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PEDIGREE ANALYSES OF THE MISSISSIPPI SANDHILL CRANE

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Abstract: The genetic status of the critically endangered Mississippi sandhill crane (*Grus canadensis pulla*) was analyzed using 2008 studbook data from the U.S. Fish and Wildlife Service managed captive breeding and release program. Since the captive breeding program was established in 1964, gene diversity has decreased 6%, average mean kinship is at the level of first cousin, and inbreeding coefficients have been observed as high as 0.375. These high inbreeding levels were correlated with lower survivorship, and an analysis of the mate suitability of current pairs at the release site revealed poor mate suitability indices. These factors may be contributing to the low survivorship and poor nest success observed in the wild. Before the results of this study can be adequately assessed and applied to management strategies, current assumptions regarding original founder relationships must be improved. I recommend applying molecular genetic analyses of the captive and released Mississippi sandhill crane population to the results of this study to increase our understanding of the population's genetic structure, and promote management decisions that result in increased genetic diversity.

Key words: captive breeding, *Grus canadensis pulla*, Mississippi sandhill crane, pedigree analysis.

The Mississippi sandhill crane (*Grus canadensis pulla*, MSC) is a critically endangered subspecies of sandhill crane, found only in Gautier, Mississippi, at the Mississippi Sandhill Crane National Wildlife Refuge (MSCNWR) and in captivity. The population of this non-migratory crane has been supplemented by a U.S. Fish and Wildlife Service managed captive breeding program since 1981, when the wild population numbers dropped below 40 individuals (Valentine and Noble 1970). There are now approximately 110 wild cranes on the refuge (S. Hereford, U.S. Fish and Wildlife Service, personal communication).

The recovery objective for the 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 the total population on the refuge has increased since the initiation of the captive breeding and release program, successful rearing of wild hatchlings has been few, and the wild population remains heavily dependent on supplemental captive breeding to remain above 100 individuals. Additionally, the population bottleneck observed prior to 1965, when the captive breeding program was first initiated, is likely to have resulted in a loss of genetic diversity. Molecular genetic studies that utilized allozyme (Dessauer et al. 1992) and microsatellite analyses (Jones 2003) reported that MSCs show nearly half the level of heterozygosity when compared to other sandhill cranes.

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). These genetic parameters are determined by estimating losses or changes to genetic variability relative to a wild founder population, which is assumed to be unrelated. Pedigree information is available for all captive cranes and for 73.6% of the wild cranes in the MSC population. I used this pedigree information and 3 pedigree analysis programs to analyze the current genetic status of the captive and released MSC population. My specific objectives were to: 1) determine the current pedigree structure of the total captive and released population, 2) compare inbreeding to survivorship, and 3) conduct analyses to make recommendations for future management.

METHODS

Data.–The data used for this study was collected from the MSC studbook, current through February 2008 (Savoie 2008). My analysis of the managed population included all the captive and released individuals.

Information on released individuals can be difficult to ascertain when working with a supplemented population, 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. Therefore, this pedigree analysis is based on 2 populations: the total population of captive and released individuals presumed alive, and the total population of captive and released individuals known to be alive as of March 2008 (S. Hereford, U.S. Fish and Wildlife Service, personal communication).

Analysis 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.

Population Management 2000 (PM2000, Pollak et al. 2005) was used to measure gene diversity (Lacy and Ballou 2001), founder genome equivalents, mean kinship (Ballou and 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 reach reproductive maturity (Mirande et al. 1996). This analysis was conducted using statistical software (SPSS Inc. 2001).

MateRx version 1.9 (Ballou et al. 2001) was used to evaluate the genetic value of mating different pairs. This program integrates 4 genetic components into a single index: the Mate Suitability Index (MSI). The 4 components are: 1) the change in population's genetic diversity associated with the pairing of 2 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 and Ballou 2004); 3) inbreeding coefficients; and 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

Table 1. Summary of March 2008 Mississippi sandhill crane captive and released population parameters as reported by PM2000.

Genetic characteristics	Current total population (presumed alive)	Current total population (known alive)
N	332	139
Founders	31	31
Living descendants	275	115
Gene diversity	0.9403	0.9400
Founder genome equivalents	8.37	8.34
Mean kinship	0.0597	0.0600
Mean inbreeding (F)	0.0074	0.0052

realistically be paired together.

RESULTS

PM2000 reported a presumed population size of 332 individuals, with 31 founders and 274.9 living descendents (individuals with incomplete ancestries are tallied as partial genetic individuals). PM2000 also reported a known population size of 139 individuals, with 31 founders and 115 living descendents (Table 1). This known population consisted of 97 cranes from the MSCNWR and 42 captive cranes. Of the cranes at the MSCNWR, 81 were reared in captivity, and 16 are unknown individuals (either founders, founder descendents, or captive-reared individuals who have lost their leg bands).

