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ANNUAL PERFORMANCE REPORT
Statewide Wildlife Research

State: Florida

Study No. 7508

Study Title: Florida Panther Genetic Restoration and Management

Period Covered: 1 July 1994 - 30 June 1995

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Abstract: Telemetry data were collected on 18 radio-collared Florida panthers (Felis concolor coryi) and six Texas cougars (F. c. stanleyana) in south Florida during the reporting period. Mortalities of instrumented panthers included three males and two females; two males died from intraspecific aggression, a female died of pleuritis from a chest puncture, another female was road killed, and the third male's cause of death remains unknown pending necropsy. No Texas cougars died during the study period. Three additional panthers were collared during capture work in 1995 bringing the number of monitored panthers to 16 by 30 June 1995. Four females denned during the past year; four neonate kittens (3 females, 1 male) were examined at two dens and two kittens were marked with transponders. Florida panthers showed spatial use patterns similar to previous years. Movements of Texas cougars were generally within habitat areas utilized by Florida panthers and the occasional movements in atypical areas such as mangroves and suburban areas were probably typical of animals exploring unfamiliar terrain. Social interactions have occurred between Texas cougars and both female and male Florida panthers.

INTRODUCTION

Florida panthers (Felis concolor coryi) are endangered by a combination of population and habitat factors. Loss and fragmentation of habitat and unregulated killing over the past two centuries have reduced and isolated populations in the southeastern United States to the point that only one population estimated at 30-50 adults exists on approximately 12,546 km² (4,844 mi²) of habitat in South Florida (Logan et al. 1994). Small population size and geographic isolation increase the chance for extinction of Florida

panthers due to demographic instability inherent in small numbers and erosion of genetic diversity from restricted gene flow and inbreeding. Genetic diversity is the basis for production of fit individuals as well as providing the ability of the population to respond to changing environmental and habitat conditions. Demographic variation has been considered important in regulating populations for many years, but the role of social and genetic factors has only recently begun to be examined as modern techniques from field biology and molecular genetics have become available. Recovery of the Florida panther is complex, but not an uncommon situation as many species face similar circumstances. A unique opportunity exists to implement conservation and management strategies for Florida panthers and evaluate results that will benefit Florida panthers as well as other rare species.

Natural exchange of genetic material occurred historically among the Florida panther population in the southeastern United States and contiguous populations of *F. c. couguar* to the north, *F. c. hipolestes* to the northwest and *F. c. stanleyana* to the west (Young and Goldman 1946). Genetic exchange between populations ceased as the coastal plain was gradually cleared and settled. Florida panthers steadily declined in abundance and distribution as a result. Gene flow occurs as individuals disperse and breed, but habitat fragmentation and insularation disrupt dispersal and natural population processes. Dispersal is not only the natural mechanism for mixing the gene pool but also minimizes inbreeding within populations. Inbreeding increases when dispersing breeders can no longer move among fragmented populations and declining population size compounds demographic and genetic factors. Implications include inbreeding depression, loss of genetic variation,

declining health, reduced survivability, lower numbers, and eventual extinction. The compounding effects of these interrelated factors, perceived as an inward spiral or vortex (Gilpin and Soule 1986), have become a cornerstone of conservation biology. A computer program (VORTEX) has been developed and widely used to predict extinction for numerous species under similar situations (see Grier 1980, Lacy and Clark 1990, and Seal and Lacy 1989).

Resident adult panthers in Florida exhibit a relatively stable social organization with slow turnover. Adult male home ranges averaged 519 km² and usually overlapped several adult female home ranges that averaged 193 km² (Maehr et al. 1991). Subadult males wander widely, whereas subadult females remain closer to natal areas and may be recruited into the breeding population more quickly than subadult males. Intraspecific aggression resulted in about 35% of the documented mortalities of radio-collared panthers in South Florida. Social organization appears to be mediated by a dominance hierarchy and mutual avoidance system (Maehr 1991) similar to that reported for western populations (Hornocker 1970, Seidensticker et al. 1973). The only notable exception is that dispersal and recruitment opportunities are limited because of availability and distribution of habitat in South Florida.

The proportion of adult females producing litters each year and average litter sizes and sex ratios for Florida panthers are similar to demographic characteristics of populations in western states (Lindzey et al. 1994). Although data on kitten and subadult survival and age-of-first reproduction are sparse, rates of reproduction and recruitment appear adequate to balance current levels of mortality. Intraspecific aggression is the leading cause of

mortality and seems to exert strong selection pressure especially on subadults and non-reproducing males. Florida panthers appear to be functioning normally in a demographic sense, but again restricted distribution and habitat in South Florida limit normal dispersal distances, recruitment, and breeding opportunities, which in turn further impedes population and genetic processes.

Genetic analysis of Florida panthers using mitochondrial DNA and nuclear markers indicated the existence of one additional genetic marker in the Everglades population; one genetic marker centered in the Big Cypress ecosystem and was considered to have descended from historic Florida panthers, and the one additional genetic marker from the Everglades ecosystem likely originated in South or Central America and was introduced from captive stock between 1957 and 1967 (O'Brien et al. 1990). Genetic health of the few remaining Florida panthers may be benefiting from the introgression of additional genetic material into the wild population (O'Brien et al. 1990). Florida panthers of Big Cypress origin have remarkably low levels of genetic variation, which is 85% less than observed in western subspecies and 65% less than seen in the Everglades panthers or the Piper captive stock from which Everglades panthers are probably derived (Roelke et al. 1993).

Florida panthers exhibit numerous morphological abnormalities and health problems that are likely associated with recent and close inbreeding. Diseases include eight viruses, 17 parasites, and nine bacterial agents that occur at relatively high prevalence (19-80%), and have caused at least six panther deaths; some infectious diseases may be related to weakened immune systems (Roelke et al. 1993). Morphological abnormalities such as cryptorchidism, malformed sperm, and atrial septal defects (ASD) also hold

serious health and reproductive implications. The genetic basis, inheritance, and etiology of associated health problems are not fully understood, but the present study provides an opportunity to evaluate the population response and adaptive significance of these interrelated factors under conditions of natural selection. The high prevalence of cowlicks (94%) and kinked tails (88%-Belden 1986) also are morphological abnormalities that most likely result from inbreeding and rapid fixation in the small panther population. Kinked tails (9%), cowlicks (27%- Roelke et al. 1993), and ASD occurred at lower frequencies in panthers from the Everglades, supporting the traits' genetic basis and the rapid influence of genetic introgression to mask the expression of these recessive characteristics. Kinked tails and cowlicks can be readily observed and used to help gauge the influences of genetic introgression, although these unusual morphological abnormalities likely have less adaptive significance than reproductive and health considerations.

Genetic diversity and health of the Florida panther population needs to be restored to ensure survival, even with adequate habitat conservation and other enhancement measures. The complex interplay of social, demographic, genetic, and health factors dictates that a timely and aggressive program be implemented to address the many problems faced by Florida panthers. A plan for genetic restoration and management of the Florida panther was developed in September 1994 (Seal 1994). Genetic restoration is a direct and immediate action that will reduce inbreeding and restore genetic variability and vitality for a healthier, more resilient population. Results from genetic restoration will enable implementation and refinement of management strategies to maintain levels of genetic diversity historically typical of the North

American population, and will restore the long-term, adaptive capacity of the panther population in South Florida.

Ultimate goals of genetic restoration are to restore historical levels of genetic diversity by imitating natural population processes, and to develop a long-term management plan to maintain a healthy population of Florida panthers in South Florida. Genetic restoration will: 1) reduce the occurrence and impact of inbreeding, 2) restore genetic variability and vitality of offspring produced and recruited as breeders into a healthier, more resilient population of Florida panthers, and 3) restore the evolutionary and adaptive potential of the South Florida population by maintaining levels of genetic diversity typical of F. concolor populations in North America. Objectives to measure accomplishment of genetic restoration and management goals include: 1) releasing appropriate animals to bolster genetic diversity in South Florida, 2) monitoring released individuals and resident panthers to evaluate translocations, 3) comparing population performance and other relevant ecological and health factors before, during, and after genetic restoration, 4) assessing genetic introgression, 5) publishing results and recommendations, and 6) developing a long-term management plan based on study results to maintain genetic diversity and health of the South Florida panther population.

The genetic restoration project is intended to imitate the natural processes of genetic exchange that originally mixed gene pools and prevented inbreeding in Florida panthers. The successful establishment and reproduction of introduced cats in this study will begin the process of genetic restoration that is important to the long-term survival and recovery of the Florida panther. Also, knowledge gained can benefit other rare species and improve

the understanding of ecological processes and appropriate conservation responses.

STUDY AREA

The study area encompassed most of southern Florida south of the Caloosahatchee River and Lake Okeechobee and west of the Atlantic Ridge which runs along the entire coast of eastern Florida, and is heavily settled (Figure 1). The area includes large blocks of low-lying public lands such as Everglades National Park, Big Cypress National Preserve, Fakahatchee Strand State Preserve, and the Florida Panther National Wildlife Refuge. Significant private lands, primarily used for cattle and crop production, lie on higher and more productive ground to the north of the public lands, and constitute some of the most important habitat for Florida panthers.

METHODS

Florida panthers and Texas cougars were captured using trained hounds, anesthetized using ketamine hydrochloride or Telazol, and fitted with radio-collars. Texas cougars were health screened and quarantined for at least 30 days before being translocated to South Florida for genetic restoration purposes (Appendix I). All animals were reinstrumented as necessary due to growth or age of transmitters. Biomedical treatments and research were conducted concurrently during re-captures to reinstrument study animals. Instrumented animals were monitored approximately every other day from fixed-wing aircraft. Locations were plotted on 7.5 minute USGS topographic maps and recorded as universal transverse mercator points.

Mating and denning behavior, aggressive encounters among males, movements and home range shifts, dispersal, survival, recruitment, displacements and replacements of individuals, and other social and ecological interactions were interpreted from radiotelemetry data and field investigations. Instrumented subadults were monitored after dispersal to document recruitment into the resident adult population. Interactions with other panthers were examined to determine factors contributing to successful or unsuccessful recruitment. Successful recruitment is considered to have occurred if a subadult establishes a home range and eventually reproduces following dispersal or independence. Death of the dispersing subadult is considered unsuccessful recruitment and establishing a home range that does not overlap with a radio-collared panther of the opposite sex is considered marginally successful.

RESULTS and DISCUSSION

Status of Instrumented Panthers

The study period began with 18 radio-instrumented panthers (7 males and 11 females) ranging in age from 3 to 15 years of age (Appendix II). Two new panthers were added as study animals during winter capture efforts; #57 was a 3 year-old female and #58 was an 8 month-old male kitten of #56. Female panther #11 was fortuitously recaptured while in the company of male panther #51 on 15 May 1995, 11 months after her radio-transmitter ceased functioning.

Five panthers died during the study period: males #12 and #26 died from aggression encounters with male #46, female #52 was struck and killed by a car, female #38 died of pleuritis from a chest puncture wound, and the cause

of death for male #42 will be determined after necropsy (Appendix III). There were 16 panthers with functioning radio-collars in south Florida as of 30 June 1995.

Locations of several radio-instrumented panthers showed spatial use patterns similar to previous years (Figures 2 and 3). The deaths of males #12 and #26 will provide the rare opportunity to observe a significant change in the social organization. Home range sizes (convex polygon method) averaged 135 km² for female panthers and 283 km² for males; female ranges were smaller than male ranges except for female #36 (Figure 4). The initiation of dispersal for male #58 away from his natal range (Figure 5) began in June 1995, when he was 14 months of age.

Four radio-instrumented panthers produced kittens during the reporting period (Appendix IV). Panther #52 denned in July 1994 on private lands east of Immokalee with unknown results. Female #52 was captured in January 1995 and appeared to still be lactating, but 2 days post-capture, #52 was struck by a car and killed. Panther #55 denned in the Turner River Unit of Big Cypress National Preserve and produced 2 female kittens (K14, K15) in April 1995. Panthers #36 and #48 denned in June 1995 and the kittens of #48 (K16♂, K17♀) were handled and permanently marked with individually numbered transponders. Transponders will be essential for documenting the progression of genetic restoration as well as providing unequivocal data on kitten survival.

