

## Extra-pair paternity in the Golden Conure (*Guaruba guarouba*) (Psittacidae: Psittaciformes) detected in captivity

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**RESUMO.** Paternidade extra-par em ararajuba *Guaruba guarouba* (Psittacidae: Psittaciformes) detectada em cativeiro. Os estudos com psitacídeos sugerem que, pelo menos os de maior porte, formem casais fiéis por toda a vida, mas ainda existem espécies pouco estudadas, como *Guaruba guarouba*, que apresenta um comportamento gregário. Foram aplicadas técnicas de "DNA fingerprinting" para o estudo do comportamento reprodutivo de *G. guarouba* em cativeiro e para determinação do sexo dos indivíduos analisados. Foi encontrado que uma mesma fêmea foi fertilizada por dois machos distintos em cativeiro e teve quatro filhotes de um e um de outro em uma mesma ninhada. Porém, não existem dados sobre esse tipo de comportamento na natureza. Portanto, são necessários mais estudos de campo para confirmar a existência de paternidade extra-par nas ararajubas. Este trabalho vem confirmar a importância do uso da técnica de "DNA fingerprinting" no estudo do comportamento reprodutivo de aves.

**PALAVRAS-CHAVE:** ararajuba, comportamento reprodutivo, "DNA fingerprinting", Psittacidae.

**ABSTRACT.** Based on field observations, parrots are believed to live in pairs and stay together for life, at least the bigger ones, and behavioral studies of the gregarious Golden Conure *Guaruba guarouba* are very scarce. In this project, DNA fingerprinting was applied to study the breeding behavior of *G. guarouba* in captivity and sex-type the individuals analyzed. Our results show that one female was fertilized by two males in captivity, and had four chicks fathered by one and one chick fathered by the other, in the same brood. Unfortunately, there is no information about breeding behavior in the wild. Further field studies are necessary to confirm that this species is not essentially monogamous, but this work reinforces the potentials of DNA fingerprinting in studies of bird breeding behavior.

**KEY WORDS:** breeding behavior, DNA fingerprinting, Golden Conure, Psittacidae.

The Golden Conure (*Guaruba guarouba*) occurs in North eastern Brazil, and lives in humid lowland rainforests and hilly upland forests; the adults exhibit yellow plumage and dark green wing-coverts tips (Forshaw 1989). It was proposed as the national bird of Brazil (Sick 1987). According to Forshaw (1989) and Sick (1993) its distribution is restricted to the states of Maranhão and Pará. However, Yamashita and França (1991) reported the sighting of six individuals at Floresta Nacional do Parque Jamari, in Rondônia in a habitat similar to where this bird was seen before. Also, P. F. Develey (pers. comm., 1993) observed three individuals at Alta Floresta, in Mato Grosso.

Forshaw (1989) considered this bird to belong to the genus *Aratinga*, but Sick (1993) based on its distinct behavior and vocalization, suggested that this species should be in the genus adopted in this study.

The Golden Conure is uncommon or rare where it is still found (Ridgely 1980) and is considered endangered (Collar *et al.* 1992). Its great demand as a cagebird and especially the destruction of the forest habitat has caused

a very considerable decline on the number of individuals, and these are still the main threats to the survival of the species. Besides this, the construction of highways, and the development and colonization caused a fragmentation in its distribution and increased the deforestation (Ridgely 1980).

Despite the fact that they live in the forest canopy, they can seek fruit at lower levels, always in small flocks. Its favorite food is "açai", *Euterpe* sp. (Arecaceae) (Sick 1993) but they can also feed on fruit, seeds or nuts from *Lecythis* (Lecythidaceae), *Anacardium* spp. and *Mangifera indica* (Anacardiaceae), *Protium* spp. (Burseraceae), *Oenocarpus bacaba* (Arecaceae), *Cecropia* spp. (Moraceae), and other trees (Collar *et al.* 1992).

The breeding season in the wild occurs from December to April, months of higher humidity, and the nest is built in a hollow in the trunk of a tree, usually 15 to 20 m above the ground. The nest usually has multiple attendants, suggesting that the breeding occurs communally, with several females contributing to the clutch (Forshaw 1989). During the reproductive season, the large Psittacidae live strictly in

pairs, and so far as known, stay together for life, but for most species of parrots appropriate studies were not performed and little is known. *Guarouba guarouba* is usually seen in flocks even during the breeding season (Sick 1993).

