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Evaluation of the Genetic Management of the Endangered Mississippi Sandhill Crane (Grus canadensis pulla)

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Evaluation of the Genetic Management of the Endangered Mississippi Sandhill Crane (*Grus canadensis pulla*)

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ABSTRACT

The genetic status of the critically endangered Mississippi sandhill crane (*Grus canadensis pulla)* was analyzed using 2009 studbook data from the U.S. Fish and Wildlife Service managed captive breeding and release program. Microsatellite DNA data provided information on shared founder genotypes, allowing for refined analysis of genetic variation in the population, and informed breeding recommendations. The genetic variation observed in the Mississippi sandhill crane was contrasted with variation observed in the Florida sandhill crane (*Grus canadensis pratensis*). Results show far less variation in the Mississippi population. Results also suggest that while gene flow no longer occurs between the two populations, the introduction of cranes from the Florida population would help to increase the observed genetic diversity of the Mississippi sandhill crane population.

Key words: captive breeding, *Grus canadensis pulla*, Mississippi sandhill crane, *Grus canadensis pratensis*, pedigree analysis, microsatellite DNA

INTRODUCTION

1.i. INTRODUCTION

The Mississippi sandhill crane (*Grus canadensis pulla*) is an IUCN Red listed critically endangered subspecies of sandhill crane. Although it is believed that the Mississippi sandhill crane population was once part of an extensive resident population of cranes in the southeastern United States (USFWS, 1991), today the Mississippi sandhill crane is found only in Gautier, Mississippi at the Mississippi Sandhill Crane National Wildlife Refuge, and in captivity. The refuge population has been supplemented by a U.S. Fish and Wildlife managed captive breeding program since 1981 when the wild population numbers dropped below 40 individuals (Valentine & Noble, 1970). There are now approximately 110 birds on the refuge (S. Hereford, U.S. Fish and Wildlife Service, personal communication).

The recovery objective for the Mississippi sandhill crane [MSC] is to "maintain a genetically viable, self-sustaining, free-living MSC population (USFWS, 1991)." To achieve this goal it is critical that the captive population used to supplement the wild population be properly managed. Although population numbers on the refuge have increased since the initiation of the captive breeding and release program, successful rearing of wild hatchlings have been few, and the wild population remains heavily dependent on supplemental captive breeding to stay above 100 individuals.

Current management of the captive MSC population relies on population pedigree information. Using pedigree information, managers are able to mate underrepresented individuals with one another in order to maintain a high level of genetic diversity in the captive

population. Pedigree information, however, does not take into account relatedness among the founders of the captive population. Given the population bottleneck observed prior to the establishment of the captive breeding program, it is likely that the founders of the captive MSC population were related to one another. This could have a significant effect on the levels of genetic diversity and the amount of inbreeding occurring in the current captive population. Previous studies on the population genetics of the Mississippi sandhill crane support this hypothesis; both allozyme (Dessauer *et al.*, 1992) and microsatellite analyses (Jones, 2003) reported that MSCs show only half the level of heterozygosity when compared to other sandhill cranes.

As a low level of gene flow has been observed between crane populations (Jones, 2005), it has been suggested that the genetic diversity of the Mississippi sandhill crane population could be increased by introducing Florida sandhill cranes (*Grus canadensis pratensis*), a sub-species of sandhill crane, to the MSC population (Jones, 2003). The impact of such an introduction, however, has not previously been assessed.

For this study I have conducted both pedigree and molecular analyses of captive and released Mississippi sandhill cranes in an effort to clarify the genetic status of the population. A breeding recommendation chart was developed based on the combination of these analyses. In addition, the variance in molecular genetic diversity between Mississippi sandhill cranes and Florida sandhill cranes was evaluated, and projected changes in the genetic diversity following the introduction of individuals from the Florida population were assessed. The ultimate goal of this study was to use the information found from these analyses these to promote management decisions that result in maintaining the genetic diversity of the endangered Mississippi sandhill crane.

1.ii. LOSS OF GENETIC VARIATION IN SMALL POPULATIONS

Individuals possess many thousands of genes, the location of which on a chromosome is referred to as its locus. Each gene may have multiple alleles, alternate forms of the gene. The variation of these alleles observed within and between populations is referred to as genetic variation. There are many ways to measure genetic variation, including allelic diversity (or allelic richness) and heterozygosity (Ballou and Foose, 1996). Allelic diversity is a measure of the number of different alleles at a given locus. High levels of allelic diversity are necessary to provide the potential for evolutionary adaption to changing environments (Fernandez, 2005). Heterozygosity refers to the percentage of loci in a population or individual for which two different alleles are found (i.e. heterozygous) (Ballou and Foose, 1996).

In small populations both allelic diversity and heterozygosity can be lost through genetic drift, the random change in the number and frequency of alleles due to chance. In addition to genetic drift, a reduction of genetic diversity in small populations is often the result of inbreeding. Inbreeding refers to the probability of identity by descent (IBD) at a given locus (Fernandez, 2005), e.g. higher inbreeding equals more likelihood of identity by descent.

USING MOLECULAR GENETICS TO MEASURE GENETIC VARIATION

The development molecular DNA technologies have enabled researchers to investigate different measures of genetic variation, such as the allelic diversity or variation observed within and among populations. Several methods for assessing genetic variation have been developed since the discovery of the structure of DNA (Watson & Crick, 1953). These include allozyme loci, mitochondrial DNA, Major Histocompatibility Complex loci, and Variable Number of Tandem Repeat (VNTR) markers. VNTR markers, which are comprised of repetitive DNA

sequences, are divided into two categories; minisatellites (between 15-70 base pairs), and microsatellites (between 2-6 base pairs) (Estoup and Angers, 1998).

1.v.ii. POPULATION ANALYSIS USING MICROSATELLITES

Microsatellite DNA are hyper-variable, single locus markers that exhibit co-dominant inheritance (Queller *et al.,* 1993). These features make them a powerful tool for inferring relationships between individuals (Queller *et al.,* 1993; Haig, 1998), but they can also be extrapolated to assess population differentiation (Balloux & Lugon- Moulin, 2002; Nybom, 2004).

Prior to individual or population level analysis using microsatellite markers the assumptions of selective neutrality and the non-random association of alleles at different microsatellite loci should be assessed. The presence of selection may be detected by a comparison of observed genotypic frequencies to those expected from the Hardy-Weinberg equilibrium principle. The Hardy-Weinberg equilibrium principle proposes that allele and genotypic frequencies will remain in equilibrium from one generation to next unless affected by disturbing influences such as mutations, non-random mating, or selection (Hardy 1908; Weinberg, 1908). Deviations from expected values of Hardy-Weinberg equilibrium can be due to a variety of causes. An excess of heterozygotes may be the result of outbreeding or a population bottleneck. Such events result in a reduction in allelic diversity, producing an observed heterozygosity that is larger than the expected heterozygosity, as expected heterozygosity is calculated by the number of alleles present (Corneut and Luikart, 1996). If an excess of homozygotes is found this may be attributed to selection at the loci being assessed, null alleles, population substructure, or inbreeding in the population (Pressoir and Berthaud, 2004).

The likelihood of each of these explanations for deviations from Hardy-Weinberg can be assessed from additional information such as pedigree knowledge (Paetkau *et al..*, 1995).

The ability to measure the level and loss of genetic variation in populations is a valuable tool when working with small populations, such as in captive breeding programs. Molecular analysis methods, such as microsatellites DNA analysis, grant managers of captive populations insights into the genetic diversity present in their population, and provide a background for making insightful management decisions.

1.ii. CAPTIVE BREEDING OF ENDANGERED AVIAN SPECIES

Over the last century captive breeding of endangered avian species, such as the California Condor (*Gymnogyps californianus*), the Guam Rail (*Rallus owstoni*), and the Mississippi sandhill crane (*Grus canadensis pulla*), has become necessary to ensure the survival of the species. Captive breeding programs, like many aspects of endangered species science, however, face serious challenges (Snyder et al., 1996). Captive populations of endangered avian species, as observed in the Whooping Crane (*G. americana*) (Lewis, 1990) and the Hawaiian Crow (*Corvus hawaiiensis*) (NRC, 1992), have experienced low numbers, high mortality, infertility, and incompatibility of mates. These problems were the result of the many difficulties and limitations of captive breeding, including increased likelihood of disease, lack of necessary husbandry knowledge, and inbreeding (Snyder *et al.,* 1996).

Inbreeding depression and the observed reduction in population fitness as a result is a significant force affecting the viability of captive populations (Leberg and Firmin, 2008). Inbreeding depression has been observed in avian species, such as the pink pigeon (*Columba mayeri*), where inbreeding reduced juvenile and adult survival and egg fertility (Swinnerton *et*

al., 2004). Inbreeding depression occurs due to the increased genomic homozygosity that is observed in inbreeding populations and results in the exposure of deleterious recessive alleles (Frankham *et al.*, 2002). In large, natural populations, selection decreases the frequency of such alleles in a process referred to as purging, resulting in decreased future inbreeding depression. In captivity, selection is often removed as a force, and therefore, purging does not occur. In an effort to restore selection in captivity, Leberg and Firmin (2008) attempted the purging of captive populations of western mosquitofish (*Gambusia affinis*) through a series of bottlenecks. Purging, however, was not an observed result in this study. Instead, the serial bottlenecks resulted in an increased probability of extinction, suggesting that avoiding inbreeding and loss of variation through minimization of kinship and avoiding small population sizes should remain the objective of captive breeding programs (Leberg and Firmin, 2008).

The ultimate goal of many captive breeding programs is the reestablishment of viable wild populations (IUCN, 1987). At the time of publication a total of 168 avian species have been reared in reintroduction programs, with more than 550 release sites (Lincoln Park Zoo, Avian Reintroduction and Translocation Database, lpzoo.org). Unfortunately, reintroduction programs have faced challenges similar to captive breeding programs. Griffith *et al.*, (1989) reported a 32% success rate from 31 reintroduction projects, and Beck *et al.*, (1994) using more stringent criteria calculated a success rate of only 11% for 145 reintroduction projects. It has even been suggested that supplementation programs may actually hinder the wild populations they are designed to help, due to the effect of a genetic supplementation load (Lynch and O'Hely, 2001). This load is based on the many genetic challenges facing captive breeding programs mentioned above, including a loss of genetic diversity due to isolation or inbreeding, accumulation of mildly deleterious alleles (through random genetic drift), or adaptation to captivity (Frankham *et al.*,

2002). Through statistical modeling Lynch and O'Hely (2001) found that this genetic supplementation load can have clear negative effects on the fitness of a wild population in just a few dozen generations. Such findings highlight the imperative of working to maintain genetic variation in captive populations.

The current goal of most captive breeding programs is to retain 90% of the genetic diversity observed in the founding population for 100 years (Frankham *et al.*, 2002). This means that the decisions made during the founding stage are extremely important, as the founding process sets the genetic characteristics for the captive population. The captive population will not maintain the same level of genetic diversity as the wild population, as the establishment of a captive breeding program inevitably leads to a population size bottleneck (Frankham *et al.*, 2002). In order to avoid the effects of this bottleneck the founding population must be sufficiently large (Frankham *et al.*, 2002). As discussed in Frankham *et al.*, (2002), Marshall and Brown (1975) recommend that the number of founders be sufficient to obtain (with a 95% certainty) all the alleles at a random locus occurring in the target population with a frequency greater than 0.05. Therefore, the number of founders necessary to prevent the loss of allelic diversity is dependent on the frequency of alleles in the wild population (i.e., the more rare alleles in the population the greater the number of founders you will need to properly maintain that genetic diversity). Unfortunately, most captive breeding programs have been established when the number of wild individuals is already too low to meet this criteria, which leads to decreased heterozygosity in the captive population, and often to severe founder effects. The effects of low founder numbers, for example, have been observed in the California condors (*G. californianus*) where 9% of the captive population carries the allele for chondrodystrophy, a lethal form of dwarfism (Ralls *et al.*, 2000).

As in the case of the California condor, many captive breeding programs are established when wild populations were extremely low and little genetic information was known about the founding population. Instead of using genetic analysis to understand the allelic diversity in their population, managers of most captive populations must rely on pedigree information in order to retain genetic diversity and avoid inbreeding in their population (Glatston, 1986). When pedigree information is complete, pedigree analyses can provide powerful methods for determining lineage structure, calculating individual inbreeding coefficients, for resolving genetic importance of specific individuals to breeding, and for recording the loss of genetic diversity over time (Haig and Ballou, 2002).

Although these analyses are useful for estimating the genetic diversity present in the current population as compared to the founding captive population, pedigree analysis alone cannot estimate true levels of genetic variation. If the founding population had low levels of genetic variation as the result of a population bottleneck, for example, the genetic variation in the current captive population will likely be much lower than as predicted by pedigree analysis. Increasingly managers of captive populations are combining molecular genetic analyses with pedigree analyses. This allows managers to compare the genetic diversity expected by analysis of the pedigree alone to the genetic variation observed in the molecular data, providing a more accurate picture of the genetic management of their population. In addition, genetic data can be valuable for filling in gaps in pedigree information, such as resolving unknown parentage, or for providing estimates of pairwise relatedness (Ivy *et al.*, 2009).

1.iv. STUDY SPECIES

There are fifteen species in the crane family (Gruidae), two of these species belong to the crowned crane subfamily (Balearicinae), and 13 belong to the typical crane subfamily (Gruinae). The fossil records for crowned cranes date back to the Eocene, 37-54 million years ago, and the two surviving species of crowned cranes, the black crowned crane (*Balearica pavonina)* and the grey crowned crane (*Balearica regulorum)* are found exclusively in Africa (Meine and Archibald, 1996). The typical cranes, which first appear in the Miocene fossil records, 5-24 million years ago, inhabit all continents except South America and Antarctica (Meine and Archibald, 1996). There are three genera of typical cranes: *Arthropoides*, *Burgeranus*, and *Grus*. Members of the *Arthropoides*, the demoiselle crane (*A. virgo*) and blue crane (*A. paradisea)* are closely genetically related to the larger wattled crane (*Bugeranus carunculatus*) (Krajewski and Fetzer, 1994; Meine and Archibald, 1996). The species in the genus *Grus* are delineated into four groups: the sandhill crane (*Grus canadensis*) of North America; the Siberian crane (*G. lecogeranus*); the "Group of Three" including the sarus crane (*G. antigone*) of India and southeast Asia, the white-naped crane (*G. vipio*) of northeast Asia, and the brolga (*G. rubicundus*) of Australia and New Guinea; and the "Group of Five," which includes the Eurasian crane (*G. grus*), the whooping crane (*G. americana*) of North America, the hooded crane (*G. monachus*) of Russia and northern China, red-crowned crane (*G. japonensis*) of east Asia, and the black-necked crane (*G. nigricollis*) of China and India (Meine and Archibald, 1996). The sandhill crane (*G. canadensis*) has been further delineated into six subspecies, three migratory subspecies: the greater sandhill crane (*G. canadensis tabida*), the lesser sandhill crane (*G. canadensis canadensis*), the Canadian sandhill crane (*G. canadensis rowani*); and three non-migratory subspecies: the Florida sandhill crane (*G. canadensis pratensis*), the Cuban sandhill

crane (*G. canadensis nesiotes*), and the Mississippi sandhill crane (*G. canadensis pulla*) (Archibald and Lewis, 1996).

Cranes are generally monogamous, staying together throughout the year and often until one bird dies (Meine and Archibald, 1996), although there has been documented evidence of extra-pair matings in sandhill cranes (Hayes *et al.,* 2006). Paired cranes will isolate themselves on their territories during the breeding season, but will gather with other groups of cranes during the non-breeding season (Meine and Archibald, 1996). Diurnal in their habits, cranes forage, rest, preen, and socialize within flocks, or tend to young during the day, and either stay on their nests during breeding season or roost with large flocks at traditional roosting sites (Meine and Archibald, 1996).

Cranes are omnivores, but their diets can vary significantly among species and with seasons, as cranes will shift their foraging strategies on a seasonal, or even daily, basis to take advantage of available food (Meine and Archibald, 1996). Sandhill cranes, for example, feed primarily on small grains during the fall through the spring, but their diet changes during nesting season, when they frequent wetland areas, to include items such as crayfish, tubers, frogs and rodents (Archibald and Lewis, 1996). Non-migratory subspecies of sandhill cranes, such as the Mississippi, also use seasonally variable wetlands, grasslands and pine savannas (Meine and Archibald, 1996).

1.v. MOLECULAR ANALYSES OF GRUIDAE

The literature on the levels of genetic variation among cranes is rapidly developing. Krajewski and Fetzner (1994) completed phylogenetic analysis across all 15 species and Jones et al. (2005, 2006) analyzed intra-specific variation among wattled (*G. carunculatus),* sarus (*G.*

antigone), and sandhill crane populations (*G. canadensis*). The sandhill crane has been analyzed by several molecular genetic analyses. Gaines and Warren (1984), Tacha *et al.,* (1986), and Dessauer *et al.,* (1992), utilized allozymes; Jarvi *et al.,* (1995) and Jarvi *et al.,* (1999) conducted MHC analyses; Rhymer *et al.,* (2001), Glenn *et al.,* (2002), and Petersen *et al.,* (2003) worked with mitochondrial DNA, and Jones et al. (2005, 2006), used microsatellite analysis. With only 3.0-7.2 alleles per locus, and 0.028-0.41 observed heterozygosity (Jones, 2005) cranes show lower genetic diversity when compared with other birds (4.9-14.1 alleles per locus, 0.43-0.85 observed heterozygosity) (Neff & Gross, 2001). The reason for this difference is unknown, but may be the result of the shorter repeat length of crane loci (7-20 repeats) in contrast with other birds (12-20 repeats) (Neff & Gross, 2001) or due to k-selected life history traits of cranes.

1.vi. DECLINE OF THE MISSISSIPPI SANDHILL CRANE

Cranes are the most endangered family of birds in the world, with 13 of its 15 species in peril (Crane Conservation Act, 2008). Although sandhill cranes (*G. canadensis*) are not endangered as a species they are threatened by the loss and degradation of wetland and breeding habitats. Loss of habitat has been especially damaging to the non-migratory subspecies of sandhill cranes, such as the Mississippi sandhill crane (MSC), which is an IUCN critically endangered subspecies, protected under the U.S. Endangered Species Act (Gee & Hereford, 1995).

The decline of the MSC population parallels the development of agriculture, industry and forestry practices in its historic range of the pine savannas in southeastern Mississippi. In 1972, when the MSC was first described as a subspecies (Aldrich, 1972) the range of the MSC ran east of the Pascagoula River to areas west of the Jackson county line, north to an east-west line 8-16

km north of VanCleave, and south to Simmons Bayou (Valentine & Noble, 1970; Aldrich, 1972; Gee & Hereford, 1995). According to Gee and Hereford (1995), during the 1800s the species was abundant enough for farmers to consider it a pest, however by the 1940s the population had dropped below 100 individuals. Over the next twenty years the suitable pine savanna habitat continued to decline, and shrunk from over 400 km^2 in 1940 to only 105 km^2 in the 1960s. By 1970 on 38-40 cranes remained in the area (Valentine & Noble, 1970). The U.S. Fish and Wildlife service listed the MSC as an endangered species under the Endangered Species Act in 1973, and in 1974 the Mississippi Sandhill Crane National Wildlife Refuge was created from land donated by the Nature Conservancy, the U.S. Department of Transportation, and the State of Mississippi (Gee & Hereford, 1995). In addition to the establishment of the Mississippi Sandhill Crane National Wildlife Refuge, in 1965 the U.S. Fish and Wildlife Service initiated a captive breeding program for the Mississippi sandhill crane. After 40 years, the breeding program continues to successfully produce offspring that are now reintroduced to the Mississippi Sandhill Crane National Wildlife Refuge.

The Mississippi sandhill crane provides an opportunity to assess the genetic status of an endangered sub-species through both pedigree and molecular analysis. Through the assessment of comprehensive pedigree records and thorough sampling of the captive population, this study aims to increase knowledge about the genetic variation present in the Mississippi sandhill crane population, and make management recommendations that result in improvement to the breeding program.

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CHAPTER 2: PEDIGREE ANALYSIS OF THE MISSISSIPPI SANDHILL CRANE

2.i. INTRODUCTION

2.i.i. *Pedigree analysis*

Captive breeding programs keep detailed information on individuals in their populations in databases called "studbooks." These databases contain a variety of information including parentage, offspring, rearing methods, medical history, etc. Pedigree analyses use studbook data to determine relationships between individuals in the captive population and use this information to make breeding decisions (Van Dyke, 2008).

Studbooks are based on the original "founder" population established when the captive breeding program was begun. These "founders" are assumed to be unrelated (Ballou & Lacy, 1995). Through pedigree analyses, genetic parameters of the current captive population are determined by estimating losses or changes to genetic variability relative to this hypothetical "founder" population (Ralls & Ballou, 2004). Pedigree analysis programs use a variety of models to make these estimations. For example, Gene-drop simulation model analyses give each founder two unique alleles that are then "dropped" down through the pedigree, assuming Mendelian inheritance, so that each descendant receives one randomly selected allele from its mother, and one randomly selected allele from its father. Several thousand iterations are performed to simulate sampling throughout the individual's genome. This model assumes no linkage, and no selection (Haig and Ballou, 2002).

Gene-drop models are used to estimate gene diversity (GD) and founder genome equivalents (FGE) of captive populations. GD is based on the probability that random sampling across the population will result in two alleles from the same locus that are identical by descent

(Lacy & Ballou, 2001), i.e. expected heterozygosity. GD is calculated using gene-drop models by counting the allele frequencies of the founder alleles remaining in the extant population. FGE refer to the number of equally represented founders that would produce the same level of gene diversity as that observed in the current population, assuming no loss of alleles (Lacy & Ballou, 2001). Gene-drop models can also estimate the potential FGE (pFGE) and potential GD (pGD). pFGE refers to the number of FGE that would be found in the captive population if the alleles of all founders still present in the population were represented equally (Ballou & Foose, 1995). pGD reflects the gene diversity that would be found in such a population.

Pedigree programs also use a matrix of additive genetic relatedness between all individuals (Ralls & Ballou, 2004) to determine kinship and inbreeding values. Mean kinship (MK) is derived from the average kinship between each individual in the population and all other individuals in the population (Ballou & Lacy, 1995). This is a useful tool for identifying genetically important individuals, as minimizing the population's mean kinship acts to maximize the retention of founder gene diversity (Miller, 1995). Inbreeding coefficients (*F*) measure the amount of kinship between parents of an individual, and are calculated as the probability that the two alleles at a locus are identical by descent due to their joint inheritance from a common ancestor. Although a loss of gene diversity is inevitable in small, captive populations due to genetic drift, population managers can use these measures of genetic diversity based on pedigree analysis to influence how quickly gene diversity is retained or lost (Van Dyke, 2008)

2.i.ii. *History of the Mississippi sandhill crane captive breeding program*

Beginning in 1965, the U.S. Fish and Wildlife service began collecting eggs from multi-clutch MSC nests at the Mississippi Sandhill Crane National Wildlife Refuge for captive propagation (Valentine & Noble, 1970). Under the management of the U.S. Fish and Wildlife, a captive breeding population was established at the Patuxent Wildlife Research Center, in Laurel, Maryland, and in 1981 the first captive reared MSC was successfully released on to the Mississippi Sandhill Crane National Wildlife Refuge (MSCNWR). There are currently 41 individuals in the captive flock which is now split between the White Oak Conservation Center in Yulee, FL, and at the Audubon Center for Research on Endangered Species, in New Orleans, LA (M. Savoie, Audubon Center for Research on Endangered Species, personal communication).

Pedigree information is available for all captive individuals in the MSC population, and as 80.77% of the birds currently on the refuge were reared in captivity, pedigree information is available for a majority of the individuals on the Mississippi Sandhill Crane Wildlife Refuge as well. Here, I use five pedigree analysis programs, SPARKS, GENES, Pedigree Viewer, Population Management 2000 and Mate RX to analyze the current genetic status of the captive/released Mississippi sandhill crane population. My specific objectives were (1) determine the current genetic structure of the total captive and released population; (2) compare inbreeding to survivorship; (3) conduct analyses to make recommendations for future management.