Status of Genetic Restoration

Eight female cougars were captured between December 1994 and May 1995 from widely dispersed locations in west Texas (Figure 6). Four cougars were flown to south Florida for release after completion of quarantine, health screening, vaccinations, and sample collections at the Houston Zoo. TX104 and TX106 were transferred to a temporary release pen in the Fakahatchee Strand State Preserve (Figure 7) during the night of 28-29 March. TX104 escaped the next day during observation and watering by forcing apart a side panel from a corner post. A quick repair with cable and pad locks secured TX106 in the release pen. An on-site press conference was held on 30 March to disseminate information about genetic restoration and provide photographic opportunities for journalists. The door of the release pen was left open after 12 days and the departure of TX106 was recorded on a remote camera at 6:58pm on 9 April.

TX101 and TX102 were flown from Houston to Fort Myers on 5 April and directly released at midnight from their air shipment crates in the Bear Island Unit of the Big Cypress National Preserve (Figure 7).

Two more female Texas cougars were transported by vehicle to South Florida on 4 May after completing health screening, vaccinations, sample collections, and quarantine at the Florida Game and Fresh Water Fish Commission's Wildlife Research Laboratory in Gainesville. TX107 was directly released near the end of 11 Mile Road in the Corn Dance Unit of the Big Cypress National Preserve (BCNP), an area referred to as Raccoon Point (Figure 7). TX103 was directly released into the Stairsteps Unit of BCNP in Lostmans Pines after a short trip via helicopter (Figure 7).

The final two cougars for release in south Florida were at the Wildlife Research Lab in Gainesville at the end of the reporting period and will be released on Long Pine Key in Everglades National Park early in the next reporting period. No significant problems were encountered during health screening, sample collection, and quarantine. Animals with cowlicks, kinked tails, and cardiac abnormalities were not used for translocation and only non-pregnant individuals were translocated to South Florida. No additional criteria were applied to animals used for translocation.

Evaluation of release methods (gradual or direct) was compromised by the escape of TX104 and no definitive conclusions could be drawn regarding the influence of the release method upon subsequent behavior of translocated animals. The escape and difficulties of re-constructing the temporary pen in dry, secure, and widely distributed sites across vast areas with limited road access resulted in all other cougars being released by the direct method. The direct method of release allowed more flexibility and efficiency in releasing translocated cougars under field conditions and proved more practical for genetic restoration purposes in south Florida. Release methods need to be tailored to the specific site and purpose of translocations and factors considered especially important were surrounding habitat conditions at a scale relevant to movements of the species being translocated.

Evaluation of translocated cougars

Locations of the six translocated Texas cougars (Figures 8 and 9) and their movements and social interactions are summarized below:

TX101 -- This female has ranged farther than any of the other released Texas cougars. She remained within a 6 mile radius of her Bear Island release site for the first month after release. This pattern shifted abruptly when she began a series of movements that essentially traced the western extent of available panther habitat in south Florida. TX101 travelled west through the Florida Panther National Wildlife Refuge into the eastern fringe of Naples and southward into Rookery Bay National Estuarine Research Reserve. After encountering inhospitable mangrove habitat, she travelled back north through the Corkscrew Regional Ecosystem Watershed and east to Labelle. Finally, TX101 ventured south to the Addition Lands and private lands in Hendry County where she has apparently begun to establish a consistent use area. This use area overlaps with male panther #45, females #36 and #56, and uncollared panthers known to use this area.

TX102 -- This cougar, released along with TX101 in Bear Island, showed a similar pattern of movements, but to a much smaller scale. She remained within a 5-6 mile radius of the release site for the first month after her release and then began more extensive movements eastward through the Addition Lands and north into private ranch land in Hendry County. TX102 returned to Bear Island briefly, but has established an area of consistent use east of the Hendry Correctional Institute, north to Rocky Lake, and east onto the McDaniel's Ranch. This area overlaps with male panthers #45 and #46, females #56, #36, and #48, and uncollared panthers also known to occupy this area.

TX103 -- TX103 moved 6 miles south into mangroves bordering the Ten Thousand Islands for the first two weeks after her release into Lostmans Pines on 4 May. She turned back north after reaching open water and returned to Lostmans Pines. She then began movements to the northwest, remaining south of Loop Road and Highway 41. TX103 has recently used a 10 square mile area to the west of Loop Road and Monroe Station in the Big Cypress National Preserve. She has been located adjacent to Highway 41, but has not been documented north of the road. No Florida panthers are currently known to use this area.

TX104 -- This female, after escaping the release pen in Fakahatchee Strand State Preserve, remained in the general area for the first month after her release, a pattern similar to TX101 and TX102. She was in the company of Florida panther female #09 in the Fakahatchee on 1 and 3 May, a pattern that has continued. These cats separated by 5 May and TX104 began westward movements into the Belle Meade area west of the southern Golden Gate Estates. TX104 has established 2 disjunct use areas, one in the Belle Meade area, bordered by I-75 to the north, CR 951 to the west, and the south Golden Gate Estates to the east. The second use area surrounds her release site in the Fakahatchee. She is often located with female #09, and overlaps with males #51 and #54.

TX106 -- This cougar was released from a holding pen into the Fakahatchee Strand State Preserve on 9 April. She has used a consistent area since her release, ranging primarily along the western Fakahatchee northward to I-75. She was near male panther #54 on 17 April and located with this 3-year-old

male on 19 April, a pattern suggestive of mating. The gestation period is approximately 90 days for these large cats, suggesting that a possible denning period may begin during the third week of July. TX106's area of use overlaps with males #51 and #54, and females #09 and #57.

TX107 -- TX107 began extensive movements immediately, travelling 9 miles to the northwest in the first three days after her release at Raccoon Point in Big Cypress National Preserve. This northwest movement continued another 10 miles until she reached Interstate 75. TX107 then turned west paralleling the south side of the Interstate to within one mile of Turner River Road. She returned southeasterly back to Raccoon Point travelling about 20 miles in six days. This cougar since has apparently settled into the central part of Big Cypress called Calusa Ranch, an area of hammocks and old-growth pines. This use area overlapped with male #42 (died 22 June 1995) and females #23 and #55.

The two Texas cougars released in FSSP (TX104 and TX106) have remained in the vicinity, moving generally within the Preserve and a similar sized block of adjacent habitat. The two animals released in the Bear Island Unit of BCNP (TX101 and TX102) have made more extensive movements, with one cat swimming to a mangrove island in Rookery Bay and skirting the fringes of two suburban areas near Naples and LaBelle without consequence; however both of these cougars appear to have settled into a large block of habitat adjacent to and northeast of the release area. Both cougars released in the southern BCNP have remained in the release areas although one cat (TX103) spent the first two weeks after release in mangroves, an unsuitable habitat (Figures 8 and 9).

Movements of translocated cougars in south Florida are generally within habitat areas utilized by resident Florida panthers and the occasional movements in atypical areas such as mangroves and suburban areas are probably typical of animals exploring unfamiliar terrain. Several of the woodlots and forested corridors used by Texas cougars have also been used by Florida panthers, and especially by dispersing subadults. Distances between consecutive locations of Texas cougars (Figure 10) showed an unusual number of longer distance movements that were more typical of patterns exhibited by male Florida panthers (Figure 11) than female Florida panthers with and without dens (Figures 12 and 13). Once these translocated cougars learn the boundaries of their new habitat they will likely move and behave in a similar manner as resident Florida panthers. Changes in movement patterns will be tested over time to evaluate the adjustment of translocated cougars to their new ecological and social environment in south Florida.

Social interactions have occurred between Texas cougars and both female and male panthers. All documented interactions have occurred in FSSP substantiating speculation that initial releases in this area would maximize social interactions and accelerate genetic exchange. No displacement of Florida panthers have occurred and no disruptions to the existing social organization have been observed. No Texas cougars have exhibited aberrant behaviors or unacceptable human interactions and none have been removed from the study. An old, non-reproducing Florida panther female #09 and TX104 have been located together on numerous occasions in FSSP. Movements of other Texas cougars have been more extensive and social interactions may have occurred with uncollared Florida panthers. TX106 was located with Florida panther male

#54 on 19 April 1995 and represented the first opportunity for mating.

Denning activity in late July will indicate the actual result of this initial social interaction between a Texas female and Florida male.

Genetic restoration of the panther population will be evaluated by comparing: 1. demographic performance, 2. morphological traits, 3. breeding history, and 4. genetic characteristics of Texas cougar-Florida panther offspring. Specific demographic components that will be monitored in the current and genetically restored Florida panther population include: 1. proportion of adult females producing litters each year, 2. average litter sizes, 3. sex ratios of litters, 4. kitten survival, 5. subadult survival to reproductive age, 6. age of first breeding, 7. recruitment into the breeding population, and 8. annual survival rates as adults.

Morphological traits and breeding history will be documented during capture activities, radiotelemetry observations, and field investigations. Evaluation of genetic introgression and characteristics will be facilitated through development of a contract with Dr. Steve O'Brien of the National Cancer Institute.

Cellular phone technology was successfully integrated with radiotelemetry to enable the efficient marking of kittens with transponders. Permanent marking of kittens with individually numbered transponders will be necessary to document kitten survival and achieve genetic restoration goals.

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LIST OF FIGURES

Figure 1. Florida panther genetic restoration study area in south Florida from 1 July 1994 to 30 June 1995.

Figure 2. Locations of female Florida panthers in south Florida from 1 July 1994 to 30 June 1995.

Figure 3. Locations of male Florida panthers in south Florida from 1 July 1994 to 30 June 1995.

Figure 4. Comparative home range sizes (convex polygon) for Florida panthers in south Florida from 1 July 1994 to 30 June 1995.

Figure 5. Locations of sub-adult male Florida panther #58 and his mother Florida panther #56 in south Florida from 10 February 1995 to 30 June 1995.

Figure 6. Capture sites of female Texas cougars translocated to south Florida for genetic restoration purposes.

Figure 7. Release sites of female Texas cougars translocated to south Florida for genetic restoration purposes.

Figure 8. Locations of three female Texas cougars (TX 101, 103, 106) released in south Florida for genetic restoration purposes from 5 April to 30 June 1995.

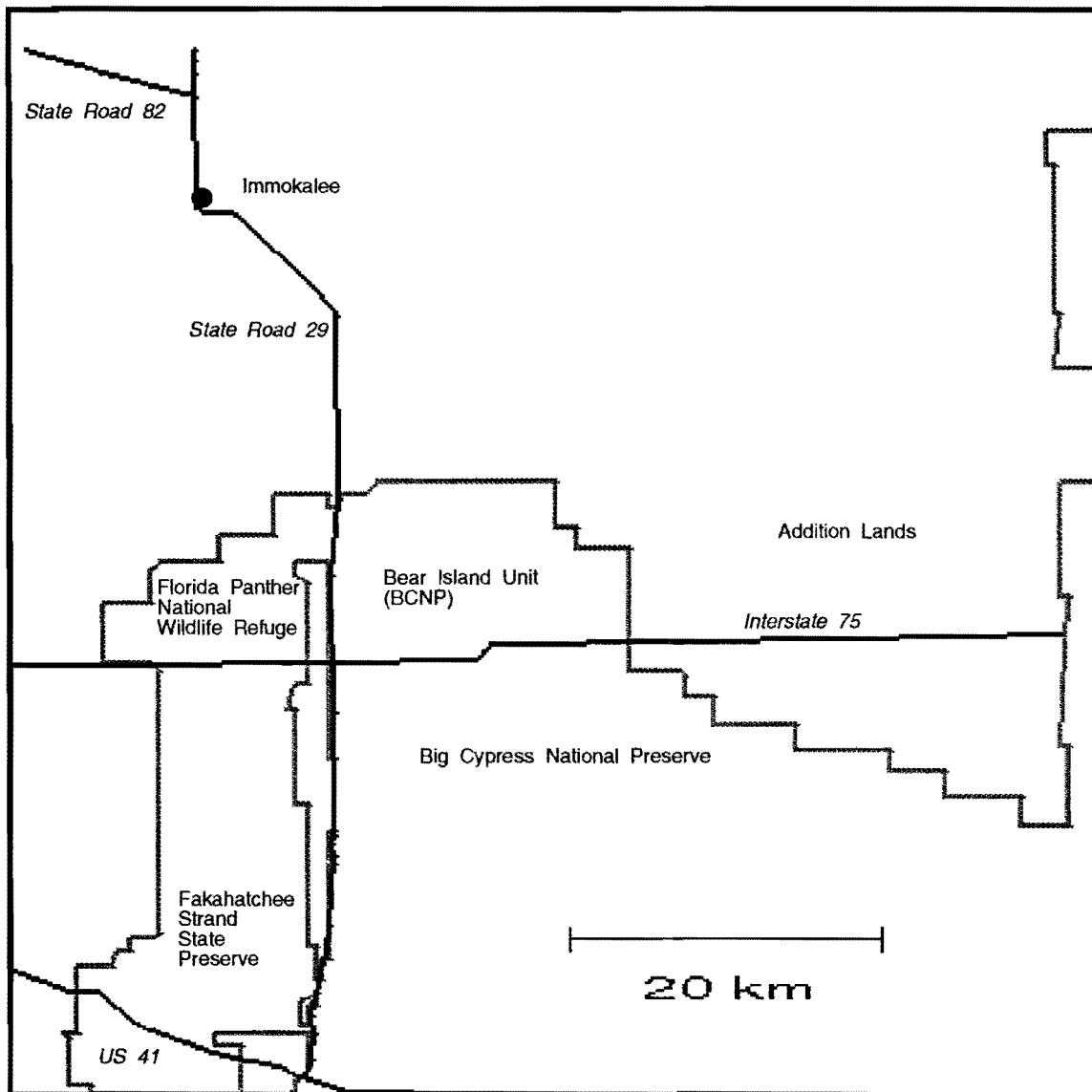
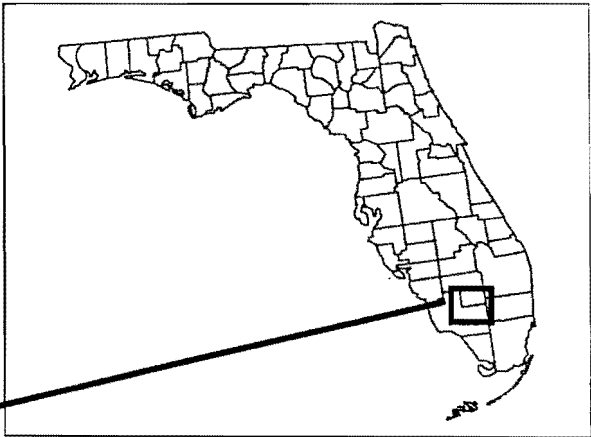
Figure 9. Locations of three female Texas cougars (TX 102, 104, 107) released in south Florida for genetic restoration purposes from 30 March to 30 June 1995.

