A Plan for Genetic Restoration and Management of the Florida Panther (*Felis concolor coryi*)

11-13 September 1994
Yulee, Florida

A PLAN FOR GENETIC RESTORATION AND MANAGEMENT OF THE FLORIDA PANTHER (*Felis concolor coryi*)

Report

Prepared by Workshop Participants

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Executive Summary

The Florida panther (*Felis concolor coryi*) is one of the most endangered large mammals in the world. The biological circumstances of geographic isolation, habitat loss, resulting small population size and associated inbreeding have resulted in significant loss of genetic variability and health of the population. The genetic variability and health of the Florida population must be restored for the taxon to survive even with adequate habitat preservation and other enhancement measures. The Florida panther is a named subspecies of the cougar, *Felis concolor coryi*, that formerly occupied southeastern United States and was contiguous with other populations of the species but presently is restricted to a small relict population in southern Florida. The range of *F. c. coryi* was contiguous with that of the Texas cougar, *F. c. stanleyana*, prior to the twentieth century, and it is likely that genetic exchange occurred between these groups because there were no dispersal barriers between their ranges.

The translocation of animals from the historically contiguous west Texas population into south Florida will restore genetic variation. The restored genetic variation will allow for more rapid adaptive evolution of the population, allowing for continued maintenance or improved demographic performance and stability, in contrast to the continued decline and instability that would be expected if the population becomes increasing homogeneous and inbred. Thus, we would expect that genetic restoration will lead to an increase in reproductive performance and survival.

A workshop was convened by the Conservation Breeding Specialist Group of the SSC/IUCN at the request of the Florida Game and Fresh Water Fish Commission to assist in the formulation of a plan for the genetic restoration and management of the Florida population of *Felis concolor*. The meeting was hosted by the White Oak Conservation Center on 11-13 September 1994. Nineteen people participated in the workshop and contributed to the final document. This is the third workshop devoted to developing genetic management strategies for restoring genetic variation to small populations which have rapidly lost genetic heterozygosity and are suffering adverse effects ascribable to this genetic loss and inbreeding. The present workshop was devoted to the needs of the Florida panther program for a genetic restoration plan.

The level of introgression required to reverse the effects of inbreeding and genetic loss in the Florida population has been estimated at 20%, or 6 to 10 effectively breeding individuals, based on the estimated current population of 30 to 50 breeding adults (Seal et al. 1992). An initial release will be made of 8 non-pregnant young adult females from the Texas population. Supplemental additions will be utilized as needed to fully achieve and maintain desired restoration results. Maintaining restored genetic health of this population will require the occasional translocation of animals to achieve an immigration rate of at least one new breeder per generation into the small population in south Florida. This level of restored genetic exchange will prevent renewed inbreeding or continued loss of genetic variability from the Florida population. Implementation of this plan will require continous monitoring and management to restore and maintain the genetic health of the population.

Objectives and Strategies

Objectives:

The objectives of the plan are to: 1) reduce occurrence of inbreeding; 2) restore genetic variability and vitality of offspring produced and recruited as breeders into a healthier, more resilient population of *F. c. coryi* and; 3) to resume the evolutionary adaptive potential of the South Florida population by restoring levels of genetic diversity to the levels typical of other subspecies of *Felis concolor* in western North America.

Strategies:

The current genetic diversity of western *Felis concolor* is most likely typical of the Florida population prior to its isolation and therefore will be used as the model for genetic restoration of *Felis c. coryi*. The Texas population of *Felis concolor* will serve as the source of genetic stock for translocation, since that population was historically contiguous with the Florida population.

The strategies to accomplish the objectives include 1) methods and locations for adding genetic variation to the Florida population, 2) selection and management of the animals to supply the genes, 3) monitoring of the genetic introgression and of its effects on the genetics and morphology of the resident Florida population, and 4) uses of the captive program for systematically measuring the effects of introgression and developing supporting methods for controlled reproduction.

1. Methods and Locations

A. Direct translocation of non-Florida (western Texas) animals into suitable unoccupied territories or potential territories in south Florida is the primary approach selected to achieve genetic restoration.

B. The potential translocation sites are in Everglades National Park (Long Pine Key), Big Cypress National Preserve (Loop Road, Turner River Unit), northern Fakahatchee Strand State Preserve, eastern Addition Lands and select private lands in Hendry County. These sites would allow the translocation of 8 female Texas animals that would have the potential to recruit and mate with Florida panther males.