The 31 founders showed variable allelic representation in the known population (Fig. 1). PM2000 reported that the proportion of genetic diversity retained from the original founder population is 0.9403 in the presumed population, and 0.9400 in the known population, with founder genome equivalents of 8.37 and 8.34, respectively. The average mean kinship for the total presumed population is 0.0597, and 0.0600 for the known population, with individual mean kinships ranging from 0 to 0.0899 (Fig 2.).

Inbreeding coefficients ranged from 0 to 0.375. Mean inbreeding of the presumed population was 0.0074, and was 0.0052 in the known population. 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. Inbreeding was significantly negatively correlated with
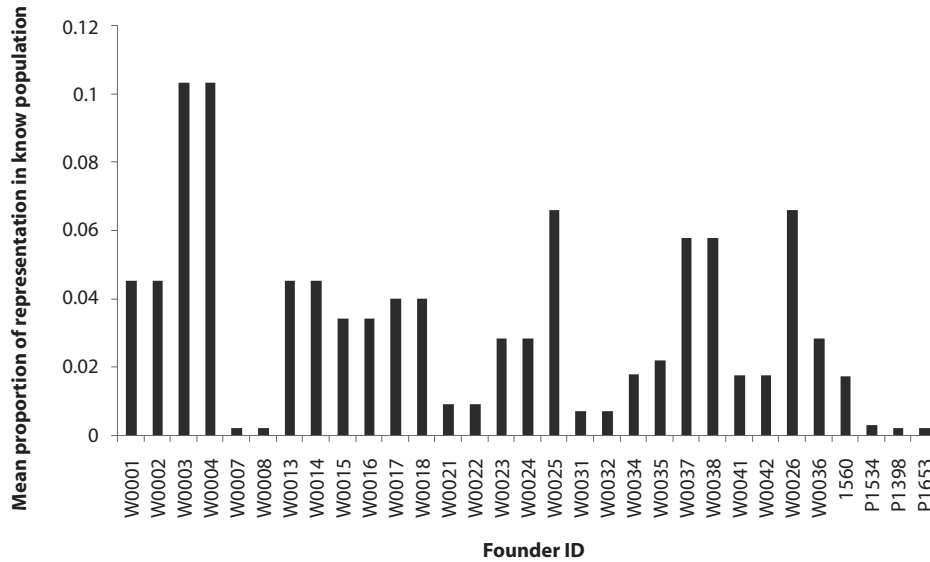
Figure 1. Proportion of alleles from the 31 founders found in the known population, as reported by PM2000.

survivorship ($n = 6$, $P < 0.001$) (Fig. 3).

MateRx analyzed the mate suitability of all possible pairs (including all presumed individuals) 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 to 7, with an average rating of 4.58. Optimal pairing was analyzed for each captive individual (Table 2). Despite pairing individuals to produce the

lowest possible MSI, the average MSI for the captive pairings was 4.19 (slightly detrimental).

DISCUSSION

Nearly 75% of the wild MSC population has been raised in captivity. 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
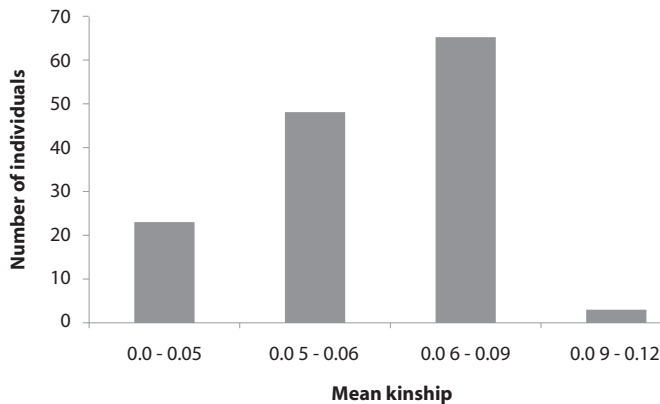

Figure 2. Mean kinship of individuals in the known population as reported by PM2000.
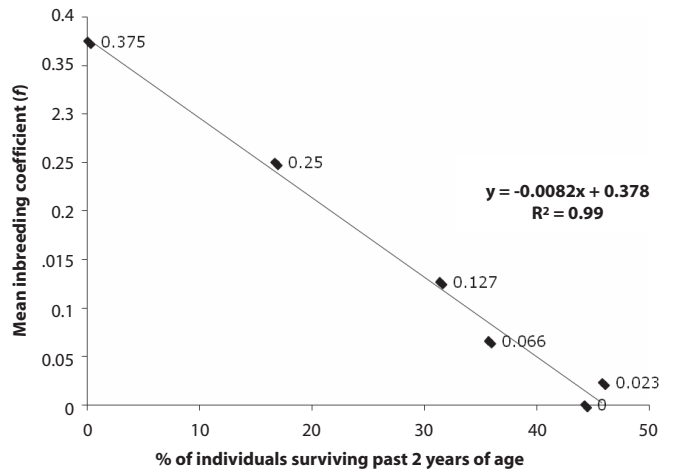