Figure 10. Distances between consecutive locations for female Texas cougars released in south Florida from 1 April 1995 to 30 June 1995.

Figure 11. Distances between consecutive locations for male Florida panthers in south Florida from 1 April 1995 to 30 June 1995.

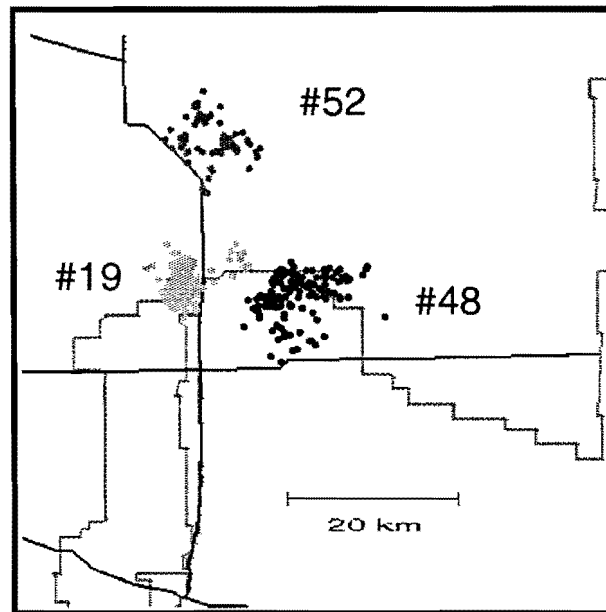
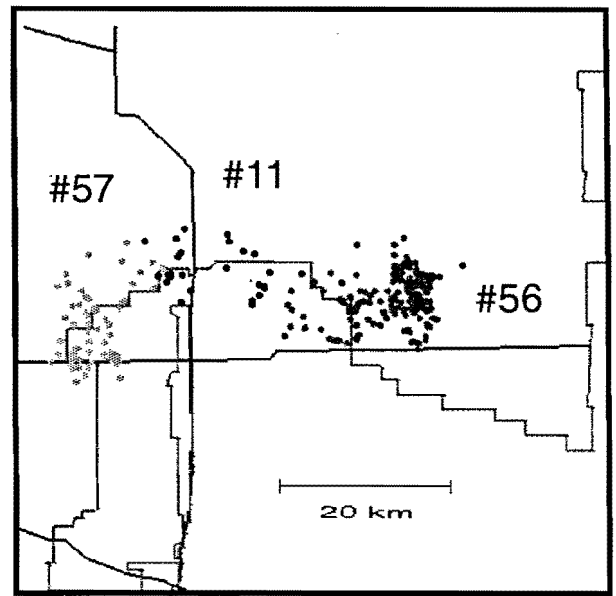
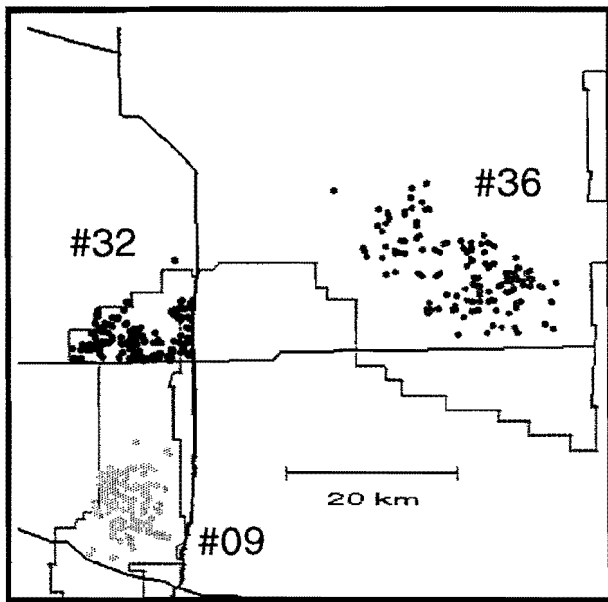
Figure 12. Distances between consecutive locations for female Florida panthers without denning activity from 1 April 1995 to 30 June 1995.

Figure 13. Distances between consecutive locations for female Florida panthers with denning activity from 1 April 1995 to 30 June 1995.



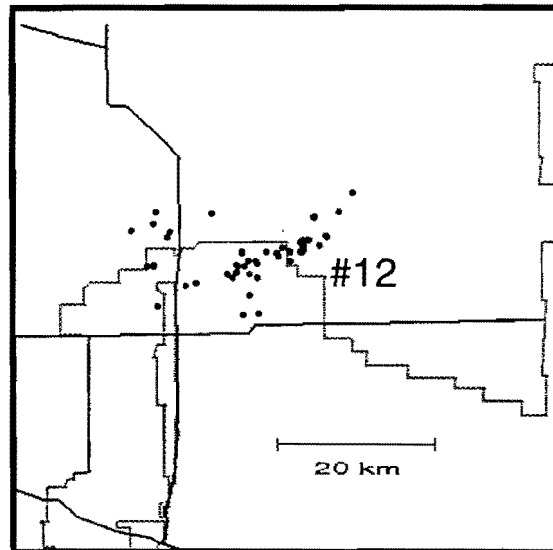
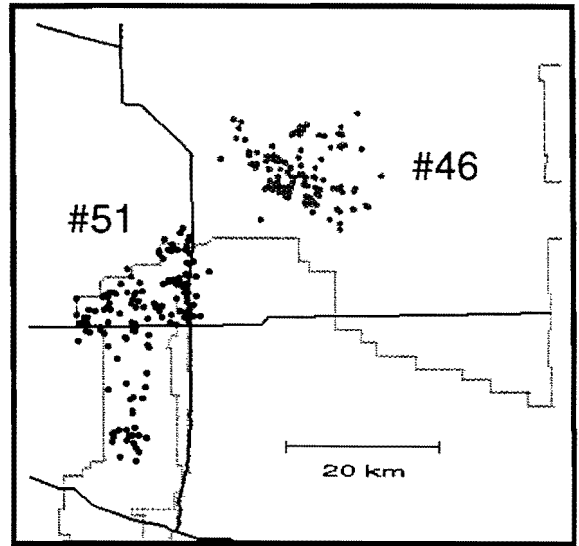
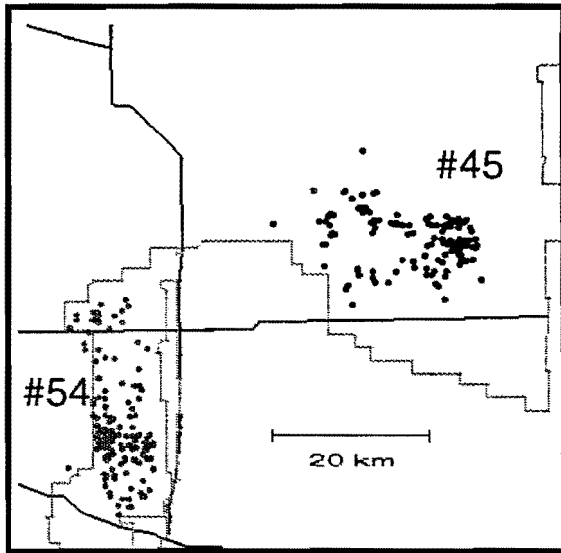
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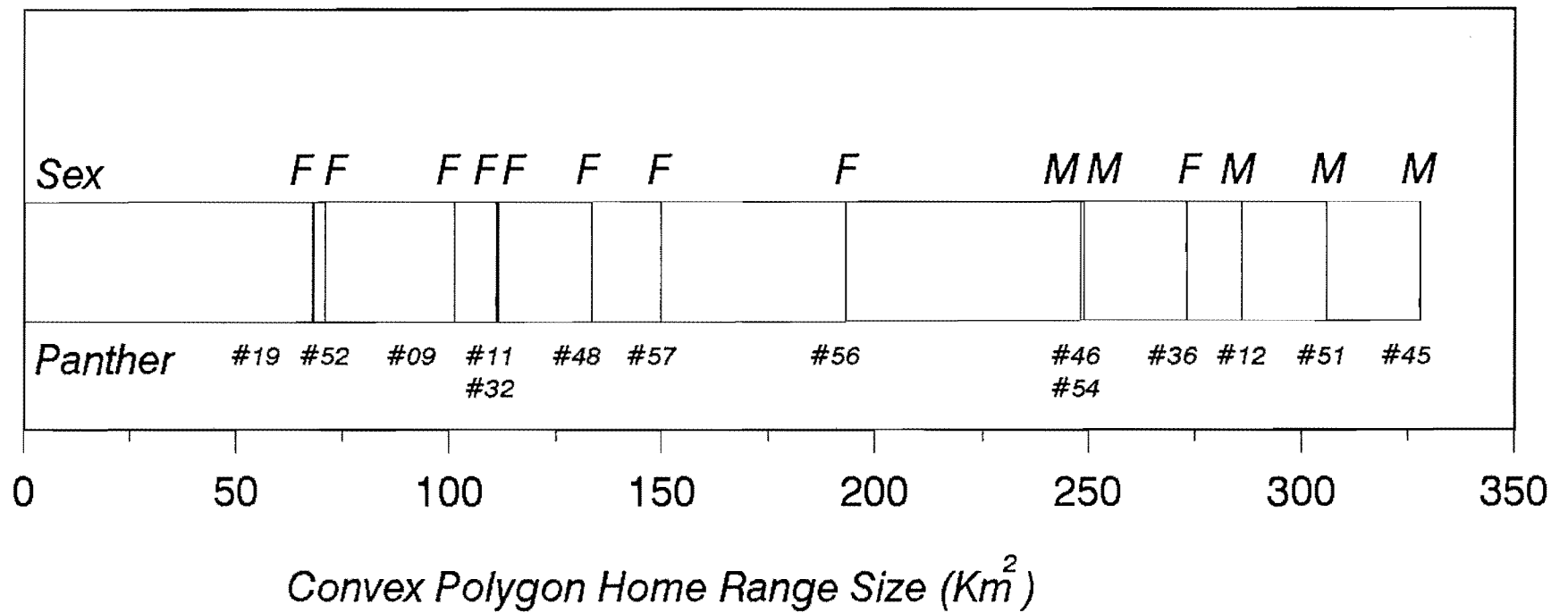
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Locations of Male Florida Panthers

