juvenile to adult harvest ratios in this study are consistent with those reported in other investigations. RICHARDS and HINE (1953) reported a range of 62–77 % juveniles in annual Wisconsin gray fox harvests. Wood (1958) reported a range of 51–69 % juveniles in annual Georgia gray fox harvests, and of 104 gray foxes collected in Florida by LORD (1961a), approximately 60 % were juveniles.

## Sex ratios

Sex ratios favoring males have been reported for gray foxes in Georgia, Florida and South Carolina (Wood 1958) and in Wisconsin (RICHARDS and HINE 1953), however, none differed significantly from 1:1. In New York, LAYNE and MCKEON (1956) reported significantly more male than female gray foxes, but later LINHART (1959) found nearly equal numbers of males and females in the population.

Investigations of the primary sex ratios of gray foxes all slightly favored males, however, no significant differences from a 1:1 ratio were noted (WOOD 1958; LAYNE and McKeon 1956).

## Reproduction

# Male reproductive cycle

Testis weights for Maryland gray foxes were greatest during February, but because no animals were available for March and April, it could not be concluded that peak testis weights occurred in February. Testis weights for gray foxes in southern Illinois were greatest in January (LAYNE 1958) and mean testis volume for Georgia gray foxes was greatest in mid-January (COOK 1974). Thus the period of peak testis weights for Maryland gray foxes occurs later than those observed for both Illinois and Georgia.

The lower mean testis weights recorded for juveniles has also been reported in other canid investigations. For red foxes in Illinois, STORM et al. (1976) found that the pattern of testis weights exhibited by juveniles was similar to that of adults, although lower juvenile weights were recorded at least through December. Similarly, DUNBAR (1973) found that the testis weights for juvenile coyotes in Oklahoma were below those recorded for adults during late summer and fall, but by February, juvenile testis weights were nearly as great as those for adults.

The pattern of spermatogenic activity for Maryland gray foxes, as evidenced by testicular and epididymal smears, is similar to those described in other gray fox investigations. In Georgia, WOOD (1958) found small quantities of sperm in 2 of 9 (22 %) juvenile males examined in November and in all of the 5 males from December through February. None of the epididymides collected from March through May contained sperm. Also in Georgia, COOK (1974) found spermatozoa in the seminiferous tubules of all male gray foxes examined in January and February and in only 43 % of those examined from March through May. He also noted abundant sperm in the epididymides of all but 1 male from December through February and that sperm remained present in March and April, but not May. Abundant sperm was found in the testes of Alabama gray foxes from mid-November to mid-April and SULLIVAN (1956) stated that the males were fertile during this period. LAYNE (1958) found sperm in the epididymides of all the gray foxes from southern Illinois examined from December through March, but in only 7 of the 15 (47 %) males examined in April. Also from southern Illinois, FOLLMAN (1967) suggested that gray fox males were fertile from December through March with the greatest number of spermatozoa occurring in February and March. His data, however, showed spermatozoa to be present in the testes for a longer period of time than those of the previously mentioned studies, probably because he employed histological techniques to determine spermatogenic activity rather than testicular and epididymal smears (COOK 1974).

# Female reproductive cycle

The onset of estrus in Maryland gray foxes was in agreement with the literature. The breeding dates estimated for gray fox vixens from Georgia and Alabama ranged from the end of December to mid-March, with the peak of breeding generally falling between late January and mid-February (Sullivan 1956; Wood 1958; Cook 1974, 1977). The mating season for gray fox vixens from southern Illinois (Layne 1958) and New York State (Sheldon 1949; Layne and McKeon 1956) were somewhat later than those noted for the southern states with the peaks occurring during mid-February and from the end of February to the first of March, respectively.

Since foxes from the more northern latitudes breed later than those from southern latitudes (SHELDON 1949; LLOYD and ENGLUND 1973), the mating season beginning in early February or late January for Maryland gray foxes would seem reasonable. This period follows those dates reported as the beginning of the gray fox mating season for Georgia and Alabama (SULLIVAN 1956; WOOD 1958; COOK 1974, 1977), and equals or

Summary of gray fox ovulation rates, litter size, mortality rates and percentages of barren females

	rate	size	litter	0	rate	barren females		
New York		35	3.66	1-7		3.3	Placental scars	SHELDON (1949)
Wisconsin		44	3.90				Placental scars	RICHARDS and HINE (1953)
New York	5.20	10	4.50 4.40	3-7	.30² .22 <sup>b</sup>	3.8	Embryo counts Placental scars	LAYNE and McKeon (1956)
Alabama		9 50 70	4.16	1-5		6.4	Embryo counts Placental scars	Sullivan (1956)
Southern Illinois	4.41	32	3.62 3.96	2–5 2–6	.14 <sup>b</sup>	2.0	Placental scars Uterine swellings	LAYNE (1958)
Georgia			4.90			7.7° 6.4 <sup>d</sup>	Embryo counts Placental scars	Wood (1958)
Florida			4.71				Placental scars	LORD (1961b)
Georgia	4.20	9	3.33		14		Placental scars Gestational sacs	Соок (1974)
Maryland	4.60	12	4.42	3–5	.39ª	45.0	Placental scars	This study

preceeds those dates reported for Illinois and New York (LAYNE 1958; SHELDON 1949; LAYNE and McKeon 1956).

Counts of placental scars were considered sufficiently accurate for the estimation of average litter size in foxes (SHELDON 1949; LAYNE 1958). However, it was generally agreed that this method would provide an estimate slightly higher than the actual average at parturition because of the inability to distinguish between resorptions or still births and live young (SHELDON 1949; WOOD 1958; HAR-RIS 1979; LAYNE 1958). MACPHERSON (1969)found that placental scar counts, from a group of arctic fox vixens with known reproductive histories, were nearly representative of the actual litter sizes.