Miyaki *et al.* (1992, 1995) carried out several studies on parrot DNA. Those authors detected female linked bands in *G. guarouba* using DNA fingerprinting, allowing sex identification in this species; they also studied the genetic variability, band segregation pattern, and parentage assignment of captive birds.

In this work DNA fingerprinting was used to study the breeding behavior of a group of *G. guarouba* kept together in captivity and to sex the individuals studied.

## METHODS

Samples of 100 µl of blood from 10 specimens of *G. guarouba*, kept in 500 µl of 100 % ethanol, from the "Flora Novaes" aviary, in Campinas, São Paulo, were used in this study. These individuals, whose sex was unknown, consisted of five presumably unrelated adults and five chicks born in captivity and from the same brood. All birds were kept together and the parents were unknown.

DNA was extracted from blood samples as described by Bruford *et al.* (1992). An aliquot from this DNA was digested with the restriction enzyme *Hae*III and purified with phenol-chloroform. The digested DNA was loaded onto a 30 x 20 cm 1 % agarose gel and run until the 2.0 kb marker had migrated to the bottom of the gel. The fractionated DNA fragments were transferred onto a nylon membrane (Hybond Nfp, Amersham) by capillary Southern blotting (Sambrook *et al.* 1989).

The membrane was pre-hybridized in 0.263M Na<sub>2</sub>HPO<sub>4</sub>, 1mM EDTA, 7 % SDS and 1 % BSA at 65 °C; and after four hs, probe 33.15 (Jeffreys *et al.* 1985) oligolabelled with [ $\alpha$ -<sup>32</sup>P]dCTP by random priming was added to the solution. After 15 hs, the membrane was washed three times for 10 min with three solutions: 0.25M Na<sub>2</sub>HPO<sub>4</sub>/1 % SDS; 2XSSC/0.1 % SDS and 1XSSC/0.1 % SDS. The membrane was exposed with two intensifying screens to an X-ray film, and then stripped and reprobed with minisatellite 33.6 (Jeffreys *et al.* 1985) as described above.

The autoradiograph was analyzed to determine the sex of each individual by presence or absence of the specific W-chromosome bands (Miyaki *et al.* 1992), and the parentage of the chicks was established by comparing each chick's patterns with the adult ones. The probability (I) for each pair sharing all the bands of the chick by chance was calculated using the formula:

$$I = [1 - (1 - X)^2]^n \quad (\text{Jeffreys } et al. 1985)$$

where n is the number of bands shared between a chick and its putative parents and X is the Index of Similarity (Wetton *et al.* 1987), calculated for an unrelated captive *G.*

*guarouba* population (Miyaki 1993 and Miyaki *et al.* 1995). The index of similarity between the chicks, chicks and their parents, and between the adults, was also calculated, using the following formula by Wetton *et al.* (1987):

$$X = \frac{2N_{AB}}{(N_A + N_B)}$$

where N<sub>AB</sub> is the number of bands shared among 2 individuals and N<sub>A</sub> and N<sub>B</sub> are the total number of bands of each individual.

## RESULTS

The profiles obtained with probes 33.6 and 33.15 are shown in figure 1. Using the W-chromosome specific bands the sexes of the adults and chicks were determined. There were two female (A2 and A5) and three male (A1, A3 and A4) adults and four male (F1, F3, F4 and F5) and one female (F2) chick.

To determine the parentage of the chicks we verified which pair of adults had all the bands present in each chick. With this analysis it was possible to find that female A5 is the mother of all chicks, and male A3 is the father of chicks F1, F2, F4 and F5, while male A4 is the father of the chick F3. These data show that the same female was fertilized by two males, having a chick fathered by one and four fathered by another, in the same brood. The probability of all bands being shared between the chicks and their putative parents by chance is shown in table 1.

The index of similarity between chicks, chicks and their parents and among the adults (assumed as unrelated) are shown on table 2. The indexes were higher between related birds than between unrelated ones, as expected.