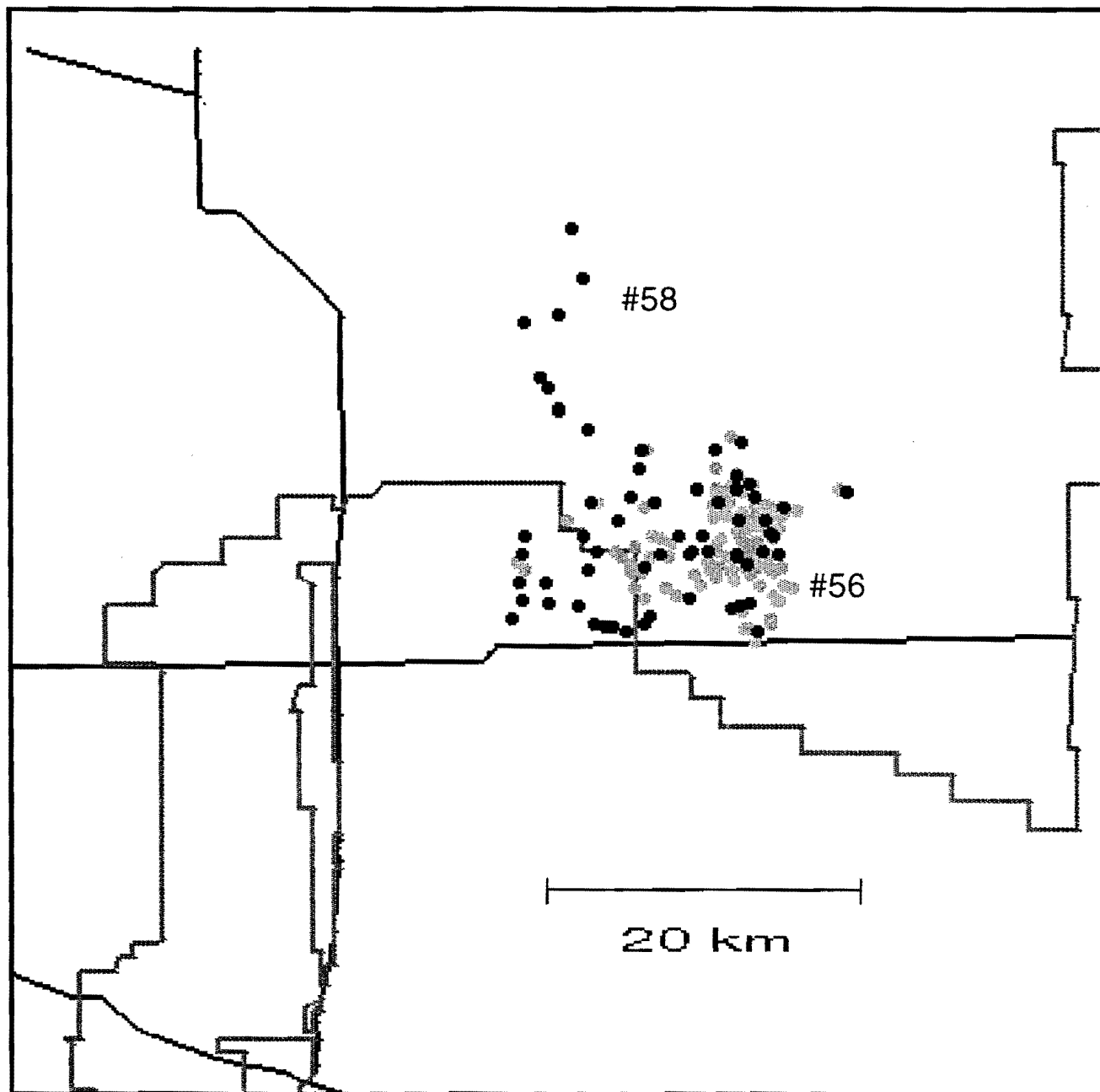
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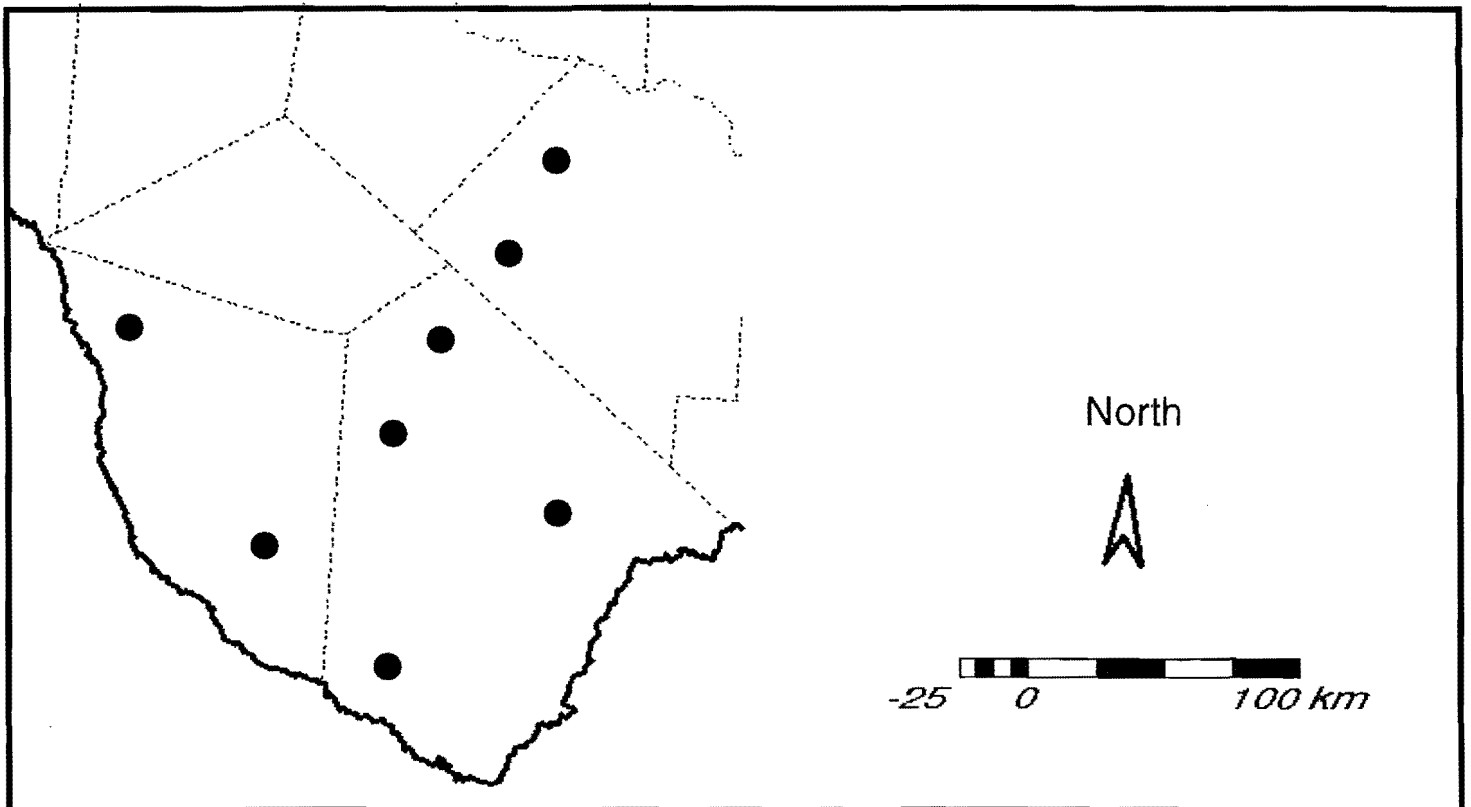
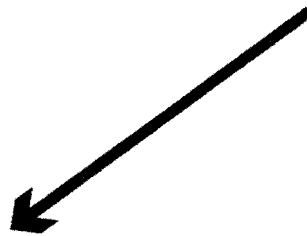
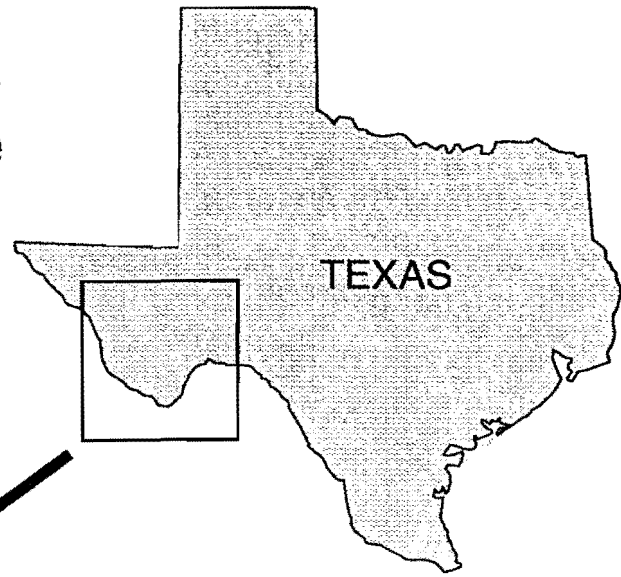


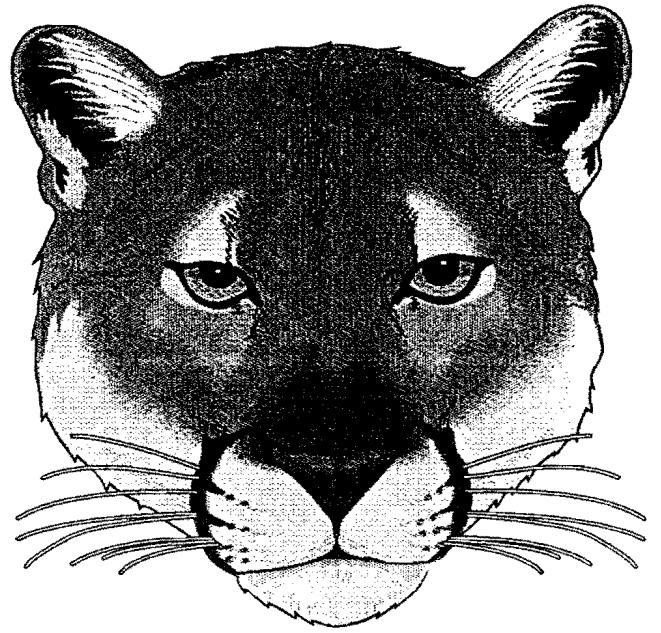
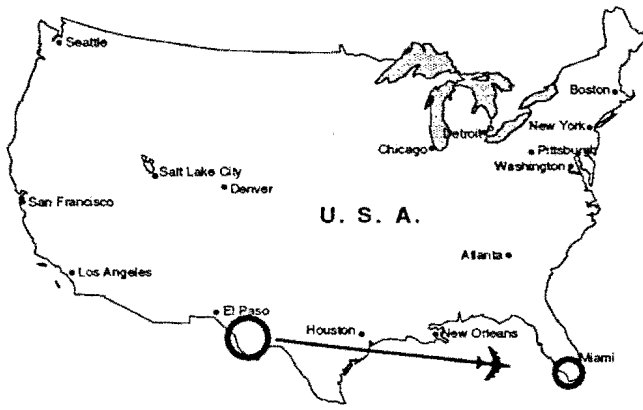
Locations of Florida Panther Sub-Adult Male #58

(And Locations of His Mother Florida Panther #56)