Figure 3. Covariation between percent of individuals surviving past 2 years of age and the mean inbreeding coefficient of those individuals for captive and released populations.
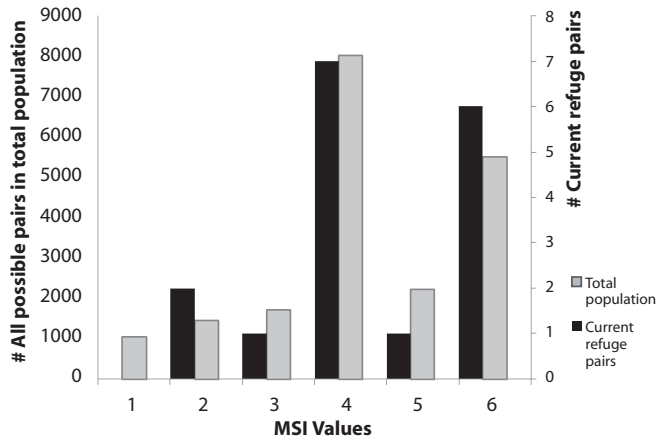
Figure 4. Distribution of mate suitability index (MSI) values for all possible pairings in the presumed population (left-hand y-axis), and the distribution of MSI values for current refuge pairs (right-hand y-axis), as reported by MateRX. A value of 1 is very beneficial to the diversity of the population; a value of 6 is very detrimental.

Table 2. Pair recommendations for captive individuals based on MateRX-reported MSI values for those pairings. Note: certain individuals have no pairings with MSI values less than 4, indicating that any offspring from that individual would be considered detrimental to the genetic diversity of the total Mississippi sandhill crane population.

Male	Female	MSI
1599	-	None < 4
1580	-	None < 4
1307	-	None < 4
1621	1758	3
1156	-	None < 4
1560	1217, 1774	1
1117	1217, 1774	2
1296	-	None < 4
1401	1217	3
1144	1138, 1681, 1794	3
1152	-	None < 4
1458	-	None < 4
1361	1774, 1592, 1217, 1708, 1787	3
1804	1787	1
-	1615	None < 4
-	1356	None < 4
-	1168	None < 6
-	1431	None < 4
1560, 1258	1774	1
1258, 1117	1138	2
1560	1592	2
1144	1681	2
1560	1217	1
-	1278	None < 4
1560, 1117, 1401	1758	2
-	1479	None < 4
1081	-	None < 4
1242	-	None < 4
1128	-	None < 4
1759	-	None < 4
-	1794	None < 4
-	1163	None < 4
1258, 1809	1708	1
-	1322	None < 4
-	1135	None < 4
1137	-	None < 4
1258	1787	1

resulted in a population size that was much higher than that of the known population size. Due to several birds residing on the refuge that have not been identified, the total MSC population is likely around 150 individuals (S. Hereford, U.S. Fish and Wildlife Service, personal communication).

The current population is derived from 31 founders. Due to unequal breeding of 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 1988). This study confirmed these outcomes in the captive MSC population: gene diversity decreased 6% and inbreeding coefficients were as high as 0.375. Additionally, the mean kinship of 0.0625 is equivalent to individuals being related, on average, at the level of first cousin (Ralls and Ballou 2004).

The difference observed in mean inbreeding for the presumed and known population 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 values observed in both populations; although

highly inbred individuals have existed in the population, they have not survived long enough to reach sexual maturity.

The primary assumption of all pedigree analyses is that founders are unrelated. The probability of related founders is likely high for the MSC population following the population bottleneck that occurred before the captive breeding program was established. Therefore, mean

kinship and inbreeding coefficients in the captive population are likely much larger than was observed in this study.

Recommendations for Future Management and Research

Before the results of the pedigree analyses conducted for this study can be adequately assessed, current assumptions regarding founder relationships in the captive and released MSC population must be improved. This can best be achieved by molecular analyses, such as microsatellite analysis, as was accomplished for whooping cranes (*Grus americana*) (Jones et al. 2002). Jones et al. (2002) used microsatellite DNA analysis to investigate the genetic relationships among founders of the whooping crane captive breeding program by using multi-locus genotyping to derive relatedness coefficients among individuals in the population. This research integrated genetic information into the traditional whooping crane studbook, allowing managers to breed individuals based on common or rare alleles within founder lines, to achieve maximum allelic diversity. Similar work on the MSC is of critical importance to the improved understanding of the captive population.

The goal of most captive breeding programs is to retain 90% gene diversity for 100 years (Frankham et al. 2002). Current gene diversity is 94% following 43 years of captive breeding. Managers must be careful with their future pairing decisions for the population and only breed individuals with the lowest mate suitability indices. This is especially important when considering the results of MateRx, which reported that a majority of all possible pairings would have a negative effect on the population's gene pool. Most of the current natural pairings on the refuge also have high (negative) mate suitability indices. This may be contributing to the low nest success on the refuge, and should be an important consideration when eggs are brought into captivity to be reared. The results of the MateRx analysis for this study may change, however, once founder relationships are established. As was observed following microsatellite analysis of the whooping crane (Jones et al. 2002), the value of certain individuals in the population may increase or decrease depending on the original patterns of relatedness of the population.

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