C. The level of introgression required to reverse the effects of inbreeding and genetic erosion is estimated at 20%, or 8 ± 2 individuals, based on the estimated current population of 30 to 50 breeding adults. The 8 introduced individuals need to produce at least 2 surviving offspring that recruit into the Florida population and mate with animals already in the Florida population. Since not all introduced individuals may survive and breed successfully, this part of the program will need to be monitored and continue until 8 introduced individuals do recruit and produce at least 2 offspring each which survive and breed in the population.

D. Maintaining restored genetic health of this population will require the occasional translocation of additional animals to achieve a recruitment rate of at least one new breeder per cougar generation into the small population in south Florida.

2. *Selection and Management*

A. Animals selected for translocation will be 2-4 year old, non-pregnant, females from widely distributed areas of the range of the source population, and without cowlicks, kinked tails, or cardiac abnormalities. Litter mates will not be selected. Otherwise, selection will not include other morphological or molecular criteria.

B. 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, animals selected for translocation will be quarantined to collect biomedical information for health screening of candidates prior to release.

3. *Monitoring*

A. The extent of introgression of Texas genes into the Florida population will be assessed by three different approaches: (1) pedigree analysis based on founder contributions from the Florida and Texas populations, (2) molecular genetic analysis of markers that show differentiation between the two populations, and (3) analysis of morphological characters that can differentiate between the populations and which may be associated with adaptation.

B. Demographic components of fitness will be monitored in the genetically restored Florida population and compared to data on the present and recent historical population. A comprehensive pedigree database (studbook) will be established for the Florida population. Pedigree analyses methods are seriously compromised when parentage is unknown. Uncertainties regarding parentage will be resolved when possible using such techniques as molecular genetics and behavioral observations.

4. *Captive Population and Research*

A. The role of the captive population will shift from one preserving subspecies variability to one consistent with current objectives for genetic restoration. Institutions holding Florida panthers in captivity will manage them in accordance with management, research, and educational objectives and needs identified in the genetic management plan and the Florida Panther Recovery Plan.

B. Actions will be undertaken to continue the development of artificial insemination (AI), so this technology can be utilized as may be appropriate to accomplish genetic restoration.

Introduction

The Florida panther (*Felis concolor coryi*) is one of the most endangered large mammals in the world. Geographic isolation, habitat loss, resulting small population size and associated inbreeding have resulted in significant loss of genetic variability and health of the population. Natural gene exchange that occurred historically between the Florida population and other contiguous populations of *Felis concolor* ceased to occur when the panther became geographically separated from other populations of the species. Gene flow occurs as individuals disperse among populations and breed. Just as dispersal is the natural mechanism for gene exchange and maintenance of genetic health within populations, those same dispersing breeders minimize the occurrence of inbreeding within populations. Inbreeding increases when dispersing breeders can no longer immigrate into the fragmented population, resulting in inbreeding depression, loss of genetic variation, declining health, reduced survivability, lower numbers and eventual extinction.

We now understand this scenario as part of the extinction process (Gilpin and Soulé 1986). It is this biological circumstance that perhaps most affects the health and survival of the Florida population. While other human related factors affect health of the panther's environment, we now understand that genetic variability and health of the Florida population must be restored for the taxon to survive even with adequate habitat preservation and other enhancement measures (Seal et al. 1992).

It is difficult to precisely describe *F. c. coryi* as it previously existed and thus design a profile upon which to base a restoration objective. The major differences observed in morphological and genetic traits between the Florida population and other populations of *Felis concolor* are likely due to the severe inbreeding and genetic bottleneck that has occurred in the Florida population (Seal et al. 1992). However, western populations of *Felis concolor* have not undergone inbreeding and genetic bottlenecks similar to that of *F. c. coryi* (Roelke et al. 1993). For these reasons, we assume that they appear today as they did at the time the Florida population became geographically isolated and are the best model for what *F. c. coryi* did look like previous to geographic isolation. Molecular genetic analysis indicate that western populations have levels of genetic diversity significantly higher than currently exist in the Florida population (Roelke et al 1993). Furthermore, there are low levels of genetic divergence among subspecies of *Felis concolor* (Roelke et al. 1993; O'Brien et al 1990). Therefore, we will use western *Felis concolor* as the model for genetic restoration of *F. c. coryi* and our objective is to restore levels of genetic diversity in the Florida population to the levels typical of western populations of *Felis concolor*.

This genetic restoration will be accomplished by reinstating gene flow from western populations into the Florida population by selectively translocating individual animals that can be recruited into the population as breeders. Such a management action seeks to mimic historic immigration into the population. The intent is not to replace, or swamp, the *F. c. coryi* gene pool with western *Felis concolor* genes. Rather, the intent is to reduce occurrence of inbreeding and restore genetic variability and vitality of offspring produced and recruited as breeders into

a healthier more resilient population of *F. c. coryi*. Furthermore, the enhanced genetic variation and gene flow will provide the necessary genetic material to restore the process of natural selection to the Florida population. Thus restoration is intended not only to resolve the current problems associated with loss of genetic variation, but also to restore evolutionary adaptive potential of the Florida population. Recovery of *F. c. coryi* will be greatly enhanced by such actions.