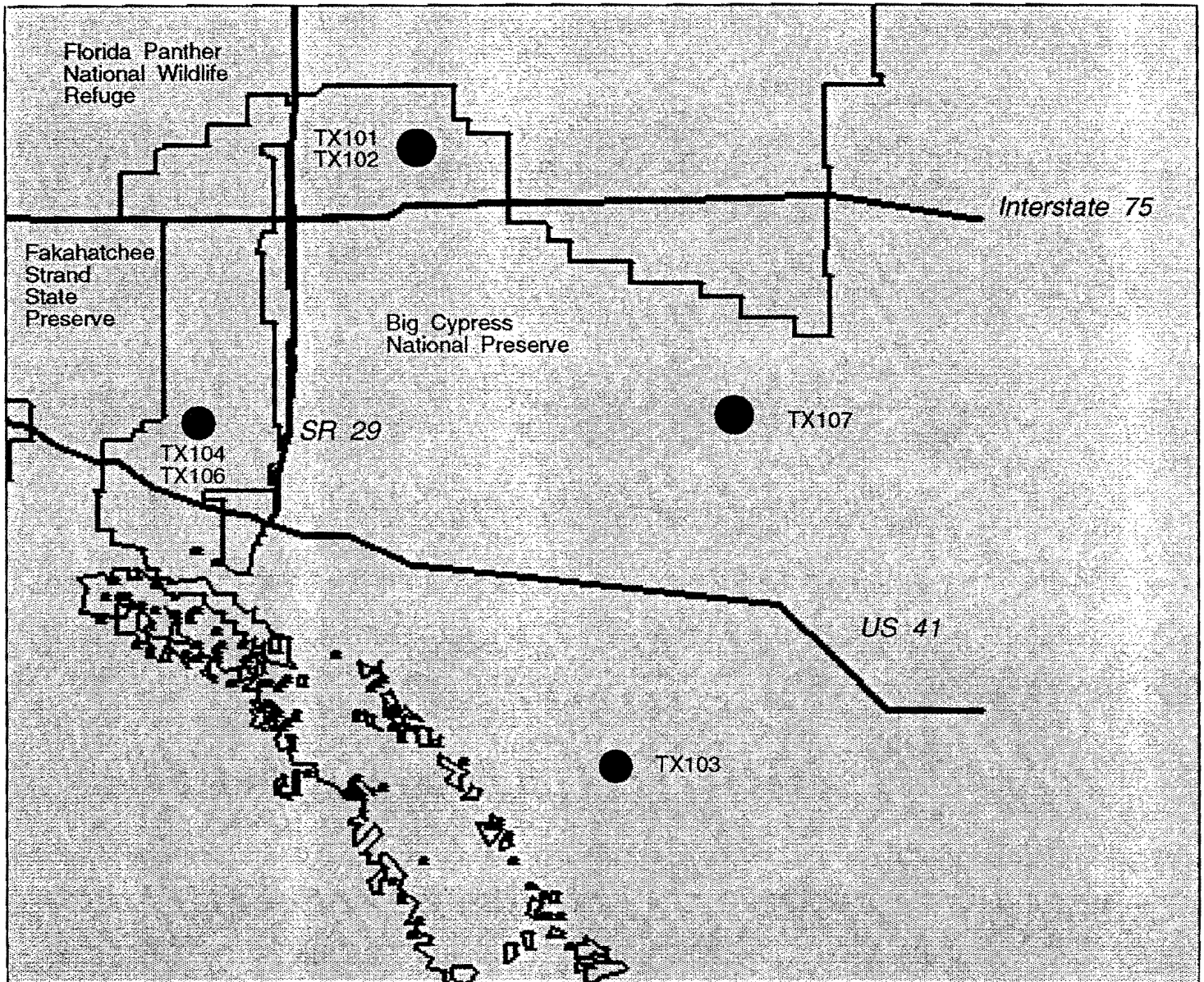


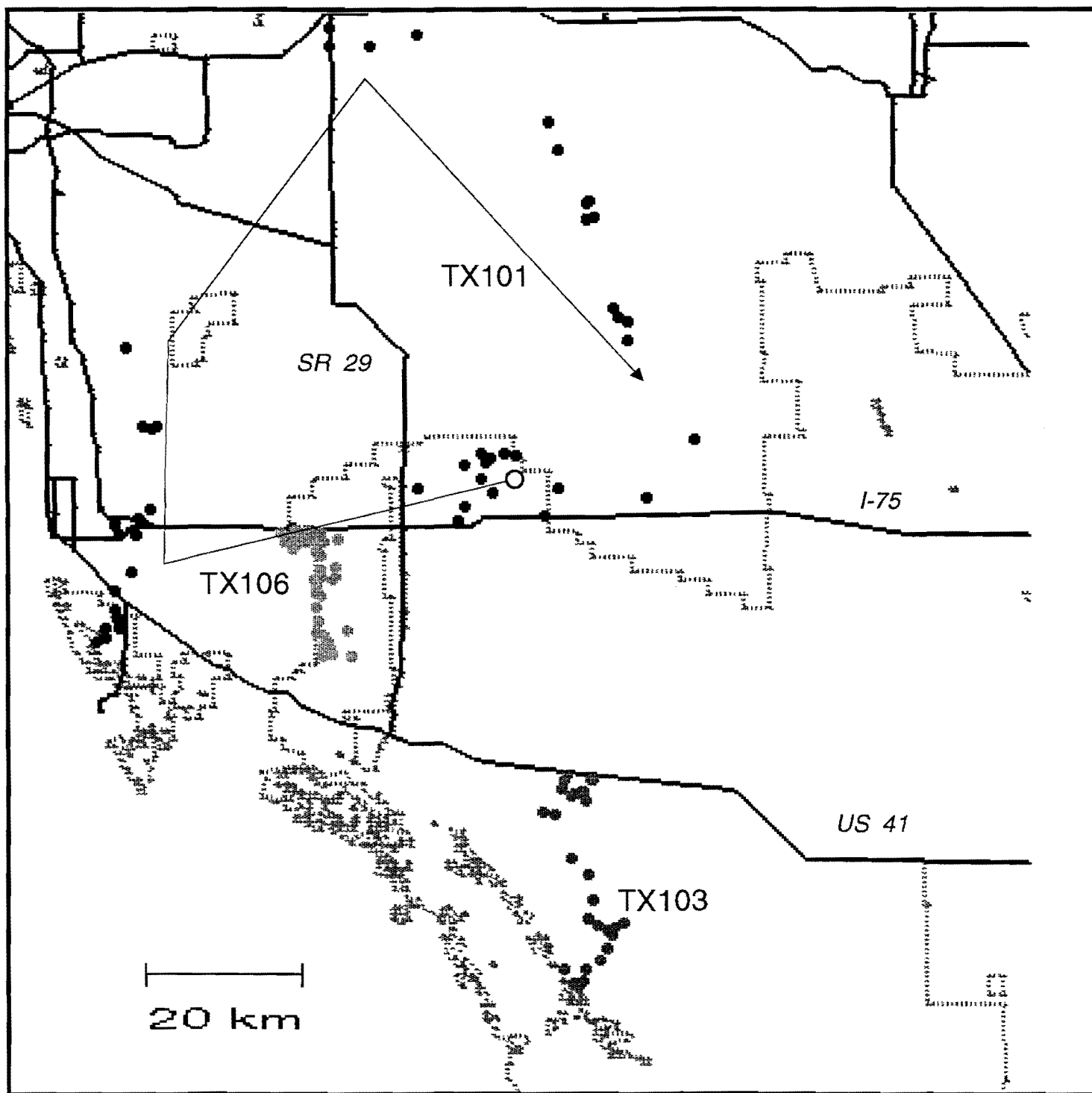
Capture Sites of the Translocated Female Texas Cougars

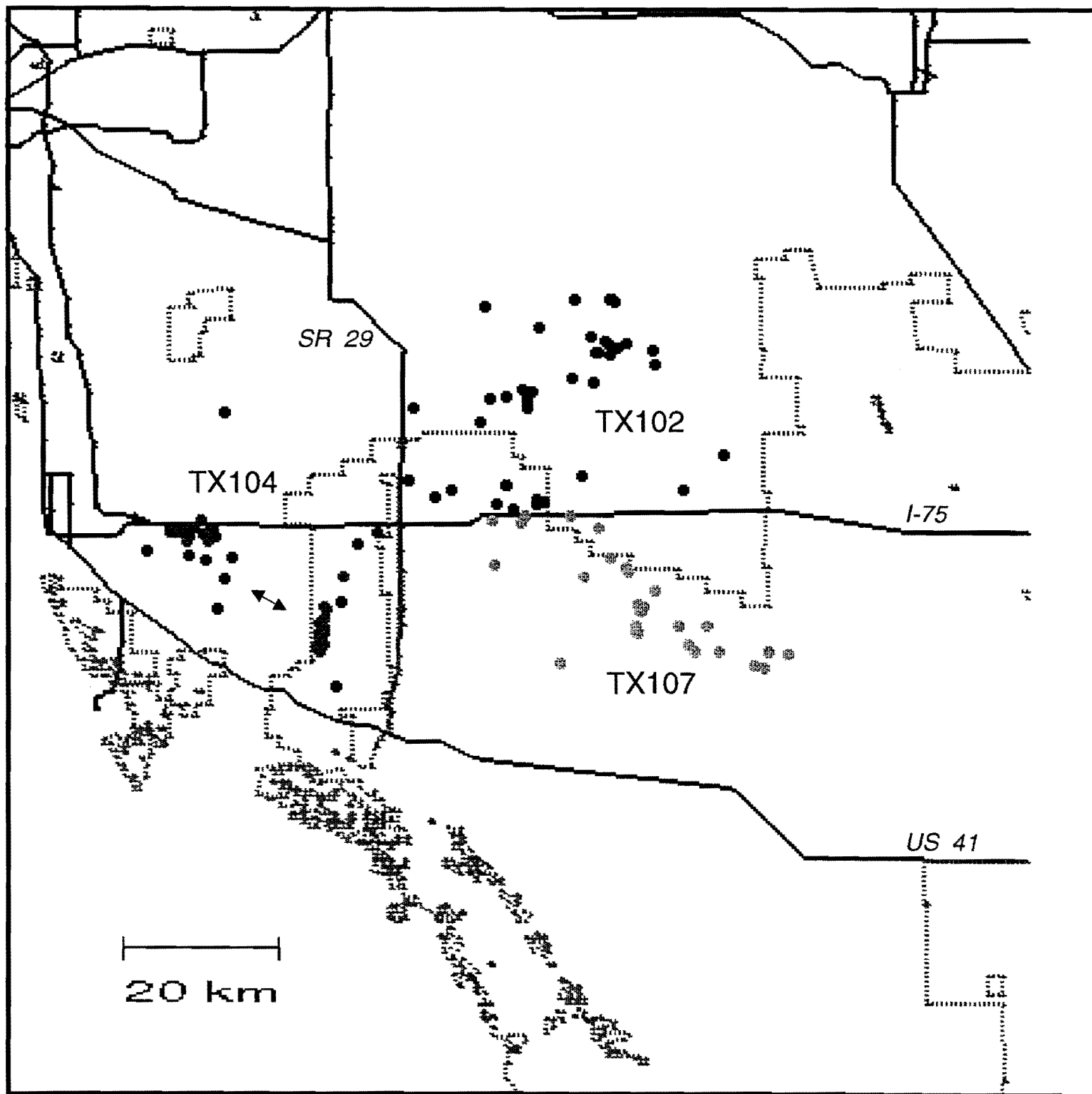




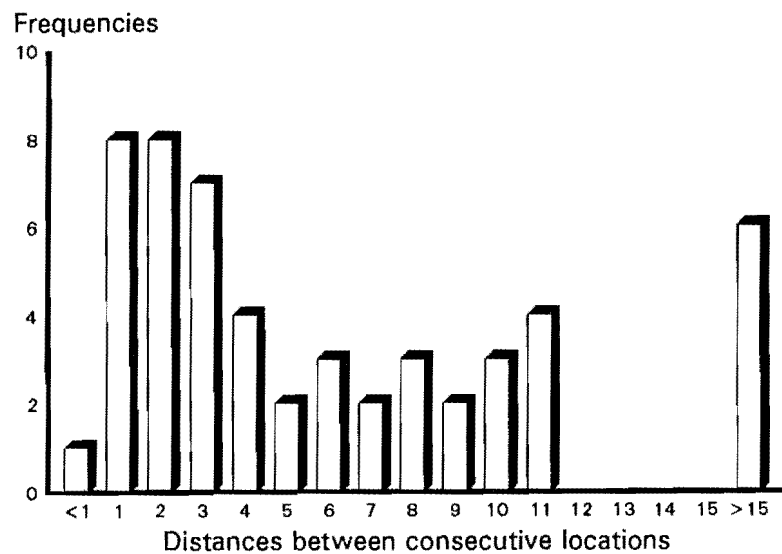
**Release Sites for
Female Texas Cougars**



