

# DNA fingerprinting in the endangered parrot *Aratinga guarouba* and other *Aratinga* species

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## ABSTRACT

The destruction of natural habitats and illegal trading are endangering many species of Brazilian parrots. Survival of some species may depend on breeding programmes in captivity. It is therefore important to be able to confirm the identity and parentage of the birds, and to maintain the genetic variability of captive populations. We studied here DNA fingerprints of the endangered *Aratinga guarouba* and five other species of *Aratinga* using two human minisatellite multilocus probes (33.6, 33.15) and the restriction enzyme *Hae*III. Hybridization with 33.6 produces individual-specific patterns with 18 to 33 bands, depending on the species. The index of similarity obtained between unrelated birds was of the same order in the endangered *A. guarouba* (0.16) as in other *Aratinga* species (0.31 to 0.12) and in wild populations of birds reported in the literature. It was possible to perform segregation analysis of the bands only in *A. aurea*, for which we studied a pedigree including five chicks. There were at least 14 unlinked loci. Also, we assigned the parentage of three *A. guarouba* from a group of seven potential parents. A novel fragment was present in two siblings and absent from the third, this was interpreted as a gonadal "mutation" in one of the parents. Hybridization with 33.15 revealed a low number of bands in all *Aratinga* species but *A. jandaya* where  $33.2 \pm 2.5$  bands were scored. However, in all species the probe 33.15 hybridizes strongly to one or several possibly W-chromosome specific bands. Multilocus fingerprints should allow individual identification, parentage assignment and probably sex determination in the genus *Aratinga*. Moreover, band sharing indices can be used to prevent consanguineous matings and to maintain the genetic diversity of these species in captivity.

## INTRODUCTION

The golden conure, *Aratinga guarouba*, is an endangered Brazilian parrot of medium size (34 cm long) with rich yellow body plumage and dark green wing tips. It inhabits the tropical rainforest in the northeast of Brazil and is endemic to an area extending from the Xingu river, Pará state, to northwestern Maranhão state (Forshaw, 1989). There is no evidence

of range contraction, but its distribution has become fragmented by road construction and forest destruction (Ridgely, 1981). Little is known of its habits in the wild where it is becoming increasingly rare, but it can still be seen in small groups in the tops of trees eating fruit, seeds, nuts and berries (Low, 1972). As in most of the psittacines, there is no sexual dimorphism. Because of its bright coloration, the golden conure has been proposed as the symbol of Brazil (Sick, 1993).

Based on some characteristics of the golden conure which are not found in the genus *Aratinga*, Sick (1993) suggested the change of its scientific name to *Guaruba guarouba*, as previously proposed.

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*A. guarouba* has been successfully bred in captivity. Usually, three to four eggs are laid between October and March and most chicks survive to adulthood. When chicks are removed and hand raised, several clutches can be produced by the same pair. In captivity, sexual maturity occurs after three to four years and longevity ranges from 20 to 30 years (N. Kawall, personal communication). In the 1990 edition of the International Studbook for the golden conure, there are 339 birds registered in captivity, but this is probably just a fraction of the true number (Schoenwald and Schoenwald, 1992).

Deforestation and capture from the wild are the main problems faced by the native populations, especially as eggs or chicks are being removed from their nests during the breeding season by traders, even though trading of wild animals is an illegal activity in Brazil. The removal of a considerable number of youngsters from the environment may lead the wild population to an extinction vortex in the near future (Gilpin and Soulé, 1986; Lacy *et al.*, 1989) since, by then, the adult breeding population will have reached the limits of longevity. Moreover, populations might experience natural fluctuations in their effective population size. It is, therefore, very difficult to quantify the immediate threat faced by the native population of parrots. However, some populations appear to be so depleted that a conservation strategy must be adopted urgently. Conservation programmes should include captive breeding as well as *in situ* protection of the birds.

Brazilian authorities have little experience in breeding parrots in captivity and because of illegal trading, laws are very restrictive towards private breeders. Indeed, it was until now very difficult to distinguish those who are genuinely dedicated to the hobby of breeding birds from those involved only in their trade. Also, there are difficulties faced by reputable breeders who wish to accredit their work, to sex their birds and to monitor their fertility.

