Note: Only occupies 5% of historic range; only 160 in wild in 2013

Reproductive Impairment in the Florida Panther: Nature or Nurture?

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Many of the remaining members of the endangered Florida panther (*Felis concolor coryi*) population suffer from one or more of a variety of physiological, reproductive, endocrine, and immune system defects including congenital heart defects, abnormal sperm, low sperm density, cryptorchidism, thyroid dysfunction, and possible immunosuppression. Mercury contamination, determined to be the cause of death of a female panther in 1989, was presented as the likely cause of thyroid dysfunction. As genetic diversity in the species was less than expected, all of the other abnormalities have been attributed to inbreeding. However, exposure to a variety of chemical compounds, especially those that have been identified as environmental endocrine disrupters (including mercury, *p,p'*-DDE, and polychlorinated biphenyls), has elicited all of the listed abnormalities in other species. A number of these contaminants are present in South Florida. An exposure pathway has been identified, and evidence presented in this paper, including the fact that there appears to be no significant difference between serum estradiol levels in males and females, suggests that many male panthers may have been demasculinized and feminized as a result of either prenatal or postnatal exposure. Thus, regardless of the effects of inbreeding, current evidence seems to indicate that environmental contaminants may be a major factor contributing to reproductive impairment in the Florida panther population. — Environ Health Perspect 103(Suppl 4):79–86 (1995)

Key words: DDE, polychlorinated biphenyls, mercury, endocrine disrupters, Florida panther, *Felis concolor*, reproductive impairment, cryptorchid, estradiol, testosterone

Introduction

There are 36 extant species of cats and, with the exception of the domestic cat, all are listed as threatened or endangered (*1*). Several reasons exist for the seemingly universal decline of felids, but we were forewarned of at least one possible cause. In 1962, Rachel Carson (*2*) reported that cats were extremely sensitive to insecticides and noted that 90% of domestic farm cats in the vicinity of Sheldon, Illinois, were killed as a result of exposure to dieldrin, an organochlorine pesticide. This pesticide was used as part of a concerted effort to eradicate the Japanese beetle. The beetle survived.

The Florida panther (*Felis concolor coryi*) is an endangered inhabitant of pine forests and oak hammocks of south central Florida (*3,4*). Currently, it is estimated that the population consists of only 30 to 50 animals comprising two genetic strains (*5*). Although the U.S. Fish and Wildlife Service (USFWS) and the Florida Game and Fresh Water Fish Commission began studying the health and life history of this animal in the early 1970s, intensive studies of the impact of contaminants on the species were not initiated until mid-1989. In July of that year, a 4-year-old radio-collared female (#27) was found dead in Everglades National Park (ENP). The cause of death was not revealed by either gross or histopathological examination, although brain tissue was too autolyzed for a definitive examination (*6*). The relatively good condition of the remainder of the carcass and lack of significant pathologic findings, when coupled with the loss of a prime breeding-age female (one of only three remaining in the ENP; the other two have died since 1989), prompted a more extensive examination. Selected tissues were analyzed for organochlorine compounds and trace elements. Whereas other contaminant residues, including selenium, *p,p'*-DDE, polychlorinated biphenyls (PCBs), oxychlordane, and *trans*-nonachlor, were detected, the only contaminant found to be present at what was then believed to be a potentially toxic concentration was mercury. Liver tissue contained 110 parts per million (ppm) mercury fresh weight (fw) and a concentration of 130 ppm mercury fw was measured in the fur (*6*). In the 1960s, liver tissues from domestic cats found dead in Minamata (Japan) contained 37 to 145 ppm mercury fw (*7*). Thus, the cause of death of Florida panther #27 was listed as mercury toxicosis (*6*).

Another female panther (#08) died in August 1988 after being removed from the wild 16 months earlier due to declining health. Necropsy showed the cause of death to be renal and hepatic failure. In addition, the animal had enlarged thyroids due to adenomatous hyperplasia (a second animal, #200, has since been diagnosed with thyroid hyperplasia: MR Dunbar, personal communication). Analyses of previously banked sera indicated that thyroid hormone concentrations in #08 were elevated above those established as normal for the domestic cat (the only point of reference at that time) and that this condition was not due to her stay in captivity (*8*). At the time of capture, the whole-blood mercury concentration was 1.17 ppm fw and was still elevated (20 ppm fw) in liver tissue at time of death (*8*).