2.ii. MATERIALS AND METHODS

2.ii.i. *Data*

The data used for this study was collected from the Mississippi Sandhill Crane Studbook, current through January 2009 (Savoie, 2009). From this studbook, which I refer to as MSC - presumed, I have created an additional studbook, the MSC – confirmed.

When working with a supplemented population, information on released individuals can be difficult to ascertain; for instance, when leg-bands are lost and individuals can no longer be identified, or when individuals die without a carcass being found. Pedigree analysis software assumes that all individuals reported in the studbook are still alive until otherwise indicated, resulting in larger population estimates than actually exist. The MSC – confirmed studbook addresses this issue by only including in the analysis individuals of the captive and released population confirmed to be alive as of March 2009 (S. Hereford, U.S. Fish and Wildlife Service, personal communication).

2.ii.ii. *Analysis*

Analyses were conducted using SPARKS Studbook Management software (Lacy, 2004), GENES 12.0 (Lacy, 1998), Statistical Package for Social Sciences (SPSS 16.0 for Mac 2007), Pedigree Viewer (Kinghorn & Kinghorn, 2006), Population Management 2000 (Lacy & Ballou, 2001), and MateRx version 1.9 (Ballou *et al.*, 2001).

2.ii.iii. *Software*

The Single Population Analysis and Records Keeping System [SPARKS] (Lacy, 2004), is the species management software program used to manage the MSC population. SPARKS was used for individual pedigree analyses, and for exporting pedigree data. GENES 12.0 (Lacy, 1998) was used to measure gene diversity (Lacy and Ballou 2001), founder genome equivalents, mean kinship (Ballou & Lacy 1995) and inbreeding. To evaluate the effect increased inbreeding levels may have on individuals, I analyzed the relationship between inbreeding coefficients and the percentage of individuals surviving past 2 years of age, the age at which MSCs can reach reproductive maturity (Mirande *et al.*, 1996). This regression analysis was conducted using the Statistical Package for Social Sciences (SPSS 16.0 for Mac 2007). Using the pedigree file exported from SPARKS, Pedigree Viewer (Kinghorn & Kinghorn, 2006) was utilized to visually display the complete population pedigree structure, as well as the pedigrees of select individuals, such as high output breeders. Following these analyses, Population Management 2000 (Lacy & Ballou, 2001) was used to model the current MSC population management goal of maintaining 90% gene diversity in the population for 100 years.

Finally, MateRx version 1.9 (Ballou *et al.*, 2001) was used to evaluate the genetic value of mating different pairs. This program integrates four genetic components into a single index: the Mate Suitability Index (MSI). The four components are (1) the change in population's genetic diversity associated with the pairing of two individuals, with preference given to those with low mean kinships; (2) the difference in mean kinships of the male and female, as it can be detrimental to pair animals with very different mean kinship due to the possibility of linking under-represented alleles with over-represented alleles (Ralls & Ballou 2004); (3) inbreeding coefficients; (4) amount of unknown ancestry in the male and female, as managers try to exclude

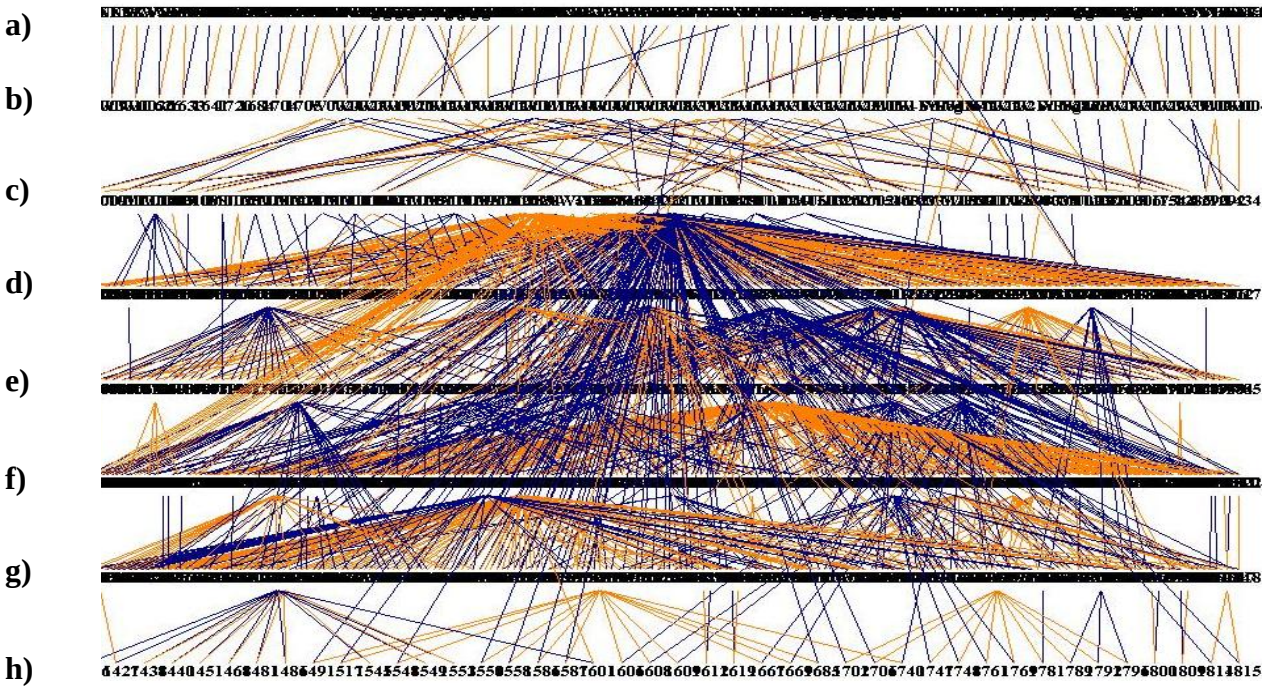
individuals with unknown pedigrees (Ballou *et al.*, 2001). The MSI ranges from 1 (very beneficial to the genetic diversity of the population) to 6 (very detrimental). Pairs known to exist in the released population were analyzed for their MSI value, but captive individuals were analyzed by MateRx to locate the best possible mate (who would result in the lowest MSI), independent of whether those birds could realistically be paired together.

2.iii. RESULTS

For the MSC – presumed studbook, GENES reported a population size of 351 individuals, with 30 founders and 282.25 living descendents (individuals with incomplete ancestries are tallied as partial genetic individuals). For the MSC – confirmed studbook, GENES reported a population size of 144 individuals, with 30 founders and 136 living descendents. This confirmed population consisted of 100 cranes from the Mississippi Sandhill Crane Wildlife Refuge and 45 captive cranes.

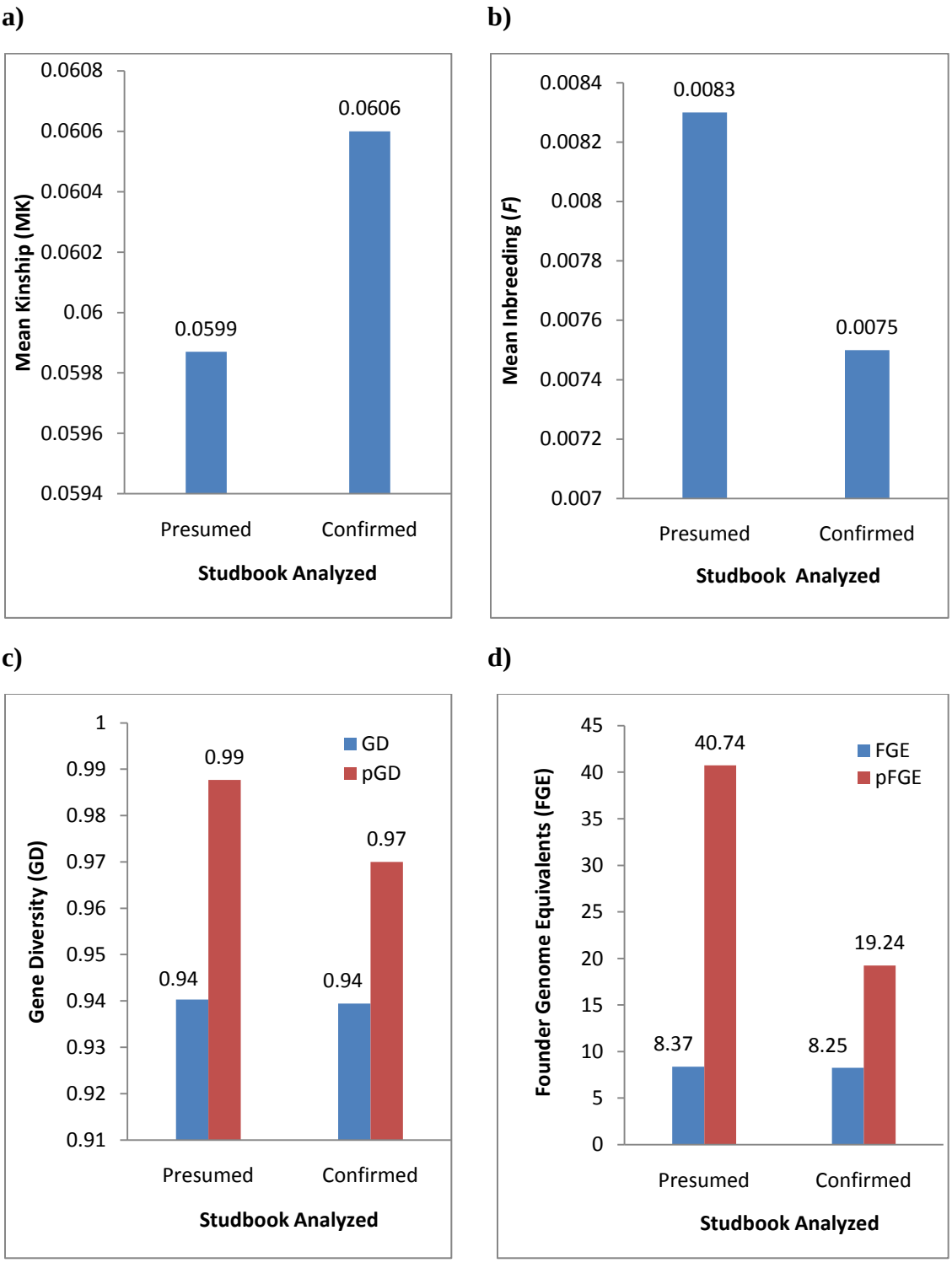
The graphical representation of the pedigree of the MSC – presumed studbook, as produced by Pedigree Viewer, reveals a 7 generation pedigree, with several non-breeders, and a few high output breeders (Fig.1). For example, Studbook #1020, fathered 112 offspring, 43 of which are still presumed alive in the population.

Figure 1. *Graphical representation of MSC - presumed studbook pedigree. (a) represents the unknown parents of the wild founders, (b) wild founders, (c) eggs first brought into captivity as basis for captive breeding program, (d) – (h) descendents of (c).*



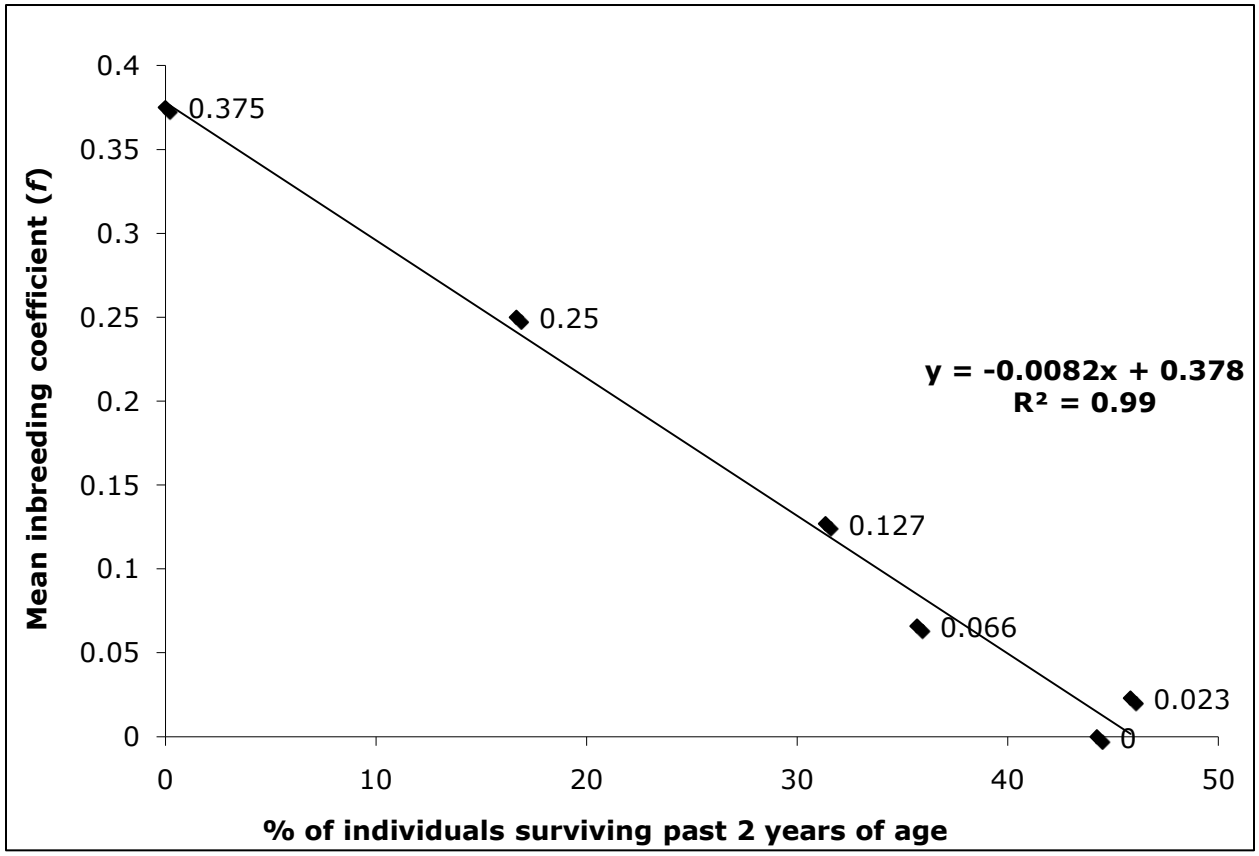
The unbalanced breeding of individuals in the MSC population has resulted in a loss of genetic diversity. The proportion of gene diversity retained from the original founder population is below 96% for both studbooks, with only 8 around founder genome equivalents (depending on which studbook is analyzed). (Fig. 2). The average mean kinship ranged from 0.060 - 0.061 (Fig. 2), with individual mean kinships in all studbooks ranging from 0 to 0.094. The potential for increasing these statistics is reported by the potential gene diversity (pGD) (0.970 - 0.99) and potential founder genome equivalents (pFGE) (19.24 - 40.74) (Fig. 2). Population Management 2000 reported similar results when management goals were modeled, estimating the need for 20 new founders in order to maintain 90% gene diversity for 100 years in the MSC – presumed studbook.

Figure 2. *Mississippi sandhill crane studbook comparisons for (a) mean kinship, (b) mean inbreeding, (c) gene diversity, (d) founder genome equivalents (FGE and pFGE). Differences in the two MSC studbooks analyzed are shown.*



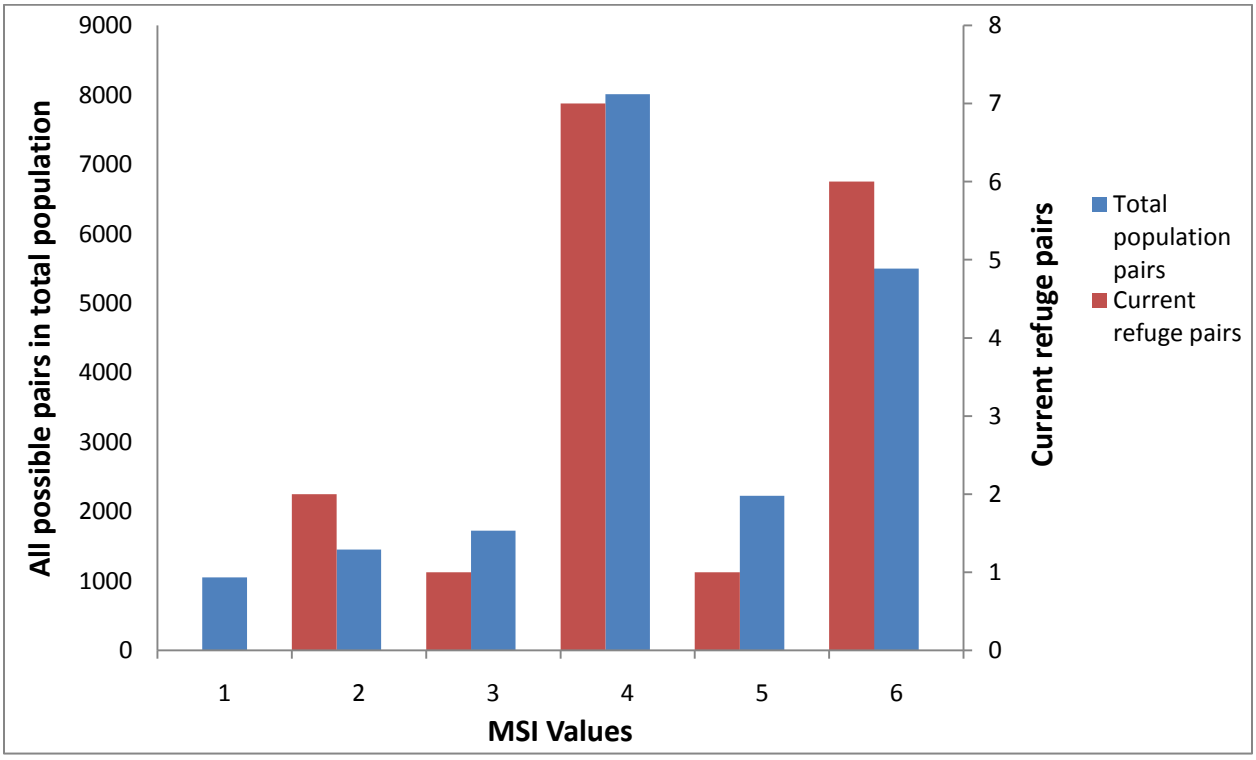
Mean inbreeding (*F*) of the studbooks ranged from 0.0075 - 0.0083 (Fig. 2), with inbreeding coefficients between 0 and 0.325. In order to study the possible effects of this inbreeding on the MSC captive and released population, I analyzed the relationship between inbreeding coefficients and the percent of individuals surviving past 2 years of age in the MSC – presumed studbook. Inbreeding was significantly negatively correlated with survivorship ($n = 6$, $p < 0.001$) (Figure 3).

Figure 3. *Covariation between percent of individuals surviving past 2 years of age and the mean inbreeding coefficient of those individuals found in the MSC – presumed studbook. ($n = 6$, $p < 0.001$)*



MateRx analyzed the mate suitability of all possible pairs (MSC – presumed studbook) in the captive and released population. The majority of these pairings (68%) would be detrimental to the population (MSI > 3) (Fig 4). The genetic value of all current mating pairs in the released population was evaluated by calculating the mate suitability indices for each pair. Values of these pairs ranged from 2 – 7, with an average rating of 4.58. Optimal pairing was analyzed for each captive individual. Despite pairing individuals to produce the lowest possible MSI, the average MSI for the captive pairings was 4.19 (slightly detrimental).

Figure 4. *Distribution of Mate Suitability Index (MSI) values for all possible pairings in the presumed population (left-hand y-axis), and the distribution of MSI values for current refuge pairs (right-hand y-axis), as reported by MateRX. A value of 1 is very beneficial to the diversity of the population, a value of 6 is very detrimental.*



2.iii.i. DISCUSSION

More than three quarters of the wild MSC population has been raised in captivity. Considering this, an understanding of the population structure of the captive MSC population is imperative if the goal of sustaining a non-supplemented wild population with high genetic diversity is to be achieved. As predicted, analysis of the presumed population resulted in a population size that was much higher than that of the confirmed population size. Due to several birds residing on the refuge that have not been identified, the total MSC population is likely around 170 individuals (S. Hereford, U.S. Fish and Wildlife Service, personal communication).

This population was derived from 19 captive founders, with 30 wild parent founders. As shown by the confirmed studbook analysis, due to unequal breeding of the wild founder lines, many fewer contributing founders would be needed to produce the same genetic diversity currently observed in the captive and released population. Unequal genetic contributions by founders often leads to greater inbreeding in future generations, and a loss of the genetic diversity originally present in the founding population (Lacy, 1989). This study confirmed this in the MSC population as, as the gene diversity decreased 6%, and inbreeding coefficients have been observed as high as 0.375. Additionally, as a mean kinship of 0.125 is comparable to half-siblings, the mean kinship of 0.06 observed in confirmed studbook population is equivalent to individuals being related to the population on average at the level of first cousin (Ralls & Ballou, 2004).

Despite high kinship levels, and the results of MateRX, which reported that a majority of all possible pairings would have a negative effect on the population's gene pool, the high number of potential founder genome equivalents in the confirmed population (MSC - Confirmed

studbook) of 19.24 indicates that equalization of founder representation may help to slow the loss of genetic diversity.

Some of the observed differences in the two studbooks assessed are a result of the different studbook designs. For example, the presumed studbook reported potential founder genome equivalents of 40.73. This is a result of the wild parents still listed as "living" in the presumed studbook, although they are no longer accounted for on the refuge, and many would be over 40 years old. The difference observed in mean inbreeding for the presumed and confirmed populations is likely the result of the inverse relationship between inbreeding and survivorship. No individuals with inbreeding coefficients higher than 0.25 survived past the first year of release. The low survival of individuals with high inbreeding coefficients also explains the relatively low mean inbreeding numbers observed in the populations; although highly inbred individuals have existed in the population, they have not survived long enough to reach sexual maturity.

The goal of most captive breeding programs is to retain 90% gene diversity for 100 years (Frankham *et al*., 2002). Given that the current gene diversity in the MSC population is 94% following only 44 years of captive breeding, that the majority of all possible pairings in the population would have a negative effect on the population's genetic diversity, and that modeling of management goals indicate the need for 20 additional founders, this goal may not be attainable for the MSC. Achievement of 90% gene diversity after 100 years may be even less likely given the primary assumption of all pedigree analyses: that founders are unrelated. When captive breeding programs are established from small isolated founding populations, as in the case of the Mississippi sandhill crane, estimates of mean kinship and inbreeding coefficients in resulting captive populations are likely much larger than are observed by pedigree analysis alone. In order

to address this issue, molecular genetic analyses were conducted on samples from Mississippi sandhill cranes to more accurately assess the genetic status of the population (See chapter 3).

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CHAPTER 3: INTEGRATION OF MOLECULAR ANALYSIS INTO PEDIGREE

3.i. INTRODUCTION

3.i.i. *Use of molecular analysis in captive management*

The minimization of kinship in captive populations can be achieved through the use of pedigree information. Pedigree knowledge alone, however, is not sufficient if pedigree information is missing or questionable (Lutaaya *et al.,* 1999) or when working with a population that has experienced a severe bottleneck before the captive population was established (Jones *et al.,* (2002). If this is the case, as it often is for endangered species, the amount of genetic diversity in the founding population is likely to have been diminished to begin with, and may result in higher levels of inbreeding and lower genetic diversity than those calculated by pedigree analyses.