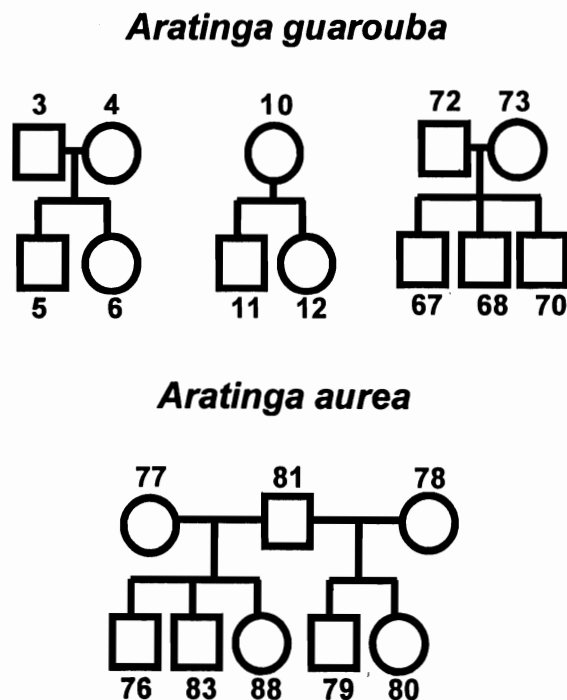
DNA fingerprinting is a powerful tool which may solve some of these problems. Sexing of *Aratinga* parrots with the human minisatellite multilocus probe 33.15 has been described previously (Miyaki *et al.*, 1992). Here, we report our results on DNA fingerprinting in *A. guarouba* and in four other non-endangered Brazilian and one Andean *Aratinga* species.

## MATERIAL AND METHODS

The blood samples were provided by four collections in São Paulo, Brazil: 25 *A. guarouba* (N.K.<sup>1</sup>,

L.M.<sup>1</sup> and P.Z.S.<sup>1</sup>), 17 *A. aurea* (N.K. and P.E.T.<sup>1</sup>), 8 *A. leucophthalmus* (P.E.T. and P.Z.S.), 6 *A. auricapilla* (P.E.T. and P.Z.S.), 4 *A. jandaya* (L.M. and P.Z.S.) and 2 *A. mitrata* (P.Z.S.). Small families (Figure 1) were available for *A. guarouba* and *A. aurea*. The sex of the birds was identified after probing with 33.15 in *A. guarouba* and *A. aurea* (Miyaki *et al.*, 1992). The sexes were not known in the other species.

The protocols used to obtain multilocus fingerprints have been described in detail elsewhere



**Figure 1** - Pedigrees of the *Aratinga guarouba* and *A. aurea* families studied.

(Bruford *et al.*, 1992). Five micrograms of genomic DNA from each bird was digested with the restriction enzyme *Hae*III. The fragments were separated by electrophoresis through a horizontal 30 cm long 1% agarose gel. Electrophoresis was stopped when a 2 kilobase (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).

A multilocus minisatellite RNA probe was prepared from 33.6 (Jeffreys *et al.*, 1985) using the plasmid prepared by Carter *et al.* (1989). The 33.6 probe was [ $\alpha$ -<sup>32</sup>P]rCTP, labelled according to the instructions

<sup>1</sup> N.K. - N. Kawall, breeder; L.M. - L. Maluf, breeder; P.Z.S. - Parque Zoológico de Sorocaba; P.E.T. - Parque Ecológico do Tietê, public organization in São Paulo where all the illegally-held wild animals which are confiscated are taken and quarantined until their subsequent destination is determined.

of the labelling kit supplier (Riboprobe, Promega). The membrane was pre-hybridized in 1X SSC, 1% SDS, 1% BSA, 0.002% sodium azide, 50  $\mu$ l DEPC at 65°C. After four hours, the probe was added to the solution and left overnight at the same temperature. The membrane was washed in 2X SSC, 0.1% SDS followed by 1X SSC, 0.1% SDS at 65°C. The filter was then autoradiographed for three days at -70°C using Fuji RX film and two intensifying screens.