Roelke (*8*) also reported other anomalies. Some animals have congenital heart defects. For example, Florida panther #20, a male, was found to have a cardiac murmur (*8*). The examining cardiologist believed that the murmur was probably due to a valvular or septal defect. The animal died within a year, and necropsy confirmed

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a septal defect (a 1-cm opening between the atria). To date, 8% of animals necropsied have been found with septal defects (MR Dunbar, personal communication).

Sperm abnormalities in the Florida panther were, and still are (*9*), the highest reported for any feline. Electroejaculation of 12 males, at least 2 of which were unilaterally cryptorchid, showed low ejaculate volume, low sperm concentration ($3–15 \times 10^6$ sperm/mm^3 semen), poor motility, and a very high proportion (92.9%) of abnormal sperm (*8*). The percentage of abnormal sperm in male panthers was 24 to 50% greater than that usually found in Texas cougars, *Felis concolor* (*1*). Moreover, 75% of Florida panther sperm exhibited severe deformity to the extent that they were classified as primary abnormalities (primary abnormalities are those abnormalities that are thought to occur during spermatogenesis as opposed to abnormalities which may arise due to external influences such as nutrition or disease) (*8*). This proportion of primary abnormal sperm is much greater than the 20 to 25% observed in either wild or captive Texas cougars (*1*) and the 27% incidence of primary abnormality observed in sperm of South African cheetahs, *Acinonyx jubatus jubatus* (*10*).

Cryptorchidism (one or both testes retained within the body cavity) has increased exponentially in male cubs since 1975 (Figure 1). Currently, 90% of the male population is cryptorchid (MR Dunbar, personal communication).

Sterility (the apparent inability to impregnate a female) has been observed in at least four panthers examined between 1978 and 1990 (*8*). Two of these had only one fully descended testis. In addition, we know of at least one apparently infertile female (#38, now deceased).

Roelke et al. (*11*) linked mercury contamination of the panther to the aquatic food chain. Panthers that subsisted mainly on a raccoon (*Procyon lotor*) diet were found to contain significantly greater levels of mercury in their tissues than those which preyed on white-tailed deer (*Odocoilus virginianus*). Raccoons, which bioaccumulate mercury (*11,12*), also concentrate lipophilic, endocrine-disrupting compounds, including methoxychlor, *p,p'*-DDE, and other organochlorine compounds, in their body fat (*12,13*).

Roelke (*8*) proposed that mercury contamination was perhaps the causal agent of hyperthyroidism, but she believed that the other anomalies were probably due to inbreeding. Even though it is known that inbreeding does occur in this population of 30 to 50 free-ranging animals (Figure 2), we suspected that environmental contaminants, especially those known to disrupt the endocrine system, might be a factor contributing to thyroid dysfunction and reproductive impairment in the Florida panther. For example, Roelke et al. (*11*) found a significant inverse correlation between the amount of mercury in whole blood of lactating females and survival of offspring to 6 months of age (Figure 3).

An abnormal hormonal environment in the mammalian testis, which, although genetically controlled, can result from exposure to endocrine-disrupting xenobiotics, including mercury, can produce all of the reproductive abnormalities listed above (*14*). Thus, we suggest that the abnormalities reported in the endangered Florida panther may be due to factors other than, or in addition to, inbreeding (e.g., the exposure of developing cubs to xenobiotic endocrine-disrupting contaminants bioaccumulated by their mother via the aquatic food chain).

Our assertions that environmental contaminant exposure may be a major cause of most, if not all, of the abnormalities noted in the Florida panther are supported by the literature and the results of a preliminary study (reported herein) designed to answer two questions: Is there evidence of abnormal estradiol/testosterone (E/T) ratios suggestive of exposure to endocrine-disrupting contaminants, as reported previously in other wildlife species (*15*)? If so, is there correlation between abnormal, or skewed, E/T ratios and the incidence of cryptorchidism in male panthers?

Materials and Methods

Since January 1985, samples of whole blood have been routinely collected from both free-ranging and captive panthers. All samples were maintained at approximately 4°C during transportation to the Florida Game and Fresh Water Fish Commission laboratory in Gainesville, Florida and

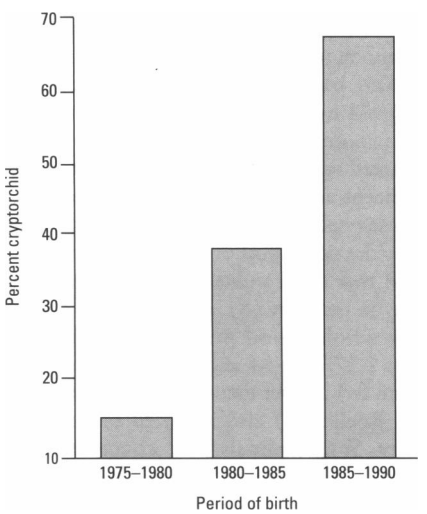

Figure 1. Percent of male offspring with undescended testes (unilaterally or bilaterally cryptorchid). From ME Roelke (*8*).