Several studies have used molecular markers to contribute to captive breeding management. In these studies molecular markers were used to for a variety of analyses, such as assessing founder relationships (Geyer *et al.,*1993; Haig *et al.,* 1994; Haig *et al.,* 1995; Gautschi *et al.,* 2003; Jones *et al.,* 2002), for pedigree reconstruction (Morin & Ryder, 1991; Bowling *et al.,* (2003), for subspecies identification (O'Brien *et al.,* 1987; Ely *et al.,* 2005), and the identification of genetically valuable individuals (Jones *et al.,* 2002; Russello & Amato 2004).

A powerful molecular tool for individual level genetic analysis is microsatellite DNA. These are single locus markers with repetitive, short sequence patterns (1-6 bp) that exhibit co-dominant inheritance (Queller *et al.,* 1993). Microsatellite loci tend to be highly polymorphic,

making them useful for the inference of relationships between individuals (Queller *et al.,* 1993; Blouin *et al.,* 1996; Haig 1998).

3.i.ii. *Relatedness estimators*

Genotypes derived from multiple microsatellite loci can be used to derive relatedness coefficients (Queller and Goodnight, 1989), which can then be applied to estimate relationships in captive populations. Several methods have been proposed for estimating relatedness from molecular markers such as microsatellites. These methods can be grouped into two classes: 1) methods that use moment estimators (MOM) to estimate pair-wise relatedness of individuals, and those that use a likelihood approach to place pairs or groups into relationship categories, such as parent-offspring, or full-sibs.

MOM and likelihood estimators work by indentifying the proportion of matching alleles within a pair. These alleles are said to be identical by state (IBS). Alleles that are IBS can be classified into two categories, those that are identical due to chance, and those that are or identical by descent (IBD) due to deriving from a common ancestor (Blouin, 2003). MOM estimators (Queller and Goodnight, 1989; Lynch and Ritland, 1999) estimate relatedness *(r)* as a continuous measure of overall IBD. Likelihood estimators (Milligan, 2003; Kalinowski *et al.,* 2006) calculate the likelihood of observing the genetic data of a given pair based on the probabilities that a pair share zero, one or two alleles at given locus that are IDB (Thomas, 2005). Although both types of estimators have been used in the management of captive breeding programs (Kozfkay *et al.*, 2008; Ivy *et al.*, 2009; Russello & Amato, 2004), each method has inherent limitations. Maximum likelihood estimators assume no inbreeding, and no population structure (Oliehoek *et al.*, 2006). Populations in need of conservation such as the Mississippi

sandhill crane (MSC), however, are often highly inbred with complex pedigree structures. MOM estimators on the hand, such as Queller and Goodnight (1989), report *(r)* values that are much more variable than can be accounted for by true variance in actual pedigrees (Van horn *et al.,* 2008), and tend to underestimate relatedness between closely related individuals (i.e. full sibs) (Frentiu *et al.,* 2008). As the Queller and Goodnight (1989) relatedness estimator measures relatedness based on the mean relatedness in the measured population these discrepancies are likely to be enhanced when working with a population with a very low level of diversity at the start, or a population with few highly related individuals in the population.

Given the lack of consensus on the accuracy of these relatedness estimators (Lynch & Ritland, 1999; van de Casteele *et al.,* 2001; Toro *et al.,* 2003;, Blouin, 2003; Oliehoek *et al.,* 2006), some researchers have instead relied on the simple calculation of the proportion of alleles shared, or allelic similarity *(s),* as a measure of how common or rare a molecular genotype is in the population (Blouin *et al.,* 1996, Jones *et al.,* 2003). While allelic similarity *(s)* cannot distinguish individuals that are IBD from those that are simply IBS, when this information is assessed in tandem with pedigree information, informative estimates of relatedness can be achieved.

3.i.iii. *Genetic analysis of the Mississippi sandhill crane*

To date three types of genetic analyses have been conducted on the Mississippi sandhill crane population: (1) allozyme analyses (Dessauer *et al.,* 1992); (2) mitochondrial DNA (Rhymer *et al.,* 2001); and (3) microsatellite DNA (Jones, 2003). These studies were focused on population differentiation among cranes, and therefore no within-population analysis has yet been applied to the pedigree of the captive Mississippi sandhill crane population. The objective

of this study was to utilize microsatellite DNA analysis to assess the level of genetic variation in the MSC, and to use this information to refine the genetic management of the population.

3.ii. MATERIALS AND METHODS

3.ii.i. *Sample collection*

In order to assess genetic variation in the MSC population, whole blood samples were collected from individuals living in captivity and on the Mississippi Sandhill Crane National Wildlife Refuge in Gautier, MS. Trapping efforts on the refuge were conducted intermittently from January 23rd 2008 to March 25th 2009. Cranes were captured using foot noose lines, a bird-catching technique from India (Hereford *et al.,* 2000). Crane foraging sites identified as potential trapping locations were baited with corn kernels. After cranes were observed feeding on the bait, noose lines were set during pre-dawn hours, and bait sites were monitored from distant blinds to minimize disturbance. When cranes feeding on the bait corn step through a foot noose their leg becomes snared and the crane is unable to fly away. Captured cranes are then restrained and hooded to limit stress while measurements are taken and blood is collected from the medial metatarsal vein. A total of 19 samples were collected in this manner. (Appendix A).

Additionally, blood samples were collected from the 19 juveniles in the 2008 cohort release, and from the captive population: 28 individuals at Audubon Center for Research on Endangered Species and 14 captive individuals at the White Oak Conservation Center. (Appendix A). For all whole blood samples, approximately 4 ml of collected blood was placed in a lysis buffer (0.1 M Tris, 0.1 EDTA, 5% SDS, 0.01M NaCl; Longmire *et al.,* 1991).

Tissue samples from 14 frozen crane carcasses and embryos were also collected and kept frozen until DNA could be extracted (Appendix A). In total, 94 samples were collected.

3.ii.ii. *Molecular genetic analysis*

DNA was extracted from the blood and tissue samples using Qiagen DNeasy Blood & Tissue Kit (QIAGEN). This extraction was completed first by digesting the histones and other proteins associated with DNA with the enzyme proteinase K. The DNA was then selectively bound to a silica-gel membrane during centrifugation, and cellular remains were washed off the DNA using a series of salt and ethanol containing buffers. Lastly, the DNA sample was eluted in buffer (Qiagen, 2006) and was kept at 20° C until use.

The 94 Mississippi sandhill samples were genotyped at 14 microsatellite loci [GRAM6, GRAM8, GRAM11, GRAM17, GRAM20, GRAM22, GRAM24, GRAM25, GRAM30, GRAM31, GRAM32a, GRAM40, GRAM42 , GRAM45] developed by Dr. Kenneth Jones from a genomic library of the whooping crane (*Grus Americana*) (Jones *et al., in prep*), a species of the same genus as the sandhill crane (Krajewski & Fetzner, 1994).

Polymerase chain reactions (PCR) were carried out in a thermocycler (Bio-Rad Icycler) in a volume of 15 microliters. PCR protocols showed optimization with 1x Promega Taq polymerase reaction buffer [Promega Corp., Madison, WI], 1.5 MgCl2, Qiagen dNTP mix [100 uM each], forward and reverse primers [0.5 uM forward, 0.005 uM reverse], one unit Promega *Taq* polymerase, and with least 25 nanograms of DNA. Additionally, in order to avoid labeling individual primers, all primers were designed with a CAG tag attached to the 5' end of the reverse primer, and a labeled CAG tag was added to the amplification reactions [0.5 uM] (Boutin-Ganache *et al.*, 2001). Each reaction was run with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 50-65°C for 30 seconds, 72° for 40 seconds, with a final extension step of 72°C for 5 minutes. PCR products were pooled together

and run against Genescan™ 500 ROX™ (red) internal size standard in an Applied Biosystems Inc. 3760 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Samples were genotyped using GeneMapper v. 4.0 (Applied Biosystems, Inc., Foster City, CA).

3.ii.iii *Microsatellite DNA analysis*

The program Micro-checker (Van Oosterhout *et al.*, 2006) was used to identify genotyping errors. The number of alleles per locus, observed heterozygosity (H_o) and expected heterozygosity (H_e) was calculated with the web-based (http://wbiomed.curtin.edu.au/genepop) version of GENEPOP (Raymond & Rousset, 1995). FSTAT (Goudet, 2001) was used to test for deviations from Hardy-Weinberg equilibrium, and to evaluate loci for linkage disequilibrium. A sequential Bonferroni test (Rice, 1989) was used to compensate for multiple comparisons.

As previously discussed, (3.i.ii) several methods exist for estimating relatedness, and there is little consensus among researchers as to the best method. In order to address this issue two relatedness estimators were used: (1) Allelic similarity *(s)*, calculated as the proportion of alleles shared (Blouin, 1996); and, (2) Queller and Goodnight's (1989) MOM relatedness estimator (*r*). Likelihood estimators (Milligan, 2003; Kalinowski, 2006) were not chosen for estimation of relatedness as these methods assume a large panmictic population. As established in Chapter 2, the MSC population does not meet this criteria.

Allelic similarity coefficients were calculated by dividing the number of allelic positions shared between two individuals by the total number of allelic positions assessed (Blouin *et al.*, 1996). A matrix of pairwise similarity coefficients was created for all sampled individuals using Microsatellite Toolkit for Excel (Park, 2001), and then reduced to reflect only the "founders" of the captive population.

MARK v.3.1. (Ritland, 2006) was used to calculate Queller & Goodnight's (1989) pairwise relatedness coefficients *(r)* (see Appendix B for equation). As with allelic similarity, a matrix of pairwise relatedness coefficients was created for all sampled individuals, and then reduced to reflect only the "founders" of the captive population.

3.ii.iv. *Studbook revision, pedigree analysis, and DNA integration*

The MSC – confirmed studbook (Chapter 2) was used as the base studbook for the integration of molecular data into pedigree analyses of the MSC population. However, while the MSC – confirmed studbook reports a more accurate picture than the historical studbook of the individuals currently found in the population, a difficulty in estimating founder relatedness persists given that this studbook is coded such that it is not the first individuals brought into captivity that represent the founding MSC population, but their wild parents, assigned by breeding territory. For the integration of microsatellite DNA data, the parentage assignment of the first individuals brought into captivity were reassigned with the "wild x wild" code, designating the starting captive population, and any individuals with unknown wild parents (such as eggs later collected from the refuge) as founders. Following the methods of Jones *et al*. (2002) molecular data was integrated into this studbook, named the MSC – DNA studbook.

As inaccurate or incomplete information can occur in pedigree record keeping that spans more than 40 years, genotypes of sampled individuals were first compared to known pedigree relationships, to identify any mislabeling or misidentification that may be present in the studbook. ML-Relate (Kalinowski *et al.*, 2006) was then used to test a priori parent/offspring hypotheses of individuals with multiple possible sires, or with multiple possible identities. This test works by evaluating two competing a priori hypotheses based on the genotypic data; that the

individuals are unrelated (null hypothesis); and that the individuals have a parent/offspring relationship (See Appendix B for test statistic).

Integration of the relatedness estimates was completed through GENES 12.0 (Lacy, 1998). Founder matrices for both *(s)* and *(r)* were entered individually into GENES, replacing the default founder matrix of zero relatedness among founders. GENES 12.0 (Lacy, 1998) was also utilized to compare the results of integrating the different founder similarity and relatedness matrices into the MSC – DNA studbook. The changes in mean inbreeding (*F*), mean kinship (MK), founder genome equivalents (FGE), potential founder genome equivalents (pFGE), and gene diversity (GD) (as described in Chapter 2) were assessed.

Finally, a breeding recommendation chart was developed for the captive MSC population based on the combined results of the pedigree analysis of the MSC – presumed studbook (the historical studbook), and the MSC – DNA studbooks with both and the allelic similarity and Q&G relatedness estimates integrated. This recommendation chart is based on a procedure for breeding recommendations developed by Dr. Ken Jones for the captive Whooping Crane (*Grus Americana*) population (Jones *et al., in prep*) in which individual pair recommendations are made based on three criteria: 1) mean kinship and inbreeding levels as calculated by pedigree analysis of the historical studbook with founder relatedness equal to zero; 2) mean kinship and inbreeding calculations of a pairing as calculated by the DNA – studbook pedigree analysis with founder allelic similarity coefficients integrated; 3) mean kinship and inbreeding calculations of a pairing as calculated by the DNA- studbook pedigree analysis with founder Q&G relatedness coefficients integrated. A step-wise analysis beginning with criteria (1) and ending with criteria (3) results in a breeding recommendation chart in which individual pairings are identified as either beneficial to the gene diversity of the population (green and

blue), harmful to the gene diversity of the population but offspring are suitable for wild release (orange and yellow), or harmful and to be avoided under all circumstances (black). See Appendix C for enumeration of chart organization.

3.iii. RESULTS

3.iii.i. *Microsatellite Analysis*

DNA fragments from 14 loci were successfully amplified (Table 1). Loci 24 and 25 were monomorphic (Table 1), and were subsequently removed from the study. The remaining 12 alleles were screened for all 94 individuals, resulting in 77 complete genotypes, and 17 individuals missing data for 1 or more loci (Appendix D). Size range, number of alleles, and expected heterozygosity were calculated (Table 1).

Table 1. *Summary of microsatellite alleles observed in samples from Mississippi sandhill crane population.*

Locus	No. of samples	Fragment Size (bp)	No. of alleles	He	Ho
GRAM6	94	235-257	5	0.80	0.83
GRAM8	90	361-393	4	0.52	0.33
GRAM11	93	256-324	8	0.70	0.79
GRAM17	84	359-395	6	0.75	0.80
GRAM20	88	379-414	4	0.51	0.55
GRAM22	92	158-170	3	0.52	0.51
GRAM24	93	355	1	0.00	0.00
GRAM25	94	147	1	0.00	0.00
GRAM30	90	157-189	7	0.78	0.98
GRAM31	92	255-259	2	0.48	0.52
GRAM32a	94	247-255	3	0.27	0.28
GRAM41	93	266-296	3	0.34	0.38
GRAM42	93	165-168	2	0.33	0.40
GRAM45	94	255-264	2	0.20	0.22

Two loci, Gram8 and Gram30, showed deviations from Hardy-Weinberg proportions, but the deviations were not significant after correcting for multiple comparisons (Table 1). All loci were in linkage equilibrium after correcting for multiple comparisons.

Allelic similarity *(s)* and Queller & Goodnight relatedness estimates *(r)* were calculated for each pair of individuals in the dataset. The *(s)* values ranged from 0.222 to 0.917, with a mean value of 0.59. The *(r)* values ranged from -0.909 to 1.00. As expected with *(r)* estimates, the mean value of *(r)* for the population was 0.00.

3.iii.ii. STUDBOOKS AND STUDBOOK INTEGRATION

Studbook/Genotype comparisons

To test the accuracy of the studbook records, the genotypes of samples were compared with expected parent/offspring genotypes. Only 2.02% (48/2376) of the alleles called did not match expected parent/offspring alleles. Given the comprehensive nature of the MSC studbook, few gaps could be filled from the molecular analyses. However, the identity of one refuge resident crane, and the probable sires of two individuals were confirmed based both on visual confirmation of genotypes, and by the ML-Relate a priori hypothesis test of parent/offspring relationships (Table 2).

Integration of relatedness estimates

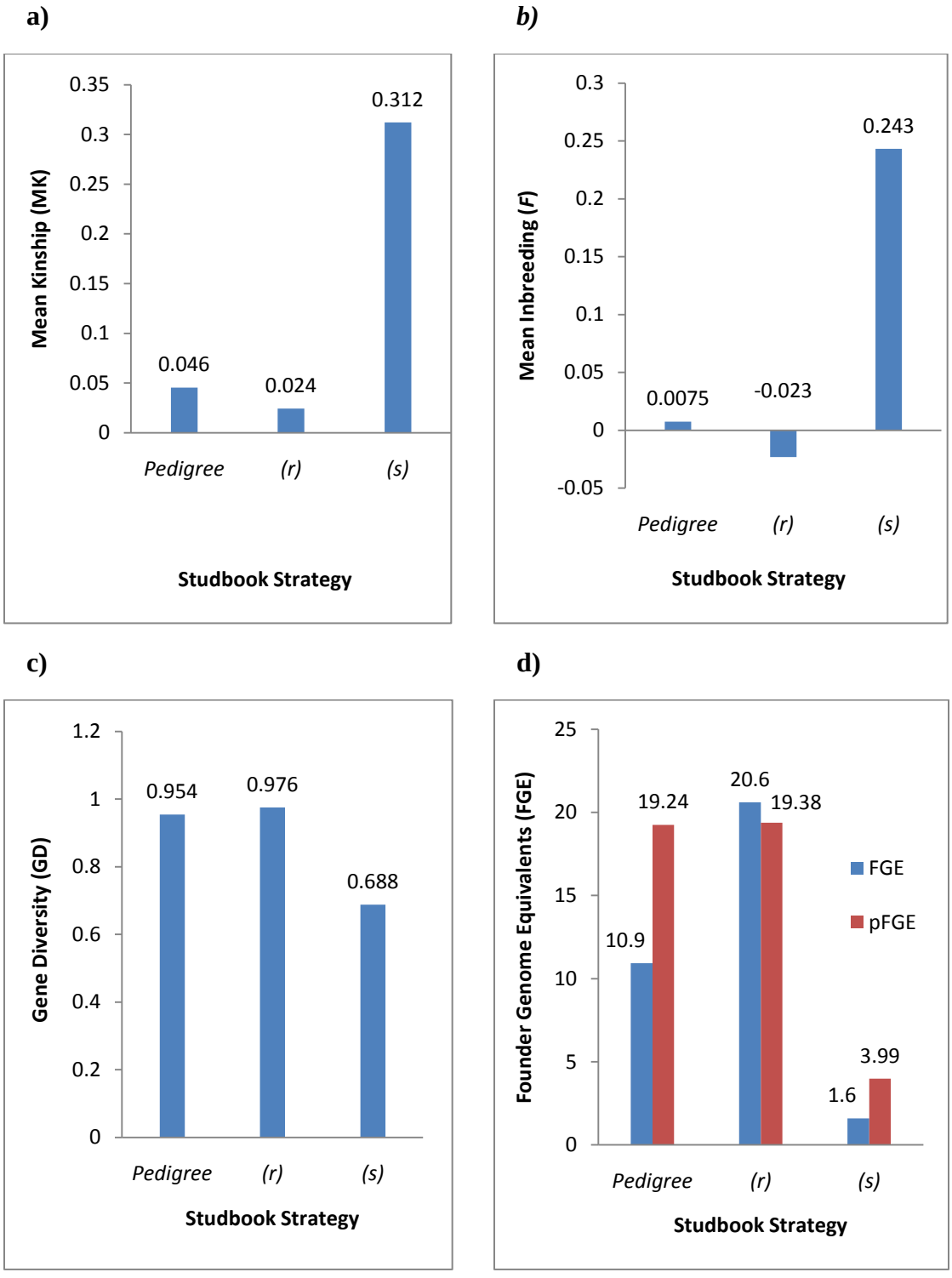
Samples were available for microsatellite analysis for 11 out of the 19 founders in the DNA studbook as reported by GENES 12.0. Based on offspring genotypes, the genotypes for 4 additional founders were estimated at 8 loci or more. For the remaining 4 founders, offspring for whom samples were available replaced their founding parents. This resulted in a final founder matrix of 20 founders.

Table 2. *Changes to studbook based on genotypic information and hypothesis testing. The P-value refers to the null hypothesis test (individuals are not related). A high P-value indicates the null hypothesis cannot be rejected. A low P-value supports the alternative hypothesis of a parent-offspring relationship.*

Individual	Unknown information	P/O relationship tested	P- value	Conclusion
1385/1223	Wild caught – ID thought to be 1385 or 1223	1034: 1223 1018: 1223 1137:1385 1163:1385	p = 0.98 p = 1.00 p = 0.064 p = 0.001	ID= 1385
1149	Multiple possible sires: 1020, 1034	1020:1149 1034:1140	p = 0.023 p = 0.936	1149 sire = 1020
1352	Multiple sires: 1040, 1152	1040:1352 1152:1352	p = 0.014 p = 0.049	1352 sire = likely 1040

Allelic similarity *(s)* and Queller & Goodnight (1989) *(r)* estimates of relatedness were calculated using the 4 estimated founder genotypes and 94 genotypes of sampled individuals. The average *(s)* for the founder population was 00.59, whereas the average *(r)* of the founders was only 0.012. The differences observed in these calculations are reflected in the results of the incorporation of the founder relatedness matrices into MSC-DNA studbook. When MSC founders were redefined as interrelated by *(s)*, the population appeared more genetically similar than as reported by studbook analysis alone (Fig 5). When MSC founders were redefined by the *(r)* estimates, however, results reported a population slightly less genetically similar than as reported by studbook analysis alone (Fig. 5).

Figure 5. *Mississippi sandhill crane studbook comparisons for (a) mean kinship, (b) mean inbreeding, (c) gene diversity, (d) founder genome equivalents (FGE and pFGE). Differences are shown for the three studbook strategies: pedigree = DNA-Studbook with founder relatedness = 0; (s)= DNA-Studbook with founder allelic similarity relatedness values incorporated; (r) = DNA-Studbook with founder Queller & Goodnight relatedness values incorporated.*



Using the output from the MSC – presumed studbook (the historical studbook), and the output from the MSC – DNA studbook for both *(s)* and *(r)* estimates, a comprehensive breeding recommendation chart was developed for the captive MSC population (See Appendix C). Out of the 412 possible pairing combinations in the captive population, only 18.20% would result in offspring that would increase the gene diversity of the population, and therefore should be kept in captivity for future breeding (green and blue highlighted pairs). 25.24% of pairings would result in offspring that would decrease the gene diversity of the captive population, but have low levels of inbreeding and are therefore suitable for release on to the Mississippi Sandhill Crane National Wildlife Refuge (orange and yellow highlighted pairs). 56.55% of the pairings result either in high inbreeding or a significant loss of gene diversity and should not be paired for any reason (black highlighted pairs). The integration of genetic variation data into the studbooks reveal several pairings that appear beneficial to the gene diversity of the population based on pedigree information alone, but are actually more genetically similar than the average pairing in the population (Table 3).

Table 3. *Inbreeding coefficients of pairings as calculated by the MSC – presumed studbook, and the MSC – DNA studbook with founder allelic similarity (s) and relatedness (r) information integrated into the analysis. Pairings listed appear beneficial to the captive population according to the MSC – presumed studbook, however microsatellite analysis reveals the pair to be more genetically similar than the average pairing in the population. The average mean kinship for the MSC – DNA (s) studbook is 0.32, the average mean kinship for the MSC – DNA (r) studbook is 0.03. Inbreeding coefficients above these average mean kinships will result in a reduction in observed heterozygosity across the DNA markers.*

Sire	Dam	MSC –presumed inbreeding (*F*)	MSC – DNA (*s*) inbreeding (*F*)	MSC-DNA (*r*) inbreeding (*F*)
1033	1217	0.000	0.335	0.60
1033	1774	0.000	0.340	0.046
1044	1163	0.000	0.375	0.114
1258	1774	0.000	0.340	0.117
1258	1138	0.000	0.335	0.031
1804	1774	0.001	0.295	0.057
1804	1138	0.016	0.335	0.067

3. iv. DISCUSSION

3.iv.i. *Microsatellite Analysis*

Twelve of 14 microsatellite DNA markers were successfully amplified and were polymorphic. The genotypic frequencies differed from panmictic expectations in two loci but were not significant after Bonferroni correction.

3.iv.ii. *Studbook Integration*

With only 2% of the alleles called varying from expected pedigree relationship calls, the historical MSC studbook appears to be relatively comprehensive and accurate. Of the mismatches, 54% can be accounted for by 4 individuals; three who have likely been mis-identified in the field (1412, 1757, 1824), and one whose sire/dam information appears to be incorrect in the studbook (1646). The remaining incongruent allele calls appear to have resulted from allelic dropout and/or incorrect allele calls.