Direct translocation of non-Florida individuals into suitable unoccupied territories or potential territories in south Florida is the primary approach selected to achieve genetic restoration of that population. The primary reasons for selecting this approach are the immediacy of introducing selected genetic material into the wild population and the elimination of possible confounding factors that might be associated with the use of individuals produced in captivity. Two other processes that will have application and aid in the genetic restoration process are captive breeding and the use of artificial insemination (AI). AI technology presently is not developed as a reliable technique in *Felis concolor*. Actions will be undertaken to continue the development of AI so this technology can be utilized as may be appropriate to accomplish genetic restoration. The specific role of a captive program to assist the genetic restoration and management of the Florida panther has been redesigned consistent with objectives for genetic restoration of the wild population (see section on Captive Population below).

The level of introgression required to reverse the effects of inbreeding and genetic loss in the Florida population has been estimated at 20%, or 6 to 10 individuals, based on the estimated current population of 30 to 50 breeding adults (Seal et al. 1992). An initial release will be made of 8 non-pregnant, young adult females from the Texas population. Supplemental additions will be utilized as needed to fully achieve and maintain desired restoration results. Maintaining restored genetic health of this population will require the occasional translocation of animals to achieve an immigration rate of at least one new breeder per generation into the small population in south Florida. This level of restored genetic exchange will prevent renewed inbreeding or continued loss of genetic variability from the Florida population.

Translocation and Monitoring

The most practical method for restoring historical levels of genetic variability in the remaining population of Florida panthers and increasing the likelihood of its long-term survival is to translocate wild non-Florida individuals into the south Florida population. The most appropriate source of genetic variation for the south Florida population is in the Texas population (*Felis concolor stanleyana*) because the range of *F. c. coryi* prior to the twentieth century was contiguous with that of the Texas cougar, *F. c. stanleyana*, to the west and it is likely that genetic exchange occurred between these groups because there were no dispersal barriers between their ranges. Female Texas animals 2-4 years of age would be the best individuals for the translocation because they would be more prone to remain near release sites, less likely to have adverse impacts on the existing population, and more easily assimilated into the existing panther population.

Selection of Animals for Translocation

The animals captured for translocation generally will not be pre-selected to conform to any genetic type, as there is no way to determine which genes presently in the Texas population would be most adaptive in Florida. However, because some genetic traits (hair whorl or cowlick, kinked tail vertebrae, and atrial septal defect) that are at high frequency in Florida but low frequency elsewhere will be used as markers of the progress of genetic restoration, any Texas animals that show those traits will be rejected. Animals with a cowlick or kinked tail will be detected at the time of capture. Atrial septal defects in candidate animals will be detected during examination in quarantine. All genetic and morphological traits presently being monitored in the Florida panthers will be measured during quarantine to provide a baseline of data. The quarantine protocols for ensuring that the translocated animals do not transfer any contagious diseases into the Florida Panther population will be the same as presently used for Florida animals, except as may be noted in specific protocols.

Animals will be selected for translocation to maximize their probability of establishment into territories and subsequent breeding. Translocated animals will be non-pregnant, young females, 2 to 4 years old. Subsequent genetic comparison of the genetically restored Florida population to the source population for genetic exchange requires that the capture locality of each animal used for translocation be documented. Moreover, the animals chosen for translocation will not include any that are known or suspected to be closely related (siblings). Translocated animals will be selected, to the extent possible, from geographically diverse areas within the range of the Texas cougar.

Identifying Potential Release Sites

Existing telemetry data coupled with field observations of uncollared panther sign reveal several potential areas for release of translocated animals. These gaps, as well as areas that are vacant but recently supported female panthers are dispersed throughout the currently occupied range of the Florida population. It should be pointed out that release of Texas animals into south Florida is not intended to merely add more individuals to the population but to restore overall health and vitality of the population to a more natural level. Intrinsic limiting factors related to habitat quality and availability may already maintain panther numbers at or near maximum density or may be the cause for fluctuating or ephemeral populations in some areas such as Everglades National Park or the southern Big Cypress National Preserve (Bass and Maehr 1991). Releases of too many additional animals into an area of already limited resources may have destabilizing or other negative effects on these tenuous and apparently recent subpopulations.