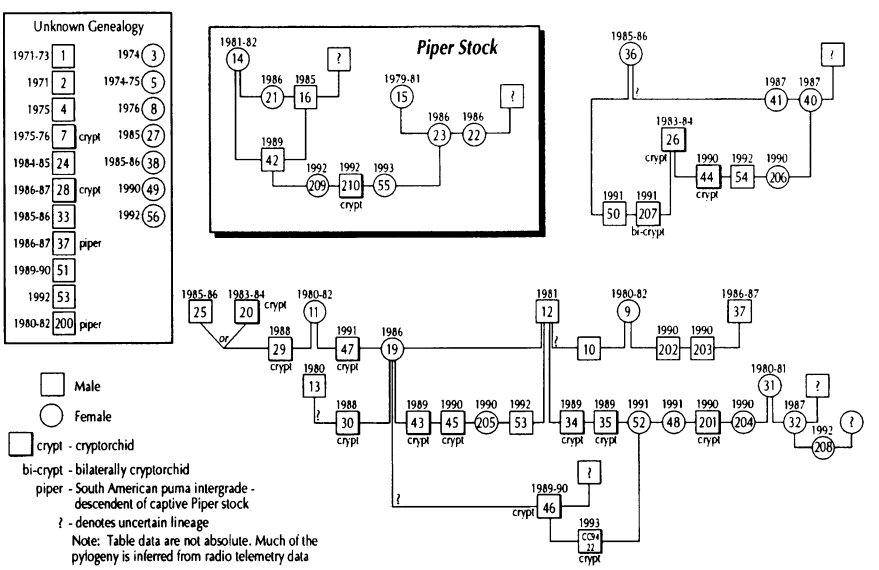

Figure 2. Pedigrees of individual wild-born Florida panthers, living and dead: field identification number inside symbols; date of birth above symbols. Data from MR Dunbar (*58*).

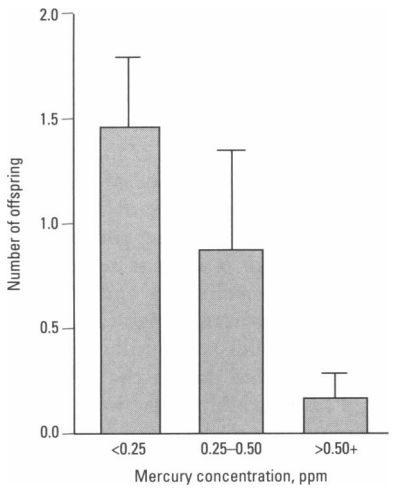

Figure 3. Average number of offspring surviving to age 6 months versus mercury concentration in whole blood of mother. From Roelke et al. (*11*).

either stored as whole blood or serum at –70°C. Thirty-two samples from 19 males and 5 females were selected for analysis. The samples selected were collected during the period beginning January 1986 and ending February 1993. Samples were selected to assure that both sexes, normal (n = 6) and cryptorchid (n = 13) males, captive (n = 2) and wild free-ranging (n = 17♂, 5♀) animals, and animals of various age classes (range = 1–12 years) were represented. As the major purpose of this preliminary study was to detect abnormal E/T ratios, location of capture and breeding status were not considered.