The membrane was stripped and reprobbed with minisatellite 33.15 (Jeffreys *et al.*, 1985), which was PCR-amplified and then oligolabelled with [ $\alpha$ -<sup>32</sup>P]dCTP. Pre-hybridization was undertaken by incubation in 0.263 M Na Phosphate pH 7.4, 1 mM EDTA, 7% SDS, 1% BSA (Westneat, 1990) at 65°C for four hours. The probe was added and hybridized overnight at 65°C. The washing and exposure conditions were as described above.

Only the scorable bands were considered. The coefficient of band sharing (index of similarity) between individuals was calculated using the formula:  $x = 2N_{AB}/(N_A+N_B)$ ; where  $N_A$  and  $N_B$  are the number of bands present in individuals A and B, respectively, and  $N_{AB}$  is the number of bands shared by A and B (Wetton *et al.*, 1987; Bruford *et al.*, 1992).

Assuming that the bands scored are independent markers, we can estimate the mean probability that all n bands in an individual's fingerprint are present in a second random individual conservatively as  $< x^n$  (Bruford *et al.*, 1992). Apparent "mutation" rates were estimated from the frequency of novel bands in the chicks with known parentage. The segregation of the bands was analysed as described by Bruford *et al.* (1992).

## RESULTS

The hybridization patterns obtained with the human minisatellite multilocus probes 33.6 and 33.15 are shown in Figure 2a and 2b. Table I presents the

mean number of bands, the mean index of similarity, the mean band frequency and the expected probability of fingerprint identity for unrelated birds detected by 33.6.

Probe 33.6 generally detected a larger number of bands than 33.15. Using 33.6, it was possible to confirm the parentage in two families of *A. guarouba* and two families of *A. aurea* and to assign the parentage of three chicks of *A. guarouba* which originated from a group of seven adult birds kept together. The mean band sharing coefficient ( $\pm$  sd) between parents and offspring was  $0.45 \pm 0.08$  for *A. guarouba* and  $0.46 \pm 0.11$  for *A. aurea*; between full siblings it was  $0.58 \pm 0.06$  for *A. guarouba* and  $0.53 \pm 0.08$  for *A. aurea*. In *A. guarouba*, 46% of the chicks' bands were of maternal origin and 53% of paternal origin and in *A. aurea* 50% were paternal. In three species, *A. guarouba*, *A. jandaya* and *A. mitrata* the values of the band sharing coefficients between some birds were similar to those expected between first order relatives (0.59 and 0.57, 0.64, respectively).