The candidate translocation sites are in Everglades National Park (Long Pine Key), Big Cypress National Preserve (Loop Road, Turner River Unit), northern Fakahatchee Strand State Preserve, eastern Addition Lands and select private lands in Hendry County. These sites would allow the translocation of 8 female Texas animals that would have the potential of interacting with Florida panther males.

Monitoring and Evaluating Translocated Animals

Monitoring of the introduced Texas animals will be incorporated into the current schedule for monitoring Florida panthers. Introductions will be evaluated by comparing survival, movements and home range sizes of introduced animals with those of Florida panthers and documenting reproductive behavior, denning and kitten rearing.

All dens of Texas females will be visited within the first month of detection. Kittens will be examined, uniquely marked, blood and other tissue samples acquired as needed, and returned to the den. Kittens will be recaptured at approximately 6 months of age and radio-instrumented; standard morphological measurements and biomedical samples will be taken. Tissue sampling for genetic analyses will be of particular importance to document introgression of the Texas genetic material into the Florida population.

The goal is to have a balanced representation from each introduced Texas animal with at least 2 individuals from each Texas x Florida intercross recruited into the population. The kittens produced through matings of Texas females with Florida males would be expected to remain with their mother for 1.5 to 2 years. Female offspring often establish home ranges that overlap their mother or share a common boundary (Maehr et al. 1991). Translocated Texas females eventually may be removed to create a vacancy for recruitment of an F1 female into the Florida population. The F1 generation will be monitored as described above to document the success of their recruitment. Any removed animals may be relocated to another location within the south Florida study area, incorporated into the captive breeding program, used as an addition to concurrent panther re-establishment studies, or returned to Texas.

Monitors of Genetic Admixture

The extent of introgression of Texas genes into the Florida population (genetic admixture) will be assessed by three methods: pedigree analysis based on founder contributions from the Florida and Texas populations, analysis of molecular genetic markers that can differentiate between the two populations, and analysis of morphological characters that are differentiated between the populations and which may be associated with adaptation. Each of these methods has inherent power for quantifying genetic introgression in populations as well as limitations in interpretation. Their usefulness and specific advantages have been demonstrated in other species and offer an important measure of the quantity of genetic introgression.

Pedigree Analysis

Analysis of a population's pedigree can be used to monitor the genetic processes within populations (Lacy et al. 1994). If information on parentage is completely known, transmission of genes from one generation to the next can be tracked assuming each offspring receives 50% of its genes from each of its parents. This approach will be used to monitor the spread of *F. c. s.* genes in the *F. c. c.* population, document any further inbreeding, and help determine the genetic origins of traits that appear in the population. Pedigree data (sex, parentage) will be

combined with life-history data (data of birth, date of death, reproductive events) to allow genetic and demographic analyses.

A computerized pedigree database (studbook) will be established and maintained for the Florida population to record parentage, sex, date of birth, location, reproductive history, and date of death of every individual in the population. Any uncertainty in the data must be clearly identified in the database. The pedigree will be maintained in a centralized location and available in a standardized format (e.g., SPARKS) to facilitate management and analyses.

Pedigree analyses methods are seriously compromised when parentage is unknown. Uncertainties regarding parentage will be resolved when possible using such techniques as molecular genetics and behavioral observations.

Molecular Markers

Molecular markers of genetic variation have emerged in recent years as powerful estimators of the extent and character of population genetic variation and differentiation. By scoring a group of highly polymorphic genetic markers, it is possible to estimate the proportion of population genetic admixture following intercrossing, such as is proposed for the Florida panther. Initially a series of markers will be identified (nuclear and mitochondrial) that differentiate between the present day Florida population and the introduced Texas animals. These markers are not thought to reflect adaptive genetic differences, rather they would be useful primarily for discriminating the proportional genetic contributions of the two closely related populations. The biological materials required for identification will include specimens (blood samples and immortalized skin culture biopsies) of living Florida animals and Texas animals used for release and intercrossing.

Two categories of molecular genetic markers will be a priority for analysis: mitochondrial DNA genes and nuclear microsatellite (di-,tri-, and tetra-nucleotide repeat) loci. Mitochondrial genes are ideal because of their rapid genetic divergence, primarily maternal inheritance, lack of recombination, and ample genetic theory for interpreting patterns of variation. Microsatellites are ideal nuclear markers because there are a large number of loci known in cats, abundant genetic variation due to high mutation rate, and their PCR format assay makes them feasible to analyze with limited amounts of tissue. Other categories of genetic loci (minisatellite or VNTR, isozymes, Y-chromosome locus introns, MHC variation) may also be considered but would not constitute a first priority.