Serum samples were analyzed for estradiol and testosterone concentrations using radioimmunoassay (RIA) procedures. For both estradiol and testosterone determinations, 200-µl samples were extracted with 5 ml diethyl ether before analysis. Each sample was analyzed in duplicate and corrected for an extraction efficiency of 92 ± 2.4% (estradiol) or 82 ± 4.3% (testosterone). Standard curves were prepared in buffer with known amounts (1, 5, 10, 25, 50, 100, 250, 500, and 1000 pg) of radioinert estradiol or testosterone. The minimum concentrations per tube that were distinguishable from 0 were 15.2 and 9.8 pg/ml for estradiol and testosterone, respectively. Cross-reactivities of the estradiol antiserum with other steroids were: 14.4% for estrone; 3.7% for estriol; < 1.0% for estradiol-17α; and 0.1% for all other steroids examined. For testosterone antiserum, cross-reactivities with other steroids were: 18.75% for 5α-dihydrotestosterone; 3.0% for 5α-androstenediol; < 1.0% for androstenedione; and 0.1% for all other steroids examined. A pooled sample (approximately 250 pg estradiol/ml or 180 pg testosterone/ml) was assayed serially in 10, 25, 50, 100, and 200 µl volumes (final volume of 200 µl with charcoal-stripped plasma). The inhibition curves for both steroids were parallel to standard curves, with the test for homogeneity of regression indicating that the curves did not differ. Further characterization of the assays involved measurement of known amounts (1, 2, 5, 10, 25, 50, 100, 250, and 500 pg) of estradiol or testosterone in 200 µl of charcoal-stripped plasma [Y = 9.27 + 1.05X; Y = the amount of estradiol measured (pg); X = the amount of estradiol added; r^2 = 0.9005]; [Y = 8.10 + 0.96X; Y = amount of testosterone measured (pg); X = amount of testosterone added (pg); r^2 = 0.8355]. Interassay and intraassay coefficients of variation were 9.4 and 10.5%, respectively, for estradiol and 9.5 and 11.5% for testosterone.

In order to assure homogeneity of variance, hormone and ratio data were log-transformed before analysis. Thus, mean values are geometric rather than arithmetic. Two or more samples were analyzed for five panthers. Only those data corresponding with the age nearest the mean age (6.0 years for ♀; 3.9 years for ♂) were used in the statistical analyses. Comparison of means was performed using either Student's t-test or one-way analysis of variance (ANOVA), as appropriate. Differences between means were detected using the Student-Newman-Keuls test with α = 0.05.

Results

Eleven of the 17 free-ranging male panthers and both captive males retained one or both testes within the body cavity; 9 retained the right testicle, 3 retained the left, and both were retained in one (#207), a captive male. The estradiol concentration in the blood of this particular animal (Table 1) was greater than that of all other males and of all females other than #11. As bilaterally cryptorchid animals have been shown to produce high estrogen levels due to the lack of a suppressive influence on estrogen production by scrotal testes (*16*), #207 was excluded from the analyses.

E/T ratios (Table 1) ranged from 0.16 to 1.47 and 0.77 to 4.03 for male and female panthers, respectively. Mean ratios are presented in Table 2. As noted, there was no significant difference ($F_{(2,19)}$ = 1.78) between mean estradiol concentrations of normal male, cryptorchid male, and female panthers. On the other hand, there were significant differences between mean testosterone concentrations ($F_{(2,19)}$ = 4.60; p < 0.05) and between E/T ratios ($F_{(2,19)}$ = 9.28; p < 0.005) of the three groups of panthers.

E/T ratios for male panthers, whether cryptorchid or normal, generally decreased with age (e.g., see data for panthers #12 and #16; Table 1) and appeared to be independent of the initial value (i.e., whether > or < 1). This was principally due to an increase in plasma testosterone concentrations with age. Estradiol concentrations either remained about the same (e.g., #16; a normal male) or decreased slightly (e.g., #12; cryptorchid) as the animal aged. A comparison of mean estradiol and testosterone concentrations of same-age (2-year-old) normal (n = 4) and cryptorchid (n = 3) males showed no significant difference (t = 0.54; t = 1.88, respectively), and there was no significant difference (t = 1.03) between E/T ratios of the two groups.

Table 1. Estradiol/testosterone (E/T) ratios measured from whole blood samples from Florida panthers. Estradiol (E) and testosterone (T) concentrations are expressed in pg/ml.

ID no.	Sex	Age[a]	E	T	Ratio
10	M	1	90	215	0.42
11	F	11	745	185	4.03
12	M[b]	5	305	250	1.22
12	M	10	240	495	0.48
12	M	12	170	585	0.29
16	M	2	225	255	0.88
16	M	3	190	255	0.75
16	M	6	270	535	0.50
16	M	7	180	615	0.29
17	M	9	90	550	0.16
20	M[b]	4	145	195	0.74
23	F	7	250	325	0.77
26	M[b]	5	110	160	0.69
26	M	9	260	495	0.53
28	M[b]	5	410	820	0.50
29	M[b]	2	445	580	0.77
29	M	3	375	575	0.65
33	M	2	150	230	0.65
34	M[b]	1	255	735	0.35
34	M	2	235	595	0.39
35	M[b]	1	275	960	0.29
38	F	7	260	115	2.26
40	F	4	490	155	3.16
42	M	2	425	780	0.54
43	M[b]	2	545	775	0.70
44	M[b]	3	275	455	0.60
45	M[b]	3	335	370	0.91
47	M[b]	1	170	630	0.27
48	F	1	340	215	1.58
51	M	2	610	415	1.47
201	M[b,c]	1	420	1200	0.35
207	M[b,c]	1	670	1260	0.53

[a]Ages are approximate (± 1 yr) for animals 11, 12, 17, 20, and 26. Ages of other animals are to nearest birthday. [b]Cryptorchid. [c]Captive.