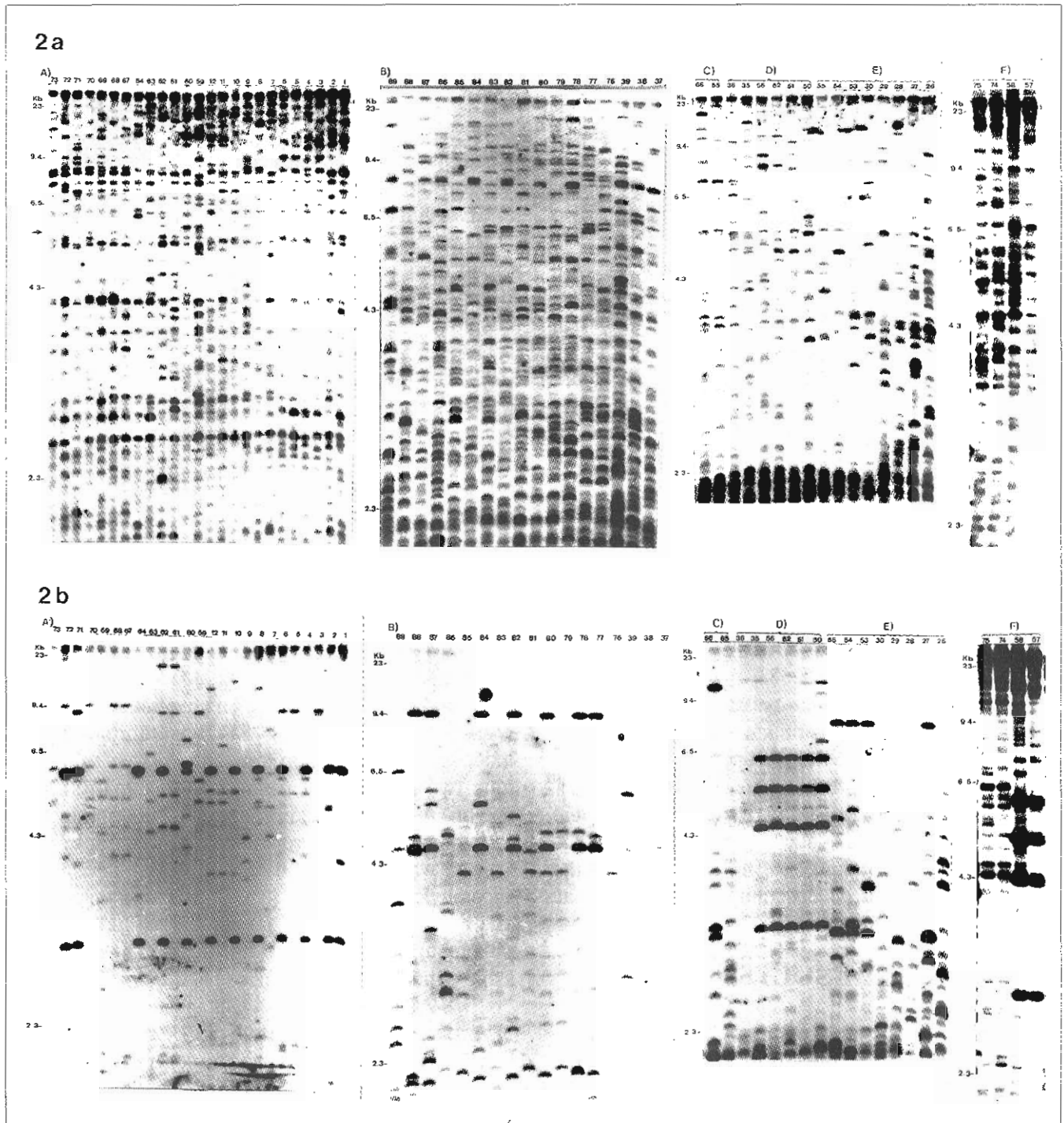
Our largest pedigree of *A. aurea* consisted of five chicks from one male and two different females. The male shared five bands in common with either of the mothers and these were excluded from the analysis. One band was present in all the chicks and two bands were absent from all. Eleven bands showed independent segregation from one another as well as from two independent pairs of co-segregating bands and one group of four co-segregating bands which was apparently allelic to two other co-segregating bands. Thus, it was possible to detect, in *A. aurea*, at least 14 separate loci with probe 33.6. Considering only 14 loci and assuming that they are completely independent, the probability of two unrelated individuals having the same band pattern by chance is less than  $10^{-13}$ .

One novel fragment was present in one family of *A. guarouba*, in which two out of the three chicks had the same unassigned band, absent in both parents, possibly representing a case of gonadal mosaicism in

**Table I** - Results of hybridization with human minisatellite multilocus probe 33.6 in unrelated individuals of *Aratinga* species.

Species	N	n $\pm$ sd	x $\pm$ sd	$x^n$	q
<i>A. guarouba</i>	25	24.0 $\pm$ 2.7	0.16 $\pm$ 0.06	$1.6 \times 10^{-19}$	0.0862
<i>A. aurea</i>	17	26.2 $\pm$ 3.5	0.12 $\pm$ 0.08	$3.3 \times 10^{-25}$	0.0598
<i>A. leucophthalmus</i>	8	18.9 $\pm$ 3.5	0.14 $\pm$ 0.08	$7.7 \times 10^{-17}$	0.0726
<i>A. auricapilla</i>	6	20.7 $\pm$ 2.1	0.19 $\pm$ 0.08	$1.2 \times 10^{-15}$	0.0993
<i>A. jandaya</i>	4	38 $\pm$ 3	0.18 $\pm$ 0.08	$1.4 \times 10^{-28}$	0.0972
<i>A. mitrata</i>	2	22.5 $\pm$ 0.7	0.311	$3.9 \times 10^{-12}$	0.1699

