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Inferred kinship patterns reveal low levels of extra-pair paternity in the endangered Neotropical Jabiru Stork (*Jabiru mycteria*, Aves: Ciconiiformes)

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Abstract The present study inferred the genetic mating system in a natural breeding population of the Jabiru Stork (*Jabiru mycteria*), a Neotropical wading bird considered endangered in part of its distribution range. Based on data from eight microsatellite loci, maximum-likelihood kinship reconstruction techniques, parentage assignment analyses and effective population size (*Ne*) estimates were applied to samples collected in the Brazilian Pantanal wetland (N = 45 nestlings from 20 nests; N = 17 shed adult feathers from 11 nests). The relationship diagnosis was determined for most of the complete clutches (86.66 %): 92.31 % were full siblings and 7.69 % were half siblings. Shed feathers collected from the nests matched the genetic parents of the offspring in 80 % of cases. Feathers collected from the ground below the nests were compatible with the putative parents in 41.67 % of cases. A mean *Ne* of 35 reproductive individuals was inferred, corresponding to an *Ne/Nc* ratio of 0.09, which is similar to the ratio found in populations of a number of different wild animals. The higher proportion of full siblings identified in the broods suggests that genetic monogamy is the prevalent mating system in the Jabiru Stork, while the detection of half siblings suggests some degree of extra-pair paternity. The present findings are in agreement with previous ecological observations of social monogamy in this species, despite the isolated evidence of extra-pair copulation events. This study also demonstrates the usefulness of a noninvasive approach to sampling adults and performing parentage and relatedness analyses in an elusive, threatened species.

Keywords Breeding behavior · Effective population size · Extra-pair fertilization · Microsatellite loci · Molecular markers

Introduction

Social monogamy is the most commonly observed mating pattern in birds (Bennett and Owens 2002) and is usually linked to a greater probability of survival of the young due to bi-parental care (Neudorf 2004). However, the use of molecular markers for the assessment of parentage patterns in natural populations of socially monogamous birds has demonstrated that genetic monogamy is a rare occurrence (Griffith et al. 2002; Ležalová-Piálková 2011). Extra-par fertilization (EPF) has been related to factors such as breeding season synchrony (Stutchbury and Morton 1995), reproductive density (Møller and Birkhead 1993), lower investment of parental care by males (Gowaty 1999) and reproductive longevity (Mauck et al. 1999).

The mating system of a species is closely related to its population dynamics and is therefore important information for management and conservation measures (Caro 2007).

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Moreover, the mating system exerts a strong influence on the effective population size (*Ne*), which is directly related to genetic variability and population viability. Therefore, the determination of the mating system and *Ne* are useful to provide inferences regarding the effects of habitat destruction and harvesting on the reduction in the level of genetic variability in a population.

Most avian biodiversity is found in the tropics, but proportionally few studies have investigated mating systems in tropical bird species (Neudorf 2004) and even fewer have addressed Neotropical birds (Macedo 2008). Moreover, detailed studies on the mating system in non-passerines and waterbirds are scarce (Macedo 2008; Miño and Del Lama 2009a). For instance, Ciconiiform and Pelecaniform waterbirds are commonly classified as socially monogamous (Bennett and Owens 2002), but ecological evidence has shown the occurrence of extra-pair copulation (EPC) in colonial ibises, spoonbills and egrets (Miño and Del Lama 2009a). Furthermore, genetic data suggest the existence of EPF in the Great Egret (*Ardea alba*), Roseate Spoonbill (*Platalea ajaja*) and Wood Stork (*Mycteria americana*) (Miño et al. 2011).

The Jabiru Stork (*Jabiru mycteria*, Aves: Ciconiiformes) is an aquatic bird found in Neotropical wetlands from southern Mexico to northern Argentina (Hancock et al. 1992). Individuals of this species are usually observed feeding alone or in groups with other species of wading birds in shallow inland waters, coastal marshes and areas of dense forest coverage, such as the Amazon region. During each breeding cycle, both sexes collaborate in building solitary nests, incubating the eggs and caring for the nestlings for nearly 6 months per year (Antas and Nascimento 1996; González 1996). The Jabiru Stork is regionally endangered in Central America and is listed by the CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) as an Appendix I species, mainly due to habitat destruction and hunting (Luthin 1987; Antas and Nascimento 1996). The demographic decline of the Central American Jabiru Stork population has likely resulted in significantly lower levels of genetic variability in comparison to South American populations (Lopes et al. 2010). Previous ecological studies on Jabiru Stork populations in Central and South America indicate that the species is socially monogamous (Kahl 1973; González 1993, 1996; Oliveira 1997). However, EPC has been reported in the Llanos (grasslands) of Venezuela (González 1996). Thus, the genetic mating system of the Jabiru Stork remains unclear.