Table 2. Mean serum hormone concentrations (95%CL) and estradiol/testosterone (E/T) ratios for normal male, cryptorchid male, and female Florida panthers. Estradiol (E) and testosterone (T) concentrations are expressed in pg/ml.

Sex/status	*n*	E	T	Ratio
Male/normal	6	198 (86–456)[a]	362 (208–628)[a]	0.54 (0.25–1.18)[a]
Male/cryptorchid	12	275 (202–369)[a]	493 (329–740)[a]	0.55 (0.41–0.74)[a]
Female	5	381 (215–676)[a]	187 (116–303)[b*]	2.04 (0.91–4.55)[b**]

Values in each column with same superscript are not significantly different from one another. *$p<0.05$; **$p<0.005$.

If cryptorchidism was, or is, due to *in utero* exposure to endocrine-disrupting chemicals, we reasoned that an investigation of the lineage of the animals selected for this study might provide additional, corroborative information. Of the six normal males, the female parent of only three (#10, #16, #42; Figure 2) is known. Two of these males (#16, #42) were offspring of the same animal (#14), a female not included in this initial study. The likely sire of #10 was #12, a cryptorchid male. We do not have information regarding the sire of #16. However, #16 was the sire as well as the half-sibling of #42 (Note: #42 and #23, a female, produced a cryptorchid male offspring in 1992; Figure 2).

Cryptorchid males were descendants of at least five different females (Figure 2). In two cases, female offspring of females that produced cryptorchid males also produced cryptorchid offspring even though, in one case, the sires (both cryptorchid) of the male and female cubs differed.

Discussion

The argument for inbreeding as the cause of reproductive impairment in the panther is based, in part, upon data obtained from electrophoretic analyses. Roelke et al. (*9*) reported that only 2 or 3 allozyme loci (depending upon the genetic strain) of 41 loci examined were polymorphic (*P*, percent polymorphism = 4.9 and 7.3 for mitochondrial DNA types A and B, respectively), and that genetic diversity in the panther population was generally less than that of other felid species. A survey of the literature does not fully support this argument.

Miththapala et al. (*17*) provided a review of genetic variation in nine species (or subspecies) representing 14 populations of large felids. The data presented were based upon the electrophoretic analysis of some 50 genetic loci. All analyses were performed in the same laboratory. The number of individuals in each population ranged from 5 to 40 and the number of loci examined ranged from 46 to 52 (mean = 49.1). In these populations, *P* ranged from 0% in the Asian lion (*Panthera leo persica*; 0 of 46 loci polymorphic) to 11% in the African lion (*P. l. leo*; 5 of 46 loci polymorphic). The mean number of polymorphic loci in the 14 populations was 2.6. Thus, genetic diversity in the Florida panther population (2–3 polymorphic loci depending on the genetic strain) ranges from slightly below to slightly above average, when other species are considered, rather than below average as reported by Roelke et al. (*9*). It should be noted, however, that other subspecies of *F. concolor* are more genetically diverse than *F. c. coryi* (e.g., 7 polymorphic allozyme loci have been detected in an *F. c. azteca* population) (*9*), but most populations studied exhibited only 2 to 3 loci with multiple alleles.

Although the impact of inbreeding most often reported in the literature is increased infant mortality (*18–20*), some of the other problems noted in male panthers have been observed in inbred lines of other species. Wildt et al. (*20*) reported that sperm count was lower in inbred than in outbred groups of foxhounds. These authors also reported that ejaculate volume, sperm motility, and testis volume seemed to be favored in outbred strains, although the differences noted were not significant.

A review of the literature (*1,21–23*), however, reveals that the Florida panther produces the least volume per ejaculate, the lowest number of sperm per ml of ejaculate, the lowest percentage of motile sperm, the highest percentage of abnormal sperm, and the highest percentage of sperm with abnormal acrosomes of any felid population or species studied.