The mean litter size for Maryland gray fox females occurred near the upper limit of the array of mean litter sizes reported in the literature (Table 9). Compensatory reproductive rates, because of higher man-caused mortality, was suggested by Schofield (1958) as the possible reason for larger red fox litters in southern Michigan than in the more northern counties.

The estimated ovulation rate based on the number of corpora lutea and corpora albicantia (4.6 per female) in the ovaries of adult Maryland gray foxes was comparable to

the ovulation rates noted in other studies (Table 9). Estimates derived from the 10 females that successfully produced litters showed a slightly higher ovulation rate (5.1 ova per female), but this figure also remained within the limits of the other reported rates. The mean number of ova produced from the 3 vixens with corpora lutea (5.7 per female) exceeded the mean rate reported in the literature (Table 9) for gray foxes and this is attributed to the small sample size.

The difference in ovulation rates between that estimated from corpora lutea (5.7 per female) and corpora albicantia (4.6 per female) is probably the result of the regression of corpora albicantia in the ovaries. Most of the vixens from which ovulation rates were determined were collected in October, November and December. It would seem logical that corpora lutea from females that failed to produce full term litters or did not lactate would experience a faster and more complete regression than those that whelped successfully.

It has been suggested that corpora albicantia normally persist up to 1.5 years following their development as corpora lutea (COOK 1977). However, the results from this study suggested that this was true for only 1 vixen, which apparently failed to produce a litter. Neither of the other 2 females with corpora lutea also possessed corpora albicantia. COOK (1977) suggested that 41 (56 %) of the 73 vixens he examined possessed ovaries with both corpora lutea and corpora albicantia.

Preimplantation mortality rates for Maryland gray fox vixens that appeared to have successfully produced litters (12 %), were lower than those rates represented in the literature (Table 9). Prenatal mortality, as estimated from the difference between the number of corpora lutea and corpora albicantia, and the number of placental scars, for 22 Maryland females (39 %) was higher than other estimates of prenatal mortality (Table 9). This is probably the result of placental scar attrition (SULLIVAN 1956) since the majority of the females from this study were collected in November and December.

The higher proportion of barren females among adult Maryland vixens (45 %) as compared with the percentages reported in other studies (Table 9) may be because the Maryland sample was obtained later in the season than data from other studies. The possible attrition of placental scars to those females collected in late fall and early winter (SULLIVAN 1956) would account for the higher rate observed.

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# Zusammenfassung

Altersbestimmung, Fortpflanzung und Sterblichkeit des Graufuchses (Urocyon cinereoargenteus) in Maryland, USA

Vier Verfahren wurden zur Altersbestimmung für 143 Graufüchse (Urocyon cinereoargenteus) angewandt, die von 1976 bis 1979 im Bundesstaate Maryland erlegt wurden. Die Altersbestimmungsmethoden waren wie folgend: 1. Zahl der jährlichen Zementablagerungen in Zähnen; 2. Grad der Verknöcherung der Epiphyse im Humerus; 3. Gewicht der ausgetrockneten Augenlinsen; und 4. Länge und Gewicht des Penisknochens. Die Daten wiesen auf einen zeitlich gleichbleibenden Altersaufbau hin. Der Jungfuchsanteil des Gesamtbestandes betrug über 50 % in jedem Jahr der

dreijährigen Untersuchung. Das Geschlechterverhältnis der erlegten Füchse war 123,4 Männchen auf je 100 Weibchen. Hodengewichte und der Grad der Spermiogenese wiesen darauf hin, daß erwachsene Rüden früher im Jahre zeugungsfähig sind als diejenigen, die zum erstenmal in die Ranzzeit kommen. Der Beginn des Östrus erfolgte Anfang Februar. Anhand von Zählungen der Plazentanarben in der Gebärmutter wurde die Wurfgröße im Durchschnitt auf 4,42 (schwankend zwischen 3 und 5) Welpen geschätzt. Die Implantationsrate beziehungsweise vorgeburtliche Mortalität betrug 88 % und 39 % im Jahre. Nichttragende Fähen umfaßten 45 % des weiblichen Bestandes.

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# SEM and carbohydrate histochemical aspects of the glands in the anal region of the pig

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#### Abstract

Investigated SEM structure and carbohydrate histochemistry of the perianal tubular skin glands and the anal glands (proctodeal glands) of 4 juvenile domestic pigs (30-40 kg). The tubular skin glands showed a secretory epithelium with apical cytoplasmic protrusions and large dilatations of the excretory ducts. The anal glands had no corresponding protrusions but the plasma membranes of the secretory cells were fenestrated.

The secretory cells and the luminal secretions of the glands contained neutral and acidic glycoproteins, with small amounts of sialic acid in the tubular skin glands and greater amounts of sialic acid in the anal glands. The reaction intensities of the PO-lectin-DAB procedures demonstrated

varying amounts of particular saccharide residues, especially in neutral glycoproteins.

The results obtained indicate an apocrine secretory mechanism in the tubular perianal skin glands and an eccrine or a holocrine-apocrine mechanism in the anal glands. Both gland types obviously contain different spectra of mucus glycoproteins. The observations are discussed in relation to the territorial scent marking behaviour of the pig.

## Introduction

In contrast to several other mammalian groups, the anal region of the pig does not show prominent glandular areas (e.g. circumanal gland, paranal sinus) (see e.g. Schaffer 1940; ORTMANN 1960; CALHOUN and STINSON 1976; NEURAND and MEYER 1982; STARCK 1982).

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