## DISCUSSION

Our results show that in a group of *G. guarouba* kept together in captivity, one of the females was fertilized by two different males. The only behavioral information available about this group is that all the adults suddenly presented an aggressive behavior towards the aviculturist (A. Novaes) and her staff during the whole breeding season. No information is available on mating, egg incubation or feeding the nestlings, and at the time of blood sampling the chicks were fully feathered and feeding by themselves among the adult birds.

Besides the parentage and sex assignment of the 10 birds by DNA fingerprinting, we were able to show that similarity indexes were higher for related than for unrelated birds, as expected. Because of the presence of two female specific fragments detected by probe 33.15, and the occurrence of four males among the chicks, the mean index of similarity between chicks and mother was lower for probe 33.15 than for probe 33.6.

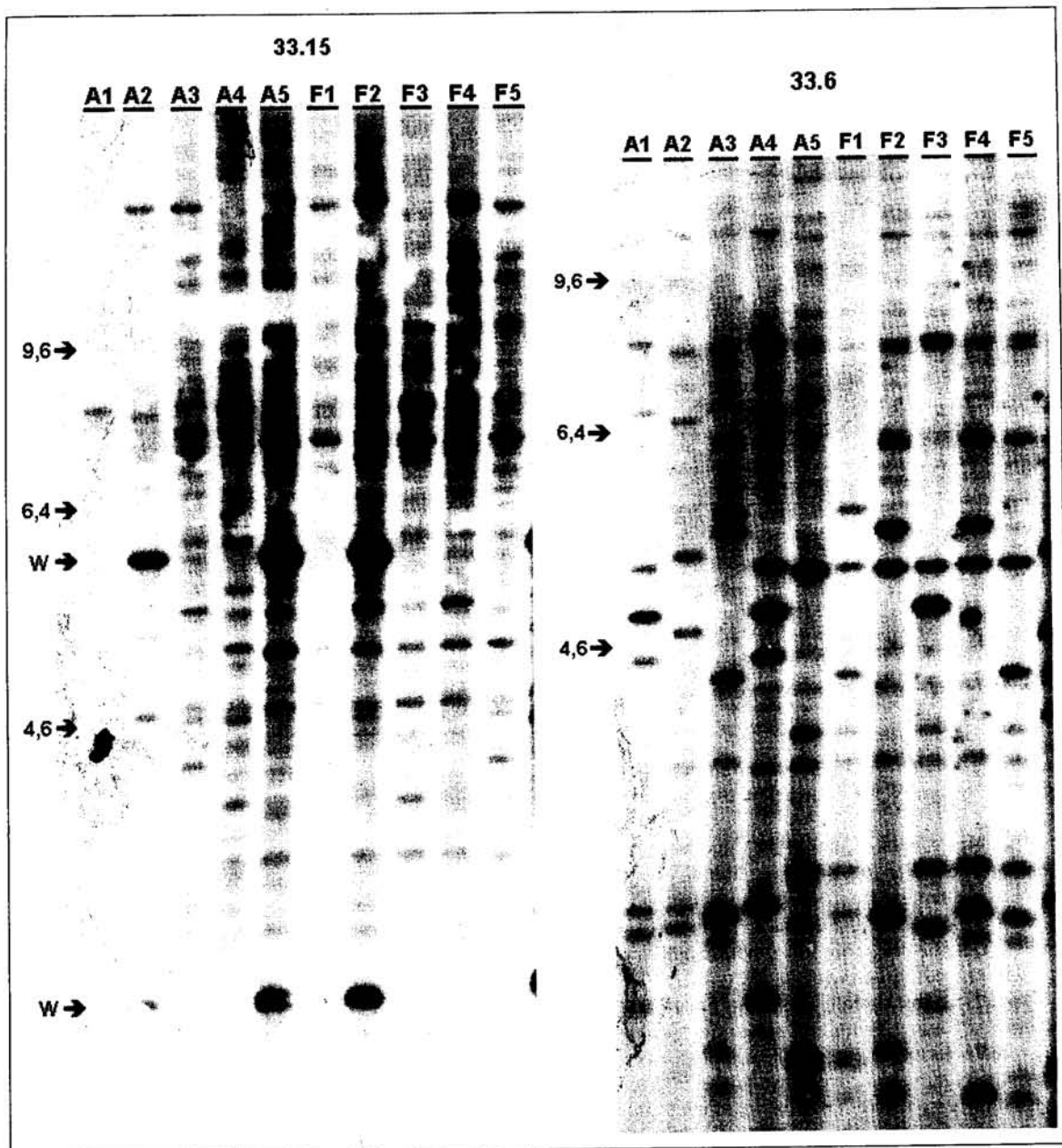


Figure 1. Autoradiographs obtained with probes 33.15 and 33.6. Molecular sizes are in kilobases (kb) and W indicates the sex linked bands. A represents the adults and F the chicks.