Cryptorchidism is a heritable trait in some species in which inbreeding has been practiced to produce purebred lines (e.g., *24,25*). Roelke et al. (*9*) believed that the condition in the panther resulted from a sex-linked recessive gene. A comparative study of *Felis concolor* populations from Florida, Texas, Colorado, Latin America, and North American zoos (*26*) indicated that cryptorchidism was only observed in 2 of more than 50 captive male cougars. The condition has never been reported in any other species of nondomestic cat (*9*), regardless of the degree of inbreeding.

Cryptorchidism in mammals is associated with three critical factors: Müllerian-inhibiting hormone, intraabdominal pressure, and androgens (*27*). No data are available on the influence of xenobiotic agents on the synthesis of Müllerian-inhibiting hormone during gonadal development. There is a clear relationship between cryptorchidism and abnormal spermatogenesis in mammals (*28*). Studies examining males with unilateral cryptorchidism have shown that the cryptorchid testis exhibits defective spermatogenesis and that sperm count is lower than in the scrotal testis (*28*). Available evidence suggests that cryptorchidism is associated with abnormal testicular function (temperature dependent) or testicular malformation, and impaired spermatogenesis is fundamental to maldescent (*28*).

Normal spermatogenesis in mammals is dependent on adequate (species specific) plasma and testicular concentrations of testosterone (*14*). Testosterone is synthesized by the testis under the control of luteinizing hormone (LH) released from the adenohypophysis under the stimulation of hypothalamic LH-releasing hormone (LH-RH). Factors that modify androgen concentrations (e.g., decreased LH or LH-RH or increased degradation of testosterone) will adversely influence the production of sperm (*14*).

Does the Florida panther exhibit unique life history characteristics that could be related to all these symptoms? The Florida panther feeds extensively in the aquatic food chain. A principal diet item of the Florida panther that is associated with the aquatic food chain is the raccoon. Raccoons in south Florida bioaccumulate a variety of pesticides and other similar compounds (*12*), including some (e.g., *p,p'*-DDE, methoxychlor) which have been shown to disrupt the endocrine system or otherwise result in reproductive impairment. Data presented by Roelke et al. (*11*) indicate that Florida panthers eat raccoons throughout their range, as mercury concentrations in panthers from each habitat area in South Florida were correlated with levels found in raccoons trapped in the same area. Schortemeyer (unpublished data) reported that approximately 12% of the average panther's diet consists of raccoons (other major diet items were feral hogs (approximately 41%), deer (approximately 27%), and armadillos [(*Dasypus novemcinctus*); nearly 8%]. Panthers bioaccumulate environmental

contaminants. For example, fat from Florida panther #27, the female found dead in 1989, contained > 57 ppm *p,p'*-DDE and 27 ppm PCBs (Table 3: USFWS, unpublished data).

Thus, having established that contaminants are present in panther habitat, that an exposure pathway exists (the raccoon), and that contaminants are present in panther tissue, only two questions remained. First, is there evidence that the physiology of the Florida panther could have been influenced by these compounds, particularly those that would affect normal steroid hormone synthesis (E/T ratios)? Second, if so, is there any correlation between E/T ratios (or concentrations of either hormone) and the rate of cryptorchidism within the population?

Very few data regarding steroid hormone concentrations in wild or captive panthers are available in the literature and there are no E/T ratio data for either sex. Serum estradiol-17β (estradiol) concentrations in the five females from this study (Table 1) ranged from 250 to 745 pg/ml. Overall, these concentrations appear to be much higher than those reported for 3 mature cougars in the London zoo (*29*), but except for Florida panther #23 (which was attended by a young cub), #38 (which exhibited elevated serum progesterone), and #40 (which had been bred 45 days before capture [no cubs produced]), we were unsure of the exact state (stage of the estrous cycle) of each female. In addition, estradiol concentrations in the zoo animals were detected using a very specific antisera whereas our analyses utilized an antisera with significant cross-reactivities with other estrogens. Thus, no valid comparisons can be made between the results from this study and that of Bonney et al. (*29*).