In the present study, the genetic mating system of the Jabiru Stork in a breeding population of the Pantanal wetland in Brazil was inferred by reconstructing kinship patterns and assigning parentage. Moreover, molecular data were used to estimate the *Ne* of the population studied. The hypothesis tested was that sexual monogamy is the prevalent mating system in this species, as suggested by ecological observations and the reproductive characteristics of the species, such as bi-parental care in the breeding season and low nest density distribution. If EPC attempts reported in previous ecological observations are frequent in this species, one would expect to find genetic evidence of EPF. Given the levels of genetic variability previously detected in the Pantanal population (Lopes et al. 2010), the estimated *Ne* is expected to be compatible with a genetically healthy population.

Methods

Sampling, DNA extraction, sexing and microsatellite genotyping

Samples were collected from Jabiru Stork nests identified in the Pantanal wetland of Brazil (state of Mato Grosso: region of the Transpantaneira roadway; state of Mato Grosso do Sul: regions of Nhecolândia and Miranda; for sampling details see Online Resource 1). Blood samples were collected from 45 nestlings in 20 nests. Three nestlings were found in 10 nests (50 %), two were found in five nests (25 %) and one was found in five nests (25 %) (Online Resource 1). Adult feathers from putative parents (N = 17, 11 nests) were collected from within the nests or the ground below the nests (Online Resource 1). The samples included three complete families (offspring and candidate father and mother), eight incomplete families (four groups of offspring-candidate mother and four groups of offspring-candidate fathers) and nine groups composed of offspring alone (Online Resource 1).

DNA was extracted from blood using a standard phenol–chloroform method (Sambrook and Russell 2001). DNA was extracted from feathers following the methods described by Miño and Del Lama (2009b). All samples were molecularly sexed based on Griffiths et al. (1998). Eight heterologous microsatellite loci were used for genotyping: WS01, WS04, WS06 (Van den Bussche et al. 1999), WS03, WS13, WS18, WS20 (Tomasulo-Seccomandi et al. 2003) and WS16 (F–5′ TTTTGGTGGGATTCATAC 3′; R–5′ GTTTAGAAAGAC TTGCCATACA 3′). Locus WS16 was HEX-labeled and WS04 was NED-labeled. The M13 method for dynamic labeling of PCR products (Schuelke 2000) with 6-FAM fluorescence was used with loci WS01 and WS06. Polymerase chain reaction (PCR) amplifications were conducted with an initial annealing temperature of 52 °C (WS01), 53 °C (WS06) 55 °C (WS16, WS18, WS20) or 60 °C (WS03, WS04, WS13) and the following parameters: one cycle at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, initial annealing temperature minus 0.5 °C per cycle

for 45 s, 72 °C for 45 s, and final extension at 72 °C for 10 min. PCR products were analyzed on an ABI 377 sequencer (Applied Biosystems, Carlsbad, CA, USA) or MegaBACE™ sequencer (GE Healthcare Life Sciences, Piscataway, NJ, USA), using GeneScan™350 ROX™ as internal size standard. Alleles were called using the Genotyper program (Applied Biosystems, Carlsbad, CA, USA) or the Genetic Profiler Software Suite v2.2 (GE Healthcare Life Sciences, Piscataway, NJ, USA). Specific control samples were used for standardizing methods to calibrate allele calling using different sequencers and software programs.