The integration of allele sharing *(s)* and relatedness *(r)* estimates of founders into the MSC - DNA studbook levels resulted in a large variation in reported levels of genetic diversity in the MSC population. For example, mean kinship (MK) increased over 500% when allele sharing estimates were introduced. While dramatic, a (MK) value of 0.31 does not accurately predict the average kinship of this population (full sib kinship = 0.25). The drastic changes in observed genetic diversity are the result of the high average allelic similarity (0.59) of the MSC population. This measurement does indicate an underlying population structure that is very similar, but without knowledge of the historic allele frequencies there is no way to differentiate between alleles IBD and IBS.

Conversely, the integration of Q&G relatedness estimates resulted in a population that appeared less genetically similar than as estimated by studbook alone. This is likely the result of the nature of the *(r)* calculation which first calculates mean *(r)* and then designates pairwise relatedness coefficients based on whether pairs or more or less similar than the mean ($-1 \leq R \leq 1$) (Hedrick, 2005). Therefore, given the low level of allelic diversity observed in the MSC population (mean $s = 0.59$), small differences in genotypes were inflated by the relatedness estimates. In other words, the changes observed in the population after the integration of *(r)* is not the result of an increase in genetic diversity of the population, but rather a decrease in the amount of diversity lost due to the fact that there was less diversity to begin with.

The integration of allelic similarity *(s)* and relatedness *(r)* into the MSC studbook reveals the care required when choosing and relying on only one relationship estimator to analyze the genetic diversity of a population, especially if that information is subsequently used to make breeding recommendations. Despite the discrepancies observed between the estimators the information revealed by them can prove useful for managers of captive populations. Estimates of relatedness, for example, provide clear indication of a good or bad pairing. If the *(r)* for a given pairing is positive, those individuals are more related than the population average, indicating a poor pairing. Thus managers can work to only pair individuals with negative *(r)* values. Estimates of allelic similarity, on other hand, can indicate the commonness or rareness of an individual's genotype in the population. As pointed out by Jones *et al.*, (2002) this information can be used to select matings that work to equalize founder allele frequencies, and can lead to heterozygosities in the population above that of the founder population.

Allelic similarity and relatedness estimates are also useful for identifying pairings that may appear unrelated according to studbook information, but are shown to be more genetically similar than the average population pairing after molecular analysis. As shown in the MSC breeding recommendation chart (Appendix C) and Table 3, the integration of genetic variation data into the MSC studbooks revealed several pairings that appear beneficial to the gene diversity of the population based on pedigree information alone, but that are actually more genetically similar than the average pairing in the population (Table 3). The pairing of these individuals would result in a loss of observed heterozygosity across the population (a loss of genetic variation), and should therefore be avoided.

The breeding recommendation chart introduced in this study combines the valuable information gleaned from both allelic similarity (*s)* and relatedness (*r*) estimators with the traditional pedigree information provided by studbook analysis. Pairing of individuals in the chart is still based primarily on mean kinship and inbreeding as estimated by the historical studbook. The decision process, however, is aided by knowledge of how the genetic variation (i.e., observed heterozygosity) in the population will change as a result of that pairing. This combined knowledge provides a powerful tool for breeding managers working to prevent inbreeding and increase genetic variation in their captive population.

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CHAPTER 4: POPULATION DIFFERENTIATION IN NON-MIGRATORY SANDHILL CRANES

4.i. INTRODUCTION

4.i.i. *Use of molecular DNA analysis and statistics to measure population differentiation*

Discrete populations of most species exhibit at least some degree of genetic differentiation (Avise, 1994). This differentiation, referred to as genetic structure, represents the distribution of genetic variation within and between groups or populations (Wright, 1951). Analysis of genetic structure is rooted in the Hardy-Weinberg principle that gene frequencies within a population will not change from one generation to the next (Hardy, 1908; Weinberg, 1908). If a population becomes divided, each of the new sub-populations will evolve its own gene frequencies via selection and drift (Wright, 1951). Therefore, by analyzing the genetic structure in two populations, the level of gene flow between those populations can be assessed.

Several approaches to the statistical description of population structure and population subdivision have been developed. One of the earliest and still implemented approaches is Sewell Wright's (1951) F-statistics; F_{IS}, F_{IT}, and F_{ST}, which describe population structure in terms of allelic correlations. F_{IS} represents the correlation between homologous alleles within individuals in a local population, and F_{IT} is the corresponding allelic correlations for the total population. The variance of allele frequency among populations is represented by F_{ST} (Wright, 1951) (See Appendix B for equation). F_{ST} hypothesizes that given the Hardy-Weinberg principle, if two populations were interbreeding they would exhibit similar gene frequencies at all neutral loci, and the variance between the frequencies would be zero (Wright, 1951). Due to the neutral loci used in microsatellite DNA analyses, Wright's F_{ST} can be easily calculated using the gene

frequencies estimated from this method. One limitation of the Wright's F_{ST}, however is the requirement of a large number of populations with equal sample sizes. To overcome this limitation Weir and Cockerham (1984) developed (θ), an estimator that provides the power of F_{ST}, but allows for a small number of populations and unequal sample sizes (See Appendix B).

F_{ST} is also commonly used as a measure of gene flow among populations (Avise, 1994). Gene flow, (i.e., the transfer of genetic material between populations), is usually expressed by the proportion of alleles in a population for each generation that is of migrant origin, known as the migration rate, *m* (Avise, 1994). As distinguishing between the effects of drift and gene flow is difficult, however, most estimates of gene flow rely of *Nm*, which is defined as the absolute number of individuals exchanged between populations per generation (Avise, 1994). Wright (1951) used F_{ST} to calculate *Nm* based on expectations for neutral alleles and equilibrium expectations (See Appendix B).

Nm has also been calculated based on the average frequency of private alleles in a population (See Appendix B). Private alleles are those that are found in only one population (Avise, 1994). Slatkin's (1985) private allele method is rooted in the theory that private alleles are only likely to attain high frequency when *Nm* is low. When enough genetic information is available F_{ST} and private allele methods should result in comparable estimates of gene flow (Slatkin & Barton, 1989).

4.i.ii. *History of sandhill cranes in the southeastern United States*

Of the 15 described species of cranes, the sandhill crane (*Grus canadensis*) is the most abundant (Meine & Archibald, 1996) and diverse, with nine designated populations and six subspecies. The lesser sandhill crane (*G.c.canadensis*), from the arctic region of North America,

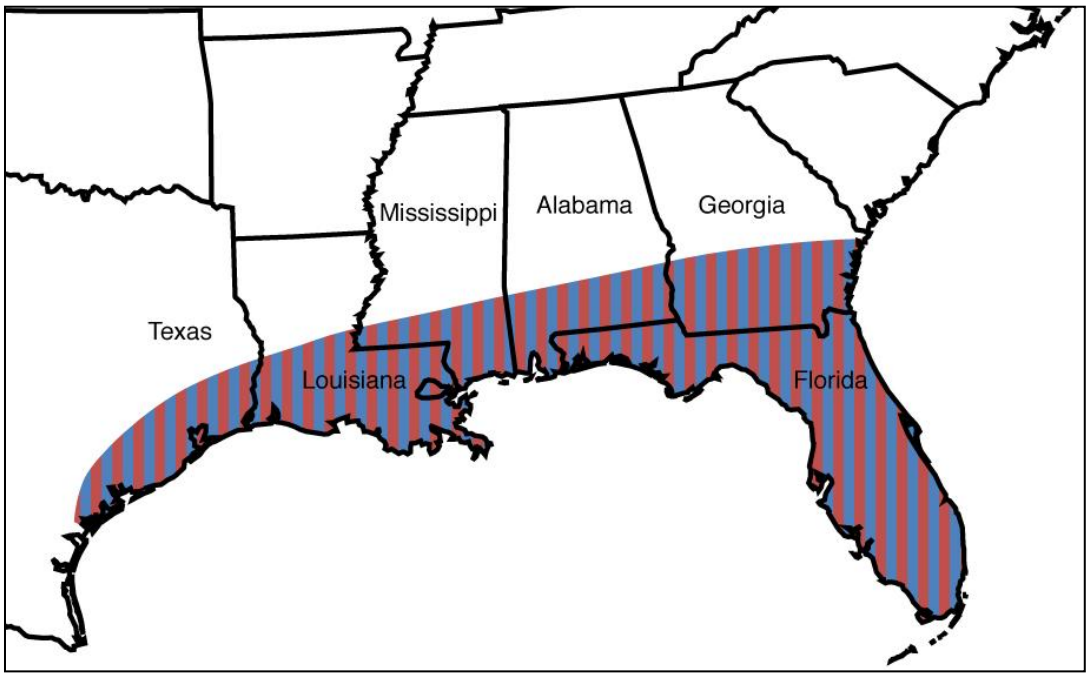
was the first sandhill to be designated a member of the crane family (*Grus canadensis*) by Brisson in 1760. In 1794 a non-migratory population of sandhills in Florida was described as the Florida sandhill crane *(G. c.* pratensis) by Meyer (1794, as found in Rhymer, 2001), and in 1854 another non-migratory population was identified on the mainland of Cuba by Poey (1854). This Cuban sandhill (*G.c. nesiotes*) was subsequently named a subspecies by Bangs and Zappey (1905). Two additional migratory subspecies, greater sandhills (*G.c.tabida*), and Canadian sandhills (*G.c.rowani*) were later defined by Walkinshaw (1965).

The final sandhill crane sub-species to be identified, the Mississippi sandhill crane (*G. c. pulla*), was first described as a non-migratory subspecies of sandhill crane in 1972 (Aldrich, 1972). In addition to the morphological distinction of having darker plumage than other sandhill cranes, the Mississippi sandhill cranes (MSC) are reported to mature earlier, and begin egg production about 6 weeks later than Florida sandhill cranes (Gee and Hereford, 1995). Although the MSC population is now considered reproductively isolated from other sandhill crane populations (Gee and Hereford, 1995; Meine and Archibald, 1996), it is believed that the MSC was once part of an extensive non-migratory population that spanned from Florida to central Texas along the Gulf coast (USFWS, 1991) (Fig. 6). Overhunting and habitat alterations (Meine & Archibald, 1996) resulted in the fragmentation of this coastal population, leading to its extirpation from Louisiana and Texas by 1919 (Oberholster, 1974). By 1960 only a small remnant population, now identified as the MSC, remained west of Florida (Aldrich, 1972) (Fig. 6).

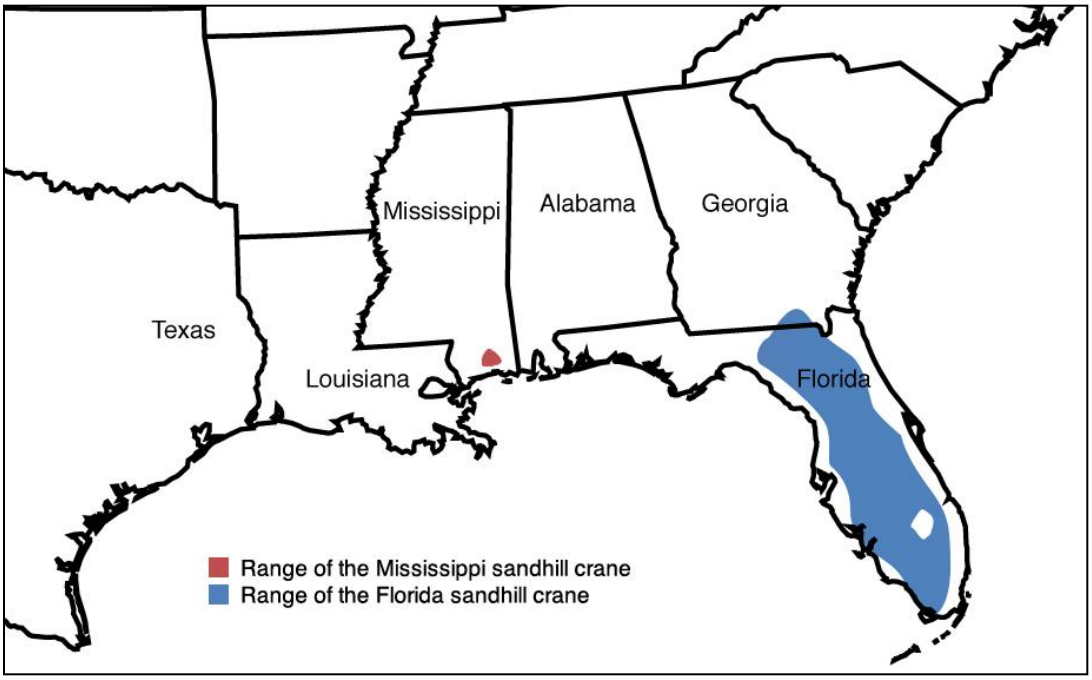
Although also listed as threatened by the USFWS (Meine & Archibald, 1996), the eastern portion of the non-migratory coastal sandhill population, the Florida population, has remained persistent in its historical range, and now spans the length of Florida from the Okefenokee

Figure 6. *a) Historical range of sandhill cranes in the southeastern United States. b) Current ranges of the Florida and Mississippi sandhill cranes.*

a)



b)



National Wildlife Refuge in southeastern Georgia to the Florida everglades (Meine & Archibald, 1996) (Fig. 6). Tacha *et al.,* (1994) estimated the population size of the Florida sandhill crane to be between 4,000 – 6,000 individuals.

4.i. iii. *Integration of Florida sandhill cranes into the Mississippi flock?*

Despite reports of reproductive isolation in the Mississippi population (Gee & Hereford, 1995), recent molecular genetic analyses of the non-migratory sandhill crane populations have not supported the distinction of the Mississippi population as a separate subspecies. Rhymer *et al.* (2001) found no significant phylogenetic divergence in the mtDNA haplotypes of the *Grus canadensis* subspecies, and suggesting that the Florida and Mississippi populations be treated as Distinct Population Segments (DPS). Both Rhymer *et al.,* (2001) and Jones (2003), however, observed relatively large allele frequency differences between the Florida and Mississippi sandhill crane populations. Jones (2003) hypothesized that the difference observed between the Florida and Mississippi populations ($F_{ST} = 0.15$) was larger than the differences between the Florida population and all other sandhill crane populations (mean FST = 0.0675) not due to true endemism, but as a result of the genetic drift and inbreeding occurring in the Mississippi population due to isolation.

In consideration of the connectivity between the historical ranges of the Mississippi and Florida populations, Jones (2003) suggested that translocations of birds from the more abundant Florida population to the Mississippi Sandhill Crane National Wildlife Refuge could be used to bolster the genetic variability of the MSCs. Given that an additional 20 MSC founders were reported to be needed to maintain 90% gene diversity in the historical MSC studbook for 100 years (Chapter 2), this suggestion is not unreasonable. Wright (1931) suggested that one migrant

individual per generation was sufficient to maintain genetic diversity and prevent inbreeding in isolated populations. Wang's (2004) research into the implications this has for conservation management found that although the number of migrants necessary can vary by sex ratio and age, between one and ten effective migrants per generation is a valid for maintaining gene flow. This would suggest that if Florida sandhill cranes were to be introduced to the Mississippi population, very low numbers of cranes would be needed to significantly increase the genetic variation observed in the MSC population.

The goal of this study was to first compare the population structure and levels of gene flow observed between the Florida and Mississippi sandhill crane populations using newly developed microsatellite markers (Jones *et al., in prep*) to the differentiation observed in Jones' 2003 study, and to then test the hypothesis that the allele frequency differences observed in the Jones' study were due to isolation and not endemism. In addition, this study aimed to observe the potential changes in genetic diversity that would be observed if small numbers of Florida sandhill cranes were integrated into the captive Mississippi population.

4.ii. MATERIALS AND METHODS

4.ii.i. *Sample Collection*

The 40 Florida sandhill crane (FSC) samples used for this study were acquired from Jones' (2003) analysis of population differentiation in cranes. Those samples were obtained from juvenile cranes fledged in Florida (Jones, 2003).

3.ii.ii. *Molecular Genetic Analysis*

As with the 45 MSC samples analyzed in Chapter 3, the 40 FSC samples were genotyped at 14 microsatellite loci [GRAM6, GRAM8, GRAM11, GRAM17, GRAM20, GRAM22, GRAM24, GRAM25, GRAM30, GRAM31, GRAM32a, GRAM40, GRAM42 , GRAM45] developed by Dr. Kenneth Jones from a genomic library of the whooping crane (*Grus Americana*) (Jones *et al., in prep*). Microsatellite amplification followed procedures as described in Chapter 3. Fluorescently labeled PCR products were pooled together and run against Genescan™ 500 ROX™ (red) internal size standard in an Applied Biosystems Inc. 3760 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Samples were genotyped using GeneMapper v. 4.0 (Applied Biosystems, Inc., Foster City, CA).

In addition to the genotypes obtained from the Florida sandhills, genotypes from the Mississippi sandhill cranes (94) were made available from Chapter 3.

3.ii.iii *Microsatellite DNA Analysis*

The program Micro-checker (Van Oosterhout *et al.,* 2004) was used to identify genotyping errors. The number of alleles per locus, observed (H_o) and expected heterozygosity (H_e) was calculated with a web-based (http://wbiomed.curtin.edu.au/genepop) version of

GENEPOP (Raymond & Rousset, 1995). FSTAT (Goudet, 2001) was used to calculate allelic richness, to test for deviations for Hardy-Weinberg, and to evaluate loci for linkage disequilibrium. A sequential Bonferroni test (Rice 1989) was used to compensate for multiple comparisons.

Pairwise multilocus F_{ST} estimates were calculated using FSTAT based on the approach of Weir & Cockerham (1984) and were tested for significance by bootstrapping. Pairwise values for the number of migrants per generation between populations (*Nm*) were calculated by GENEPOP (Raymond & Rousset, 1995) using Slatkin's private allele method (Slatkin, 1985; Barton & Slatkin, 1986) and Wrights (1951) *Nm*.

In order to test the hypothesis that the differentiation observed between Mississippi and Florida sandhill cranes in Jones' 2003 study of gene flow between sandhill cranes was the result of genetic drift and inbreeding caused by the isolation of the Mississippi population, and not endemism, FSC genotypes were compared to two separate datasets: 1) all MSCs sampled; and, 2) founders and wild caught MSC samples only.

Using Microsatellite Toolkit for Excel (Park, 2001), allelic similarity coefficients *(s)* were calculated for within the Florida population, and between the Florida and Mississippi populations. The two FSC individuals with the lowest average relatedness to all individuals in the MSC population were identified. A matrix of pairwise similarity coefficients was created for all sampled individuals (both Florida and Mississippi) and then was reduced to reflect only the "founders" of the captive MSC population with the addition of the two FSC individuals described above.

3.ii.iv. *Pedigree Analysis Following the Integration of Florida Sandhill Cranes*

A founder relatedness matrix, including the addition of the two FSC individuals with the lowest average *(s)* was integrated into the MSC – DNA studbook as utilized in Chapter 3. Integration of the *(s)* estimates was completed through GENES 12.0 (Lacy, 1998), replacing the default founder matrix of zero relatedness among founders.

GENES 12.0 (Lacy, 1998) was utilized to compare the results of integrating the FSC added founder similarity matrices into the MSC – DNA studbook. However, new additions to a studbook remain "potential founders," and do not affect population statistics until they have generated offspring in the population. In order to assess potential changes to the MSC population after the introduction of two FSCs, the program Population Management 2000 (Lacy & Ballou, 2001) was used to hypothetically pair the two introduced FSCs with two individuals in the captive MSC population, and produce offspring. GENES 12.0 (Lacy, 1998) was used to calculate the changes in mean inbreeding (*F*), mean kinship (MK), founder genome equivalents (FGE), potential founder genome equivalents (pFGE), and gene diversity (GD) (as described in Chapter 2).

Finally, in order to assess whether the same results observed after introducing FSC into the MSC population could be achieved simply by improving the pairing of individuals currently in the captive MSC population, the changes in mean inbreeding (*F*), mean kinship (MK), founder genome equivalents (FGE), potential founder genome equivalents (pFGE), and gene diversity (GD) (as described in Chapter 2) were calculated by GENES 12.0 (Lacy, 1998) after using Population Management 2000 (Lacy & Ballou, 2001) to hypothetically mate the two optimal (as identified by lowest average allelic similarity) MSC pairs. The results of this analysis were then

compared to the current population statistics, as well as the predicted population statistics after the introduction of the FSC cranes.

4.i. RESULTS

4.iii.i. *Microsatellite Analysis*

DNA fragments from 14 loci were successfully amplified (Table 4). Once again, Loci 24 and 25 were monomorphic (Table 4), and were subsequently removed from the study. The remaining 12 alleles were screened for all 40 Florida sandhill crane samples, resulting in 30 complete genotypes, and 10 individuals missing data for 1 or more loci (Appendix D). Size range, number of alleles, and expected and observed heterozygosity and were calculated (Table 4).

Table 4. *Summary of microsatellite alleles observed in Florida sandhill crane population.*

Locus	No. of samples	Fragment Size (bp)	No. of alleles	He	Ho
GRAM6	40	231-275	10	0.89	0.88
GRAM8	38	361-401	9	0.80	0.47
GRAM11	40	248-324	13	0.90	0.70
GRAM17	35	359-395	6	0.75	0.80
GRAM20	39	374-430	12	0.86	0.74
GRAM22	39	158-174	4	0.53	0.54
GRAM24	31	355	1	0.00	0.00
GRAM25	30	147	1	0.00	0.00
GRAM30	34	157-189	8	0.81	0.85
GRAM31	40	255-259	2	0.28	0.32
GRAM32a	40	243-259	5	0.65	0.72
GRAM41	33	260-296	4	0.53	0.67
GRAM42	40	162-171	4	0.57	0.60
GRAM45	40	255-264	2	0.05	.05

Although three loci, Gram8, Gram11, and Gram41 showed deviations from Hardy-Weinberg proportions, none were significant after correcting for multiple comparisons (adjusted nominal level 5%) (Table 4). All loci were in linkage equilibrium after correcting for multiple comparisons.

While the MSC population reported only 2 private alleles, the FSC population reported 30 (Table 5). When measurements of diversity are compared with the Mississippi population the Florida population reports significantly ($p < 0.05$) higher gene diversity (H_e) and allelic richness than the Mississippi population (Table 6). The difference in observed heterozygosity (H_o) however, was not significant at the 0.05 level.

Table 5. *Table of alleles found in only one population (i.e. private alleles).*

Locus	Allele	Frequency	Found
GRAM6	231	0.113	Florida
GRAM6	243	0.088	Florida
GRAM6	247	0.175	Florida
GRAM8	361	0.010	Mississippi
GRAM8	369	0.053	Florida
GRAM8	381	0.039	Florida
GRAM8	385	0.066	Florida
GRAM8	389	0.105	Florida
GRAM8	397	0.118	Florida
GRAM8	401	0.026	Florida
GRAM11	248	0.014	Florida
GRAM11	252	0.125	Florida
GRAM11	264	0.137	Florida
GRAM11	296	0.075	Florida
GRAM11	304	0.088	Florida
GRAM11	316	0.013	Florida
GRAM17	383	0.014	Florida
GRAM30	157	0.021	Mississippi
GRAM20	374	0.026	Florida
GRAM20	382	0.013	Florida
GRAM20	390	0.218	Florida
GRAM20	398	0.244	Florida
GRAM20	402	0.154	Florida
GRAM20	410	0.039	Florida
GRAM20	418	0.039	Florida
GRAM20	430	0.026	Florida
GRAM30	181	0.088	Florida
GRAM32a	243	0.05	Florida
GRAM32a	259	0.063	Florida
GRAM41	250	0.061	Florida
GRAM42	162	0.038	Florida
GRAM42	171	0.038	Florida

Table 6. *Descriptive statistics for Florida and Mississippi sandhill crane populations derived from multilocus microsatellite DNA genotypes.*

Population	Average number of genotypes	Alleles per polymorphic locus	Allelic Richness	He	Ho
Mississippi	87.5	4.08	4.07	0.52	0.55
Florida	41.5	6.58*	6.51*	0.54*	0.611

* = significant difference between populations ($p < 0.05$)

A significant ($p < 0.05$) F_{ST} value of 0.137 was observed for pairwise comparisons between all Florida and Mississippi sandhill cranes sampled. Estimates of *Nm* for the same comparison were 0.62 for Slatkin's (1985) private allele method, and 1.57 based on F_{ST}. The variance observed in allele frequencies between the two populations decreased significantly ($p < 0.05$) when only samples from captive MSC founders and wild-hatched Mississippi sandhills were included in the analysis (Table 6).