Whereas we found no significant difference between mean testosterone concentrations of normal and cryptorchid male panthers (Table 2), Barone et al. (*23*) found that testosterone concentrations in cryptorchid Forida panthers (270 ± 0.11 pg/ml) were significantly lower ($p < 0.05$) than in noncryptorchid males (610 ± 0.11 pg/ml). A more recent study (*26*) reported no significant difference between testosterone levels of five populations of cougars (including 16 Florida panthers, 7 of which were unilaterally cryptorchid). The mean testosterone concentration observed, which involved a total of 65 animals, was 720 pg/ml, which the authors thought to be low (but which was greater than mean concentrations measured in our study). They concluded that the differences noted in semen quality (the Florida panther exhibited the lowest testicular volume, ejaculate volume, sperm motility, sperm concentration, and percent structurally normal sperm) were apparently not due to differences in steroid hormone concentrations but were more likely mediated at the testicular level. Direct exposure to a variety of environmental contaminants has been shown to cause one or more of these abnormalities. These include benomyl (*30–32*), carbenzadim (*33,34*), chlordecone (*35*), methoxychlor (*31*), methylmercury (*36*), 2,3,7,8-tetrachlorodibenzo- *p*-dioxin (TCDD) (*37*), and some PCB congeners (*38*). Exposure to several chemicals, including fenarimol, methylmercury, and TCDD, has resulted in decreased male fertility (*37,39,40*).

Even more notable, however, is the fact that there was no significant difference between estradiol concentrations in the three groups of panthers: normal males, cryptorchid males, and females. In addition, E/T ratios for 2 of the 19 males examined were greater than 1.0 and were near unity for several others (Table 1). The data also suggest that estradiol concentrations in female panthers, with the exception of #23 which seemed to be somewhat masculinized (E/T ratio < 1: Table 1), may have been above normal when compared with London zoo animals. These data suggest that steroid hormone concentrations and E/T ratios may have been abnormal in both male and female panthers. If testosterone levels were abnormally low, as suggested by Barone et al. (*26*), this would account for the abnormal ratios observed in males used for this study.

Demasculinization and feminization of males resulting from exposure to exogenous estrogens, or other endocrine-disrupting compounds, has been shown in species as diverse as mice (*41,42*), alligators (*15*), and turtles (*15,43*). Guillette et al. (*15*) reported significantly greater estradiol concentrations, when compared to controls, in female alligators in Lake Apopka (Florida), apparently resulting from prenatal exposure to *p,p'*-DDE, a potent antiandrogen (LE Gray, personal communication).

Prenatal exposure of rats to the antiandrogenic metabolites of the fungicide vinclozolin exhibited hypospadias, cleft phallus, and suprainguinal (cryptorchid) ectopic testes (*44,45*). In addition, male mammals lacking adequate concentrations or recognition of plasma androgen (lack of androgen receptors) exhibit a high incidence of cryptorchidism (*27*). Male offspring of female rats exposed to atrazine during pregnancy and lactation exhibited reduced androgen (5α-androstane-3α, 17β-diol and 5α-dihydrotestosterone) concentrations in the pituitary at 21 days of age (*46*). Pyrethrins and bioallethrin, a synthetic pyrethroid, have been shown to be efficient competitors for androgen binding sites in human tissue. The binding of testosterone to sex hormone-binding globulin was inhibited by 60 and 43% by pyrethrins and bioallethrin, respectively (*47*).

Based upon the results obtained from the comparison of two small samples (four cryptorchid and three normal panthers) of 2-year-old animals, we were unable to detect any correlation between cryptorchidism and abnormal E/T ratios. However, this does not indicate that such a correlation does not exist but merely that our small data set does not show it. Barone et al. (*23*) noted that testosterone concentrations were lower ($p < 0.05$) in cryptorchid than in noncryptorchid Florida panthers, but it appears that the panthers tested were of varying ages, which may have influenced the results of the comparison.

Likewise, the information regarding the lineage of cryptorchid animals is inconclusive, neither fully substantiating nor refuting our original hypothesis. Female offspring of cryptorchid-producing females may produce cryptorchids due to the fact that they too were exposed to the same or similar chemicals as their mothers. This is quite possible given the relatively short (16 km) dispersal distance (*3*) of female panthers. In contrast, based upon current information, we cannot rule out the effects of inbreeding either. However, one of the major arguments for inbreeding as the cause of the suite of symptoms shown in the panther was based upon the fact that some of these symptoms were only observed in the pure Florida panther strain. The other strain,

Table 3. Organochlorine compounds found in liver tissue (µg/g lipid fresh weight) from three Florida panthers. Samples analyzed in 1990 (U.S. Fish and Wildlife Service, unpublished data).

ID	Moisture, %	*p,p'*-DDE	PCB-1254	Oxychlordane	*t*-nonachlor
07	77.19	5.45	7.32	<0.0098	<0.0098
27	75.23	57.65	27.06	2.00	4.82
G8420	75.00	7.86	10.14	1.86	2.43

an introgressed hybrid (*5*) stock commonly referred to as the Piper stock, was slightly more genetically diverse (3 polymorphic loci as opposed to 2 in the purebred strain) and, until recently, had shown only low sperm count and abnormal sperm. But, as noted in Figure 3, male #210, a Piper animal born in 1992, has only one fully descended testis.