An estimate of the quantity of population genetic exchange can be calculated by quantifying the extent of variation attributable to Texas vs. Florida genetic markers in subsequent generations. They will reflect an overall estimate of admixture because the markers are not the genes subject to adaptation or selective pressure. The proportion of genes subject to adaptive pressures and that may have been retained from the Florida animals may be different and should

not be presumed identical to genetic marker frequencies. Sufficient polymorphic markers should be assayed to allow unambiguous genetic identification of each animal translocated from Texas and their offspring.

Morphological Characters

The Florida panther was originally described by Cory (1896) and named as a distinct subspecies by Bangs (1889). The original taxonomic description of *F. c. coryi* was based largely on geographical distribution and cranial morphology, specifically the occurrence of a distinctive broad frontal region of the skull with rather broad and highly arched nasals (Bangs 1989; Goldman, 1946). Belden (1986) reported two additional morphological characters consistently observed in Big Cypress Swamp panthers: a ridge or whorl of hairs similar to a cowlick on the mid-dorsal thorax and a 90 degree angle or kink in the posterior tail vertebrae. An analysis of 636 *F. concolor* specimens, including 35 Florida panthers, collected from 1896 to 1987, observed an 83% incidence of the cowlick in Florida panthers compared with 4.8% in other subspecies (Wilkens et al. in press). The kinked tail occurred in 100% of 18 examined specimens of Florida panther from Big Cypress Swamp for which post cranial skeletons were available (Belden, 1986; Wilkens et al., in press). The majority of these specimens postdate the demographic reduction of *F. c. coryi*. The tail kink and cowlick were likely less frequent in the historic outbred ancestors of *F. c. coryi*. Although tail kinks, cowlicks, and a shortened skull have been used in taxonomic descriptions of the Florida population, all of these characteristics may be consequences of inbreeding and consequently they should not be regarded as traits that should be deliberately preserved in future populations of Florida panthers.

Morphological traits are an important component of the monitoring program for three reasons. First, perhaps more than any other kind of trait, morphology defines the essence of *Felis concolor*. The maintenance of species characteristics can be assessed by routinely recording morphological traits during population recovery. Thus, miniaturization for example, would be an undesirable consequence of genetic drift during recovery. Such evolution outside the normal limits of *Felis concolor* variation could be detected by monitoring body size. Second, some morphological traits may be adaptations to local conditions in Florida. Such traits should be monitored even though their status as adaptations may be questionable. Pelage color should be monitored, for example, because dark coloration is suspected to be an adaptation to environments such as are found in south Florida. Third, some morphological traits are reflections of inbreeding. Such traits (e.g., kinked tails, cowlicks or hair whorls) are likely to have recessive inheritance and should be monitored with the expectation that they will be masked by introgression (i.e., not be expressed in first generation intercrosses of Florida and Texas animals). Although recessive deleterious traits should not be expressed in first generation intercrosses, they probably will be expressed in later generations in a minority of intercross descendants. This decline in frequency of expression is a normal and desired result.

Monitoring morphological traits will allow the documentation of the combined effects of genetic exchange and adaptation to the Florida habitats via natural selection. Morphological traits

will be scored under four circumstances: (1) shortly after birth, (2) at approximately 6 months of age, when animals are under anesthesia while being fitted with radio-collars, (3) at each handling of the animals for other purposes, and (4) after death. Routine weight and other measurements will be made (body mass, body length, tail length, chest girth, neck girth, shoulder height, hind foot length, ear length) on kittens, young adults and at necropsy. Kinking of the tail, cowlicks and other unusual characteristics will also be scored. All wild and captive animals that die and can be recovered will be prepared as museum specimens (skin, skull and skeleton). Pelage characteristics and skull measurements (including bilateral asymmetries) will be recorded from such specimens.

Variation in most morphological traits is affected by environmental circumstances as well as inheritance. Although environmental contributions to variation within populations can sometimes be substantial, genetic effects are often the predominant influence on morphological traits (Mousseau and Roff 1987). The monitoring of morphological traits is based on the reasonable assumption that at least part of any differences detected may be genetic. Detailed studies of morphological inheritance in *Felis concolor* are not available; therefore, the possible inheritance of traits must be decided on a trait-by-trait basis by comparison with results for closely related species (e.g., domestic cat) or more distantly related vertebrates (e.g., hoofed stock, humans). Thus, white flecking that has been observed on some *Felis concolor* hides is caused by tick scars and is almost certainly nongenetic. Conformational traits on the other hand (e.g., measurements of body parts) are usually highly heritable in domestic animals and so may be capable of rapid evolution in *Felis concolor.*

Each individual will be assigned a unique identification number that is used in all circumstances to insure that museum vouchers can be matched up with tissue samples, medical records, etc. Marking with passive integrated transponders may be the most infallible way to accomplish this identification. Such transponders will be injected into a body region (e.g., ear cartilage) that is permanently attached to the hide of the specimen. Ear tattooing is a good backup, but not a substitute for a transponder. In addition, site of origin and breeding history of the animal will be documented for each specimen. The site of origin for individual Texas animals that are used in translocations is especially critical because Texas populations may differ in genetic constitution, pathogen load, etc. The site of capture for each Texas animal will be established.