N - number of individuals; n - mean number of bands, sd - standard deviation; x - mean band sharing coefficient;  $x^n$  - probability of two unrelated individuals sharing the same band pattern; q - mean band frequency (Jeffreys *et al.*, 1985).



**Figure 2** - Multilocus DNA fingerprints of 62 individuals of six *Aratinga* species: A) *A. guarouba*, B) *A. aurea*, C) *A. mitrata*, D) *A. aunicapilla*, E) *A. leucophthalmus*, F) *A. jandaya*, obtained with human minisatellite multilocus probe 33.6 (a) and 33.15 (b). The unassigned band found in chicks 68 and 70 is shown (→).

one of the parents. The origin of this fragment could be explained by recombination, DNA slippage, etc. A total of 144 chick's bands were scored in our *A. guarouba* pedigrees.

The patterns observed after hybridization with probe 33.15 were generally less complex, with three to 17 bands detected, depending on the species (Figure 2b). However, in *A. jandaya* the mean number of bands

was much higher ( $33.2 \pm 3.5$ ). Given the low number of bands usually detected with this probe, the data on band sharing coefficients and the probability of two individuals showing the same pattern by chance are only presented for *A. jandaya* ( $0.16 \pm 0.01$  and  $3.8 \times 10^{-27}$ , respectively).

The segregation pattern of the bands detected with probe 33.15 was studied in the same family of *A.*

*aurea*. Among the 11 paternal bands, three were non-informative and two were linked. Thus, it was possible to detect at least six separate loci. Only one of the bands was neither segregating nor in linkage with the bands detected by probe 33.6. So, the results with probe 33.15 did not add significant information to that provided by probe 33.6 in this species.

Interestingly, 33.15 hybridized more strongly to some bands in some individuals of all *Aratinga* species (Figure 2b). Such hybridization has been shown previously to be sex-specific in *A. guarouba* and *A. aurea* (Miyaki *et al.*, 1992) and therefore, possibly also in *A. mitrata*, *A. leucophthalmus*, *A. auricapilla* and *A. jandaya*.

## DISCUSSION

DNA fingerprinting is being applied as a powerful tool for identification and paternity testing in humans (Jeffreys *et al.*, 1985) and in different animals including birds (eg. Wetton *et al.*, 1987; Burke and Bruford, 1987). Some official organizations for the protection of endangered species are making use of DNA fingerprints to either prove illegal trade or legal

breeding success (Mathé *et al.*, 1993). This method can also be valuable in the estimation of consanguineous relationship between individuals and can be potentially used to monitor the genetic variability of captive birds (Brock and White, 1992).

We present our data on the application of human minisatellite multilocus probes 33.6 and 33.15 in studies of the endangered conure *A. guarouba* and five other species of *Aratinga*. Band sharing coefficients and probabilities of two unrelated individuals sharing the same band pattern were estimated using the minisatellite multilocus probe 33.6 in all species and 33.15 only in *A. jandaya*. Interestingly, the band sharing coefficients obtained were in accordance with data found in other wild birds (Tables I and II). This may indicate that the captive populations of *A. guarouba* studied here are still of high genetic variability. The low estimated probability of fingerprint identity between random unrelated individuals emphasizes the potential utility of probe 33.6 in individual identification and parentage analysis, both of which are very important for the control of captive birds.

Linkage between bands has been reported in different species of birds (e.g. Hanotte *et al.*, 1992) including the Hispaniolian parrot (Brock and White,

**Table II** - Band sharing coefficients (x) and mean number of bands (n) detected in different species of birds using minisatellite multilocus probes.