Population genetic parameters, effective population size estimates and power of set of microsatellite markers in relatedness analyses

To minimize bias in subsequent parentage and relatedness analyses, population genetic parameters were estimated for a sample of 29 previously genotyped unrelated Jabiru Storks collected from different isolated nests in the Pantanal wetland. Observed (*Ho*) and expected (*He*) heterozygosity, number of alleles per locus, allele frequencies and tests for departure from the Hardy–Weinberg Equilibrium were computed using the GenAlEx v. 6.3 program (Peakall and Smouse 2006). Tests for departure from linkage equilibrium were performed using the Fstat v. 2.9.3.2 program (Goudet 1995). Critical significance levels were corrected following the Bonferroni procedure (Rice 1989). The power of the set of markers for the parentage analyses was estimated by computing the probability of identity (P_I) (Waits et al. 2001) and exclusionary probability (P_E) with i) both parents known, ii) one parent known and iii) neither parent known (Jamieson and Taylor 1997), using the GenAlEx program. The effective population size (*Ne*) was estimated based on the method described by Wang (2009) and implemented on the Colony v. 2.0 program (Jones and Wang 2010).

Queller and Goodnight's (1989) relatedness index (hereafter denoted *rQG*) was chosen for the relatedness analyses after the evaluation of its performance with the genetic data using the iRel online application (Gonçalves da Silva and Russello 2011). To minimize the probability of falsely excluding true relationships in subsequent relatedness analyses due to biased observed *r*-values, the iRel was used to identify the cut-off relatedness values specific to the present sample (Russello and Amato 2004). The performance of the set of markers was evaluated by assessing the change in relatedness estimates as additional loci were added to the analyses, performing a rarefaction procedure with the web-based platform Re-Rat (Schwacke et al. 2005), as described in Lima et al. (2011).

Relatedness, kinship reconstruction and parentage analyses

Pairwise *rQG* values were computed for all pairs of supposed siblings (blood samples collected from nestlings within the same nest) and for all putative parent-offspring (PO) pairs. Initial analyses were based on 45 offspring and 17 adults from 20 supposed families (Online Resource 1).

Kinship analyses aimed to classify each one of the 35 pairs of putative siblings as full siblings (FS), half siblings (HS) or unrelated (UR) using the multiple-step approach proposed by Miño et al. (2011). First, the *rQG* pairwise values were estimated using GenAlEx and the cut-off values method (Blouin et al. 1996) was applied to classify pairs in relationship categories, based on *rQG* values. The midpoints between the means of the distribution of the pairwise relatedness estimates of each simulated relationship category were taken as cut-off values (Blouin et al. 1996). Next, a maximum likelihood (ML) relationship hypothesis was determined for each nestling pair using ML-Relate (Kalinowski et al. 2006). The significance of the relationship hypotheses was evaluated by computing the probability of the nestlings being related according to the ML relationship versus the a priori expected relationship under the assumption of monogamy (FS). Finally, full-sibling pairs were reconstructed using the ML method (Wang 2004) implemented in Colony, which applies a full-pedigree algorithm to reconstruct kinship patterns. Population allele frequencies previously estimated from 29 unrelated Jabiru Storks were loaded as input. The simulation parameters in Colony were as follows: 'monogamy' for females and males, 'very long' run, 'full likelihood' analysis, 'high likelihood' precision, no updating allele frequencies, no prior sibship size, genotyping errors and mutation rates of 0.01, no inbreeding model.

The goal of the parentage analyses was to determine whether the feathers collected in and below the nests could be assigned to the genetic parents of the offspring sampled in each nest. For parentage assignment, the genotypes of putative parents were first visually compared to those of offspring to identify mismatching alleles/loci. Parentage assignments were then performed using a likelihood-based approach in Cervus v. 3.0 (Marshall et al. 1998), with corrected equations (Kalinowski et al. 2007). LOD scores were calculated separately for father-offspring and mother-offspring pairs. Those with the highest LOD scores were identified as potential parent-offspring pairs. Critical delta scores (difference in log-likelihood ratio scores between the two most likely candidate parents) were calculated at the 95 % level of confidence by simulating 10,000 parent-offspring pairs based on allele frequencies derived from the study population. As the level of relatedness (*r*) among same-sex candidate parents can influence the accuracy of

the parentage analysis (Marshall et al. 1998), pairwise values of relatedness among all breeding females and among all breeding males (estimated via the *rQG* index in GenAlEx) were included as input in the Cervus analyses. Parentage patterns were also reconstructed using Colony.

Results

Population genetic variability, effective population size and power of microsatellites in relatedness analyses

The number of alleles per locus ranged from two to seven and *He* ranged from 0.142 to 0.747 (Online Resource 2). There was no evidence of linkage disequilibrium or departure from the Hardy–Weinberg Equilibrium after the Bonferroni correction. The P_I was 0.0002. The P_E was 0.972 with both parents known, 0.880 with one parent known and 0.997 with neither parent known, indicating that the set of microsatellites used exhibited reasonable power to distinguish individual samples and exclude parents. The mean effective population size estimated from the sibship reconstruction was 35 Jabiru Storks (95 % CI: 21–60).