Observations in the wild during the mating season suggest that parrots live in pairs that stay together for life (Sick 1993). With the availability of molecular marker techniques and especially using the human multilocus minisatellite probes developed by Jeffreys *et al.* (1985), it is possible to study the breeding behavior of many birds, determine paternity of the chicks, and detect that some birds considered as monogamous can present extra-pair fertilizations (review in Birkhead and Möller 1992). Field observations are not always accurate enough to detect

intra-brood parasitism or extra-pair paternity (Miyaki 1993). Burke and Bruford (1987) detected an unexpected extra-pair copulation in a House Sparrow (*Passer domesticus*) family, and Burke *et al.* (1989) found extra-pair parentage within two out of three different mating systems in dunnocks (*Prunella modularis*). Breeding behavior studies using DNA techniques have not been applied to wild parrot populations, but studies by Miyaki *et al.* (1995) with seven *G. guarouba* individuals that lived in the same cage showed that three chicks of two successive broods were

Table 1. Parentage relationships and probabilities (I) of the bands shared between the chicks and the pair occurring by chance, using minisatellite probes 33.15 and 33.6.

Chick	Mother	Father	Probabilities I	
			33.15	33.6
F1	A5	A3	$3,26 \times 10^{-4}$	$1,66 \times 10^{-5}$
F2	A5	A3	$1,31 \times 10^{-5}$	$1,92 \times 10^{-4}$
F3	A5	A4	$1,31 \times 10^{-5}$	$1,66 \times 10^{-5}$
F4	A5	A3	$2,64 \times 10^{-6}$	$2,21 \times 10^{-3}$
F5	A5	A3	$2,64 \times 10^{-6}$	$4,89 \times 10^{-6}$

Table 2. Mean index of similarity and standard error between the adults, the chicks, the chicks and their fathers, and the chicks and their mother, calculated for both 33.15 and 33.6 probes.

	Probe 33.15	Probe 33.6
Adults	$0.190 \pm 0.127$	$0.330 \pm 0.109$
Chicks	$0.543 \pm 0.098$	$0.419 \pm 0.079$
Chicks and fathers	$0.437 \pm 0.091$	$0.475 \pm 0.157$
Chicks and mother	$0.365 \pm 0.094$	$0.570 \pm 0.147$

born from the same pair. In the present study, a female was fertilized by two of the three males present in the same cage.

The aviculturist L. Maluf (1988) observed the mating behavior of another group of five adult *G. guarouba* in captivity and reported their sudden aggressivity trying to protect the mated pair and its chicks during the breeding season. Despite the poor information about the *G. guarouba* breeding behavior in the wild, the available data suggests a communally protecting behavior towards the chicks. Carlos Yamashita (pers. obs.) reports that when the flocks feel threatened the whole group goes into the nest, which makes the capture of this bird relatively easy. Such gregarious behavior in other bird species increases tendencies toward cuckoldry, since colonially nesting species have been found to have a higher rate of extra-pair copulations than solitary ones (Birkhead and Möller 1992).

It was not possible to observe if the males helped to feed the chicks, as reported in *Campylorhynchus nuchalis* (Passeriformes) (Rabenold *et al.* 1990) since the *G. guarouba* chicks were already feeding by themselves by the time this work was being done.

This work reinforces the potentials of applying DNA fingerprinting on studies of breeding behavior of birds, since field and captivity observations are not always conclusive. The data presented here suggest that the gregarious behavior of *G. guarouba* might be related to a

non monogamic mating system, as in other colonially and gregarious breeders (Birkhead and Möller 1992), which could not be tested due the lack of suitable data. Since the observations described here could be a consequence of an artificial captive situation, with the presence of more than one breeding male, further studies are necessary to establish the mating system and reproductive behavior of these birds in the wild.

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