Species	Probe	n ± sd	x ± sd
<i>Taeniopygia guttata</i> (Birkhead <i>et al.</i> , 1990)	33.6	25	0.167 ± 0.014
	33.15	34	0.161 ± 0.013
<i>Prunella modularis</i> (Burke <i>et al.</i> , 1989)	33.15	15	0.24 ± 0.10
<i>Passer domesticus</i> (Burke and Bruford, 1987)	33.6	6	0.28
	33.15	15	0.17
<i>Ficedula hypoleuca</i> (Gelter and Tegelström, 1992)	33.15	15.2 ± 2.0	0.023
<i>Agelaius phoeniceus</i> (Gibbs <i>et al.</i> , 1990)	33.15	23.3 ± 0.37	0.23 ± 0.012
<i>Cygnus olor</i> (Meng <i>et al.</i> , 1990)	pSPT19.6	23.7 ± 2.0	0.283
<i>Cygnus cygnus</i> (Meng <i>et al.</i> , 1990)	pSPT19.6	17.7 ± 1.6	0.283
<i>Cygnus columbianus</i> (Meng <i>et al.</i> , 1990)	pSPT19.6	18.6 ± 2.1	0.204
<i>Passerina cyanea</i> (Westneat, 1990)	33.15	18.6 ± 5.6	0.22 ± 0.09
	33.6	13.9 ± 3.4	0.28 ± 0.10
<i>Merops apiaster</i> (Jones <i>et al.</i> , 1991)	33.15	23.7 ± 2.8	0.193 ± 0.079
	33.6	24.2 ± 0.8	0.204 ± 0.071

1991). In *A. aurea* it was possible to show that among 29 scored bands detected by probe 33.6 there were at least 14 segregating loci. This is a conservative estimate of the number of segregating loci, since the probability of finding two unlinked fragments co-segregating by chance in a total of 24 bands is 0.17 for a pedigree including five chicks. Based on this information, the probability of obtaining an identical band pattern in two random individuals is  $< 10^{-13}$ . In the absence of segregation data in other species of *Aratinga*, we cannot exclude the possibility that a large proportion of the bands detected in these species are linked together. Nevertheless, the large number of bands detected in each species as well as the low level of band sharing observed between unrelated individuals suggest that the human minisatellite probe 33.6 will be informative for individual identification and parentage assignment in all the species of *Aratinga* studied. The lack of segregation data for other *Aratinga* species is a consequence of the difficulty of obtaining large families because of the small number of eggs and low fledging success typical of these species in captivity.

The band sharing coefficients between parents and offspring were higher ( $0.45 \pm 0.08$  in *A. guarouba* and  $0.46 \pm 0.11$  in *A. aurea*), as expected, than those between unrelated birds ( $0.16 \pm 0.06$  in *A. guarouba* and  $0.12 \pm 0.08$  in *A. aurea*). However, the values obtained between some individuals of previously unknown relationship were similar to those expected between related individuals. So, two of the *A. guarouba*, two *A. mitrata* and two *A. jandaya* from Parque Zoológico de Sorocaba might have been captured in the wild from the same nest and so may possibly be siblings. This result illustrates how multilocus fingerprints can be used for monitoring the genetic variability of captive endangered parrots and how it can assist the breeders in their choice of less related pairs for mating. Multilocus fingerprints should also provide a way to distinguish chicks bred in captivity (by comparison with their parents) from the ones taken from the wild.

Our results with probe 33.15 show that it is less useful than probe 33.6 for identification and parentage analysis since it detects a small number of bands in most of the species studied. We found the same result in four species of *Ara* studied previously (Miyaki *et al.*, 1993). Brock and White (1991) have also determined that this probe detects many linked bands and the number of loci analysed was very low. However, this probe can be useful for sex determination as some of the fragments obtained are W-chromosome linked in *A. guarouba* and *A. aurea* (Miyaki *et al.*, 1992) and possibly also in *Ara* (Miyaki *et al.*, 1993). The sexes of the individuals belonging to the other *Aratinga* species

were unknown but we can predict that the strong fragments detected by 33.15 in some individuals are also W-specific in these species.