Performance analyses for the study population indicated that the *rQG* index exhibited low variance and did not deviate from the expected value for an ideal panmictic population, except for the PO category (Online Resource 3). Misclassification rates ranged from 12.1 % (PO-UR) to 36.2 % (HS-FS), showing that, as expected, non-adjacent categories were more likely to be distinguished successfully (Online Resource 4). The observed *rQG* values within nests ranged from −0.02 to 1 (Online Resource 5). There was little change in the *rQG* estimator after the eighth locus was added (average difference between seventh and eighth loci: 0.014) (Fig. 1), suggesting that the relatedness estimates would have changed little with the use of more loci.

Patterns of kinship and parentage

Kinship patterns were reconstructed for 35 nestling pairs sampled in 15 nests, following the method described by Miño et al. (2011). A final diagnosis was reached for 33 nestling pairs (94.28 %), most of which were classified as full siblings (87.87 %) and 12.13 % were classified as half siblings. Among the 15 nests analyzed, a relationship diagnosis was established for all nestling pairs (complete clutches) in 13 nests (86.66 %) (Table 1). Nests N10 and N11 (13.34 % of the total) had a final relationship diagnosis for only 2/3 of the nestling pairs; as the final diagnosis of kinship was not reached for the complete clutch, these nests were excluded from the computations of frequencies of monogamy and of EPF. Among the 13 nests

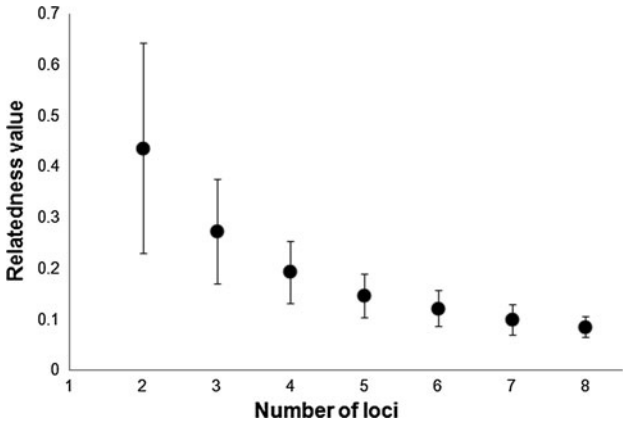

Fig. 1 Rarefaction analysis of Jabiru Stork in the Pantanal wetland, Brazil, showing relationship between number of loci used and mean difference between consecutive genetic relatedness estimates. Standard deviations for 1,000 simulations are shown

with a diagnosis for the complete clutch, 12 (92.31 %) were composed only of full siblings, in agreement with the expectation under assumption of a monogamous mating system (Fig. 2a). The presence of half siblings together with full siblings suggests the occurrence of EPF in nest N13 (7.69 % of the nests with a final diagnosis) (Fig. 2b).

Among the total 29 putative PO comparisons using shed feathers, 18 (62.07 %) had a PO relationship confirmed by all analytical methods employed (Table 2; Fig. 3). Four out of five shed feathers found within nests (80 %) were confirmed as compatible with the genotypes of nestlings sampled in those nests. Seven out of 12 feathers (58.33 %) collected on the ground below nests were excluded as putative parents of the nestlings sampled in those nests. In two (N15 and N17) out of the three nests in which shed feathers from both sexes were collected, the genotypes of both adults were compatible with those of the nestlings, thereby supporting the hypothesis of genetic monogamy (Fig. 3).