Table 7. *Pairwise estimates of F_{ST} and Nm based on microsatellite DNA genotypes.*

	F_{ST} (Weir & Cockerham, 1984)	*Nm* (Slatkin, 1985)	*Nm* (F_{ST}, Wright, 1951)
	Florida		
Mississippi – all samples	0.137	0.62	1.57
Mississippi – founders and wilds only	0.094*	0.97	2.41

* = Significant pairwise comparison

The average allelic similarity for each pair of individuals within the Florida population ((s) = 0.46) was significantly less ($p < 0.05$) than the average similarity for each pair of individuals within the Mississippi population ((s) = 0.59). The average allelic similarity for all Florida and Mississippi samples combined was 0.50. The lowest average allelic similarity of an individual Florida sample to all Mississippi samples was 0.17.

3.iii.ii. *Studbooks and Studbook Integration*

The two individuals with the lowest average pairwise allelic similarity to the Mississippi samples (samples 313 and 320, average (s) = 0.31 and 0.37, respectively) were selected for hypothetical integration into the captive MSC population. The pairwise allelic similarity of these individuals to the "founders" were added to the "founder" matrix of the MSC – DNA studbook. This resulted in a final founder matrix of 22 founders.

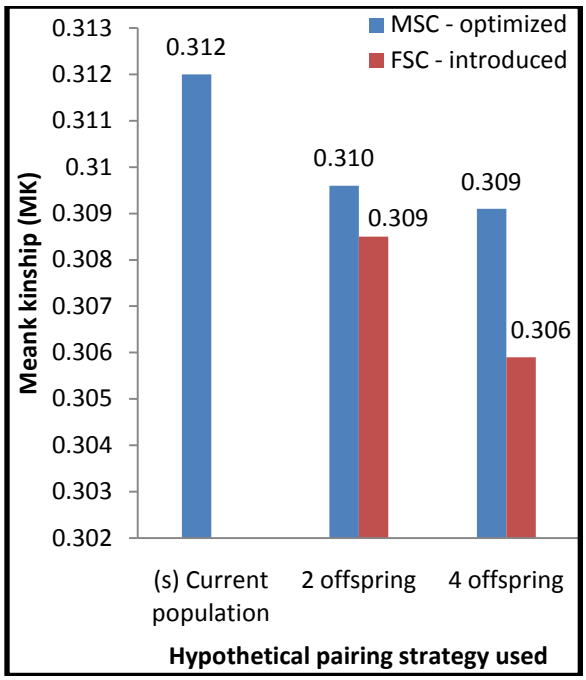
When the founders of this new MSC population including two FSC were redefined as related based on allelic similarity (s) the hypothetical pairing and successful mating of individuals 313 and 320 with two individuals from the captive MSC population (individuals 1708 and 1479) resulted in a decrease in mean kinship and mean inbreeding in the total MSC population, and corresponding increases in gene diversity (GD) and founder genome equivalents (FGE) (Figure 7). One offspring per pair resulted in a 0.96% decrease in MK, a 2.5% decrease in *F*, a 0.29% increase in GD and a 1.2% increase in FGE. The hypothetical production of two offspring per pair resulted in a 1.9% MK decrease, a 2.8% decrease in F, a 0.87% increase in GD, and a 1.9% increase in FGE (Figure 7).

The two captive MSC individuals with the lowest average pairwise allelic similarity to the other Mississippi samples were identified (samples 1560 and 1787, average (s) = 0.51 and

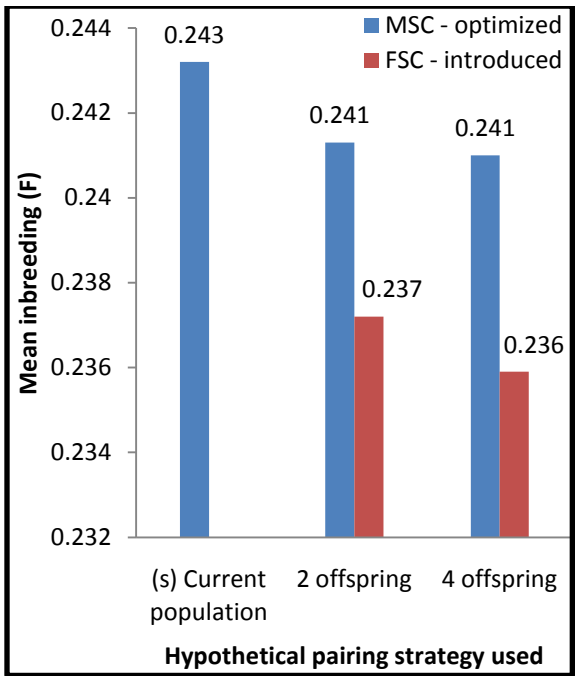
0.54, respectively) and hypothetically mated with two additional low kinship individuals in the captive MSC flock (1809 and 1135). As with the FSC introduction and pairing, these optimal MSC pairings resulted in a decrease in mean kinship and mean inbreeding in the total MSC population, and corresponding increases in gene diversity (GD) and founder genome equivalents (FGE) (Fig. 6). However these changes were not as significant as the changes observed after the FSC introduction. The hypothetical production of two offspring per pair resulted in a 0.96% MK decrease, a 0.82% decrease in F, a 0.44% increase in GD, and a 0.62% increase in FGE (Figure 7).

Figure 7. *Comparison of GENES results of MSC – DNA (s) studbook following the hypothetical pairing of 1) MSC – optimized = 2 optimal MSC pairings as identified by average allele sharing and mean kinship statistics; 2) FSC – introduced = pairing of 2 FSCa with two MSCs. Comparisons are shown for (a) mean kinship, (b) mean inbreeding, (c) gene diversity, (d) founder genome equivalents (FGE) following the successful production of 2 and 4 offspring.*

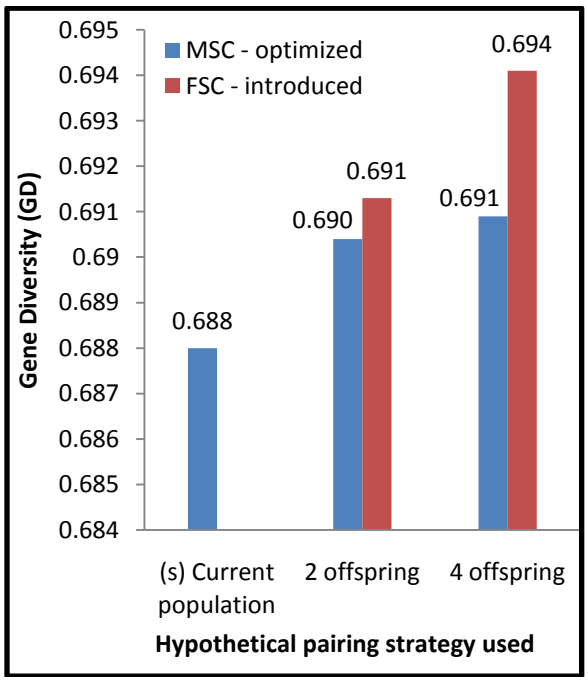
a)



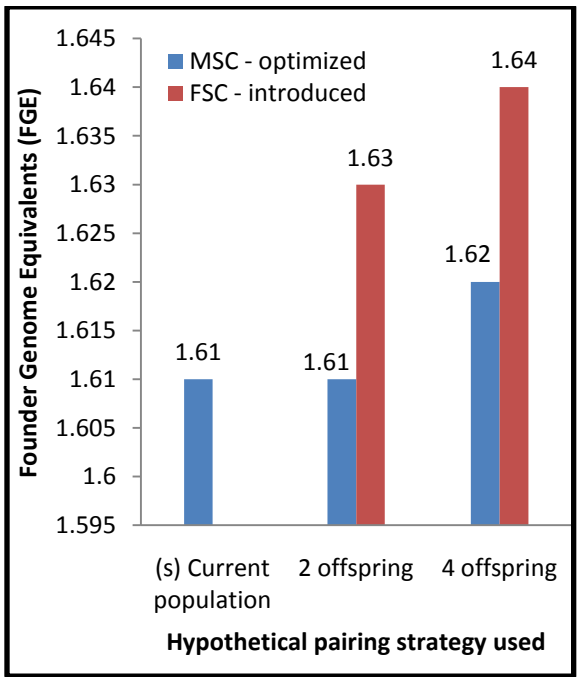
b)



c)



d)



4.iv. DISCUSSION

4.iv.i. *Microsatellite Analysis*

As with the analysis of the Mississippi sandhill crane samples, 12 out 14 DNA markers were successfully amplified and were polymorphic (Table 4). The genotypic frequencies differed from panmictic expectations in three loci but were not significant after Bonferonni correction. Although not significant, the heterozygote deficiency seen at these loci is likely the result of sampling scheme. The samples from the Florida population used for this study were obtained from the research of Jones (2003), in which the Florida population was observed outside of Hardy-Weinberg equilibrium expectations. Jones (2003) suggested the variance between observed and expected heterozygosity was the result of sampling across subpopulations. The sampling scheme for the Florida samples used in this and the 2003 study were collected from nest sites at two different locations within Florida. Thus the decrease in heterozygosity in the total population was a consequence of different allele frequencies in the subpopulations, also known as the Wahlund effect (Wahlund,1928).

4.iv.ii. *Population differentiation and integration*

Private alleles were found in both the Mississippi and Florida populations. In the Mississippi population no unique allele was held at a frequency higher than 10%. The Florida population held several private alleles above 10% (Table. 5). Given the significantly higher allelic richness of the Florida population (Table 6) and the bottleneck experienced by Mississippi population, the high numbers of private alleles in the Florida population are not surprising. This high number, however, has likely led to the variances observed in estimates of *Nm*, as the private allele method of *Nm* calculation relies on the number of private alleles in a population.

The variance observed in the microsatellite DNA allele frequencies (FST = 0.137) (Table 7) between the Florida and Mississippi populations were comparable to the variance observed for the same comparison in Jones' (2003) study (FST = 0.15). However, when comparisons were made between the Florida population and only founders and wild hatched birds in the Mississippi population the allelic differentiation decreased 34% (Table 7). This supports Jones' (2003) hypothesis that the differentiation observed between the Mississippi population and other populations of sandhill cranes is the result of isolation and inbreeding. If the lower level of gene flow observed between the Mississippi population and other populations of cranes was the result of endemism, we would expect to see the similar levels of allele frequency variance between the Florida samples and the wild-hatched/founders samples as we see for all Mississippi samples. It has been established (Chapters 2&3) that high levels of inbreeding are present in the captive MSC population (Chapters 1&2). The differences observed when only wild-hatched/founder samples are assessed suggest that this inbreeding has led to further isolation of the Mississippi population.

Higher allelic richness has also led to lower mean allelic similarity (0.46) in the Florida population. With the hypothetical integration of two individuals from the Florida population into the Mississippi population with the lowest *(s)* values an overall increase in genetic diversity was observed (Fig. 7). An overall increase in genetic diversity was also observed following the breeding of the optimal MSC pairs. This increase, however, was not as large as the change seen after the introduction of the FSCs, and the impact of the pairing appears to taper off after the first two offspring were introduced to the population (Figure 7). The introduced Florida cranes, on the other hand, continued to increase the genetic diversity of the MSC population with the

production of more offspring, highlighting the impact the introduction of just a few cranes from the Florida population could have on Mississippi population.

One danger that must be considered before such an introduction, however, is the possibility of outbreeding depression. Outbreeding depression is the reduced fitness of offspring that results from the mating of two genetically divergent individuals (Tallmon *et al.,* 2004). Orangutans (*Pongo* pygmaeous), for example, were once managed in captivity as one species. After Warren *et al.,* (2001) reported molecular genetic evidence that the Sumatran and Borneo populations were two distinct groups the captive breeding program began preventing interbreeding between the two populations. In this study, however, Warren *et al.*, (2001) found evidence that the populations diverged approximately 1.1 million years ago. The Mississippi population is believed to have been isolated for less than 100 years, suggesting that outbreeding depression would be an unlikely consequence of the introduction of Florida sandhill cranes.

Overall this study supports the findings of Rhymer (2001) and Jones (2003), that while the Mississippi population of sandhill cranes shows little evidence of current gene flow with the Florida population, this is the result of geographic isolation caused by anthropogenic events, and gene flow is likely to have occurred in the recent past. The results of this study suggest that the introduction of individuals from the Florida population would result in an increase in the genetic variation of the Mississippi sandhill crane population. Hybridization of crane species and subspecies has been observed both in the wild and in captivity (Johnsgard, 1984), but further research is needed to assess the behavioral and biological impacts of such an introduction. Additional research is also suggested into the levels of gene flow between the Mississippi population and other migratory populations of sandhill cranes. Both Rhymer (2001) and Jones (2003) found less allele frequency variation between the Mississippi sandhills and migratory

populations, than observed between the Mississippi and Florida populations. Further study may reveal that the introduction of greater sandhill cranes (*G. c. tabida*), for example, into the Mississippi captive breeding program, will represent a pairing more analogous to historical gene flow.

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CHAPTER V

5.i. CONCLUSIONS

Pedigree analysis of the Mississippi sandhill crane (MSC) captive and released population revealed a population with reduced genetic diversity due to unequal breeding of founder lines. The historical MSC studbook (MSC – presumed) was edited to more accurately reflect true population size (MSC-confirmed). Regardless of the studbook analyzed, more than 6% of the gene diversity present at the establishment of the captive breeding program has been lost. At the current pace and without the addition of at least 20 new MSC founders to the historical studbook, the levels of gene diversity in this population will not meet the goal of most captive breeding programs of 90% gene diversity after 100 years (Frankham *et al*., 2002).

The primary assumption of all pedigree analyses is that founders are unrelated. Given the population bottleneck experienced by the MSC population prior to the establishment of the captive breeding program, it was hypothesized that this assumption was invalid. Microsatellite DNA analysis of samples from both captive and wild-hatched Mississippi sandhill cranes, supported this hypothesis, finding low allelic richness and high allelic similarity. Resulting estimates of relatedness based on these measures of genetic variation in the founding population were integrated into the studbook analyses.

The integration of relatedness estimates into the MSC pedigree analyses exposed the difficulties and careful consideration necessary when choosing a relatedness estimator. Considering the increasing use of molecular markers to assess relationships both in-situ and ex-situ, this study recommends further research into the variation observed between estimators.

For captive breeding programs with detailed studbook records available, the breeding recommendation chart developed by Jones *et al.* (*in* prep) used for this study provides a powerful alternative to relying on either molecular markers or studbook analysis alone for breeding decisions. The development and comparison of the utility of similar breeding recommendation charts for other captive populations with molecular data available is suggested.

Finally, this study found allele frequency variation between the Florida and Mississippi populations similar to those observed in previous studies (Rhymer *et al.*, 2001; Jones, 2003). When the analysis was limited to founders and wild-hatched MSC less differentiation was observed than when the entire Mississippi population was considered, suggesting that inbreeding and isolation has been the source of genetic structure in the MSC population. While the practical application of integrating Florida sandhill cranes with the Mississippi sandhill cranes needs to be addressed in further studies, the hypothetical integration conducted in this study highlights the impact that the introduction of just a few birds could have on the Mississippi population.

Before such drastic measures are implemented, however, it is recommended that managers of the captive MSC population begin making pairing decisions based on the chart introduced in this study. Doing so is the first step towards breeding management decisions that will result in the maintenance, if not promotion, of genetic diversity of the endangered Mississippi sandhill crane.

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APPENDIX A: SAMPLE INFORMATION

Sample information including date collected, type of sample collected, studbook number of sample individual, location of sample individual, quality of sample, and sample individuals history, i.e. captive, released or wild.

Date	Sample Type	Studbk #	Loc. ID	Location	Quality	History
3/3/2009	Frozen liver tissue	1020	Stubby	ACRES	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1024	038402	ACRES	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1033	037602	ACRES	Good	Captive
1/9/2009	.5 ml whole blood in 5 ml buffer	1081	940347	Yulee	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1117	038411	ACRES	Good	Captive
1/2/2009	.5 ml whole blood in 5 ml buffer	1128	940345	Yulee	Good	Captive
1/6/2009	.5 ml whole blood in 5 ml buffer	1135	940352	Yulee	Good	Captive
1/8/2009	.5 ml whole blood in 5 ml buffer	1137	940349	Yulee	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1138	038508	ACRES	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1144	297	ACRES	Good	Captive
3/3/2009	Liver tissue sample - frozen	1152	038512	ACRES	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1156	038516	ACRES	Good	Captive
2/26/2009	L. wing tissue - frozen	1162	861	USFWS	Poor	Released
2/26/2009	Breast tissue - frozen	1162	861	USFWS	Poor	Released
1/8/2009	.5 ml whole blood in 5 ml buffer	1163	940350	Yulee	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1168	038656	ACRES	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1217	038918	ACRES	Good	Captive
1/13/2009	.5 ml whole blood in 5 ml buffer	1242	980303	Yulee	Good	Captive
1/23/2008	3 drops whole blood in 1.5ml buffer	1255	920	USFWS	Good	Released
2/26/2009	Liver tissue sample - frozen	1257	922	USFWS	Acceptable	Released
1/8/2009	.5 ml whole blood in 5 ml buffer	1258	940355	Yulee	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1278	039043	ACRES	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1296	196	ACRES	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1307	039092	ACRES	Good	Captive
1/2/2009	.5 ml whole blood in 5 ml buffer	1322	940359	Yulee	Good	Captive
2/26/2009	Liver tissue sample - frozen	1326	105	USFWS	Acceptable	Released
2/18/2009	.5 ml whole blood in 5 ml buffer	1356	039105	ACRES	Good	Captive
2/18/2009	.5 ml whole blood in 5 ml buffer	1361	039181	ACRES	Good	Captive
10/16/2008	3 drops whole blood in 1.5ml buffer	1385	202 (UB)	USFWS	Good	Released
2/17/2009	.5 ml whole blood in 5 ml buffer	1401	039202	ACRES	Good	Captive
2/26/2009	Liver tissue sample - frozen	1412 - ?	292 - ?	USFWS	Acceptable	Released
2/19/2009	.5 ml whole blood in 5 ml buffer	1431	039251	ACRES	Good	Captive
11/4/2008	.5 ml whole blood in 5 ml buffer	1440	314	USFWS	Good	Released
2/19/2009	.5 ml whole blood in 5 ml buffer	1458	039325	ACRES	Good	Captive
1/8/2009	.5 ml whole blood in 5 ml buffer	1479	950354	Yulee	Good	Captive
2/26/2009	Liver tissue sample - frozen	1528	501	USFWS	Acceptable	Released
2/13/2009	.5 ml whole blood in 5 ml buffer	1534	503	USFWS	Good	Released
2/18/2009	.5 ml whole blood in 5 ml buffer	1560	039706	ACRES	Good	Captive
2/18/2009	.5 ml whole blood in 5 ml buffer	1580	039798	ACRES	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1586	818	USFWS	Good	Released
2/17/2009	.5 ml whole blood in 5 ml buffer	1599	039819	ACRES	Good	Captive
12/15/2008	.5 ml whole blood in 5 ml buffer	1611	954	USFWS	Good	Released

Date	Sample Type	Studbk #	Loc. ID	Location	Quality	History
2/18/2009	.5 ml whole blood in 5 ml buffer	1615	039910	ACRES	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1621	039916	ACRES	Good	Captive
1/27/2009	.5 ml whole blood in 5 ml buffer	1624	961	USFWS	Good	Released
2/26/2009	Liver tissue sample - frozen	1640	403	USFWS	Acceptable	Released
2/26/2009	Liver tissue sample - frozen	1646	052	USFWS	Acceptable	Released
2/19/2009	.5 ml whole blood in 5 ml buffer	1681	030220	ACRES	Good	Captive
11/25/2008	.5 ml whole blood in 5 ml buffer	1701	334	USFWS	Good	Released
1/9/2009	.5 ml whole blood in 5 ml buffer	1708	Y55321	Yulee	Good	Captive
12/12/2008	.5 ml whole blood in 5 ml buffer	1726	163	USFWS	Good	Released
12/5/2008	.5 ml whole blood in 5 ml buffer	1751	442	USFWS	Good	Released
10/6/2008	3 drops whole blood in 1.5ml buffer	1757	459	USFWS	Good	Released
2/19/2009	.5 ml whole blood in 5 ml buffer	1758	275	ACRES	Good	Captive
12/31/2008	.5 ml whole blood in 5 ml buffer	1759	Y45005	Yulee	Good	Captive
2/19/2009	.5 ml whole blood in 5 ml buffer	1774	030503	ACRES	Good	Captive
1/9/2009	.5 ml whole blood in 5 ml buffer	1787	Y55309	Yulee	Good	Captive
12/31/2008	.5 ml whole blood in 5 ml buffer	1794	Y55315	Yulee	Good	Captive
2/17/2009	.5 ml whole blood in 5 ml buffer	1804	030502	ACRES	Good	Captive
1/6/2009	.5 ml whole blood in 5 ml buffer	1809	Y75305	Yulee	Good	Captive
12/4/2008	.5 ml whole blood in 5 ml buffer	1819	30801	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1820	Y85001	USFWS	Good	Released
12/9/2008	.5 ml whole blood in 5 ml buffer	1823	030803	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1824	Y85004	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1825	Y85005	USFWS	Good	Released
12/9/2008	.5 ml whole blood in 5 ml buffer	1827	030805	USFWS	Good	Released
12/9/2008	.5 ml whole blood in 5 ml buffer	1828	30806	USFWS	Good	Released
11/4/2008	.5 ml whole blood in 5 ml buffer	1830	030808	USFWS	Good	Released
12/9/2008	.5 ml whole blood in 5 ml buffer	1830	030808	USFWS	Good	Released
12/9/2008	.5 ml whole blood in 5 ml buffer	1831	030809	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1832	030810	USFWS	Good	Released
12/9/2008	.5 ml whole blood in 5 ml buffer	1833	030811	USFWS	Good	Released
2/18/2009	.5 ml whole blood in 5 ml buffer	1834	030812	ACRES	Good	Captive
12/1/2008	.5 ml whole blood in 5 ml buffer	1835	030813	USFWS	Good	Released
2/19/2009	.5 ml whole blood in 5 ml buffer	1836	030814	ACRES	Good	Captive
12/1/2008	.5 ml whole blood in 5 ml buffer	1837	030815	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1838	030816	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1840	Y85006	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1841	030818	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1842	030819	USFWS	Good	Released
12/1/2008	.5 ml whole blood in 5 ml buffer	1843	030820	USFWS	Good	Released
2/19/2009	.5 ml whole blood in 5 ml buffer	1844	030821	ACRES	Good	Captive
12/1/2008	.5 ml whole blood in 5 ml buffer	1845	030822	USFWS	Good	Released
2/26/2009	Rotted egg membrane - frozen	DP Nest 08	none	USFWS	Poor	Wild
5/27/2009	Rotten yolk and tissue – frozen	HW 09	none	USFWS	Acceptable	Wild
2/26/2009	Rotten yolk and tissue - frozen	LG Egg 08	none	USFWS	Poor	Wild
5/27/2009	Rotten yolk and tissue – frozen	Vick 09	None	USFWS	Acceptable	Wild

Date	Sample Type	Studbk #	Loc. ID	Location	Quality	History
2/26/2009	Liver tissue sample – frozen	WH04	W-04	USFWS	Poor	Wild
11/5/2008	.5 ml whole blood in 5 ml buffer	WH09	W-9	USFWS	Good	Wild
9/16/2008	3 drops whole blood in 1.5ml buffer	WH25	W-25	USFWS	Good	Wild
10/22/2008	.5 ml whole blood in 5 ml buffer	WH36	W-36	USFWS	Good	Wild
2/29/2008	3 drops whole blood in 1.5ml buffer	WH37	W-37	USFWS	Good	Wild
9/17/2008	3 drops whole blood in 1.5ml buffer	WH38	W-38	USFWS	Good	Wild
10/22/2008	3 drops whole blood in 1.5ml buffer	WH40	W-40	USFWS	Good	Wild
10/22/2008	3 drops whole blood in 1.5ml buffer	WH41	W-41	USFWS	Good	Wild

APPENDIX B: Test Statistics

Queller and Goodnight relatedness calculation (Queller & Goodnight, 1989) as used by MARK v 3.1 (Ritland, 2006):

Given individuals $X = \{x_1, x_2, \ldots x_L\}$ and $Y = \{y_1, y_2, \ldots y_L\}$ the estimator is given by:

$$r_{\mathrm{QG}}(\mathrm{X},\mathrm{Y}) = \frac{\sum_{l-1}^{L}(\delta_{ac} + \delta_{ad} + \delta_{bc} + \delta_{bd} - p_a - p_b - p_c - p_d)}{\sum_{l-1}^{L}(2 + \delta_{ab} + \delta_{cd} - p_a - p_b - p_c - p_d)},$$

where δ_{xy} is defined as $\delta_{x,x} = 1$ and $\delta_{x,y-x} = 0$ (the Kronecker delta), and the population frequencies of $\{a,b,c,d\}$ alleles are represented by $\{p_a, p_b, p_c, p_d\}$.

