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GENETICAL ANALYSIS OF WOOD STORK (*MYCTERIA AMERICANA*) POPULATIONS USING NUCLEAR AND MITOCHONDRIAL DNA MARKERS

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Resumo. – Análise genética das populações de Cabeças-secas (*Mycteria americana*) utilizando marcadores de DNA nuclear e mitocondrial. – Nesse estudo foi determinada a estrutura genética das colônias reprodutivas de Cabeça-seca (*Mycteria americana*) do Pantanal brasileiro, a partir do conjunto de dados levantados nos mesmos locos de DNA nuclear (alozimas e microssatélites) estudados na população norte americana. Não foi observada diferenciação genética entre as colônias brasileiras nas análises baseadas nos marcadores nucleares. Esses resultados foram interpretados como conseqüentes da ocorrência de intenso fluxo gênico entre as colônias brasileiras. Baixa diferenciação foi detectada entre as populações da América do Norte e do Sul. Esses resultados foram explicados supondo: 1) fluxo gênico histórico e contemporâneo intermediado por membros das colônias localizadas entre essas duas populações, 2) histórico populacional comum durante última glaciação, pois mudanças climáticas restringiram a distribuição dessa espécie à uma região geográfica pequena, promovendo maior contato entre essas populações. Um fragmento de 390/460 pares de base da região controladora do DNA mitocondrial foi seqüenciado e, pela primeira vez, estudado nessa espécie. Seqüências do DNA mitocondrial foram utilizadas na determinação do nível de diferenciação genética entre colônias do Pantanal. O teste de AMOVA revelou baixa subdivisão populacional entre as colônias. Os processos evolutivos responsáveis pelo padrão de variação genética observado foram investigados utilizando Nested Clade Analyses (NCA). Os eventos de expansão populacional e fluxo gênico restrito com isolamento pela distância foram detectados pela NCA como ocorrências que afetaram historicamente a população do Pantanal.

Abstract. – In this study, the genetic structure of the Wood Stork (*Mycteria americana*) breeding colonies of the Brazilian Pantanal was determined using the same nuclear loci (allozymes and microsatellites) studied in the North American population. No genetic differentiation was observed among Brazilian colonies in the analyses based on nuclear markers. These results were interpreted as a consequence of intense gene flow among Brazilian colonies. Low genetic differentiation was detected between North and South American populations. These results were explained supposing: 1) historical and contemporary gene flow intermediated by members of the colonies located between these two populations, and 2) historical gene flow, during the last glaciation, when these populations were closer than at the present time, a condition that favored the exchange among their members. A fragment of 390/460 base pairs of the mitochondrial DNA control region was sequenced and studied for the first time in this species. Mitochondrial DNA sequences were used to determine the level of genetic differentiation among Pantanal colonies. The AMOVA revealed low genetic subdivision among colonies. The evolutionary processes responsible for the observed genetic variation patterns were investigated using the nested clade analyses (NCA). The range expansion events associated with restricted gene flow due to isolation by distance were detected by NCA as occurrences which historically affected the Pantanal population. *Accepted 6 January 2004*.

Key words: Wood Stork, *Mycteria americana*, genetic differentiation, mitochondrial DNA, microsatellites, allozymes.

INTRODUCTION

The Wood Stork (*Mycteria americana*) is a colonial aquatic bird belonging to the Family Ciconiidae (Ciconiiformes order). It is a wading bird adapted to wet-dry climatic cycles such as those observed in wetlands. This species inhabits subtropical and tropical regions of the American continent and its breeding range extends from the southern United States to northern Argentina (AOU 1998). The Wood Stork breeding population size in the southeastern United States have suffered drastic reduction, from 60,000 individuals in the 1930s to nearly 5000 in 1978 (Ogden *et al.* 1987). The drop in the United States population size has been attributed to the loss of wetland habitats. The species has been classified, in the United States, as endangered since 1984 (USFWS 1996). In the Brazilian Pantanal wetlands, during the dry season, large and relatively stable Wood Stork breeding colonies are found. The Pantanal region, situated along the Paraguay River and its tributaries, is one of the largest wetlands of the world (150,000 km^2). Approximately 80% of the Pantanal area is located in central-western Brazil and it contains the characteristics of three primary vegetative domains: Amazon rainforest, cerrado of central Brazil, and the chaco vegetation of Bolivia and Paraguay. The climate is tropical with two very distinctive seasons: a flooded season (October–March), and a dry season (April–September). The Pantanal region is one of the most significant breeding grounds for birds in South America, a pristine wetland as yet not presenting significant disturbance or habitat degradation (Alho *et al.* 1988).

The genetic structure of populations can be determined by estimating the genetic diversity within and among subpopulations. Such genetic information can be useful in conservation management, e.g., in identifying appropriate demographic conservation units or, perhaps, in assessing source-sinks populations (Haig & Avise 1996). Genetic studies performed to investigate the patterns of population differentiation in North American Wood Stork colonies were conducted by Stangel *et al.* (1990) using allozyme loci, and Van Den Bussche *et al.* (1999) using microsatellites and DNA fingerprinting. Both studies showed low genetic differentiation levels among distinct geographic colonies. These results were explained by high gene flow among colonies as well as by the recent colonization of southeastern United States.