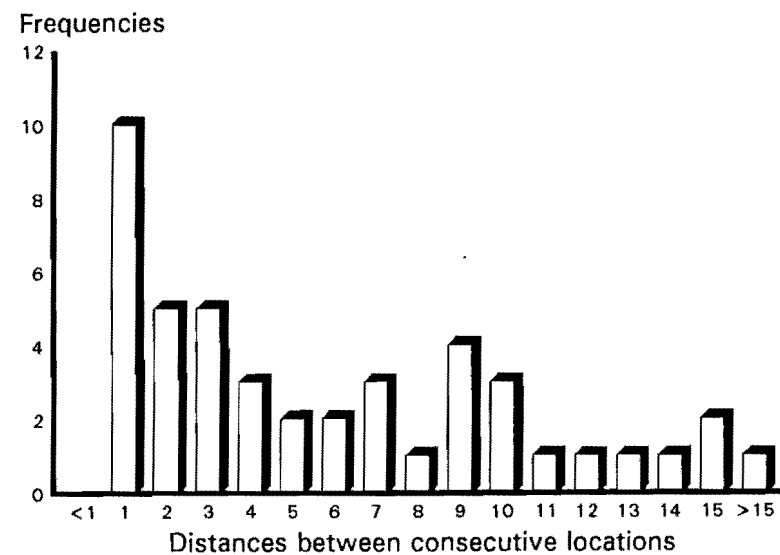




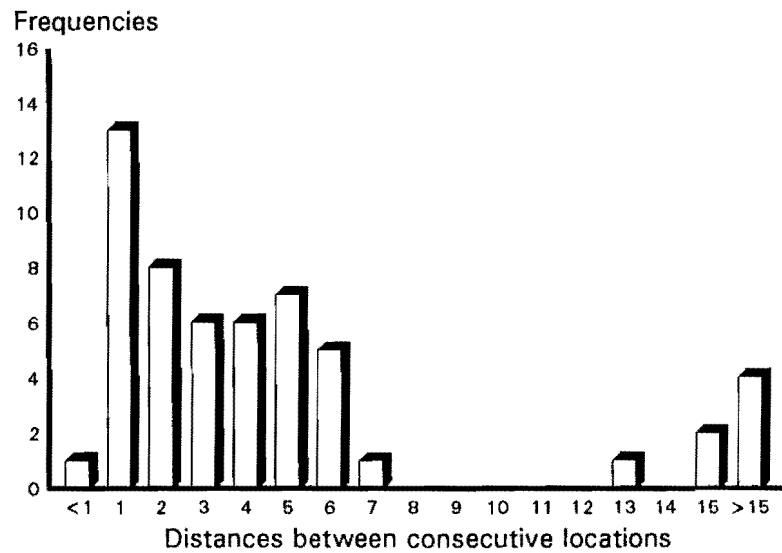
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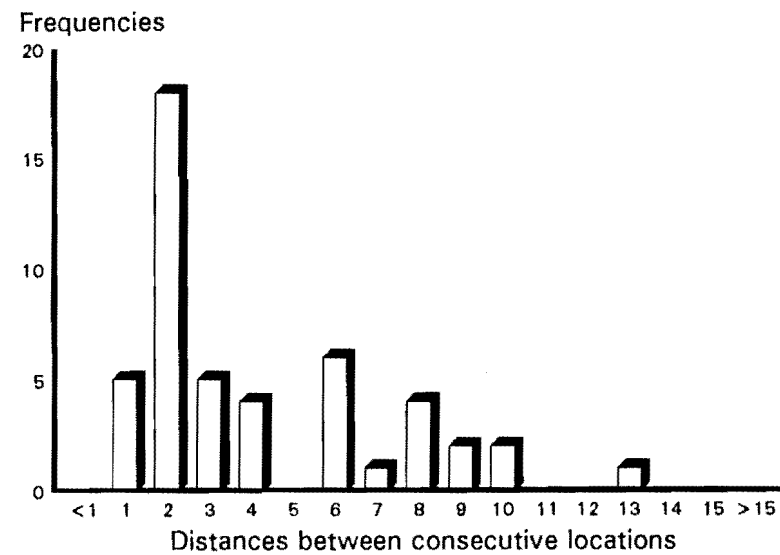
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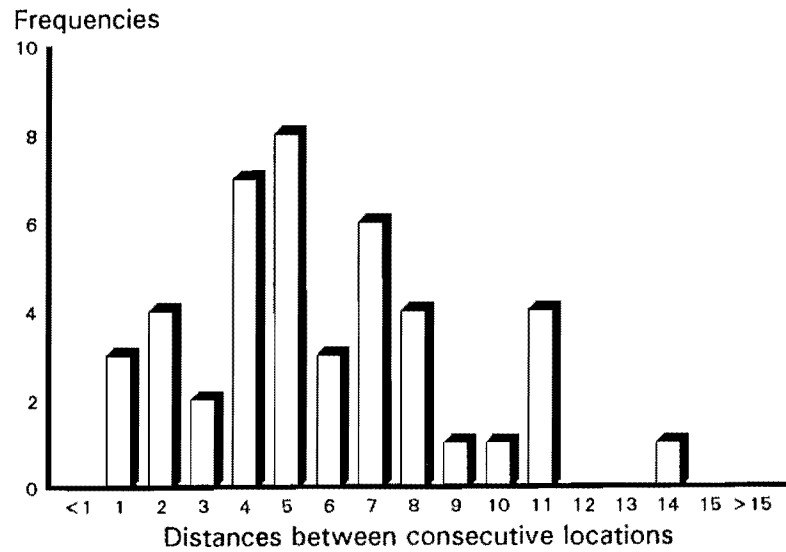
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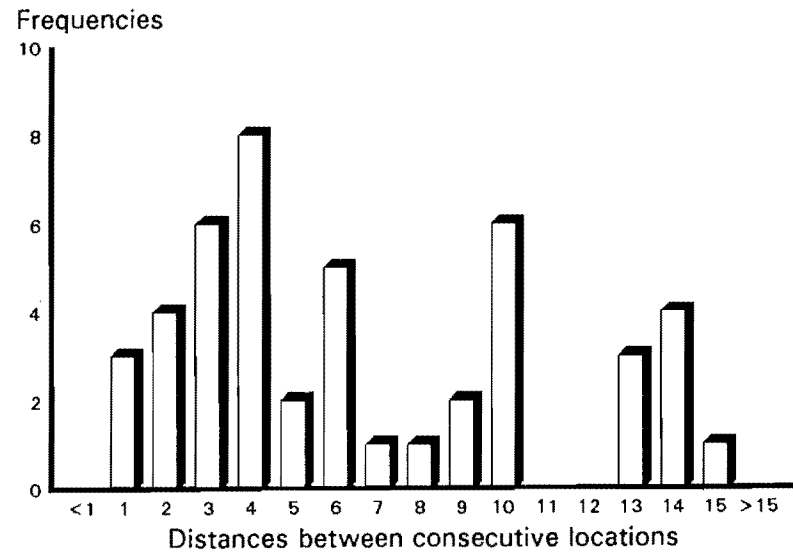
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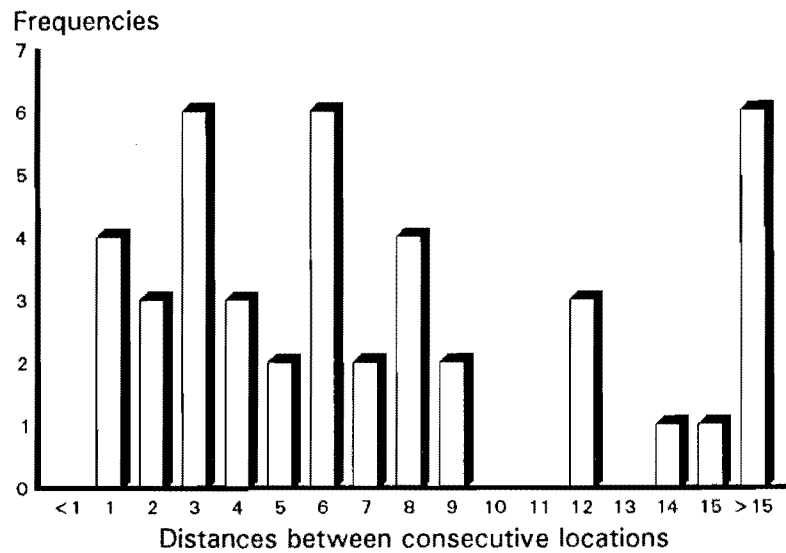
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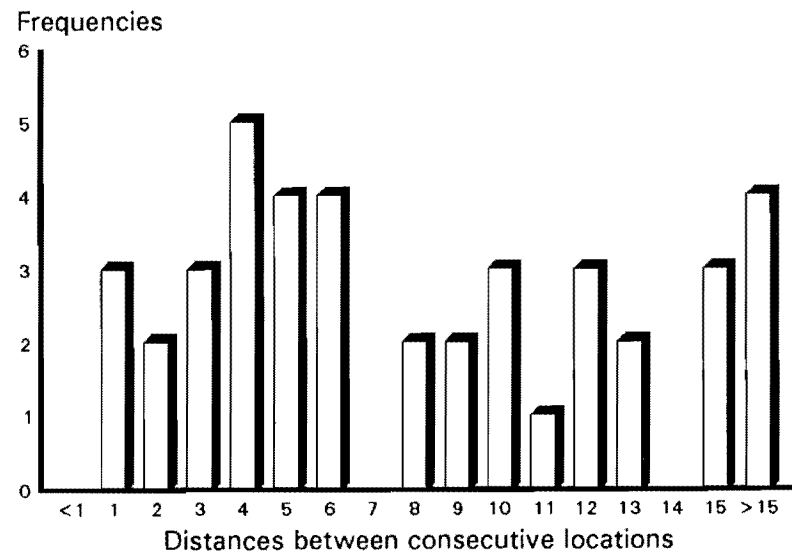
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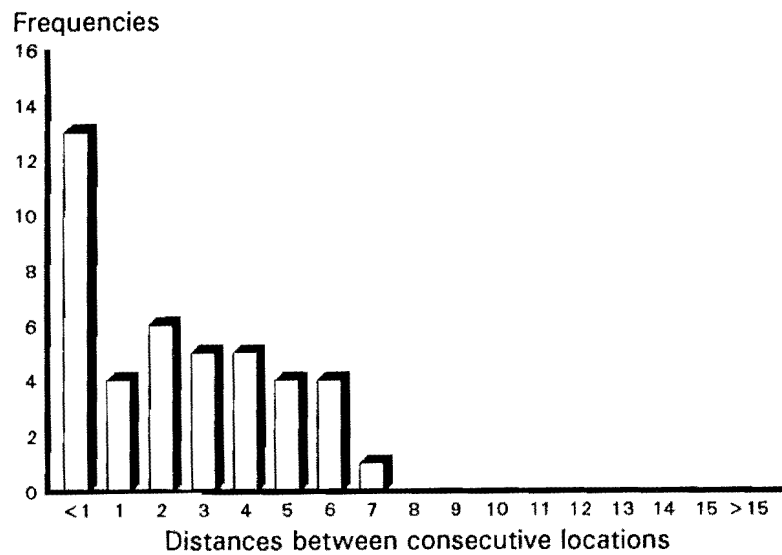
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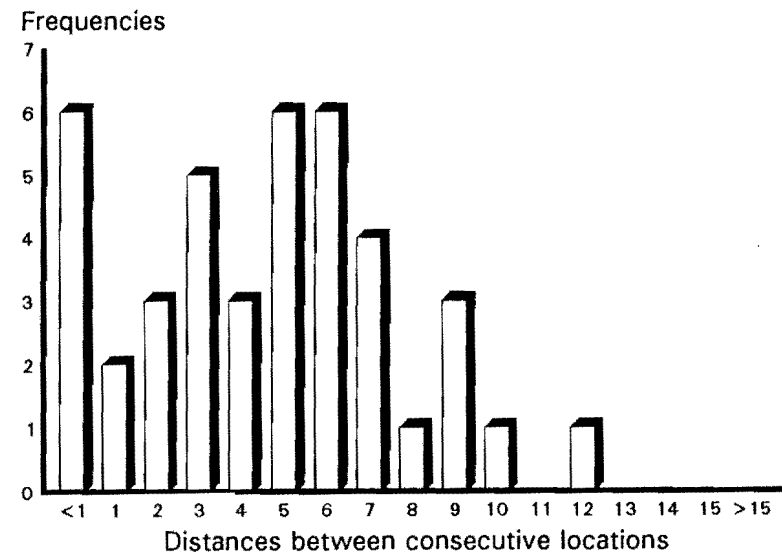
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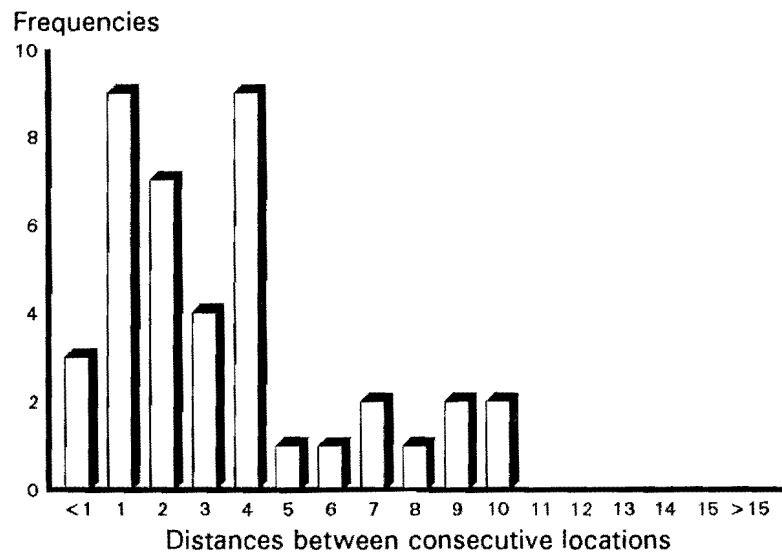
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#36



#57



LIST OF APPENDICES

Appendix I. Pre-release health protocol, sample collection, and selection criteria for cougars from west Texas being translocated to south Florida.