Monitoring of Maladaptive Traits

Population declines and associated inbreeding in the Florida panther have resulted in significant losses in genetic variability and viability. The population exhibits multiple physiological abnormalities that are likely a consequence of recent close inbreeding. This inbreeding has resulted in a high incidence of maladaptive traits which include reproductive and medical abnormalities. Furthermore, the Florida panther has suffered from numerous health problems and infectious diseases that may be a consequence of a defective immune system. The goal of the genetic restoration is to reverse the consequences of inbreeding and monitor the reduction in frequency of the maladaptive traits.

Maladaptive traits

1. Reproductive abnormalities

The occurrence of detrimental reproductive traits includes a high incidence of cryptorchidism, morphologically-abnormal sperm per ejaculate and malformed sperm acrosomes.

a. Cryptorchidism: Cryptorchidism is heritable and is suspected to result from a sex-limited recessive (or possible dominant) autosomal gene in several domestic species: dog, sheep, swine, and cat (Romagnoli, 1991; Burns & Fraser, 1964; Claxton & Yeates, 1972; McPhee & Buckley, 1934). Sixty-five percent of free-ranging Florida panthers of Big Cypress genetic lineage are cryptorchid, while there has been only 1 cryptorchid male of 7 males in the Everglades population (Dunbar, 1994). Cryptorchidism has not been observed in medical examinations of over 40 free-ranging cougars captured in Texas, Colorado, British Columbia, or Chile, and was observed in only two of more than 50 captive males, one in Chile and one in a US zoo (Roelke et al., 1993). Circulating testosterone concentrations were lower in male Florida panthers with only one descended testicle than in those with two, whereas testosterone levels in Florida panthers with two normally descended testicles were no different from males in other cougar populations (Barone et el., 1994). In addition, cryptorchid male Florida panthers tend to produce fewer motile sperm per ejaculate than normal males: 0.54×10^6 compared with 2.19×10^6 (Barone et al., 1994). A high incidence of cryptorchidism has been recently observed in the last two decades, a period in which there have been a number of documented matings between close relatives (Roelke et al., 1993). These observations document the rapid rise toward genetic fixation of a maladaptive genetic trait in a small population that continues to inbreed.

b. Spermatozoal Traits: Semen quality and endocrine and reproductive functions have been shown to be adversely affected in some inbred lines of several species, including mice, cats, 2 lion subspecies and cheetahs (Wildt et al., 1994). Comparative reproductive analyses of seminal traits in five feline species, revealed that Florida panthers males display some of the poorest seminal quality traits ever recorded for any felid species or subspecies (Barone et el., 1994). Total motile sperm per ejaculate in Florida males is 18-38 times lower than in other cougars, 30-270 times lower than in other felids and 30 times lower than in the cheetah. Although cougars and other large felids tend to produce high proportions of morphologically abnormal sperm, the Florida panther has a significantly greater frequency of malformed spermatozoa (average 93.5% per ejaculate) than any other subspecies; particularly noteworthy was a 42% incidence of acrosomal defects, a trait that renders sperm deficient in fertilization potential (Barone et al., 1994).

2. Medical defects

a. Atrial septal defects (ASD): Atrial septal defects, termed patent foramen ovale, have been found in 5 out of 55 documented Florida panther mortalities from 1972 through July 1994 (Roelke et al., 1993; Dunbar, personal communication). This defect may have contributed to

death in 4 out of 5 of these cases. ASD has been observed in several species including humans (ASD is the most common congenital heart defect in human adults) (Braunwald, 1992), but has not previously been observed in cougars and only rarely in domestic cats (Bolton & Lui, 1977). The etiology of ASD is not well understood, but certain cases in humans suggest an autosomal dominant mode of inheritance (Lynch et al., 1978; Mascia et al., 1987). Heart murmurs have been detected in Florida panthers, sometimes at high frequencies; however, the relationship between ASD and heart murmurs is not clear. Recently, hypo- and hyper-vitaminosis A have been ruled out as potential causes of cardiac abnormalities in Florida panthers (Dunbar, 1994). The etiology of the cardiac defects is unknown, however, there may be a genetic explanation.

Assessment and Surveillance of Clinical Health and Reproductive Status

Health Screening and Quarantine of Candidates for Translocation

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 (Wolff and Seal 1993). 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 is a list of clinical health parameters and treatments that will be assessed during quarantine to select individuals for translocation.