This equation can be stated more simply as:

$$r_{\mathrm{QG}}(\mathrm{X},\mathrm{Y}) = \frac{\sum\sum\sum(Py - P)}{\sum\sum\sum(Px - P)},$$

where P is the population frequency of the allele shown at the current locus and position, Px is the frequency of the current allele in the current individual (0.5 or 1.0, depending on whether the individual is heterozygous or not), and Py is the frequency of the current allele in the individual's partner (i.e. the individual being compared to) (Beebee & Rowe, 2008).

A priori relationship hypothesis test (Kalinowski et al., 2006):

Given that *k*-coefficients, k_0, k_1, k_2 represent the probabilities that two individuals share zero, one or two alleles at a locus the test statistic, Λ, is equal to:

$$\bigwedge = \text{Ln}\left[\frac{L(K_{(Putative}\ )}{L\ (K_{(Alternative}\ )}\right],$$

where $K_{(Putative)}$ represents the *k*-coefficients for the putative relationship being tested, and $K_{(Alternative)}$ represents the *k*-coefficients for an alternative hypothesis (unrelated individuals). Genotypes for the alternative hypothesis are simulated for the pair being tested in two steps. First, the number of alleles IBD is chosen from K, and then given K the genotypes for the alternative hypothesis are chosen. This simulation is performed a large number of times (>1000). The *P* value is equal to the proportion of times that the simulated Λ is greater than or equal to the observed Λ. A small *P* value indicates the alternative hypothesis can be rejected.

***Wright's F_{ST} statistic* (Wright, 1951):**

Given V_p represents the variance of allele frequencies among populations, and $\bar{p}$ the observed mean allele frequency:

$$F_{ST}\ = \frac{V_p}{\bar{p}(1-\bar{p})}.$$

Weir and Cockerham's (1984) calculation of F_{ST} (Θ_{ST}):

Given $\sigma_T^2 = \sigma_B^2 + \sigma_W^2 + \sigma_I^2$, where σ_T^2 represents the total variance of allele frequency within a population, σ_B^2 represents the between subpopulation variance in allele frequency, σ_W^2 represnts the allele frequency variance between individuals within a subpopulation, and σ_I^2 represents the between gametes within individuals variance in allele frequency. $\boldsymbol{\Theta_{ST}}$ can be estimated from

$$\boldsymbol{\theta_{ST}} = \frac{\sum_i \sum_u \boldsymbol{\sigma_B^2}}{\sum_i \sum_u \boldsymbol{\sigma_T^2}},$$

where the variances in allele frequency are summed over all alleles *i* and all loci *u*.

Wright's Nm statistic (1951) as calculated from F-statistics:

$$Nm \cong \frac{(1 - F_{\mathrm{ST}})}{4F_{\mathrm{ST}}}.$$

Slatkin's (1985) Nm statistic as calculated from private alleles:

$$\ln [p(1)] = -0.505 \ln (Nm) - 2.440,$$

,

where $[p(1)]$ represents the average frequency of private alleles.

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APPENDIX C: Mississippi Sandhill Crane Breeding Recommendation Chart

C.i. CHART ORGANIZATION

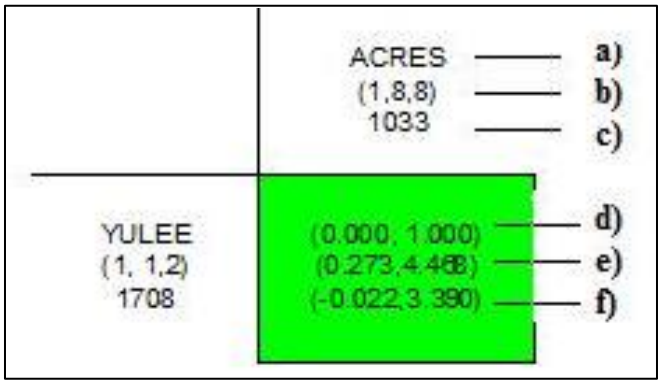
The chart is first organized by the mean kinship rank of individuals in the population as calculated by the MSC – presumed studbook (i.e., individuals at the top left of the table have the mean kinship values (MK) in the population). Males are listed across the top of the chart, and females are listed going down the left hand side of the chart. Red lines indicate the average mean kinship for the entire population (0.0599). Individuals above and to the left of the red lines report lower MK values than the population average, individuals below and to the right of the red lines report higher MK values than the population average.

Organizing the chart in order of MK, produces three categories of pairings; rare with rare (top left quadrant); rare with common (bottom left and top right quadrants); and common with common (bottom right quadrant). Individual pairings are then color coded according to the quality of the pairing, as indicated by inbreeding coefficients and Mate Suitability index values (see Chapter 2 for description of MSI calculation) as reported by the MSC – presumed studbook and listed on the top row of individual pairing cells. (See Figure 7 for a description of the information found in each cell). Pairings highlighted in green and blue represent the rare with rare pairings. These are individuals with few ancestors (low MK) and rare genotypes. Offspring from these pairings would increase the gene diversity of the population, and are therefore suitable for being kept in captivity. Offspring from pairings highlighted in yellow are pairings between genetically mismatched birds (i.e., common with rare). These offspring along with the third category, common with common (highlighted in orange), should not be kept in captivity as they would decrease the population's gene diversity. Orange and yellow offspring, however, are suitable for refuge release. Pairings that would result in inbreeding coefficients larger than the

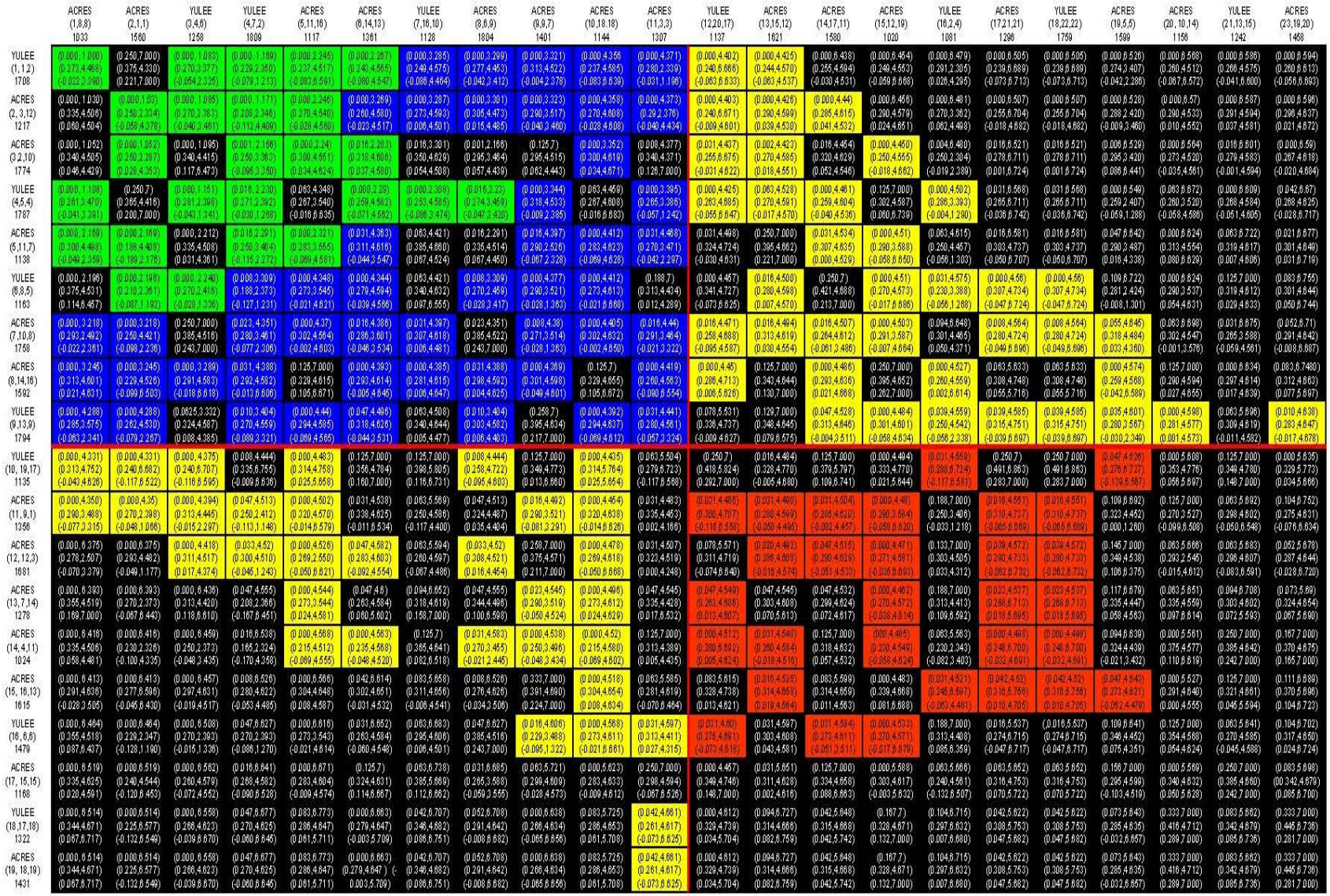
average mean kinship, or with MSI ratings above 4 are highlighted in black. These are pairings that should not occur for any reason.

Once the quality pairings have been color coded according to information reported by the historical studbook the information obtained from the DNA studbooks is incorporated into the breeding recommendations. This is done by assessing the inbreeding levels indicated by the MSC – DNA (*s*) studbook, and the MSC – DNA (*r*) studbook. Any pairings that report higher inbreeding values than the average MK value for the individual studbook (0.32 for (*s),* and 0.03 for (*r)*) are blacked out, as these pairings would result in a reduction in observed heterozygosity across the DNA markers.

Figure 7. ***Close up and description of data included in chart cells. a)*** *Represents the location of the captive individual (ACRES = Audubon Center for Research on Endangered Species, New Orleans, LA; YULEE = White Oak Conservation Center, Yulee, FL).* ***b)*** *Indicates the three mean kinship ranks as calculated by the three studbook strategies (MK rank of MSC – presumed, MK rank of MSC - DNA allelic similarity (s), and MK rank of MSC – DNA Queller and Goodnight (r), respectively).* ***c)*** *Indicates the studbook number of the captive individual.* ***d)*** *The first row in a pairing cell indicates the inbreeding coefficient, and Mate Suitability index (MSI) rating of the potential pairing as calculated by the MSC – presumed studbook. As described in Chapter 2 the MSI rating indicates the quality of a pairing on a 1-6 scale). For the cell shown below, the pairing of studbook number 1033 (male) with studbook number 1708 (female) would result in offspring with an inbreeding coefficient of 0.000, and a MSI rating of 1.000.* ***e)*** *The second row in a pairing cell lists the inbreeding coefficient and MSI rating for the given pairing as calculated by the MSC – DNA (s) studbook.* ***f)*** *The third row in a pairing cell lists the inbreeding coefficient and MSI rating of a given pairing as calculated by the MSC – DNA (r) studbook.*



Mississippi Sandhill Crane Breeding Recommendation Chart



Mississippi Sandhill Crane Breeding Recommendation Chart: Quadrant 1 – *Rare with Rare pairings*

	ACRES (1,8,8) 1033	ACRES (2,1,1) 1560	YULEE (3,4,6) 1258	YULEE (4,7,2) 1809	ACRES (5,11,16) 1117	ACRES (6,14,13) 1361	YULEE (7,16,10) 1128	ACRES (8,6,9) 1804	ACRES (9,9,7) 1401	ACRES (10,18,18) 1144	ACRES (11,3,3) 1307
YULEE (1,1,2) 1708	(0.000,1.000) (0.273,4.468) (-0.022,3.390)	(0.250,7.000) (0.375,4.330) (0.221,7.000)	(0.000,1.083) (0.270,3.377) (-0.054,2.325)	(0.000,1.169) (0.229,2.360) (-0.079,1.213)	(0.000,2.245) (0.237,4.517) (-0.083,6.591)	(0.000,2.267) (0.243,4.565) (-0.080,4.547)	(0.000,3.285) (0.249,4.575) (-0.086,4.464)	(0.000,3.299) (0.277,4.453) (-0.042,3.412)	(0.000,3.321) (0.313,4.522) (-0.004,2.378)	(0.000,4.356 (0.237,4.585) (-0.083,6.639)	(0.000,4.371) (0.280,2.339) (-0.031,1.196)
ACRES (2,3,12) 1217	(0.000,1.030) (0.335,4.506) (0.060,4.504)	(0.000,1.03) (0.250,2.334) (-0.058,4.378)	(0.000,1.085) (0.270,2.383) (-0.040,2.461)	(0.000,1.171) (0.208,2.346) (-0.112,4.409)	(0.000,2.246) (0.270,4.540) (-0.028,4.560)	(0.000,3.269) (0.260,4.580) (-0.023,4.517)	(0.000,3.287) (0.273,4.593) (0.006,4.501)	(0.000,3.301) (0.305,4.473) (0.015,4.485)	(0.000,3.323) (0.290,3.517) (-0.040,3.460)	(0.000,4.358) (0.270,4.608) (-0.028,4.608)	(0.000,4.373) (0.29,2.376) (-0.040,4.434)
ACRES (3,2,10) 1774	(0.000,1.052) (0.340,4.505) (0.046,4.429)	(0.000,1.052) (0.250,2.287) (0.028,4.353)	(0.000,1.095) (0.340,4.415) (0.117,6.473)	(0.001,2.166) (0.250,3.363) (-0.095,3.350)	(0.000,2.24) (0.300,4.551) (0.034,4.624)	(0.016,2.283) (0.318,4.606) (0.037,4.580)	(0.016,3.301) (0.350,4.629) (0.054,4.508)	(0.001,2.166) (0.295,3.464) (0.057,4.439)	(0.125,7) (0.295,4.515) (0.062,4.443)	(0.000,3.352) (0.300,4.619) (0.034,4.671)	(0.008,4.377) (0.340,4.371) (0.126,7.000)
YULEE (4,5,4) 1787	(0.000,1.108) (0.261,3.470) (-0.041,3.391)	(0.250,7) (0.365,4.416) (0.200,7.000)	(0.000,1.151) (0.281,2.398) (-0.043,1.341)	(0.016,2.230) (0.271,2.392) (-0.030,1.268)	(0.063,4.348) (0.267,3.540) (-0.016,6.635)	(0.000,2.29) (0.259,4.582) (-0.071,4.562)	(0.000,2.308) (0.253,4.585) (-0.086,3.474)	(0.016,2.23) (0.274,3.459) (-0.047,3.420)	(0.000,3.344) (0.318,4.533) (-0.009,2.385)	(0.063,4.459) (0.267,4.608) (-0.016,6.683)	(0.000,3.395) (0.265,3.386) (-0.057,1.242)
ACRES (5,11,7) 1138	(0.000,2.169) (0.300,4.498) (-0.049,2.359)	(0.000,2.169) (0.188,4.408) (-0.189,2.176)	(0.000,2.212) (0.335,4.508) (0.031,4.361)	(0.016,2.291) (0.250,3.464) (-0.115,2.272)	(0.000,2.321) (0.283,3.555) (-0.069,4.581)	(0.031,4.363) (0.311,4.616) (-0.044,3.547)	(0.063,4.421) (0.385,4.660) (0.067,4.524)	(0.016,2.291) (0.335,4.514) (0.067,4.460)	(0.016,4.397) (0.290,2.526) (-0.067,2.328)	(0.000,4.412) (0.283,4.623) (-0.069,4.628)	(0.031,4.468) (0.270,3.471) (-0.042,2.297)
YULEE (6,8,5) 1163	(0.000,2.196) (0.375,4.531) (0.114,6.467)	(0.000,2.196) (0.210,2.361) (-0.087,1.192)	(0.000,2.240) (0.270,2.416) (-0.028,1.336)	(0.008,3.309) (0.188,2.373) (-0.127,1.231)	(0.000,4.348) (0.273,3.545) (-0.021,4.621)	(0.000,4.344) (0.279,4.594) (-0.039,4.566)	(0.063,4.421) (0.340,4.632) (0.097,6.555)	(0.008,3.309) (0.270,2.459) (-0.028,3.417)	(0.000,4.377) (0.290,3.521) (-0.028,1.363)	(0.000,4.412) (0.273,4.613) (-0.021,6.668)	(0.188,7) (0.313,4.434) (0.012,4.289)
ACRES (7,10,8) 1758	(0.000,3.218) (0.293,2.492) (-0.022,2.361)	(0.000,3.218) (0.250,4.421) (-0.098,2.236)	(0.250,7.000) (0.385,4.516) (0.243,7.000)	(0.023,4.351) (0.280,3.461) (-0.077,2.306)	(0.000,4.37) (0.302,4.564) (-0.002,4.603)	(0.016,4.386) (0.286,3.601) (-0.046,3.534)	(0.031,4.397) (0.307,4.618) (0.006,4.481)	(0.023,4.351) (0.385,4.522) (0.243,7.000)	(0.008,4.38) (0.271,3.514) (-0.028,1.363)	(0.000,4.405) (0.302,4.632) (-0.002,4.650)	(0.016,4.44) (0.291,3.464) (-0.021,3.322)
ACRES (8,14,16) 1592	(0.000,3.246) (0.313,4.601) (0.021,4.631)	(0.000,3.246) (0.229,4.526) (-0.099,6.503)	(0.000,3.289) (0.291,4.583) (-0.018,6.618)	(0.031,4.388) (0.292,4.582) (-0.013,6.606)	(0.125,7.000) (0.329,4.615) (0.105,6.671)	(0.000,4.393) (0.293,4.614) (-0.005,4.646)	(0.000,4.385) (0.281,4.615) (0.006,4.647)	(0.031,4.388) (0.298,4.592) (0.004,4.625)	(0.000,4.369) (0.301,4.598) (-0.049,4.601)	(0.125,7) (0.329,4.655) (0.105,6.672)	(0.000,4.419) (0.260,4.563) (-0.090,6.554)
YULEE (9,13,9) 1794	(0.000,4.288) (0.285,3.575) (-0.063,2.341)	(0.000,4.288) (0.262,4.530) (-0.079,2.267)	(0.0625,3.332) (0.324,4.587) (0.008,4.385)	(0.010,3.404) (0.270,4.559) (-0.089,3.321)	(0.000,4.44) (0.294,4.585) (-0.069,4.565)	(0.047,4.496) (0.318,4.626) (-0.044,3.531)	(0.063,4.508) (0.340,4.644) (0.005,4.477)	(0.010,3.404) (0.303,4.582) (0.006,4.403)	(0.258,7) (0.395,4.634) (0.217,7.000)	(0.000,4.392) (0.294,4.637) (-0.069,4.612)	(0.031,4.441) (0.280,4.561) (-0.057,3.324)

Mississippi Sandhill Crane Breeding Recommendation Chart – Quadrant 2 – *Common with Rare pairings*