Regarding the other abnormalities noted in the population, prenatal exposure to nitrofen, an herbicide, has resulted in septal defects in rat neonates (*48*). Suppression of the immune system has been reported to result from exposure to a variety of compounds including atrazine, captan, carbofuran, 2,4-D, DDT, diazinon, endosulfan, fenitrothion, lindane, malathion, mercuric chloride, methyl parathion, and several of the synthetic pyrethroids (*49*). Exposure to metribuzin (*50*), mercuric chloride (*51*), hexachlorobenzene (*52*), DDE, and dieldrin (*53*) has resulted in altered thyroid function in a variety of organisms.

Thus, we are faced with the following: *a*) the Florida panther is on the verge of extinction, with only 30 to 50 animals remaining; *b*) the remaining panther habitat has been impacted by agricultural chemicals, many of which have been shown to disrupt the endocrine system or otherwise result in reproductive impairment; *c*) the raccoon provides a pathway for exposure of the panther; *d*) data presented herein provide evidence that the panther has been exposed; *e*) the population is affected by a suite of symptoms that, taken as a whole, appear to be unique to the panther; and *f*) all of the symptoms expressed by members of the population have been shown to result, in other species, from exposure to one or more of the chemicals present within the system. Most of the abnormalities noted have also been associated with vitamin A deficiency, which may also be associated with a raccoon diet; (MR Dunbar, personal communication).

Fox (*54*) suggested that when managers are faced with a serious and urgent problem for which there appears to be no certain cause due to a lack of causal data, it is essential that they "draw together all the disparate threads of evidence and make them into a coherent whole" so that decisions which are both scientifically and socially acceptable can be made. In the case of the Florida panther, it has been decided that inbreeding (believed to be the cause of decreased genetic diversity) is the cause of the problems observed and that the only solution is the introduction of additional genetic diversity in the form of eight female Texas cougars (*F. c. stanleyana*). But, whereas this action will provide additional genetic diversity (*F. c. stanleyana* is polymorphic at 4 allozyme loci, 3 of which differ from *F. c. coryi*) (*9*), what will be the overall outcome after these new animals have had sufficient time to accumulate significant contaminant loads (e.g., adult bald eagles that have migrated to the Great Lakes shoreline exhibit reproductive impairment after feeding on fish and other food items from the lakes for 2 years or more) (*55*)?

In addition to this important question, there are several others that must also be addressed. What concentrations of estradiol and testosterone are normal for males of a given age? How do steroid hormone concentrations vary seasonally in female panthers? Is there any correlation between E/T ratios or cryptorchidism and abnormal spermatozoa, sperm motility, thyroid dysfunction, or immune system suppression? Guillette et al. (*15*) reported 50% mortality of juvenile alligators hatched from eggs obtained from a population exposed to dicofol. We know that raccoons provide a pathway for exposure of the panther population to agricultural chemicals. We also know that there is a long history of agricultural chemical use in the area that includes critical panther habitat (*56*). Furthermore, many of the most commonly used pesticides have been associated with developmental or reproductive abnormalities. Could such exposure (in addition to mercury) also lead to early mortality of panther cubs as has been demonstrated in birds (*57*) and alligators (*15*)?

Additional research is proposed that will provide answers to many of these questions. We propose that the following be undertaken: *a*) blood samples and tissue biopsies should be collected from all female Texas cougars prior to their release into existing panther habitat; *b*) similar samples should be collected from one or more suitable control or reference populations; *c*) tissue biopsies should be collected from the introduced female cougars annually for at least 3 years; *d*) all serum and tissue samples, including those collected earlier from both captive and free-ranging panthers, should be analyzed for thyroid and steroid hormone concentrations or for chemical residues, as appropriate; *e*) blood samples should be collected from all newborn males, including the male offspring of the introduced cougar females, at ages 6 to 8 months and 16 to 18 months, for determination of hormone concentrations; *f*) semen should be collected from all male offspring at age 24 to 36 months; and *g*) normal immune response for *F. concolor* must be determined for comparison with immune response in *F. c. coryi*. Selected tissues from raccoons collected during 1993 and 1994 are currently being analyzed for chemical residues. The data gained from these investigations should further elucidate the impact of endocrine-disrupting chemicals on the Florida panther.

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