1. Complete physical examination with emphasis on cardiac evaluation. Further cardiac assessment including ECG, chest radiography and ultrasonography will be performed if heart murmurs are detected.

2. Serum chemistry and hematological evaluation.

3. Evaluation for internal and external parasites.

4. Vaccinate and apply appropriate therapy to prevent introduction of pathogens and to ensure animal survival post-release.

5. Screen for various bacterial, viral, rickettsial and protozoal diseases that may include but not be limited to FIP, FIV, FeLV, panleukopenia, calicivirus, rhinotracheitis, rabies, brucellosis, and toxoplasmosis.

If future translocation programs include the release of males, a complete reproductive evaluation including semen analysis and sperm morphology assessment will be conducted.

Clinical Health and Reproductive Assessment of Captive and Free-ranging F. concolor in the

Restoration Program

Since the population of Florida panthers has suffered from numerous fatal infectious agents, the following is a partial list of clinical health and reproductive parameters and treatments that will be assessed in captive and free-ranging *F. concolor* including intercrosses.

1. Complete physical examination with emphasis on cardiac evaluation. Further cardiac assessment including ECG, chest radiography and ultrasonography may be performed if heart murmurs are detected.

2. Serum chemistry and hematological evaluation.

3. Evaluation for internal and external parasites.

4. Vaccinate and apply appropriate therapy to prevent introduction of pathogens and to ensure animal survival.

5. Screen for various bacterial, viral, rickettsial and protozoal diseases that may include but not be limited to FIP, FIV, FeLV, panleukopenia, calicivirus, rhinotracheitis, rabies, brucellosis, and toxoplasmosis.

6. Toxicological screen of fat and blood for heavy metals, organochlorines, PCBs and others.

7. Assess reproductive traits and semen quality in adults.

8. Nutritional screen (trace minerals and others).

9. Evaluation of metabolic parameters (T3, T4, cortisol, etc).

10. Evaluation of reproductive hormones (estradiol, testosterone, FSH, LH, etc.)

11. Evaluation of immune system function (i.e., CD4:CD8 lymphocyte ratio, etc.)

12. Evaluate causes of adult and kitten mortality through complete pathologic examination.

Monitoring Demographic and Fitness Parameters during Genetic Restoration

The existing Florida Panther population is threatened by demographic problems as well as genetic problems (for example, mortality rates are unacceptably high, and habitat is not sufficient to allow expansion of the population to a more stable size; Seal and Lacy 1989). The translocation of non-Florida animals into south Florida will restore genetic variation and,

therefore, may increase fecundity and survival. In addition, the restored genetic variation will allow for more rapid adaptive evolution of the population, allowing for maintained or improved demographic performance and stability, in contrast to the continued decline and instability that might be expected if the population becomes increasing homogeneous and inbred. Thus, we would expect that genetic restoration will lead to an increase in reproductive performance and survivability.

Outbreeding depression, a possible negative consequence that could result from introgression, has been considered, but was rejected as an implausible outcome of the planned genetic restoration. For example, the outcrossed panthers in the Everglades subpopulation showed a reversal of some of the effects of inbreeding seen in the Big Cypress segment of the population (O'Brien et al. 1990), and showed no evidence of outbreeding depression. The proposed intercrossing for genetic restoration would be between populations that are much more similar genetically, and would more recently have exchanged genes via natural migration, than were the subspecies that were crossed in the Piper stock that was subsequently released successfully into the Everglades (Roelke et al. 1993). Outbreeding depression would be unprecedented for a cross between such closely related and recently diverged mammalian populations as the Florida and Texas *F. concolor*. It will be necessary to monitor demographic parameters of the intercrossed population to assure that damaging effects of the genetic restoration did not occur, and to document the likely beneficial effects of genetic restoration.

The planned genetic restoration consists of an initial introduction of sufficient animals so that approximately 20% of the breeders in the population will consist of migrants from the Texas population. This will be followed by further genetic management that results in at least 1 translocated animal recruiting into the breeding population per generation (about 6 years). This rate of genetic mixture would not be so great as to cause genetic swamping of locally adapted traits presently in the south Florida population. After the initial genetic restoration, any locally adapted gene would still persist at a frequency of about 80%, and a majority of the next generation would consist of animals that are homozygous for any Florida-adapted genes. Natural selection would further increase the frequency of non-Florida genes that were advantageous, and would keep low the frequency of any non-Florida genes that may be maladaptive in the south Florida habitats. Ongoing gene flow at a rate of at least 1 new breeder recruited per generation (perhaps 2-4% of the breeding population) would over many generations lead to the replacement of locally adapted traits only if the selective advantage of the locally adapted trait over the non-local variant of the gene was less than the rate of immigration (2-4%). The low probability of loss of genetic traits that have a slight adaptive advantage in the Florida environment would likely be much more than offset by the substantial advantage gained by the reversal of the deleterious effects of inbreeding already seen in the population.