	ACRES (1,8,8) 1033	ACRES (2,1,1) 1560	YULEE (3,4,6) 1258	YULEE (4,7,2) 1809	ACRES (5,11,16) 1117	ACRES (6,14,13) 1361	YULEE (7,16,10) 1128	ACRES (8,6,9) 1804	ACRES (9,9,7) 1401	ACRES (10,18,18) 1144	ACRES (11,3,3) 1307
YULEE (10,19,17) 1135	(0.000,4.331) (0.313,4.752) (-0.043,4.626)	(0.000,4.331) (0.240,6.682) (-0.117,6.522)	(0.000,4.375) (0.240,6.707) (-0.116,6.595)	(0.008,4.444) (0.335,6.755) (-0.009,6.636)	(0.000,4.483) (0.314,4.758) (0.025,5.658)	(0.125,7.000) (0.356,4.784) (0.160,7.000)	(0.125,7.000) (0.398,5.805) (0.116,6.731)	(0.008,4.444) (0.258,4.722) (-0.095,4.603)	(0.125,7.000) (0.349,4.773) (0.013,6.660)	(0.000,4.435) (0.314,5.764) (0.025,5.654)	(0.063,5.504) (0.279,6.723) (-0.117,6.568)
ACRES (11,9,1) 1356	(0.000,4.350) (0.290,3.488) (-0.077,3.315)	(0.000,4.35) (0.270,2.398) (-0.048,1.066)	(0.000,4.394) (0.313,4.445) (-0.015,2.297)	(0.047,4.513) (0.250,2.412) (-0.113,1.148)	(0.000,4.502) (0.320,4.570) (-0.014,6.579)	(0.031,4.538) (0.338,4.625) (-0.011,6.534)	(0.063,5.569) (0.250,4.586) (-0.117,4.400)	(0.047,4.513) (0.324,4.487) (0.035,4.404)	(0.016,4.492) (0.290,3.521) (-0.081,3.291)	(0.000,4.464) (0.320,4.638) (-0.014,6.626)	(0.031,4.483) (0.335,4.463) (0.002,4.166)
ACRES (12,12,3) 1681	(0.000,6.375) (0.278,2.507) (-0.070,3.379)	(0.000,6.375) (0.293,4.482) (-0.049,1.177)	(0.000,4.418) (0.311,4.517) (0.017,4.374)	(0.033,4.52) (0.300,4.510) (-0.045,1.243)	(0.000,4.526) (0.269,2.550) (-0.050,6.621)	(0.047,4.582) (0.283,4.603) (-0.092,4.554)	(0.063,5.594) (0.260,4.597) (-0.067,4.486)	(0.033,4.52) (0.308,4.521) (0.016,4.464)	(0.258,7.000) (0.375,4.571) (0.211,7.000)	(0.000,4.478) (0.269,4.618) (-0.050,6.668)	(0.031,4.507) (0.323,4.519) (0.000,4.248)
ACRES (13,7,14) 1278	(0.000,6.393) (0.355,4.519) (0.169,7.000)	(0.000,6.393) (0.270,2.373) (-0.067,6.443)	(0.000,6.436) (0.313,4.420) (0.118,6.610)	(0.047,4.555) (0.208,2.366) (-0.167,6.451)	(0.000,4.544) (0.273,3.544) (0.024,4.581)	(0.047,4.6) (0.263,4.584) (0.060,5.602)	(0.094,6.652) (0.318,4.619) (0.158,7.000)	(0.047,4.555) (0.344,4.496) (0.100,6.598)	(0.023,4.545) (0.290,3.519) (-0.050,4.524)	(0.000,4.496) (0.273,4.612) (0.024,4.629)	(0.047,4.545) (0.335,4.428) (0.017,6.532)
ACRES (14,4,11) 1024	(0.000,6.416) (0.335,4.506) (0.058,4.481)	(0.000,6.416) (0.230,2.326) (-0.100,4.335)	(0.000,6.459) (0.250,2.373) (-0.048,3.435)	(0.016,6.538) (0.165,2.324) (-0.170,4.358)	(0.000,4.568) (0.215,4.512) (-0.069,4.555)	(0.000,4.563) (0.235,4.568) (-0.048,4.520)	(0.125,7) (0.365,4.641) (0.082,6.518)	(0.031,4.583) (0.270,3.455) (-0.021,2.445)	(0.000,4.538) (0.250,3.496) (-0.048,3.434)	(0.000,4.52) (0.215,4.580) (-0.069,4.602)	(0.125,7.000) (0.313,4.389) (0.005,4.435)
ACRES (15,16,13) 1615	(0.000,6.413) (0.291,4.636) (-0.028,3.505)	(0.000,6.413) (0.277,6.596) (-0.045,6.430)	(0.000,6.457) (0.297,4.631) (-0.019,4.517)	(0.008,6.526) (0.280,4.622) (-0.053,4.485)	(0.000,6.566) (0.304,4.648) (0.008,4.587)	(0.042,6.614) (0.302,4.651) (-0.031,4.532)	(0.083,6.658) (0.311,4.656) (-0.006,4.541)	(0.008,6.526) (0.276,4.626) (-0.034,3.506)	(0.333,7.000) (0.391,4.690) (0.224,7.000)	(0.000,4.518) (0.304,4.654) (0.008,4.634)	(0.063,5.585) (0.281,4.619) (-0.070,6.464)
YULEE (16,6,6) 1479	(0.000,6.464) (0.355,4.518) (0.087,6.437)	(0.000,6.464) (0.229,2.347) (-0.128,1.190)	(0.000,6.508) (0.270,2.393) (-0.015,1.336)	(0.047,6.627) (0.270,2.393) (-0.086,1.270)	(0.000,6.616) (0.273,3.543) (-0.021,4.614)	(0.031,6.652) (0.263,4.584) (-0.060,4.548)	(0.063,6.683) (0.295,4.606) (0.006,4.501)	(0.047,6.627) (0.385,4.516) (0.243,7.000)	(0.016,4.606) (0.229,3.488) (-0.095,1.322)	(0.000,4.568) (0.273,4.611) (-0.021,6.661)	(0.031,4.597) (0.313,4.411) (0.027,4.316)
ACRES (17,15,15) 1168	(0.000,6.519) (0.335,4.625) (0.020,4.591)	(0.000,6.519) (0.240,4.544) (-0.120,6.453)	(0.000,6.562) (0.260,4.579) (-0.072,4.552)	(0.016,6.641) (0.268,4.582) (-0.090,6.528)	(0.000,6.671) (0.283,4.604) (-0.009,4.574)	(0.125,7) (0.324,4.631) (0.114,6.667)	(0.063,6.738) (0.385,5.669) (0.112,6.662)	(0.031,6.685) (0.265,3.588) (-0.059,3.555)	(0.063,5.721) (0.299,4.609) (-0.028,4.573)	(0.000,5.623) (0.283,4.633) (-0.009,4.612)	(0.250,7.000) (0.298,4.594) (-0.067,6.526)
YULEE (18,17,18) 1322	(0.000,6.514) (0.344,4.671) (0.067,6.717)	(0.000,6.514) (0.225,6.577) (-0.132,6.549)	(0.000,6.558) (0.266,4.623) (-0.039,6.670)	(0.047,6.677) (0.270,4.625) (-0.060,6.645)	(0.083,6.773) (0.286,4.647) (0.061,5.711)	(0.000,6.663) (0.279,4.647) (0.003,5.709)	(0.042,6.707) (0.346,4.682) (0.086,6.751)	(0.052,6.708) (0.291,4.642) (-0.008,6.682)	(0.000,6.638) (0.266,4.634) (-0.065,6.656)	(0.083,5.725) (0.286,4.653) (0.061,5.708)	(0.042,4.661) (0.261,4.617) (-0.073,6.625)
ACRES (19,18,19) 1431	(0.000,6.514) (0.344,4.671) (0.067,6.717)	(0.000,6.514) (0.225,6.577) (-0.132,6.549)	(0.000,6.558) (0.266,4.623) (-0.039,6.670)	(0.047,6.677) (0.270,4.625) (-0.060,6.645)	(0.083,6.773) (0.286,4.647) (0.061,5.711)	(0.000,6.663) (0.279,4.647) (-0.003,5.709)	(0.042,6.707) (0.346,4.682) (0.086,6.751)	(0.052,6.708) (0.291,4.642) (-0.008,6.682)	(0.000,6.638) (0.266,4.634) (-0.065,6.656)	(0.083,5.725) (0.286,4.653) (0.061,5.708)	(0.042,4.661) (0.261,4.617) (-0.073,6.625)

Mississippi Sandhill Crane Breeding Recommendation Chart – Quadrant 3 – *Common with Rare pairings*

	YULEE (12,20,17) 1137	ACRES (13,15,12) 1621	ACRES (14,17,11) 1580	ACRES (15,12,19) 1020	YULEE (16,2,4) 1081	ACRES (17,21,21) 1296	YULEE (18,22,22) 1759	ACRES (19,5,5) 1599	ACRES (20, 10,14) 1156	YULEE (21,13,15) 1242	ACRES (23,19,20) 1458
YULEE (1,1,2) 1708	(0.000,4.402) (0.240,6.666) (-0.063,6.633)	(0.000,4.425) (0.244,4.570) (-0.063,4.537)	(0.000,6.438) (0.255,4.594) (-0.030,4.531)	(0.000,6.454) (0.249,4.553) (-0.059,6.668)	(0.000,6.479) (0.291,2.305) (0.026,4.295)	(0.000,6.505) (0.239,6.689) (-0.073,6.713)	(0.000,6.505) (0.239,6.689) (-0.073,6.713)	(0.000,6.526) (0.274,3.407) (-0.042,2.286)	(0.000,6.568) (0.260,4.512) (-0.067,6.572)	(0.000,6.586) (0.266,4.575) (-0.041,6.600)	(0.000,6.594) (0.260,6.613) (-0.056,6.693)
ACRES (2,3,12) 1217	(0.000,4.403) (0.240,6.671) (-0.009,4.601)	(0.000,4.426) (0.290,4.599) (0.039,4.530)	(0.000,4.44) (0.285,4.615) (0.041,4.532)	(0.000,6.456) (0.290,4.579) (0.024,4.651)	(0.000,6.481) (0.270,3.362) (0.062,4.498)	(0.000,6.507) (0.255,6.704) (-0.018,4.682)	(0.000,6.507) (0.255,6.704) (-0.018,4.682)	(0.000,6.528) (0.288,2.420) (-0.009,3.460)	(0.000,6.57) (0.290,4.533) (0.010,4.552)	(0.000,6.587) (0.291,4.594) (0.037,4.581)	(0.000,6.596) (0.296,4.637) (0.021,4.672)
ACRES (3,2,10) 1774	(0.031,4.437) (0.255,6.675) (-0.031,4.822)	(0.002,4.423) (0.270,4.585) (0.018,4.551)	(0.016,4.454) (0.320,4.629) (0.052,4.546)	(0.000,4.450) (0.250,4.555) (-0.018,4.662)	(0.004,6.480) (0.250,2.304) (-0.019,2.389)	(0.016,6.521) (0.278,6.711) (0.001,6.724)	(0.016,6.521) (0.278,6.711) (0.001,6.724)	(0.006,6.529) (0.295,3.420) (0.086,6.441)	(0.000,6.564) (0.273,4.520) (-0.035,4.561)	(0.016,6.601) (0.279,4.583) (-0.001,4.594)	(0.000,6.59) (0.267,4.618) (-0.020,4.684)
YULEE (4,5,4) 1787	(0.000,4.425) (0.263,4.685) (-0.055,6.647)	(0.063,4.528) (0.270,4.591) (-0.017,4.570)	(0.000,4.461) (0.259,4.604) (-0.040,4.536)	(0.125,7.000) (0.302,4.587) (0.060,6.739)	(0.000,4.502) (0.286,3.393) (-0.004,1.290)	(0.031,6.568) (0.265,6.711) (-0.036,6.742)	(0.031,6.568) (0.265,6.711) (-0.036,6.742)	(0.000,6.549) (0.259,2.407) (-0.059,1.288)	(0.063,6.672) (0.260,3.520) (-0.058,4.586)	(0.000,6.609) (0.268,4.584) (-0.051,4.605)	(0.042,6.67) (0.268,4.625) (-0.028,6.717)
ACRES (5,11,7) 1138	(0.031,4.498) (0.324,4.724) (-0.030,4.831)	(0.250,7.000) (0.395,4.662) (0.221,7.000)	(0.031,4.534) (0.307,4.635) (0.000,4.529)	(0.000,4.51) (0.290,3.588) (-0.058,6.650)	(0.063,4.615) (0.250,4.457) (-0.056,1.303)	(0.016,6.581) (0.303,4.737) (-0.050,6.707)	(0.016,6.581) (0.303,4.737) (-0.050,6.707)	(0.047,6.642) (0.290,3.487) (0.016,4.338)	(0.000,6.624) (0.313,4.554) (0.080,6.629)	(0.063,6.722) (0.319,4.617) (0.007,4.606)	(0.021,6.677) (0.301,4.649) (0.031,6.719)
YULEE (6,8,5) 1163	(0.000,4.457) (0.341,4.727) (-0.073,6.625)	(0.016,4.500) (0.280,4.598) (0.007,4.570)	(0.250,7) (0.421,4.688) (0.213,7.000)	(0.000,4.51) (0.270,4.573) (-0.017,6.686)	(0.031,4.575) (0.230,3.388) (-0.056,1.268)	(0.000,4.56) (0.307,4.734) (-0.047,6.724)	(0.000,4.56) (0.307,4.734) (-0.047,6.724)	(0.109,6.722) (0.281,2.424) (-0.008,1.301)	(0.000,6.624) (0.290,3.537) (0.054,4.631)	(0.125,7.000) (0.319,4.612) (0.029,4.633)	(0.083,6.755) (0.301,4.644) (0.050,6.744)
ACRES (7,10,8) 1758	(0.016,4.471) (0.258,4.688) (-0.095,4.587)	(0.016,4.494) (0.313,4.619) (0.030,4.554)	(0.016,4.507) (0.264,4.612) (-0.061,3.486)	(0.000,4.503) (0.291,3.587) (-0.007,4.664)	(0.094,6.648) (0.301,4.465) (0.050,4.371)	(0.008,4.564) (0.280,4.724) (-0.049,6.696)	(0.008,4.564) (0.280,4.724) (-0.049,6.696)	(0.055,4.645) (0.318,4.484) (0.033,4.360)	(0.063,6.698) (0.302,4.547) (-0.001,3.576)	(0.031,6.675) (0.265,3.588) (-0.059,4.561)	(0.052,6.71) (0.291,4.642) (-0.008,6.687)
ACRES (8,14,16) 1592	(0.000,4.46) (0.286,4.713) (0.006,5.626)	(0.125,7.000) (0.343,4.644) (0.130,7.000)	(0.000,4.486) (0.293,4.636) (0.021,4.668)	(0.250,7.000) (0.395,4.652) (0.262,7.000)	(0.000,4.527) (0.260,4.559) (0.002,6.614)	(0.063,5.633) (0.308,4.748) (0.055,5.716)	(0.063,5.633) (0.308,4.748) (0.055,5.716)	(0.000,4.574) (0.259,4.568) (-0.042,6.589)	(0.125,7.000) (0.290,4.594) (0.027,4.655)	(0.000,6.634) (0.297,4.614) (0.017,4.639)	(0.083,6.7480) (0.312,4.663) (0.077,5.697)
YULEE (9,13,9) 1794	(0.078,5.531) (0.336,4.737) (-0.009,4.627)	(0.129,7.000) (0.348,4.645) (0.079,6.575)	(0.047,4.528) (0.313,4.646) (-0.004,3.511)	(0.000,4.484) (0.301,4.601) (-0.058,4.634)	(0.039,4.559) (0.250,4.542) (-0.056,2.338)	(0.039,4.585) (0.315,4.751) (-0.039,6.697)	(0.039,4.585) (0.315,4.751) (-0.039,6.697)	(0.035,4.601) (0.280,3.567) (-0.030,2.349)	(0.000,4.598) (0.281,4.577) (0.001,4.573)	(0.063,5.696) (0.309,4.619) (-0.011,4.582)	(0.010,4.638) (0.283,4.647) (-0.017,4.678)

Mississippi Sandhill Crane Breeding Recommendation Chart – Quadrant 4 – *Common with Common pairings*

	YULEE (12,20,17) 1137	ACRES (13,15,12) 1621	ACRES (14,17,11) 1580	ACRES (15,12,19) 1020	YULEE (16,2,4) 1081	ACRES (17,21,21) 1296	YULEE (18,22,22) 1759	ACRES (19,5,5) 1599	ACRES (20, 10,14) 1156	YULEE (21,13,15) 1242	ACRES (23,19,20) 1458
YULEE (10,19,17) 1135	(0.250,7) (0.418,5.824) (0.292,7.000)	(0.016,4.484) (0.328,4.770) (-0.005,4.680)	(0.125,7.000) (0.379,5.797) (0.109,6.741)	(0.000,4.494) (0.333,4.770) (0.021,5.644)	(0.031,4.559) (0.288,6.724) (-0.117,6.581)	(0.250,7) (0.491,6.863) (0.283,7.000)	(0.250,7.000) (0.491,6.863) (0.283,7.000)	(0.047,4.626) (0.276,6.727) (-0.139,6.587)	(0.000,5.608) (0.353,4.776) (0.056,5.697)	(0.125,7.000) (0.349,4.780) (0.148,7.000)	(0.000,5.635) (0.329,5.773) (0.034,5.666)
ACRES (11,9,1) 1356	(0.031,4.486) (0.300,4.707) (-0.116,6.558)	(0.031,4.490) (0.280,4.599) (-0.050,4.495)	(0.031,4.504) (0.285,4.620) (-0.082,4.467)	(0.000,4.48) (0.290,3.584) (-0.068,6.620)	(0.188,7.000) (0.250,3.406) (-0.033,1.218)	(0.016,4.551) (0.310,4.737) (-0.065,6.669)	(0.016,4.551) (0.310,4.737) (-0.065,6.669)	(0.109,6.692) (0.323,4.452) (0.000,1.260)	(0.125,7.000) (0.270,3.527) (-0.099,6.508)	(0.063,5.692) (0.298,4.602) (-0.050,6.548)	(0.104,6.752) (0.275,4.631) (-0.076,6.634)
ACRES (12,12,3) 1681	(0.078,5.571) (0.311,4.719) (-0.074,6.640)	(0.020,4.492) (0.286,4.608) (-0.016,4.574)	(0.047,4.515) (0.290,4.629) (-0.051,4.533)	(0.000,4.471) (0.271,4.581) (-0.035,6.693)	(0.133,7.000) (0.303,4.505) (0.033,4.312)	(0.039,4.572) (0.290,4.733) (-0.062,6.732)	(0.039,4.572) (0.290,4.733) (-0.062,6.732)	(0.145,7.000) (0.349,4.538) (0.106,6.375)	(0.063,5.666) (0.293,2.545) (-0.015,4.612)	(0.063,5.683) (0.296,4.607) (-0.083,6.591)	(0.052,5.678) (0.287,4.644) (-0.028,6.720)
ACRES (13,7,14) 1278	(0.047,4.549) (0.263,4.686) (0.013,4.607)	(0.047,4.545) (0.303,4.608) (0.070,5.613)	(0.047,4.532) (0.299,4.624) (0.072,4.617)	(0.000,4.462) (0.270,4.572) (-0.038,4.614)	(0.188,7.000) (0.313,4.413) (0.109,6.592)	(0.023,4.537) (0.268,6.713) (0.018,5.695)	(0.023,4.537) (0.268,6.713) (0.018,5.696)	(0.117,6.679) (0.335,4.447) (0.058,4.563)	(0.063,5.651) (0.335,4.559) (0.097,6.614)	(0.094,6.708) (0.303,4.602) (0.072,5.593)	(0.073,5.69) (0.324,4.654) (0.067,5.690)
ACRES (14,4,11) 1024	(0.000,4.512) (0.280,6.692) (0.005,4.624)	(0.031,4.548) (0.260,4.684) (-0.018,4.516)	(0.125,7.000) (0.318,4.632) (0.057,4.532)	(0.00,4.485) (0.230,4.549) (-0.068,4.624)	(0.063,5.563) (0.230,2.343) (-0.082,3.403)	(0.000,4.498) (0.248,6.700) (-0.032,4.691)	(0.000,4.498) (0.248,6.700) (-0.032,4.691)	(0.094,6.639) (0.324,4.439) (-0.021,3.432)	(0.000,5.561) (0.375,4.577) (0.110,6.619)	(0.250,7.000) (0.385,4.642) (0.242,7.000)	(0.167,7.000) (0.370,4.675) (0.165,7.000)
ACRES (15,16,13) 1615	(0.083,5.615) (0.328,4.738) (0.013,4.621)	(0.016,4.526) (0.314,4.658) (0.019,4.564)	(0.083,5.599) (0.314,4.659) (0.011,4.563)	(0.000,4.483) (0.339,4.668) (0.081,6.688)	(0.031,4.521) (0.246,6.597) (-0.063,4.481)	(0.042,4.52) (0.316,5.756) (0.010,4.705)	(0.042,4.52) (0.316,5.756) (0.010,4.705)	(0.047,4.543) (0.273,4.621) (-0.062,4.479)	(0.000,5.527) (0.291,4.640) (0.000,4.555)	(0.125,7.000) (0.321,4.661) (0.045,5.594)	(0.111,6.689) (0.370,5.696) (0.104,6.723)
YULEE (16,6,6) 1479	(0.031,4.60) (0.275,4.681) (-0.073,4.618)	(0.031,4.597) (0.303,4.608) (0.043,4.581)	(0.031,4.584) (0.273,4.611) (-0.061,3.511)	(0.000,4.533) (0.270,4.571) (-0.017,6.679)	(0.188,7.000) (0.313,4.408) (0.085,6.359)	(0.016,5.537) (0.274,6.715) (-0.047,6.717)	(,0.016,5.537) (0.274,6.715) (-0.047,6.717)	(0.109,6.641) (0.346,4.452) (0.075,4.351)	(0.125,7.000) (0.354,4.568) (0.054,4.624)	(0.063,5.641) (0.270,4.585) (-0.045,4.588)	(0.104,6.702) (0.317,4.650) (0.024,6.724)
ACRES (17,15,15) 1168	(0.000,4.457) (0.349,4.746) (0.148,7.000)	(0.031,5.651) (0.311,4.628) (0.002,4.616)	(0.125,7.000) (0.334,4.658) (0.088,6.663)	(0.000,5.588) (0.303,4.617) (-0.003,5.632)	(0.063,5.666) (0.240,4.561) (-0.132,6.507)	(0.063,5.652) (0.316,4.753) (0.070,5.722)	(0.063,5.652) (0.316,4.753) (0.070,5.722)	(0.156,7.000) (0.295,4.599) (-0.103,4.519)	(0.000,5.569) (0.340,4.632) (0.050,5.628)	(0.250,7.000) (0.385,4.660) (0.242,7.000)	(0.083,5.698) (00.342,4.679) (0.085,6.700)
YULEE (18,17,18) 1322	(0.000,4.612) (0.329,4.739) (0.034,5.704)	(0.094,6.727) (0.314,4.666) (0.082,6.759)	(0.042,5.648) (0.315,4.668) (0.042,5.742)	(0.167,7) (0.328,4.671) (0.132,7.000)	(0.104,6.715) (0.297,6.632) (0.007,6.680)	(0.042,5.622) (0.308,5.753) (0.047,5.682)	(0.042,5.622) (0.308,5.753) (0.047,5.682)	(0.073,5.643) (0.285,4.635) (-0.032,6.657)	(0.333,7.000) (0.416,4.712) (0.289,7.000)	(0.083,5.662) (0.342,4.679) (0.085,6.736)	(0.333,7.000) (0.445,6.736) (0.281,7.000)
ACRES (19,18,19) 1431	(0.000,4.612) (0.329,4.739) (0.034,5.704)	(0.094,6.727) (0.314,4.666) (0.082,6.759)	(0.042,5.648) (0.315,4.668) (0.042,5.742)	(0.167,7) (0.328,4.671) (0.132,7.000)	(0.104,6.715) (0.297,6.632) (0.007,6.680)	(0.042,5.622) (0.308,5.753) (0.047,5.682)	(0.042,5.622) (0.308,5.753) (0.047,5.682)	(0.073,5.643) (0.285,4.635) (-0.032,6.657)	(0.333,7.000) (0.416,4.712) (0.289,7.000)	(0.083,5.662) (0.342,4.679) (0.085,6.736)	(0.333,7.000) (0.445,6.736) (0.281,7.000)

APPENDIX D: MICROSATELLITE DNA GENOTYPES

Mississippi sandhill cranes:

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
1018	272 / 268	373 /?	255 / 263	359 /?	?/?	158 / 166	355 / 355	147 / 147	169 / 157	255 / 259	247 / 255	266 / 266	168 / 165	255 / 264
1020	268 / 268	373 / 377	251 / 251	359 / 375	394 / 414	170 / 170	355 / 355	147 / 147	161 / 169	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
1024	272 / 308	377 / 377	251 / 263	359 / 363	394 / 394	158 / 158	355 / 355	147 / 147	165 / 173	255 / 255	247 / 247	266 / 269	165 / 165	264 / 264
1033	272 / 308	373 / 393	251 / 263	375 / 391	394 / 394	158 / 166	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1034	268 / 308	373 / 377	251 / 255	375 / 363	394 / 414	158 / 166	355 / 355	147 / 147	169 / 161	255 / 259	247 / 247	266 /?	168 / 165	264 / 264
1036	272 / 268	377 / 361	251 /?	363 / 375	394 /?	158 /?	355 / 355	147 / 147	165 / 169	255 /?	247 / 247	266 / 296	165 / 168	264 / 264
1081	268 / 276	373 / 393	267 / 263	363 / 375	414 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 255	247 / 255	266 / 266	165 / 165	255 / 264
1117	268 / 272	373 / 373	251 / 255	375 / 359	394 / 414	158 / 170	355 / 355	147 / 147	161 / 169	255 / 259	247 / 255	266 / 266	165 / 168	255 / 264
1128	268 / 308	373 / 377	251 / 263	?/?	394 / 394	158 / 166	355 / 355	147 / 147	169 / 173	255 / 255	247 / 247	266 / 296	165 / 168	264 / 264
1135	268 / 272	?/?	251 / 251	375 / 375	394 / 414	158 / 166	355 / 355	147 / 147	161 / 169	255 / 255	247 / 247	266 / 296	165 / 168	264 / 264
1137	268 / 272	373 / 361	251 / 255	375 / 375	394 / 394	158 / 158	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
1138	256 / 308	373 / 377	255 / 263	391 / 391	394 / 414	158 / 170	355 / 355	147 / 147	169 / 173	255 / 255	247 / 247	266 / 269	165 / 168	264 / 264
1144	268 / 268	373 / 373	251 / 263	359 / 375	394 / 414	170 / 166	355 / 355	147 / 147	161 / 169	259 / 259	247 / 247	266 / 266	168 / 168	264 / 264
1149	268 / 308	377 / 393	251 / 263	359 / 375	394 / 414	170 / 158	355 / 355	147 / 147	161 / 169	259 / 255	247 / 247	266 / 269	165 /?	255 / 264
1151	272 / 276	373 / 373	255 / 263	363 /?	394 / 414	170 / 170	355 / 355	147 / 147	165 /?	255 / 255	247 / 247	266 /?	165 / 165	255 / 264
1152	308 / 308	377 / 377	251 / 251	359 / 359	394 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
1156	268 / 276	377 / 393	251 / 263	363 / 391	394 / 394	170 / 158	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264
1162	276 / 260	393 / 393	255 / 263	363 / 359	414 / 414	158 / 158	355 / 355	147 / 147	169 / 173	255 / 255	255 / 247	266 / 266	165 / 165	255 / 264
1163	256 / 308	373 / 393	251 / 263	359 / 359	394 / 394	158 / 158	355 / 355	147 / 147	173 / 177	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1168	268 / 272	373 / 373	255 / 263	375 / 363	394 / 394	166 / 158	355 / 355	147 / 147	161 / 165	255 / 259	247 / 247	266 / 269	165 / 165	264 / 264
1217	276 / 308	373 / 373	251 / 251	359 / 391	414 / 414	158 / 158	355 / 355	147 / 147	165 / 173	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1242	308 / 308	373 / 377	251 / 255	359 / 375	394 / 414	158 /?	355 / 355	147 / 147	161 / 165	255 / 259	247 / 247	?/?	165 / 165	264 / 264