Discussion

The present study offers the first inferences regarding parentage and kinship patterns in a natural population of the Neotropical Jabiru Stork using information on polymorphic microsatellite loci. Samples from complete broods of nestlings and their putative parents were analyzed to identify first-order relatives (FS or PO relationships). Kinship patterns among nestlings with a final diagnosis revealed that a high proportion of nests (92.31 %) were composed only of full siblings. The parentage analyses allowed matching the genetic parents of the offspring to feathers collected within the nests or on the ground below the nests. Collectively, our results suggest the prevalence

Table 1 Kinship patterns reconstructed for Jabiru Stork nestlings sampled in nests following methods described by Miño et al. (2011). For each nestling pair analyzed, the following are shown: Queller and Goodnight's pairwise relatedness value (*rQG*), most likely relationship category (ML-R) indicated by the maximum likelihood method implemented in ML-RELATE, probability value of hypothesis test [$P(H_P/H_A)$] to establish the significance of the ML-R category (H_P: putative hypothesis; H_A: alternative hypothesis: a P value < 0.01 indicates that H_P fits the genetic data better than H_A), full-sibling groups reconstructed in COLONY and the final relationship determined by considering agreement among all analytical methods employed. All hypothesis tests were conducted simulating 10,000 genotype pairs. UR: unrelated; FS: full siblings; HS: half siblings; NA: not available; '_' indicates a lack of a final diagnosis of relationship category due to inconsistencies among the methods applied

Nest #	Nestling pair	*rQG*	ML-R	$P(H_P/H_A)$[a]	COLONY output[b]	Final relationship
N01	J01–J02	0.900	FS	0.0000	FS	FS
N02	J03–J04	0.577	FS	0.0181	FS	FS
N02b	J45–J46	0.671	FS	0.0021	FS	FS
N04	J47–J48	0.626	FS	0.0081	FS	FS
N04	J47–J49	0.183	HS	0.1838	FS	FS
N04	J48–J49	0.660	FS	0.0122	FS	FS
N08	J09–J10	0.461	FS	0.0531	FS	FS
N09	J11–J12	0.608	FS	0.0179	FS	FS
N09	J11–J13	0.575	FS	0.0248	FS	FS
N09	J12–J13	0.699	FS	0.4120	FS	FS
N10	J16–J17	−0.020	UR	0.0489	NA	–
N10	J16–J18	0.310	HS	0.2374	FS	HS
N10	J17–J18	0.106	HS	0.3770	NA	HS
N11	J19–J20	0.015	UR	0.1349	NA	–
N11	J19–J21	0.563	FS	0.0380	FS	FS
N11	J20–J21	0.532	FS	0.0094	FS	FS
N12	J24–J25	0.859	FS	0.0021	FS	FS
N12	J24–J26	0.859	FS	0.0024	FS	FS
N12	J25–J26	1.000	FS	0.0001	FS	FS
N13	J27–J28	0.144	HS	0.1785	NA	HS
N13	J27–J29	0.770	FS	0.0000	FS	FS
N13	J28–J29	0.014	HS	0.1143	NA	HS
N14	J30–J31	0.667	FS	0.0228	FS	FS
N14	J30–J32	0.710	FS	0.0171	FS	FS
N14	J31–J32	0.549	FS	0.1167	FS	FS
N15	J33–J34	0.273	HS	0.1953	FS	FS
N15	J33–J35	0.233	HS	0.1278	FS	FS
N15	J34–J35	0.766	FS	0.0001	FS	FS
N16	J36–J37	0.760	FS	0.0196	FS	FS
N16	J36–J38	0.875	FS	0.0092	FS	FS
N16	J37–J38	0.446	FS	0.1371	FS	FS
N18	J40–J41	0.239	HS	0.1041	FS	FS
N18	J40–J42	0.511	FS	0.0474	FS	FS
N18	J41–J42	0.801	FS	0.0075	FS	FS
N19	J43–J44	0.540	FS	0.0141	FS	FS

[a] In all cases, H_P was established as the ML-R category and tested against an H_A of UR or FS. For example, for pair J47-J49, the H_P of HS was tested against an H_A of FS, but the test result was non-significant

[b] Only successfully reconstructed full siblings are available in COLONY output

of monogamy as the genetic mating system in this species, which is in agreement with previous ecological observations (Kahl 1973; González 1993, 1996); we also inferred that EPF may be present at a low level.