The analysis of the genetic structure of the Pantanal Wood Stork breeding colonies had two main objectives: 1) to determine the genetic structure among Pantanal colonies using both nuclear and mitochondrial markers; 2) to compare the genetic structure between North and South America populations using nuclear markers.

METHODS

Eight Wood Stork breeding colonies from the Brazilian Pantanal were sampled in 1997, 1999, and 2000 (Fig.1): Mimoso (MI, 16°09'S–55°48'W), Tucum (TU, 16°26'S–56°03'W), Porto da Fazenda (PF, 16°27'S–56°07'W), Fazenda Ipiranga (FI, 16°25'S–56°36'W), Baia de Gaíva (BG, 16°39'S–57°10'W), Baia Bonita (BB, 18°40'S–56°26'W), Fazenda Retirinho (FR, 19°50'S–56°02'W), and Rio Vermelho (RV, 19°36'S–56°51'W). Growing feathers and blood samples (approx. 0.5 ml) were collected from nestlings.

Allozymes. In total, 234 nestlings from five colonies (PF, BG, BB, RV and FR) were analyzed in 17 allozyme loci, described by Stangel *et al.* (1990), and in 5 new loci not previously studied in this species (Lopes 2002, Del Lama *et al.* 2002).

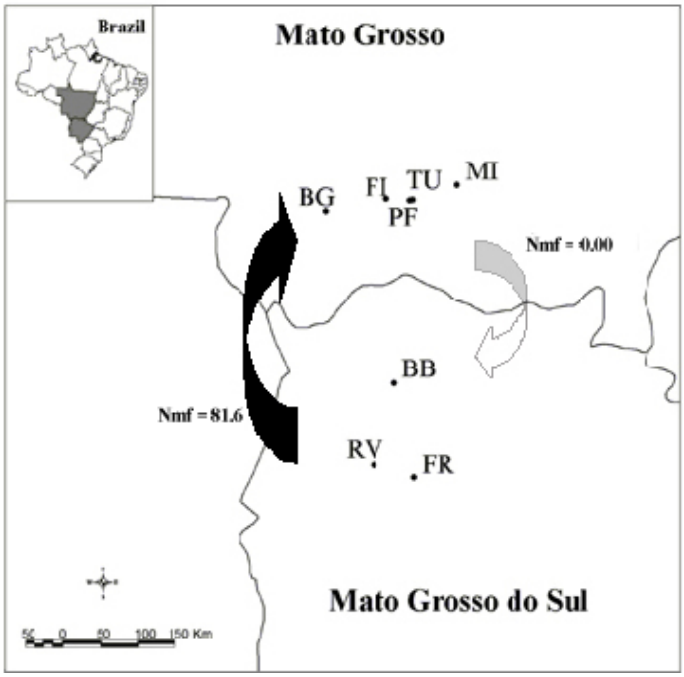

FIG. 1. Map showing sample sites of the Wood Stork Pantanal population (MI = Mimoso, TU = Tucum, PF = Porto da Fazenda, FI = Fazenda Ipiranga, BG = Baia de Gaíva, BB = Baia Bonita, FR = Fazenda Retirinho, and RV = Rio Vermelho). The arrows are indicating the estimated number of females migrants per generation (Nmf) between north and south Pantanal colonies.

Microsatellites. DNA was extracted from 197 individuals belonging to the seven Wood Stork breeding colonies (PF, TU, FI, BG, BB, RV and FR). Genotypes in four microsatellite loci (Van Den Bussche *et al*. 1999) were identified (Rocha 2002, Rocha *et al*. 2004).

Mitochondrial DNA. Total genomic DNA was extracted from 62 nestlings belonging to the eight colonies sampled (7–8 individuals/colony). A fragment of 390/460 base pair (bp) of the mtDNA control region was amplified and sequenced for each individual using the primers developed in this study (Lopes 2002, Lopes *et al*. in prep.).

Data analyses. Levels of genetic differentiation and gene flow among subpopulations were assessed by the estimation of Fst (Wright 1943) and Nm (number of migrants per generation, Slatkin 1981). The analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was used to investigate the patterns of population substructure with mtDNA data. The average gene flow between regions based on the coalescent theory and the time to most recent common ancestor (TMRCA) were calculated with just the mtDNA sequence data. The nested clade analysis (NCA) was used to investigate the evolutionary processes responsible for the observed genetic structure patterns in the mtDNA. The NCA approach tests the geographical association of haplotypes and can discriminate between the effects of contemporary gene flow and nonrecurrent historical events (e.g., range expan-

sion and fragmentation) (Templeton *et al.* 1992, Templeton *et al.* 1995).