Appendix II. Comprehensive list of all radio-instrumented Florida panthers in south Florida from 10 February 1981 to 30 June 1995.

Appendix III. Summary of Florida panther mortalities and injuries in south Florida from 8 March 1978 to 30 June 1995.

Appendix IV. List of all known dens of radio-instrumented female Florida panthers in south Florida from June 1985 to June 1995.

Appendix I. Pre-release health protocol, sample collection, and selection criteria for cougars from west Texas being translocated to south Florida.

October 24, 1994

Mike R. Dunbar, MS, DVM

The threat of infectious disease in translocation programs is an issue of major concern because of the inherent risks involved with the introduction of pathogens into wild populations of naive animals. Conversely, introducing naive animals into populations harboring potential lethal organisms also has potential consequences. Therefore, biomedical information is needed for health screening of candidates prior to release. The following protocol will be used to assess the health and assure the release of healthy wild-caught cougars (Felis concolor stanleyana) from western Texas into southern Florida to co-mingle and breed with established Florida panthers (F. c. coryi).

This protocol also contains a list of samples and data to be obtained from each animal to be used in various research projects involved with genetics, toxicology, nutrition, parasitology, and others.

Finally, this document will serve as a treatment protocol to assure the release of healthy animals that will have the best chance of survival and least chance of transferring disease and parasites to the existing population in South Florida.

- I. Quarantine: Texas cougars destined for translocation from West Texas to South Florida will be isolated from all other animals upon capture, quarantine, and transportation. Animals will be quarantined for a period of at least 30 days, possibly extending to 60 days. Close observation shall be made to note appetite, attitude, behavior, and

condition of each animal. The attending veterinarian will take appropriate samples to diagnose any abnormal condition and administer appropriate therapy (note: it is important to make a definitive diagnosis of any abnormal condition before initiating any treatment).

Housing and feeding shall conform to established procedures for large captive felids. Human contact shall remain at a minimum.

- II. Physical Examination and Selection Criteria: Upon quarantine, each animal will receive a complete physical examination including body weight, measurements, dental exam (prophylaxis if needed), note of any congenital abnormalities, pregnancy determination (palpation, ultrasound, serum progesterone level), presence of heart murmurs (if positive, additional evaluation may include ECG, ultrasound, chest radiography, echogram, and other procedures if needed), and analysis of immune system function (optional).

Any animal with cowlicks, kinked tails, congenital cardiac abnormalities, or pregnant will be excluded from the project.

Near the end of the quarantine period, each animal will receive an additional follow-up examination, as deemed appropriate.

- III. Sample Collection: During the initial examination appropriate biomedical samples will be collected and the following tests and procedures will be conducted and evaluated:

1. Vaginal smear (evaluate cell types to determine stage of estrus, presence of infection, or abnormal cell types)
2. Complete blood count (CBC)
3. Serum Chemistry (panel)

4. Serology (initially and 2 weeks later, if needed)
 - a. Feline infectious peritonitis virus (FIP)/Feline enteric corona virus (FECV)
 - b. Feline immunodeficiency virus (FIV)/puma lentivirus (PLV) (by Western Blot)
 - c. Feline leukemia virus (FeLK)
 - d. Feline panleukopenia virus (FPLV)
 - e. Feline calicivirus (FCV)
 - f. Feline viral rhinotracheitis virus (FVRV)
 - g. Pseudorabies virus (PRV)
 - h. Canine distemper virus (CDV)
 - i. Feline syncytia-forming virus (FeSFV)
 - j. Brucella (abortus, suis)
 - k. Toxoplasma gondii
 - l. Rabies (titer)
5. Culture (Bac-T and Virology) (swab)
 - a. nasal
 - b. oro-pharyngeal/tonsillar crypt
 - c. rectal
 - d. fecal (Salmonella sp., Campylobacter sp., etc.)
6. Parasites:
 - a. Blood parasites (Microfilaria, Cytauxzoon felis, Haemobartonella sp.) (In EDTA whole blood or methanol fixed blood smears)

- b. fecal (flotation, sedimentation, and direct smear). On flotation, pay particular attention for presence of Cylicospirura subequalis. If present, use appropriate treatment and recheck.
 - c. Perform Baermann test for presence of lungworm, especially for Vogeloides felis. If present, treat appropriately and recheck.
 - d. Identify representative samples of ectoparasites, remove, and preserve.
 - e. Perform eye exam for presence of Metathelazia californica. If present, use appropriate treatment, remove and preserve in alcohol for identification. Recheck.
- 7. Toxicology: (blood in heparin and fat (or muscle) biopsy)
 - a. Heavy metal screen
 - b. Organochlorine screen
 - c. PCB (total)
 - d. others as needed
- 8. Nutritional
 - a. Trace mineral screen
- 9. Metabolic
 - a. T_3 and T_4
 - b. Cortisol (optional)
- 10. Genetics (blood and tissue biopsy)
- 11. Reproductive hormone
 - a. Estradiol

- b. Testosterone
 - c. FSH (optional)
 - d. LH (optional)
- 12. Urinalysis
 - 13. Hair (2.0 + gm desirable - freeze for storage)
 - 14. Radiographs - survey (optional)
 - 15. Bank (store) 10-20 mls serum (freeze)

IV. Treatment:

- 1. Vaccines: During quarantine and after collection of samples at two weeks (if needed), each animal will receive vaccinations with killed virus vaccines for the following:
 - a. FVR
 - b. FCV
 - c. FPLV
 - d. rabies
- 2. Anti-parasite treatment:
 - a. Each animal will be sprayed with an appropriate paracidal chemical for ticks, fleas, and lice. (Chemical will not be a known estrogenic or anti-androgenic insecticide such as Chlorpyrophos or synthetic pyrethroid.)
 - b. Ivermectin (S.Q.)
 - c. Praziquantel
 - d. Note: Evaluate effectiveness with fecal analysis (eggs/oocysts count, Baermann, eye exam).

- V. A health certificate will be issued for each animal by a licensed (state of Texas), accredited veterinarian not exceeding 30 days prior to entering the state of Florida.
- VI. Complete necropsies will be performed on all animals that die during shipment or quarantine.

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Appendix II. Comprehensive list of all radio-instrumented Florida panthers in south Florida from 10 February 1981 to 30 June 1995.

Cat #	Sex	Capture Date	Age	Birth Date	Parents	Use Area	Death Date	Cause of Death
1	M	2/10/81	10	-	-	Fakahatchee	12/14/83	vehicle (SR 84 mm18)
2	M	2/20/81	10	-	-	Fakahatchee	11/29/84	panther
3	F	1/23/82	9	-	-	Fakahatchee	1/17/83	capture
4	M	1/27/82	7-8	-	-	Fakahatchee	4/18/85	vehicle (SR 84 mm17)
5	F	2/23/82	7-8	-	-	Fakah. Conserv. Club	11/23/82	unknown
6	M	2/27/82	6-8	-	-	Raccoon Pt. (North Swamp)	4/16/82	unknown
7	M	3/2/82	6-7	-	-	Raccoon Pt.- Fakahatchee	10/26/85	vehicle (SR 29 C. prison)
8	F	3/25/84	9-10	-	-	Fakahatchee	8/20/88	liver failure, old age
9	F	1/26/85	3-4	-	-	Fakahatchee	-	-
10	M	1/15/86	5 mos.	8/85	#09, ?	GG Estates, Fakahatchee	1/27/87	panther #12
11	F	1/21/86	4-5	-	-	Bear Island, Price's	-	-
12	M	1/28/86	5	-	-	Bear Is., FPNWR, FSSP	11/7/94	panther #46
13	M	2/27/86	4-5	-	-	Bear Is. to Alico	12/14/87	vehicle (SR 29 Sunniland)
14	F	12/07/86	5-6	-	-	Everglades	6/20/91	unknown
15	F	12/13/86	5-6	-	-	Everglades	6/10/88	panther
16	M	1/12/87	12-14 mos.		#14, ?	Everglades to Stairsteps	-	-
17	M	1/20/87	6-7	-	-	Gum Swamp to Nobles	7/20/90	unknown
18	F	1/22/87	7-8	-	-	Gum Swamp, Scofield's, BCSIR	10/1/90	panther (#28?)
19	F	2/9/87	9 mos.	5/86	#11, #12	Bear Is., Prices, FPNWR	-	-

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Cat #	Sex	Capture Date	Age	Birth Date	Parents	Use Area	Death Date	Cause of Death
20	M	3/10/87	3-4	-	-	Alico to Bear Island	8/24/88	heart defect
21	F	3/16/87	12-14 mos.	-	#14,?	Everglades	-	in captivity
22	F	3/18/87	5-6 mos.	-	#15,?	Everglades	7/??/91	infection
23	F	3/18/87	5-6 mos.	-	#15,?	Everglades, Raccoon Point	-	-
24	M	1/30/88	3-4	-	-	Highlands Co.	8/22/88	unknown
25	M	2/16/88	4-5	-	-	FPNWR	8/26/88	infection from panther fight
26	M	3/1/88	5-6	-	-	BCSIR, Nobles to Bear Is.	7/8/94	panther #46
27	F	4/11/88	2-3	-	-	Everglades	7/23/89	mercury
28	M	11/29/88	1.5	-	-	Ft. Myers, Lake Hicpochee, Gum Swamp to Nobles	9/25/92	panther (#26?)
29	M	1/3/89	6.5 mos.	5/88	#11, #20	Bear Island, Gum Swamp	5/27/92	pseudorabies
30	M	1/6/89	9 mos.	3/88	#19, #13	Bear Is., Prices, FPNWR, FSSP	1/29/90	panther #37
31	F	1/12/89	7-9	-	-	FPNWR	3/3/94	vehicle (SR 29 Sunniland)
32	F	2/3/89	2-2.5	-	(#31?)	FPNWR	-	-
33	M	3/5/89	1.5-2	-	-	Loop Rd. to Gum Swamp	11/25/89	rabies
34	M	1/8/90	10 mos.	3/89	#31, #12?	FPNWR, BCSIR, Gum Swamp	11/15/93	esophageal puncture
35	M	1/15/90	10 mos.	3/89	#31, #12?	Regency Farms	1/22/90	bacterial infection from capture
36	F	1/27/90	4-5	-	-	Nobles	-	-
37	M	1/30/90	3-4	-	-	FSSP, FPNWR, Bear Is.	11/26/90	vehicle (SR 29 Miles City)
38	F	2/8/90	4.5	-	-	Raccoon Pt., Cons. Area 3b	8/4/94	pleuritis in chest