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
1255	272 / 260	377 / 377	251 / 255	391 / 395	394 / 414	158 / 158	355 / 355	147 / 147	161 / 169	255 / 255	247 / 247	266 / 266	165 / 168	264 / 264
1257	268 / 272	373 / 377	251 / 263	?/?	?/?	170 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 165	255 / 264
1258	260 / 268	373 / 373	255 / 263	359 / 395	394 / 414	158 / 170	355 / 355	147 / 147	161 / 173	255 / 255	247 / 255	266 / 266	165 / 168	264 / 264
1278	308 / 308	373 / 373	263 / 263	359 / 391	394 / 414	158 / 158	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264
1296	268 / 272	373 / 373	251 / 251	359 / 375	394 / 414	166 / 158	355 / 355	147 / 147	169 / 169	259 / 255	247 / 247	266 / 266	165 / 168	264 / 264
1307	256 / 272	373 / 373	263 / 263	359 / 363	394 / 414	158 / 166	355 / 355	147 / 147	165 / 177	255 / 259	247 / 247	266 / 269	165 / 165	255 / 264
1322	276 / 308	377 / 377	251 / 263	391 / 359	394 / 394	170 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1326	260 / 268	373 / 373	251 / 267	359 / 375	379 / 414	158 / 158	355 / 355	147 / 147	161 / 169	255 / 259	247 / 251	266 / 269	165 / 168	264 / 264
1352	308 / 308	373 / 377	251 / 263	359 / 391	394 / 394	158 / 170	355 / 355	147 / 147	?/?	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264
1356	268 / 272	373 / 373	251 / 255	359 / 391	394 / 414	158 / 158	355 / 355	147 / 147	161 / 169	255 / 259	247 / 255	266 / 269	165 / 165	255 / 264
1361	256 / 272	373 / 373	251 / 255	359 / 363	394 / 394	158 / 158	355 / 355	147 / 147	157 / 161	255 / 255	247 / 255	266 / 266	165 / 165	264 / 264
1385	256 / 268	373 / 393	251 / 263	359 / 375	394 / 394	158 / 158	355 / 355	147 / 147	165 / 177	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264
1401	260 / 268	373 / 393	235 / 255	359 / 375	394 / 414	158 / 158	355 / 355	147 / 147	165 / 189	255 / 259	247 / 247	266 / 296	165 / 168	264 / 264
1412	268 / 268	393 / 393	235 / 255	359 / 363	414 / 414	158 / 158	355 / 355	147 / 147	161 / 169	255 / 259	251 / 255	266 / 296	165 / 165	264 / 264
1431	268 / 276	377 / 377	251 / 263	375 / 391	394 / 394	158 / 158	355 / 355	147 / 147	169 / 161	255 / 259	247 / 247	266 / 269	165 / 165	255 / 264
1440	276 / 276	373 / 377	251 / 255	359 / 363	414 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 255	266 / 266	165 / 168	264 / 264
1458	268 / 272	377 / 377	251 / 251	363 / 375	394 / 394	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1479	272 / 308	373 / 373	251 / 267	391 / 391	394 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 266	165 / 165	255 / 264
1528	268 / 308	373 / 373	251 / 263	?/?	394 / 394	158 / 170	355 / 355	147 / 147	161 / 169	255 / 259	247 / 255	266 / 266	165 / 165	264 / 264
1534	260 / 276	373 / 373	251 / 255	359 / 359	394 / 414	158 / 158	355 / 355	147 / 147	165 / 173	259 / 259	247 / 247	266 / 296	165 / 165	264 / 264
1560	260 / 268	373 / 373	263 / 267	363 / 375	394 / 414	158 / 158	355 / 355	147 / 147	165 / 189	259 / 259	251 / 255	266 / 296	165 / 165	264 / 264
1580	268 / 308	373 / 393	251 / 263	359 / 375	394 / 394	158 / 158	355 / 355	147 / 147	169 / 177	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
1586	268 / 308	373 / 373	251 / 263	?/?	414 / 414	158 / 170	355 / 355	147 / 147	161 / 169	255 / 259	247 / 247	266 / 266	165 / 168	255 / 264
1599	272 / 276	373 / 373	263 / 263	363 / 363	394 / 394	158 / 170	355 / 355	147 / 147	165 / 177	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
1611	268 / 308	373 / 373	251 / 255	359 / 375	394 / 394	158 / 158	355 / 355	147 / 147	169 / 177	259 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1615	268 / 268	393 / 393	251 / 255	359 / 375	394 / 394	158 / 170	355 / 355	147 / 147	165 / 169	259 / 259	247 / 247	266 / 269	165 / 165	264 / 264
1621	256 / 268	377 / 377	251 / 255	375 / 391	394 / 414	158 / 170	355 / 355	147 / 147	161 / 173	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
1624	272 / 308	373 / 377	251 / 263	359 / 363	394 / 394	?/?	355 / 355	147 / 147	161 / 169	255 / 255	247 / 247	266 / 266	165 / 165	255 / 264
1640	268 / 308	?/?	235 / 251	?/?	?/?	158 / 158	355 / 355	147 / 147	169 / 189	255 / 255	247 / 247	266 / 296	165 / 168	255 / 264
1646	260 / 276	?/?	235 / 251	391 /?	394 / 414	158 / 158	355 / 355	147 / 147	173 / 189	259 / 255	247 / 255	266 / 296	165 / 168	264 / 264
1681	268 / 276	373 / 373	255 / 263	359 / 363	414 / 414	158 / 170	355 / 355	147 / 147	165 / 189	255 / 255	247 / 247	266 / 296	165 / 168	264 / 264
1701	268 / 272	373 / 373	251 / 263	363 / 375	394 / 414	158 / 158	355 / 355	147 / 147	169 / 177	259 / 259	247 / 247	266 / 269	165 / 165	255 / 264
1708	260 / 268	373 / 373	251 / 267	375 / 391	394 / 414	158 / 158	355 / 355	147 / 147	165 / 173	259 / 259	247 / 255	266 / 266	165 / 165	264 / 264
1726	268 / 272	373 / 373	251 / 263	359 / 391	414 / 414	170 / 170	355 / 355	147 / 147	169 / 173	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1751	276 / 308	377 / 377	263 / 263	359 / 391	394 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1757	268 / 276	373 / 373	251 / 255	359 / 363	414 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 255	266 / 266	165 / 168	264 / 264
1758	272 / 308	373 / 373	251 / 263	363 / 391	394 / 414	170 / 170	355 / 355	147 / 147	161 / 169	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264
1759	268 / 272	373 / 373	251 / 251	375 / 375	394 / 414	158 / 166	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 296	165 / 168	264 / 264
1774	256 / 260	373 / 373	255 / 263	359 / 359	?/?	158 / 166	355 / 355	147 / 147	169 / 173	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1787	268 / 268	373 / 373	255 / 263	359 / 359	394 / 414	158 / 158	355 / 355	147 / 147	165 / 173	255 / 255	247 / 255	266 / 296	165 / 168	255 / 264
1794	256 / 260	373 / 373	255 / 263	359 / 391	394 / 414	158 / 170	355 / 355	147 / 147	173 / 189	255 / 255	247 / 247	266 / 296	165 / 168	264 / 264
1804	260 / 272	373 / 373	251 / 263	391 / 395	414 / 414	158 / 170	355 / 355	147 / 147	161 / 169	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
1809	260 / 268	373 / 393	251 / 255	363 / 375	394 / 414	170 / 170	355 / 355	147 / 147	161 / 169	259 / 259	247 / 247	266 / 266	165 / 168	255 / 264
1819	260 / 308	373 / 373	235 / 263	359 / 375	394 / 394	158 / 158	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 296	165 / 165	264 / 264
1820	256 / 268	373 / 393	251 / 251	359 / 375	394 / 414	158 / 158	355 / 355	147 / 147	165 / 177	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1823	256 / 268	373 / 393	251 / 263	359 / 375	394 / 414	158 / 158	355 / 355	147 / 147	169 / 177	259 / 259	247 / 247	266 / 269	165 / 165	264 / 264
1824	?/?	393 / 393	255 / 255	391 /?	?/?	166 / 166	?/?	147 / 147	?/?	255 / 259	247 / 247	?/?	165 / 168	264 / 264
1825	268 / 272	373 / 373	251 / 255	391 / 395	394 / 414	170 / 170	355 / 355	147 / 147	161 / 169	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
1827	272 / 276	373 / 377	251 / 255	363 / 391	394 / 414	158 / 158	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 269	165 / 165	264 / 264
1828	268 / 272	393 / 393	251 / 255	359 / 391	394 / 414	158 / 158	355 / 355	147 / 147	161 / 189	255 / 255	247 / 255	266 / 269	165 / 168	264 / 264
1830	268 / 276	373 / 373	235 / 263	375 / 391	394 / 394	158 / 158	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 296	165 / 168	264 / 264
1831	268 / 268	373 / 393	235 / 255	359 / 359	414 / 414	158 / 158	355 / 355	147 / 147	161 / 189	255 / 259	247 / 247	266 / 269	165 / 168	255 / 264
1832	256 / 272	373 / 373	263 / 263	359 / 363	414 / 414	158 / 166	355 / 355	147 / 147	169 / 177	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1833	256 / 260	373 / 373	255 / 263	359 / 359	394 / 414	158 / 158	355 / 355	147 / 147	173 / 177	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1834	268 / 272	373 / 393	251 / 263	363 / 375	394 / 414	158 / 170	355 / 355	147 / 147	165 / 169	259 / 259	247 / 247	266 / 269	165 / 165	264 / 264
1835	272 / 272	373 / 373	251 / 263	359 / 363	414 / 414	158 / 166	355 / 355	147 / 147	165 / 177	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
1836	256 / 268	373 / 373	251 / 255	359 / 391	394 / 414	158 / 166	355 / 355	147 / 147	161 / 169	255 / 259	247 / 255	266 / 269	165 / 165	264 / 264
1837	260 / 272	393 / 393	255 / 263	363 / 375	394 / 394	158 / 158	355 / 355	147 / 147	169 / 177	259 / 259	247 / 247	266 / 269	165 / 165	264 / 264
1838	268 / 272	373 / 377	251 / 263	363 / 375	394 / 414	158 / 158	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 266	165 / 165	264 / 264
1840	256 / 272	373 / 373	251 / 255	375 / 391	414 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 255	247 / 247	266 / 266	165 / 168	264 / 264
1841	268 / 308	373 / 393	251 / 255	375 / 391	394 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 296	165 / 165	264 / 264
1842	256 / 260	393 / 393	251 / 251	375 / 375	394 / 414	158 / 170	355 / 355	147 / 147	?/?	255 / 259	247 / 247	266 / 266	165 / 165	255 / 264
1843	260 / 268	373 / 373	251 / 255	359 / 359	394 / 414	158 / 166	355 / 355	147 / 147	161 / 169	255 / 259	247 / 247	266 / 296	165 / 165	264 / 264
1844	260 / 272	373 / 373	251 / 255	359 / 359	394 / 414	158 / 166	355 / 355	147 / 147	?/?	259 / 259	247 / 247	266 / 266	165 / 165	255 / 264
1845	256 / 268	373 / 373	251 / 255	359 / 359	414 / 414	158 / 166	355 / 355	147 / 147	169 / 177	255 / 259	247 / 255	266 / 266	165 / 168	264 / 264
DPN Est08	260 / 308	373 / 377	255 / 263	363 / 391	394 / 414	158 / 166	355 / 355	147 / 147	165 / 177	255 / 255	247 / 255	266 / 266	165 / 165	264 / 264
HW 09	308 / 308	373 / 373	263 / 263	?/?	?/?	158 / 170	355 / 355	147 / 147	177 / 177	?/?	247 / 251	266 / 266	?/?	264 / 264
LGE G8	268 / 268	373 / 373	251 / 251	?/?	394 / 394	158 / 158	355 / 355	147 / 147	169 / 177	255 / 259	247 / 247	266 / 266	165 / 168	264 / 264
VIC K8	256 / 272	373 / 393	251 / 263	359 / 363	394 / 414	158 / 170	355 / 355	147 / 147	165 / 169	255 / 259	247 / 247	266 / 266	165 / 165	264 / 264
WH 04	272 / 272	?/?	251 / 255	?/?	?/?	166 / 170	355 / 355	147 / 147	?/?	?/?	247 / 247	266 / 266	?/?	264 / 264
WH 09	268 / 300	373 / 373	251 / 251	375 / 391	379 / 394	158 / 170	355 / 355	147 / 147	157 / 161	255 / 255	247 / 255	266 / 266	165 / 168	255 / 264

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
WH 25	268 / 276	373 / 393	235 / 235	359 / 395	394 / 414	158 / 158	355 / 355	147 / 147	165 / 169	259 / 259	247 / 251	296 / 296	165 / 165	264 / 264
WH 36	300 / 308	377 / 377	267 / 267	359 / 363	394 / 394	158 / 158	355 / 355	147 / 147	169 / 189	255 / 255	247 / 251	266 / 269	165 / 168	264 / 264
WH 37	276 / 308	373 / 373	251 / 263	363 / 391	394 / 414	158 / 158	355 / 355	147 / 147	161 / 165	255 / 255	247 / 255	266 / 266	165 / 165	264 / 264
WH 38	276 / 308	373 / 373	251 / 263	363 / 391	394 / 414	158 / 158	355 / 355	147 / 147	169 / 173	255 / 255	247 / 255	266 / 266	165 / 165	264 / 264
WH 40	268 / 268	373 / 377	251 / 267	359 / 363	394 / 394	158 / 166	355 / 355	147 / 147	161 / 169	255 / 259	247 / 255	266 / 269	165 / 168	255 / 264
WH 41	308 / 324	373 / 373	251 / 263	387 / 391	406 / 414	158 / 166	355 / 355	147 / 147	157 / 165	255 / 255	247 / 255	266 / 266	165 / 165	264 / 264

Florida sandhill crane genotypes

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
304	256 / 268	373 / 397	247 / 263	363 / 395	390 / 394	170 / 174	?/?	?/?	?/?	255 / 255	247 / 251	266 / 269	165 / 171	264 / 264
305	252 / 264	373 / 373	259 / 263	359 / 359	398 / 398	158 / 166	?/?	?/?	165 / 173	255 / 259	251 / 255	266 / 266	165 / 171	264 / 264
306	252 / 260	373 / 373	235 / 247	359 / 391	390 / 402	158 / 170	?/?	?/?	173 / 177	255 / 259	255 / 259	?/?	168 / 168	264 / 264
308	272 / 272	373 / 397	247 / 275	359 / 395	414 / 430	158 / 166	355 / 355	?/?	173 / 173	255 / 259	247 / 251	266 / 269	165 / 165	264 / 264
309	264 / 264	385 / 397	251 / 267	363 / 363	390 / 390	166 / 166	?/?	?/?	165 / 181	255 / 255	247 / 247	?/?	168 / 168	264 / 264
310	272 / 296	373 / 377	243 / 251	359 / 363	418 / 430	158 / 166	355 / 355	?/?	169 / 173	255 / 259	247 / 251	?/?	165 / 165	264 / 264
311	260 / 272	381 / 381	263 / 263	?/?	398 / 414	158 / 166	?/?	?/?	161 / 173	255 / 259	251 / 255	?/?	165 / 165	264 / 264
312	272 / 272	373 / 373	263 / 275	359 / 395	398 / 414	158 / 166	?/?	?/?	165 / 173	255 / 259	251 / 255	266 / 269	165 / 165	264 / 264
313	256 / 308	373 / 389	243 / 275	363 / 363	398 / 398	166 / 166	355 / 355	147 / 147	169 / 177	255 / 255	243 / 251	266 / 269	168 / 168	264 / 264
315	308 / 308	373 / 373	263 / 267	383 / 391	402 / 406	158 / 158	355 / 355	147 / 147	165 / 173	255 / 255	247 / 247	260 / 269	168 / 168	264 / 264
316	260 / 260	369 / 369	263 / 267	363 / 395	398 / 406	166 / 170	355 / 355	147 / 147	173 / 177	255 / 255	247 / 247	266 / 269	165 / 168	264 / 264
320	260 / 304	377 / 377	231 / 231	363 / 395	398 / 398	?/?	355 / 355	147 / 147	?/?	255 / 255	243 / 247	?/?	165 / 165	264 / 264
322	252 / 252	381 / 389	247 / 267	359 / 391	374 / 398	158 / 166	355 / 355	147 / 147	165 / 169	255 / 259	247 / 259	?/?	165 / 165	264 / 264
323	252 / 308	397 / 397	251 / 267	359 / 395	378 / 398	158 / 174	355 / 355	147 / 147	169 / 173	255 / 255	251 / 259	266 / 266	162 / 165	255 / 264

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
328	264 / 308	377 / 389	231 / 259	359 / 363	406 / 406	158 / 166	355 / 355	147 / 147	169 / 173	255 / 259	247 / 259	266 / 269	165 / 168	264 / 264
330	296 / 296	389 / 397	247 / 251	363 / 395	390 / 410	166 / 170	?/?	147 / 147	161 / 173	255 / 259	247 / 251	?/?	168 / 168	264 / 264
331	264 / 272	373 / 373	263 / 263	359 / 363	398 / 402	166 / 166	?/?	147 / 147	165 / 173	255 / 255	247 / 251	266 / 269	165 / 168	264 / 264
333	252 / 260	385 / 389	235 / 247	363 / 391	390 / 394	166 / 166	?/?	147 / 147	177 / 189	255 / 255	247 / 251	266 / 266	162 / 168	264 / 264
334	256 / 260	373 / 397	251 / 251	359 / 363	390 / 398	158 / 170	355 / 355	147 / 147	165 / 181	255 / 255	247 / 251	266 / 269	168 / 168	264 / 264
340	252 / 260	385 / 389	235 / 247	363 / 391	390 / 394	166 / 166	355 / 355	147 / 147	?/?	255 / 255	247 / 251	266 / 269	162 / 168	264 / 264
341	256 / 260	377 / 377	263 / 267	359 / 363	390 / 398	158 / 166	355 / 355	147 / 147	165 / 173	255 / 255	247 / 251	260 / 266	165 / 165	264 / 264
343	268 / 268	373 / 373	263 / 267	363 / 391	378 / 402	158 / 166	355 / 355	147 / 147	161 / 173	255 / 255	247 / 247	266 / 266	165 / 168	264 / 264
344	296 / 316	393 / 401	267 / 275	363 / 395	374 / 402	158 / 158	355 / 355	147 / 147	?/?	255 / 259	247 / 255	266 / 266	165 / 171	264 / 264
345	300 / 300	373 / 377	247 / 263	359 / 363	390 / 410	166 / 166	355 / 355	147 / 147	169 / 177	255 / 255	247 / 247	260 / 266	165 / 168	264 / 264
346	264 / 324	369 / 373	231 / 243	363 / 395	402 / 402	158 / 166	355 / 355	147 / 147	161 / 169	255 / 255	251 / 251	266 / 269	165 / 165	264 / 264
347	260 / 272	377 / 377	243 / 263	391 / 395	414 / 414	158 / 166	355 / 355	147 / 147	173 / 189	255 / 255	247 / 251	266 / 266	165 / 165	264 / 264
353	264 / 324	369 / 373	231 / 243	363 / 395	402 / 402	158 / 166	355 / 355	?/?	161 / 169	255 / 255	251 / 251	266 / 269	165 / 165	264 / 264
355	248 / 272	373 / 373	263 / 275	391 / 395	382 / 406	166 / 166	355 / 355	147 / 147	165 / 173	255 / 259	247 / 255	266 / 269	165 / 168	264 / 264
359	296 / 304	377 / 377	243 / 247	?/?	406 / 418	166 / 166	355 / 355	147 / 147	169 / 169	255 / 255	247 / 251	266 / 296	165 / 168	264 / 264
361	260 / 264	373 / 373	231 / 255	363 / 363	398 / 402	166 / 166	355 / 355	147 / 147	?/?	255 / 255	247 / 247	266 / 266	165 / 168	264 / 264
362	260 / 264	397 / 397	263 / 267	359 / 359	390 / 398	166 / 166	355 / 355	147 / 147	173 / 185	255 / 255	243 / 259	266 / 266	165 / 168	264 / 264
363	304 / 304	?/?	259 / 267	359 / 359	?/?	158 / 166	355 / 355	147 / 147	165 / 165	255 / 259	243 / 247	266 / 269	165 / 168	264 / 264
364	304 / 304	393 / 393	259 / 267	391 / 395	390 / 390	166 / 166	355 / 355	147 / 147	165 / 177	255 / 255	247 / 251	266 / 269	165 / 168	255 / 264
366	260 / 264	373 / 373	231 / 255	363 / 363	398 / 402	166 / 166	355 / 355	147 / 147	173 / 173	255 / 255	247 / 247	266 / 266	165 / 168	264 / 264
367	296 / 304	377 / 377	243 / 247	359 / 391	406 / 418	166 / 166	355 / 355	147 / 147	?/?	255 / 255	247 / 251	269 / 269	165 / 168	264 / 264
368	260 / 260	373 / 389	231 / 251	363 / 395	390 / 410	166 / 166	355 / 355	147 / 147	181 / 181	255 / 259	247 / 247	260 / 266	165 / 168	264 / 264
372	252 / 308	?/?	247 / 255	?/?	390 / 390	158 / 166	355 / 355	147 / 147	173 / 181	255 / 255	247 / 251	266 / 266	165 / 168	264 / 264
373	252 / 268	385 / 385	247 / 247	375 / 391	398 / 406	166 / 170	355 / 355	147 / 147	177 / 181	255 / 255	247 / 251	266 / 269	165 / 168	264 / 264

ID #	Gram 11	Gram 8	Gram 6	Gram 17	Gram 20	Gram 22	Gram 24	Gram 25	Gram 30	Gram 31	Gram 32a	Gram 41	Gram 42	Gram 45
374	260 / 264	373 / 401	231 / 255	?/?	398 / 402	166 / 166	355 / 355	147 / 147	165 / 173	255 / 255	247 / 247	266 / 269	165 / 168	264 / 264
375	252 / 308	373 / 389	247 / 255	?/?	390 / 406	166 / 166	355 / 355	?/?	165 / 173	255 / 255	247 / 251	266 / 269	165 / 168	264 / 264

VITA

Jessica Renee Henkel graduated from Stony Brook University in New York with a B.A. in English. Following several years working in science publishing at Cambridge University Press, Jessica returned to school to pursue a research career in biology. Jessica's research interests include the ecology and conservation biology of disturbed ecosystems. She also enjoys educating young members of the community about wetlands and wetland loss through her work with Bayou Rebirth, a non-profit organization in New Orleans, Louisiana. In the future, Jessica hopes to continue her work in both the academic and non-profit arenas. She lives in Uptown New Orleans with her husband, Thom.