The demographic components of fitness that will be monitored in the genetically restored Florida population are those that are being monitored presently and include:

(1) proportion of the number of adult females producing litters each year,
(2) average litter size,

(3) average litter sex ratio,
(4) kitten survival through 12 months,
(5) subadult survival to reproductive age,
(6) recruitment of adults into the population of territorial breeders,
(7) age of first breeding,
(8) annual adult survival.

These aspects of demography have been monitored for a number of years and they will be maintained in a studbook database, so that a baseline exists for comparison to the future genetically restored population.

Some of these demographic parameters may well improve following genetic restoration, as effects of previous inbreeding are reversed, and it is unlikely that any components of fitness would decrease as a result of the planned genetic management. In the unlikely event that the monitoring reveals a decrease in overall fitness in the inter-crossed progeny or their descendants, it would be important to examine the causes of the fitness depression and evaluate whether inter-crossed panthers should be removed from the wild population. Any outbreeding depression would be greatest in either the first or second generation of outcrossing. It would be possible at that time, if such did occur, to remove inter-crossed panthers from the wild population in south Florida. Even if some inter-crossed descendants remained in the wild, any decreased viability or fertility would eliminate genes that are poorly adapted to the Florida habitats within a few generations, and would lead to the evolution of new coadapted (and non-inbred) genomes. Thus, there is little risk that any maladaptive traits could "swamp" locally adapted traits or cause long-term damage to population viability.

The Role of the Captive Population in the Genetic Restoration of the Florida Panther

The implementation of a genetic restoration program for *Felis concolor coryi* will have a dramatic impact on the original aspects of the captive breeding program. The need to establish a large reservoir of identified *Felis concolor coryi* to maintain a specific level of genetic diversity as a safeguard from extinction will no longer be required (Seal et al. 1989.)

The role of the captive population will shift from one of preservation of the subspecies variability to one consistent with current objectives for genetic restoration. Institutions holding Florida panthers in captivity will manage them in accordance with management, research and educational objectives and needs identified in the genetic management plan and the Florida Panther Recovery Plan.

Production of Various Generation Types To Assess Intercross Effetcs

The number of individuals needed from various generational types (F_1, F_2, backcross) to assess fully the effect of intercrossing far exceeds the space presently available in the captive

population. A rough estimate is that 20-30 offspring are minimally needed for each generational type for adequate evaluation of the effects of intercrossing on, for example, survival rates. While these data can not be generated in the short term, they can be accumulated in time, at a rate compatible with the space available in the captive population. Initial strategies will be to produce 2-3 litters each of reciprocal F_1 (i.e., Texas female x Florida male and Florida female x Texas male) and backcross of F_1 to Florida parents (B_F), since these are the generational types most likely to occur first in the wild population. These intercrosses will provide enough initial data to detect problems that could significantly compromise the program (e.g., sterility). However, reciprocal F_2 and F_1 backcrossed to Texas parents (B_T) also will be produced to provide data for comparative purposes. The eventual goal is to increase the numbers in each generational type over time.

The primary objectives of the captive population will be:

1. Continue research on reproductive physiology, health and husbandry concerns.

2. Specific research on assisted reproduction to improve the reliability of AI techniques.

3. Assess results of introgression undertaken in captivity as previously outlined under Monitors of Genetic Admixture, Monitoring of Maladaptive Traits, Assessment and Surveillance of Clinical Health, and Monitoring Demographic and Fitness Parameters during Restoration.

4. Assess the impact of introgression in captivity on maternal behavior.

5. Continue development of techniques that improve the success rates of large carnivore release programs.

6. Production of F_1 animals for reintroduction into identified habitat for expansion of the species into its historical range outside south Florida when feasible and provide introgressed panthers to other institutions, when release is not feasible, for further research and public education needs.

7. Educate the public regarding panthers in Florida, their importance in the natural system and the recovery program.

Each *Felis concolor* in captivity that is part of this program will be evaluated as to health and reproductive fitness and assigned a specific role in the program. Production of F_1 cats will be based specifically on needs of genetic restoration. Offspring that do not have a specific role or have fulfilled their role in the genetic restoration program will be placed in accordance with each institutions animal disposition policy. A serious effort must be made to produce only introgressed offspring that have a specific role in the restoration program due to limited space in participating institutions.

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