The application of multilocus fingerprints in the genetic management of captive populations of parrots is multiple: it offers the necessary information to accredit successful breeders, giving them the opportunity of preventing the extinction of endangered species; it provides a tool for sexing some species; it can be used to monitor the genetic diversity of captive birds, potentially increasing the efficiency of their survival and reproductive performance; and it also offers a powerful forensic tool for the monitoring of illegal trading.

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## RESUMO

Muitas espécies de psitacídeos estão ameaçadas de extinção devido à destruição dos habitats e do tráfico ilegal. Programas de reprodução em cativeiro podem ser a salvação de muitas destas espécies. Para o sucesso de tais programas, é necessário identificar as aves individualmente, evitar os acasalamentos consanguíneos e manter a variabilidade genética das populações cativas. Neste trabalho foi aplicada a técnica de "DNA fingerprinting" ou "identificação individual pelo DNA" na espécie ameaçada *Aratinga guarouba* e em outras cinco espécies do gênero *Aratinga*. Foram utilizadas duas sondas de mini-satélite multilocos humano (33.6 e 33.15) e a enzima de restrição *HaeIII*. A hibridação com a sonda 33.6 resultou em padrões de bandas específicos de cada indivíduo contendo de 18 a 33 bandas, dependendo da espécie. O índice de similaridade obtido entre aves não aparentadas de *A. guarouba* (0,16) foi comparável aos obtidos nas demais espécies de *Aratinga* (0,31 a 0,12) e em diversas espécies de aves selvagens já estudados. Foi possível realizar a análise de segregação de bandas apenas em *A. aurea* onde foi estudado um pedigree contendo cinco filhotes, pelo menos 14 locos não ligados foram identificados. Também foi possível determinar a filiação de três *A. guarouba* mantidos em um bando de sete pais

em potencial. Foi encontrado um fragmento mutado em dois irmãos e ausente no terceiro, este fato foi interpretado como uma mutação gonadal em um dos pais. A hibridação com a sonda 33.15 detectou um número menor de bandas em todas as espécies de *Aratinga*, com exceção de *A. jandaya* onde  $33,2 \pm 2,5$  bandas foram observadas. No entanto, em todas as espécies a sonda 33.15 hibrida fortemente com uma ou mais bandas ligadas ao cromossomo W. Esta técnica permite a identificação individual, a confirmação da filiação e a determinação do sexo no gênero *Aratinga*. E ainda, o coeficiente de bandas em comum pode ser utilizado para prevenir cruzamentos entre parentes e manter a diversidade genética destas espécies mantidas em cativeiro.