Sampling and methodological design

Our sample included blood from nestlings and feathers shed from adults. A number of studies have demonstrated

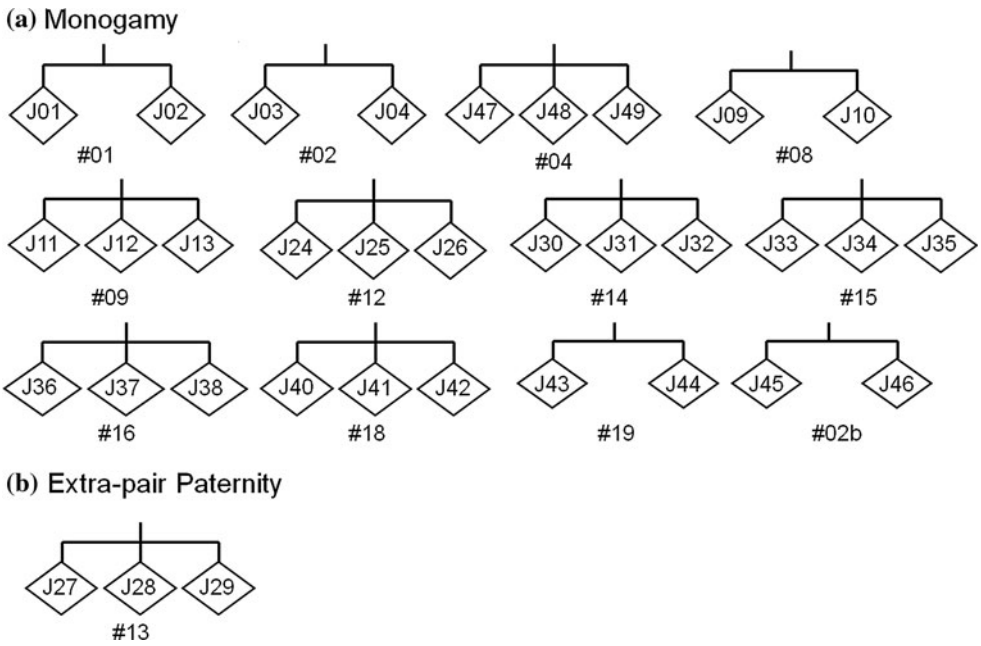

Fig. 2 Summary of kinship patterns diagnosed for Jabiru Stork nestlings from same broods following approach proposed by Miño et al. (2011). Twelve nests suggested monogamy (**a**) and one nest indicated extra-pair paternity (**b**). Sample and nests codes are as in Online Resource 1. Please refer to Table 1 for detailed results on kinship patterns

that shed feathers are a good source of DNA (e.g., Pearce et al. 1997), including a previous investigation on the Jabiru Stork that examined the population structure over its entire distribution range (Lopes et al. 2010). In the present study, feathers found within nests proved to be more valuable for parentage assignment than those found below nests. Thus, greater sampling efforts should be invested to collect these feathers. This non-invasive approach provided an efficient strategy for sampling adults to investigate the mating system in the endangered Jabiru Stork, the adults of which are elusive and extremely difficult to catch. Future studies could use this methodological design to address questions related to other understudied reproductive aspects of the Jabiru Stork, such as nest-site fidelity (Kahl 1973; González 1993).

As recommended by Van Horn (2008), to carry out of a series of parallel analyses rather than relying on a single method proved to be adequate for kinship classification, as the misclassification rates for *r*-values were rather high in this study. Moreover, the final classification of nestling pairs in a relationship category was achieved if and only if all the applied analytical methods were congruent regarding the relationship identified for each pair. The use of this rather conservative approach was aimed to increase the reliability of the inferred kinship patterns (Miño et al. 2011).

Jabiru Stork mating system based on genetic data

The Jabiru Stork has traits that may be associated with the prevalence of social and genetic monogamy, such as a long life span, the need for bi-parental care to raise the altricial chicks over a long dependence period and a low nest-density distribution (Bennett and Owens 2002). The Jabiru Stork also exhibits behavior related to long-lasting pair bonds, such as nest reuse in successive years (Kahl 1973; González 1993) and pairing outside the breeding season (Oliveira 1997). Moreover, given the high energy and time costs associated with reproduction in this species, González (1996) suggests (based on data collected from the Llanos of Venezuela) that fewer than 50 % of breeding pairs that reproduce in one season could also be active in the following season and that approximately 25 % of pairs are reproductively successful in subsequent breeding seasons. Therefore, behavioral characteristics and ecological constraints may place selective pressure on the species, reinforcing monogamy (Johnson and Burley 1998).