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Cat #	Sex	Capture Date	Age	Birth Date	Parents	Use Area	Death Date	Cause of Death
39	M	3/19/90	3-4	-	-	Everglades	6/18/90	pyrothorax
40	F	2/26/90	1.5-2	-	(#36?)	Nobles, Bear Island	-	-
41	F	2/28/90	1.5-2	-	(#36?)	Nobles, BCSIR	9/21/90	panther #28
42	M	3/6/90	11 mos.	5/89	#14, #16	Everglades, Raccoon Pt.	-	-
43	M	5/1/90	9.5 mos.	7/89	#19, #12	FPNWR, Nobles	11/1/91	panther #26
44	M	4/30/91	6 mos.	11/90	#40, #26	Bakers, Naples, ENP, Raccoon Pt.	7/6/93	panther #42
45	M	5/8/91	6 mos.	11/90	#19, #12	FPNWR, Nobles, BCSIR	-	-
46	M	1/30/92	2-2.5	-	-	Bear Island, Gum Swamp, Okaloacoochee Slough	-	-
47	M	2/21/92	6 mos.	7/91	#11, #12	Bear Island, Belle Meade, FSSP	2/19/93	panther #51
48	F	2/24/92	4 mos.	10/91	#31, #12	FPNWR, Bear Island	-	-
49	F	2/25/92	2	-	(#36?)	Nobles	-	-
50	M	3/4/92	8 mos.	5/91	#36, #26	Nobles, Allico, Devils Garden	12/6/93	vehicle (CR 846 5 mi E of Immokalee)
51	M	3/26/92	3	-	-	FSSP, FPNWR	-	-
52	F	5/5/92	6 mos.	10/91	#31, #12	FPNWR, Sadie Cypress	-	-
53	M	2/10/93	10 mos.	4/92	#19, #12	FPNWR	2/26/93	panther (uncollared ♂)
54	M	2/10/93	10 mos.	4/92	#40, ?	Bakers, FPNWR, FSSP, Deep Lake	-	-
55	F				#23, #42	Raccoon Pt.	-	-

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Cat #	Sex	Capture Date	Age	Birth Date	Parents	Use Area	Death Date	Cause of Death
56	F	2/3/94	2-3	-	-	Nobles, Bakers	-	-
57	F	1/31/95	3	-	-	FPNWR	-	-
58	M	2/8/95	8 mos.	4/94	#56, ?	Addition Lands	-	-

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Appendix III. Summary of Florida panther mortalities and injuries in south Florida from 8 March 1978 to 30 June 1995.

ID#	DATE	SEX	AGE AT DEATH	LOCATION
Road Kills				
G 80-4	12-23-79	F	1.5-2.5	SR 29 just No. of Alligator Alley
G 80-15	07-02-80	M	1.5-2.5	SR 29 near Sunniland
G 81-19	04-19-81	F	2-3	SR 29 near Copeland
G 83-22	03-19-83	M	2-3	US 27 - Palmdale
#01	12-14-83	M	12-14	Alligator Alley mm 18
G 84-26	11-18-84	F	8-10	Alligator Alley mm 16
G 85-BNZ	01-10-85	F	18-24 mos.	Alligator Alley mm 16
#04	04-18-85	M	12+	Alligator Alley mm 17
#07	10-26-85	M	10	SR 29, 4 mi. So. of Alligator Alley
—	11-15-86	F	4-5	Alligator Alley mm 16.5
#13	12-14-87	M	6-8	SR 29 - Sunniland
RK-850	01-25-89	M	3	CR 850 near Immokalee
RK-846	06-18-90	M	10 mos.	CR 846, 1 mi. E. of 833 - Hendry Co.
#37	11-26-90	M	4-5	SR 29, 1/2 mi. No. of I-75 - Collier Co.
Kitten of #11	02-04-91	F	9 mos.	SR 29, 6 mi. No. of I-75 - Collier Co.
Kitten of #19	11-09-92	F	7 mos.	SR 29 - Sunniland
—	08-09-93	M	2-3	Daniels Rd. 1 mi. E. of I-75
#50	12-06-93	M	2.5	CR 846 - 5 mi. E. of Immokalee
Kitten of #52	02-28-94	M	8 mos.	3 mi. No. along County Line Rd
#31	03-03-94	F	12-14	SR 29 - Sunniland
#52	1/14/95	F	3.3	CR 846 4 m E Immokalee, (Dupree Road)
Road Injuries				
Big Guy	11-02-84	M	-	US 41, 1/4 mi. E. of Turner River Rd.
—	05-12-85	F	-	CR 951, 2 mi. No. of US 41
#20	06-17-87	M	-	CR 858, .8 mi. E. of SR 29
#28	11-29-88	M	-	Near Daniels Rd. at Ft. Myers Airport
—	04-07-92	M	-	Alico Rd. - 1/3 mi. E. of I-75

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ID #	DATE	SEX	AGE AT DEATH	LOCATION
Shootings				
G 78-65	03-08-78	M	2-3	L-28, Dade County
Canal Point	05-22-83	F	adult	L-8 canal near White Belt Ranch, Palm Bch Co.
G 83-75	12-01-83	M	3-6	Seminole Indian Reservation, Hendry Co. (James Billie)
G 84-20	10-30-84	F	2-3	Corbett WMA, Palm Bch. Co. (Elmer Brooker)
G 85-CS	03-26-85	F	2-3	CSSP, Collier Co. (skeleton)
#09	??-??-86	F	not fatal	Golden Gate Estates, So. of SR 84, Collier Co.
Intraspecific Aggression				
#02	10-27-84	M	14+	FSSP, panther fight
#10	01-27-87	M	16-20 mos.	Mud Lake Strand - by adult male panther
#25	08-26-88	M	4-5	FPNWR - bacterial infection from panther fight
#30	01-29-90	M	22 mos.	Killed by adult male #37 - FSSP
#41	09-26-90	F	2	Hendry Co. W. of Seminole Reservation - killed by male #28
#18	10-03-90	F	9	Hendry Co. So. of CR 846 near Rock Lake - killed by male #28
#43	11-01-91	M	2	Indian Reservation, Hendry Co. - killed by adult male #26
#28	09-25-92	M	5.5	Indian Reservation, Hendry Co. - possibly killed by male #26
#47	02-19-93	M	18 mos.	Killed by male #51 in FSSP
#53	02-26-93	M	11 mos.	Killed by uncollared male No. of FPNWR
#44	07-06-93	M	2.5	Killed by adult male #42 - Raccoon Point
#26	07-08-94	M	11-12	4 mi. E. Hendry Prison - killed by male #46 - punctured skull
#12	11-08-94	M	13-14	Shouts property, Hendry County - died of infected injury from fight with #46
Other or Unknown Causes				
#06	04-16-82	M	6-7	NE BCNP - unknown cause
#03	01-17-83	F	9+	FSSP - capture mortality

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ID#	DATE	SEX	AGE AT DEATH	LOCATION
#05	11-18-83	F	8-9	Fakahatchee Conserv. Club - unknown cause
#08	08-20-88	F	13-15	Gainesville - liver failure (old age)
#24	08-22-88	M	3-5	Glades Co. near Palmdale - unknown
#20	08-24-88	M	4-5	Bear Island - congenital heart defect
#33	11-25-89	M	3	2 mi. NW of Hendry Prison - rabies
#35	01-24-90	M	10 mos.	Gainesville - bacterial infection
#17	07-23-90	M	9	Addition lands near Tangerine Camp - unknown
#29	05-27-92	M	4	Hendry Co., Gum Swamp - pseudorabies
#34	11-15-93	M	5	SE Hendry Co. next to L28 interceptor - bacterial infection from lacerated esophagus
#38	8-4-94	F	9	Monument Unit - pleuritis from chest puncture
#42	6-22-95	M	6	Raccoon Point - unknown

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Appendix IV. List of all known dens of radio-instrumented female Florida panthers in south Florida from June 1985 to July 1995.

Panther #	Den Date	Location	UTM Coordinates	Habitat	# of Kittens	Kittens handled	Sire
09	6/85	Golden Gate Estates	?	?	1	#10 ♂	unknown
	6/87	upper FSSP ¹	454.4, 2891.3	hardwood hammock	1 ♀	-	#12
	5/89	FSSP	461.1, 2878.6	mixed swamp	?	-	unknown
	7/90	FSSP	462.3, 2882.2	mixed swamp	2	#202 ♂, 203 ♂	#37
	6/93	FSSP	456.0, 2873.8	hardwood hammock	unsuccessful?	-	#51
11	5/86	Bear Island	468.1, 2896.4	pine/palmetto	3	#19 ♀	#12
	5/88	Bear Island	468.1, 2896.4	pine/palmetto	1?	#29 ♂	#20
	4/90	Bear Island	469.9, 2898.7	pine/palmetto	1?	♀ (roadkill)	#12
	7/91	Price's	469.8, 2907.1	hardwood hammock	1?	#47 ♂	#12
	5/93	Bear Island	468.9, 2896.2	hardwood hammock	?	-	#12?
14	4/89	ENP-LPK	537.2, 2799.2	hardwood hammock	2	#42	#16
	3/91	ENP-LPK	536.9, 2808.0	hardwood hammock	unsuccessful		#16
15	5/88	ENP-LPK	525.1, 2807.2	hardwood hammock	unsuccessful		#16
19	3/88	Price's	468.1, 2906.0	hardwood hammock	4	#30 ♂	#13
	7/89	FPNWR ³	460.6, 2893.7	hardwood hammock	1?	#43 ♂	#12

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Panther #	Den Date	Location	UTM Coordinates	Habitat	# of Kittens	Kittens handled	Sire
	11/90	Rock Spring Island	460.6, 2902.7	pine/palmetto	2	#205 ♀, #45 ♂	#12
	3/92	NE Hog Pond	459.8, 2900.7	pine/palmetto	2	1 ♀ (roadkill), #53 ♂	#12
	5/94	FPNWR	464.6, 2902.9	pine/palmetto	2	K12 ♀, K13 ♀	#517
31	3/89	Catherine Island	454.0, 2907.0	pine/palmetto	3	#34 ♂, #35 ♂	#12
	7/90	Regency Farms	459.9, 2903.9	pine/palmetto	2	#201 ♂, #204 ♀	#12
	9/91	Barfields	464.7, 2906.0	hardwood hammock	2	#48 ♀, #52 ♀	#12
32	3/89	Catherine Island	457.1, 2898.6	pine/palmetto	unsuccessful?	-	unknown
	5/92	FPNWR	457.4, 2897.4	pine/palmetto	1	#208 ♀	#12
36	3/90	BCSIR ^a	492.5, 2906.2	hardwood hammock	?	-	unknown
	5/91	Addition Lands	491.1, 2899.0	pine/palmetto	2	#207 ♂, #50 ♂	#26
	10/93	N of BCSIR	489.1, 2909.4	pine/palmetto	1	-	#26 or #34
	6/95	Canoe Lake Strand	502.8, 2901.3	pine/palmetto	?	-	#45?
40	11/90	Baker's Grade	487.1, 2896.3	pine/palmetto	2	#206 ♀, #44 ♂	#26
	3/92	Baker's Grade	485.0, 2897.7	pine/palmetto	2	#54 ♂ (K01 ^a), K02 ♂	#28?
	6/93	Baker's Grade	486.9, 2896.9	pine/palmetto	3	K03 ♂, K04 ♀, K05 ♀	#26
48	10/93	Bear Island	475.9, 2901.6	pine/palmetto	3	K06 ♂, K07 ♀, K08 ♀	#12
	6/95	Dozier Hammock	482.1, 2903.6	pine/palmetto	2	K16 ♂, K17 ♀	unknown

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Panther #	Den Date	Location	UTM Coordinates	Habitat	# of Kittens	Kittens handled	Sire
52	7/93	Sadie Cypress	467.8, 2918.6	mixed swamp	2	1♂ (roadkill)	#46
	7/94	Sadie Cypress	469.6, 2919.0	cypress swamp	?	-	unknown
55	4/95	BCNP	483.7, 2871.8	pine/palmetto	2	K14 ♀, K15 ♀	#42
56	4/94	Baker's Grade	485.8, 2897.3	pine/palmetto	3	K09 ♂, K10 ♀, K11 ♂	unknown

- 1 Fakahatchee Strand State Preserve.
- 2 Kittens removed from wild into captive breeding program.
- 3 Florida Panther National Wildlife Refuge.
- 4 Kittens handled as neonates at den.
- 5 Big Cypress Seminole Indian Reservation.
- 6 Kittens marked with individually numbered transponders.