## REFERENCES

- Birkhead, T.R., Burke, T., Zann, R., Hunter, F.M. and Krupa, A.P.** (1990). Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* 27: 315-324.
- Brock, M.K. and White, B.N.** (1991). Multifragment alleles in DNA fingerprints of the parrot *Amazona ventralis*. *J. Hered.* 82: 209-212.
- Brock, M.K. and White, B.N.** (1992). Application of DNA fingerprints to the recovery program of the endangered Puerto Rican parrot. *Proc. Natl. Acad. Sci.* 89: 11121-11125.
- Bruford, M.W., Hanotte, O., Brookfield, J.F.Y. and Burke, T.** (1992). Single-locus and multilocus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations - A Practical Approach* (Hoelzel, A.R., ed.). Oxford Press, New York, pp. 225-269.
- Burke, T. and Bruford, M.W.** (1987). DNA fingerprinting in birds. *Nature* 327: 149-152.
- Burke, T., Davies, N.B., Bruford, M.W. and Watchwell, B.J.** (1989). Parental care and mating behaviour of polyandrous dunnocks. *Prunella modularis* related to paternity by DNA fingerprinting. *Nature* 338: 249-251.
- Carter, R.E., Wetton, J.H. and Parkin, D.T.** (1989). Improved genetic fingerprinting using RNA probes. *Nucleic Acids Res.* 17: 5867.
- Forshaw, J.M.** (1989). *Parrots of the World*. Lansdowne Editions, Willoughby, pp. 672.
- Gelter, H.P. and Telgelström, H.** (1992). High frequency of extra-pair paternity in Swedish pied fly-catchers revealed by allozyme electrophoresis and DNA fingerprinting. *Behav. Ecol. Sociobiol.* 31: 1-7.
- Gibbs, H.L., Weatherhead, P.J., Boag, P.T., White, B.N., Tabak, L.M. and Hoysak, D.J.** (1990). Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. *Science* 250: 1394-1397.
- Gilpin, M.E. and Soulé, M.E.** (1986). Minimum viable populations: process of species extinction. In: *Conservation Biology: the Science of Scarcity and Diversity* (Soulé, M.E., ed.). Sinauer, Sunderland, pp. 19-34.
- Hanotte, O., Bruford, M.W. and Burke, T.** (1992). Multilocus DNA fingerprints in gallinaceous birds: general approach and problems. *Heredity* 68: 481-494.
- Jeffreys, A.J., Wilson, V. and Thein, S.L.** (1985). Hypervariable minisatellite regions in human DNA. *Nature* 314: 67-73.
- Jones, C.S., Lessells, C.M. and Krebs, J.R.** (1991). Helpers-at-the-nest in European Bee-eaters (*Merops apiaster*) a genetic analysis. In: *DNA Fingerprinting: Approaches and Applications* (Burke, T., Dolf, G., Jeffreys, A.J. and Wolff, A.R., eds.). Birkhäuser Verlag, Basel, pp. 169-192.
- Lacy, R.C., Flesness, N.R. and Seal, U.S.** (1989). Puerto Rican parrot populations viability analysis. In: *Report to the US Fish and Wildlife Service. Captive breeding specialist group, Species Survival Commission, IUCN, Apple Valley, Minnesota*, pp. 129.
- Low, R.** (1972). *The Parrots of South America*. John Gifford Ltd., London, pp. 290.
- Mathé, J., Eisenmann, C. and Seitz, A.** (1993). Paternity testing of endangered species of birds by DNA fingerprinting with non-radioactive labelled oligonucleotide probes. In: *DNA Fingerprinting: State of the Science* (Pena, S.D.J., Chakraborty, R., Epplen, T.J. and Jeffreys, A.J., eds.). Birkhäuser Verlag, Basel, pp. 387-393.
- Meng, A., Carter, R.E. and Parkin, D.T.** (1990). The variability of DNA fingerprints in three species of swan. *Heredity* 64: 73-80.
- Miyaki, C.Y., Hanotte, O., Wajntal, A. and Burke, T.** (1992). Sex typing of *Aratinga* parrots using the human minisatellite probe 33.15. *Nucleic Acids Res.* 20: 5235-5236.
- Miyaki, C.Y., Hanotte, O., Wajntal, A. and Burke, T.** (1993). Characterization and applications of multilocus DNA fingerprints in Brazilian endangered macaws. In: *DNA Fingerprinting: State of the Science* (Pena, S.D.J., Chakraborty, R., Epplen, J.T. and Jeffreys, A.J., eds.). Birkhäuser Verlag, Basel, pp. 395-401.
- Ridgely, R.S.** (1981). The current distribution and status of mainland neotropical parrots. In: *Conservation of New World parrots - Proceedings of the International Committee for Bird Protection, Parrot Working Group meeting* (Pasquier, R.F., ed.). Washington, DC: Smithsonian Institution Press, pp. 233-384.
- Sambrook, J., Fritsch, E.F. and Maniatis, T.** (1989). *Molecular Cloning - a Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, vols. 1, 2, and 3.
- Schoenwald, R. and Schoenwald, J.** (1992). Experiences in breeding the golden conure. *AFA Watchbird* 19: 9-11.
- Sick, H.** (1993). *Birds in Brazil, a Natural History*. Princeton University Press, Princeton, pp. 703.
- Westneat, D.F.** (1990). Genetic parentage in the indigo bunting: a study using DNA fingerprinting. *Behav. Ecol. Sociobiol.* 27: 67-76.
- Wetton, J.H., Carter, R.E., Parkin, D.T. and Walters, D.** (1987). Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327: 147-149.

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