Despite widespread genetic monogamy, a low degree of extra-pair paternity was also detected in the Jabiru Stork, as suggested by the presence of half siblings in nest N13. This finding is in agreement with previous field observations describing extra-pair copulation activity in this species: a male copulated repeatedly with two different females in a nest in the Llanos of Venezuela, although the couple abandoned the nest 3 weeks after the EPC event (González 1996). While EPC does not always result in EPF, these behaviors are linked. Indeed, EPC behavior in the European Great Cormorant (*Phalacrocorax carbo sinesis*) detected through ecological observations was associated with the occurrence of EPF (Kortlandt 1995). For the Jabiru Stork, the lack of more detailed ecological data on EPC hinders accurate predictions regarding EPF, but is an indication that it is possible.

Table 2 Parentage patterns reconstructed for Jabiru Stork adult feathers and nestlings sampled in the Pantanal wetland, Brazil. For each supposed parent-offspring pair, the following are shown: Queller and Goodnight's pairwise relatedness value (*rQG*), the most likely relationship category (ML-R) indicated by the maximum likelihood method implemented in ML-RELATE, results of parentage assignment carried out using the program CERVUS and results of parentage analyses conducted using the program COLONY. UR: unrelated; HS: half siblings; PO: parent-offspring. Feathers collected within nests are shown in bold; the remaining feathers were collected from the ground below nests (see Online Resource 1 for details on sampling)

Nest	Pair	*rQG*	ML-R	Parentage CERVUS[a]	Parentage COLONY
N03	J05–**EC08-1A**	0.838	PO	Confirmed paternity	Confirmed paternity
N03	J05–EC08-2A	0.093	UR	Excluded paternity	Excluded paternity
N05	J06–**CD18A**	0.257	PO	Confirmed maternity	Confirmed maternity
N06	J07–**CD19-2A**	0.034	UR	Excluded paternity	Excluded paternity
N06	J07–CD19-1A	0.068	UR	Excluded paternity	Excluded paternity
N07	J08–CD20-1A	0.096	UR	Excluded paternity	Excluded paternity
N07	J08–CD20-2A	0.065	UR	Excluded paternity	Excluded paternity
N07	J08–CD20-3A	−0.178	UR	Excluded maternity	Excluded maternity
N08	J09–CD32A	−0.105	UR	Excluded maternity	Excluded maternity
N08	J10–CD32A	0.076	UR	Excluded maternity	Excluded maternity
N12	J24–TP05A	0.1932	UR	Excluded maternity	Excluded maternity
N12	J25–TP05A	0.199	UR	Excluded maternity	Excluded maternity
N12	J26–TP05A	0.199	UR	Excluded maternity	Excluded maternity
N14	J30–FI07A	0.660	PO	Confirmed paternity	Confirmed paternity
N14	J31–FI07A	0.592	PO	Confirmed paternity	Confirmed paternity
N14	J32–FI07A	0.655	PO	Confirmed paternity	Confirmed paternity
N15	J33–**FI08-1A**	0.032	PO	Confirmed maternity	Confirmed maternity
N15	J34–**FI08-1A**	0.222	PO	Confirmed maternity	Confirmed maternity
N15	J35–**FI08-1A**	0.029	PO	Confirmed maternity	Confirmed maternity
N15	J33–**FI08-2A**	0.502	PO	Confirmed paternity	Confirmed paternity
N15	J34–**FI08-2A**	0.578	PO	Confirmed paternity	Confirmed paternity
N15	J35–**FI08-2A**	0.666	PO	Confirmed paternity	Confirmed paternity
N16	J36–FI09A	0.396	PO	Confirmed maternity	Confirmed maternity
N16	J37–FI09A	0.487	PO	Confirmed maternity	Confirmed maternity
N16	J38–FI09A	0.473	PO	Confirmed maternity	Confirmed maternity
N17	J39–FI10-1A	0.505	PO	Confirmed paternity	Confirmed paternity
N17	J39–FI10-2A	0.255	PO	Confirmed maternity	Confirmed maternity
N19	J43–PA12A	0.229	PO	Confirmed paternity	Confirmed paternity
N19	J44–PA12A	0.352	PO	Confirmed paternity	Confirmed paternity

[a] CERVUS simulation parameters were as follows: 95 % of loci typed, genotyping error rate of 0.01, minimum of 6 typed loci per individual, 10 % of candidate mothers and 15 % of candidate fathers sampled in the population. Information on adult relatedness was included in the simulations, as follows: 24 % of females were related to other females with $r > 0.25$; 20 % of males were related to other males with $r > 0.25$. Additional analyses in which these parameters were slightly altered did not change the results