RESULTS AND DISCUSSION

Genetic differentiation among Pantanal colonies. The genetic structure of the Pantanal Wood Stork breeding colonies was determined using the same allozyme markers studied by Stangel *et al.* (1990). No differentiation was detected among Brazilian colonies (Fst = 0.005). The estimated number of migrants per generation based on Fst (Nm = 48.8) and on private alleles (Nm = 11.3) suggests high gene flow occurring among colonies (Lopes 2002, Del Lama *et al.* 2002). The study was developed with the same microsatellite loci reported as polymorphic in the North American population (Van Den Bussche *et al.* 1999), and exhibited no significant differences among Pantanal subpopulations (Fst = 0.022). As the Fst value did not differ significantly from zero, Pantanal subpopulations can be considered as a single population. The estimated migration rate based on microsatellites (Nm = 11.11) confirmed the high gene flow level among Pantanal colonies (Rocha 2002, Rocha *et al.* 2004). This result suggests a high gene flow level among birds of neighboring breeding colonies, and it is comparable to that found in the North American colonies.

Population genetic studies based on mtDNA often reveal information different from that found with nuclear markers. In this first study with mtDNA genetic marker in the Wood Stork colonies, 19 haplotypes were identified in the sample of 62 analyzed individuals. The most frequent haplotype (H1) represents 61.3% of all sequences and it is present in the eight Pantanal colonies studied. The analysis of molecular variance (AMOVA) revealed a low subdivision among populations in the total sample (ϕst = 0.03364; Nm_f = 14.34). Analysis of population subdivision based on mtDNA agrees with data from nuclear markers showing that subpopulations of Wood Stork in the Pantanal have low genetic differentiation. This lack of geographical structuring among birds from different colonies is expected as a result of intense gene flow. Estimates of gene flow between colonies located in north and south Pantanal areas, calculated by the maximum-likelihood method, showed that the migration between colonies is strictly unidirectional, from southern to northern subpopulations (Fig.1). As mtDNA is only maternally inherited, the dispersal pattern observed can be interpreted as the result of either female-biased or female-male mediated gene flow among colonies.

Events of range expansion as well as restricted gene flow with isolation by distance were determined by NCA to explain the observed haplotype distribution pattern among colonies. These results suggest that the Wood Stork Pantanal population originated in a small population of founders that expanded in size. The highest number of haplotypes found in the south Pantanal colonies and the south-north direction of the gene flow indicate that the southern colonies are older than the northern ones. According to this supposition the founders may have become established first in the south Pantanal region, and then expanded their range to the northern one (Lopes *et al.* in prep.).

Climate changes in the Pantanal region during the glaciation period may have limited the establishment of Wood Stork breeding colonies in this region. During glaciation, the birds probably shifted to the Equator region where changes in rainfall were not so extreme. These movements may have favored interchanges among South American colony members and homogenized the gene pool from which the Pantanal population originated. The TMRCA, determined through coalescence analyses, was approximately 10,470 years before present. This estimated

time derived from coalescence analyses coincides with the estimated time elapsing to the end of the last glacial period (approx. 10,000). This result corroborates the theory of non establishment of Wood Storks colonies in the Pantanal region during the last glaciation.

In summary, nuclear and mitochondrial markers revealed no genetic differentiation among Pantanal colonies in the analysis based on Fst. Otherwise, the NCA based on mtDNA showed some population structuring, indicating that the Wood Stork Pantanal population underwent a recent expansion and that the geographic distance can restrict the gene flow.

Genetic differentiation between Pantanal and North-American colonies. Genetic studies performed to investigate the population differentiation patterns between North and South American Wood Stork colonies using nuclear markers (allozymes and microsatellites) showed lacking or low genetic differentiation between them. Genetic analysis based on allozymic data showed no genetic differentiation. This result was explained by supposing that gene flow is caused by interchange between individuals of North and South American colonies (Del Lama *et al.* 2002). Limited differentiation between United States and Pantanal colonies was found using data from microsatellite markers. Such results can be explained: 1) by historical and contemporary gene flow intermediated by members of colonies located between both populations, following a stepping-stone migration model, and 2) by an historical gene flow during the last glaciation when both populations were closer than at the present time, a condition that favored the exchange among their members. Differentiation between North and South American populations was not previously detected by allozymes because these markers are not sensitive enough to detect such small differences (Rocha *et al.* 2004).

Conservation implications. The Wood Stork population is an ecological indicator of wetland health. The genetic analysis of the mtDNA sequences in the Pantanal Wood Stork population showed an unidirectional gene flow from southern colonies towards the northern ones. This pattern of migration indicates that the colonization of the south Pantanal occurred first. According to our results, the conservation of this region is more critical than that of the northern Pantanal because the major genetic sources for this species, and probably of other wading birds, are located in the south.

The genetic differentiation level within and among populations is an important component to be taken into account in conservation decisions. In the case of the Wood Storks of the American continent, because of the low genetic differentiation level presented by nuclear markers among colonies, they should be considered as a single population. As a consequence, the conservation strategies affecting them need to be reviewed.

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