One may suppose that reproductive synchrony is the most likely ecological explanation contributing to EPF in the Jabiru Stork, as proposed for several other birds (Griffith et al. 2002; Stutchbury and Morton 1995). Breeding synchrony is closely related to hydrological conditions of habitats occupied by Jabiru Stork throughout its distribution range (Kahl 1973; González 1996; Antas and Nascimento 1996). However, since mating strategies can vary among different populations of a species, among individuals within populations and between breeding seasons, depending on ecological and/or social factors (Neudorf 2004), future fine-scaled and long-term studies, applying both ecological and genetic approaches, should be carried out to determine the level of EPF in other populations of the Jabiru Stork.

Effective population size, mating system and implications for conservation

Parentage analyses based on molecular markers in the Jabiru Stork were used to estimate the *Ne*, which exerts a strong influence on the level of genetic variability in

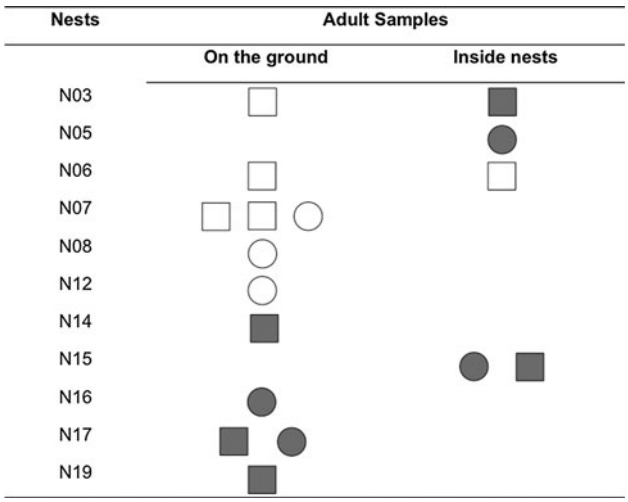

Fig. 3 Summarized parentage patterns for each Jabiru Stork nest with adult feathers available (candidate parents). *Circles* represent females, *squares* represent males, *white* and *grey* colors represent parent-offspring exclusion or confirmation, respectively

natural populations. Genetic estimates of *Ne* identified a mean of 35 reproductively active adults in the Pantanal wetland regions studied (Transpantaneira, Miranda and Nhecolândia), which represent the first attempt to better characterize this population. Surveys of Jabiru Stork nests available for these same areas (Antas and Nascimento 1996; Oliveira 1997) estimated that approximately 382 individuals (census size: *Nc*) were reproducing between 1988 and 1997. Taking into account our *Ne* estimate, this would render a *Ne/Nc* ratio of 0.09, which is similar to the ratio typically observed in wild populations across several taxa, estimated to be approximately 0.11 (Frankham 1995). The population size estimated for the Jabiru Stork population in the Pantanal wetland is within the range of the minimum size considered necessary for long-term survival of many wildlife populations (Whitlock 2000). The estimation of the *Ne/Nc* ratio enables researchers to infer additional parameters based on either of the ratio components. The long-term monitoring of the *Ne/Nc* ratio could help in the drafting of management strategies to increase the *Ne* and, consequently, the level of genetic variability in a population (Luikart et al. 2010). Future long-term studies on the Jabiru Stork population in the Pantanal wetland would allow the assessment of the *Ne/Nc* ratio over time and the determination of whether or not the population size remains stable.

A predominant monogamous mating system in the Jabiru Stork may contribute to the maintenance of the levels of genetic diversity in the population, as both sexes have the same opportunity to pass their genes onto the next generation (Caro 2007). On the other hand, occurrence of EPF contributes to increase the within-family genetic diversity allowing a more effective selection and, consequently, affecting positively the population in terms of fitness (Holman and Kokko 2013). Given that low population densities might be correlated with low EPF rates (Griffith et al. 2002), it would be expected that less dense populations, such as Jabiru Stork from Central America, may show even lower rates of EPF or absence of this behavior. The data presented here would allow comparisons to declining Jabiru Stork populations, such as those in Central America, which have been demonstrated to harbor lower genetic variability (Lopes et al. 2010). Overall, the present study contributes information about the genetic mating system and population-level status of Jabiru Storks, which can help to guide future management and conservation strategies.

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