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Genetic variability in the Red-tailed Amazon (*Amazona brasiliensis*, Psittaciformes) assessed by DNA fingerprinting

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RESUMO. **Variabilidade genética do papagaio-de-cara-roxa (*Amazona brasiliensis*, Psittaciformes) avaliada pela técnica de *DNA fingerprinting***. O papagaio-de-cara-roxa (*Amazona brasiliensis*) é uma espécie de psitacídeo ameaçada de extinção e endêmica de uma estreita faixa litorânea da Floresta Atlântica do sudeste do Brasil. No presente trabalho, utilizamos a técnica de *DNA fingerprinting* para estimar a variabilidade genética dentro e entre dois grupos de papagaios de localidades diferentes: 15 aves de Ilha Comprida (sudeste do Estado de São Paulo) e seis de Guaraqueçaba (nordeste do Paraná). Os resultados obtidos revelaram que as aves de Ilha Comprida e Guaraqueçaba apresentam níveis de variabilidade genética estatisticamente similares, sugerindo que as aves destes dois grupos podem constituir uma única população. Os índices de similaridade observados entre todas as aves (x = 0,223±0,091 e 0,335±0,088 com as sondas 33.6 e 33.15, respectivamente) indicam que esta espécie apresenta níveis moderados de variabilidade genética e baixo endocruzamento. Do ponto de vista da conservação, os resultados obtidos indicam que esta espécie está mais ameaçada pela perda de hábitat e pelo tráfico ilegal do que pelo endocruzamento. Portanto, as estratégias de conservação devem ser direcionadas para minimizar os efeitos das atuais ameaças a esta espécie, preservando assim a variabilidade genética existente.
PALAVRAS-CHAVE: *Amazona brasiliensis*, DNA fingerprinting, variabilidade genética, conservação, Psittacidae

ABSTRACT. The Red-tailed Amazon (*Amazona brasiliensis*) is a threatened parrot endemic to the Atlantic Forest in the narrow coastal plain of southeastern Brazil. In the present study, we used multilocus DNA fingerprinting to assess the genetic variability both within and between two red-tailed amazon groups from different localities: 15 birds from Ilha Comprida (southeastern São Paulo State) and six birds from Guaraqueçaba (northeastern Paraná). The results revealed that birds from Ilha Comprida and Guaraqueçaba have statistically similar levels of genetic variability, suggesting that birds of these two groups should be represent only one population. The similarity index observed among all birds (x = 0.223±0.091 and 0.335±0.088 for 33.6 and 33.15 probes, respectively) indicate that species has moderate levels of genetic variability and low levels of inbreeding. For conservation purposes, the results presented here indicate that this species is more threatened by habitat destruction and pouching to illegal traffic than by inbreeding. Thus, efforts should be directed to lessen these current threats, preserving its present genetic variability
KEY WORDS: *Amazona brasiliensis*, DNA fingerprinting, genetic variability, conservation, Psittacidae

The Red-tailed Amazon (*Amazona brasiliensis*) is a parrot endemic to the Atlantic Forest in southeastern Brazil. It occurs from southeastern São Paulo State to northeastern Santa Catarina (Scherer-Neto 1989, Martuscelli 1995) in a narrow area localized between the Serra do Mar and the Atlantic coast which totals 4,760 km^2 (Figure 1, Stattersfield and Capper 2000). This species occurs in forests from lowland up to 700 m elevation, using habitats below 200 m for feeding and breeding (Scherer-Neto 1989, Martuscelli 1995). Breeding areas are mostly located on small estuarine islands rarely on the mainland (Stattersfield and Capper 2000). A complex mosaic of vegetation characterizes its habitat: mangrove, flooded, seasonally flooded, sand-plain, and transitional forests (Martuscelli 1995).

This species is considered vulnerable by the International Union for Conservation of Nature and Natural Resources (IUCN, Baillie *et al*. 2004) and is included in the official list of threatened species in Brazil (IBAMA 2004, *http://www.mma.gov.br/port/sbf/fauna/index.cfm*). Habitat loss (more intense in São Paulo state) and trapping are the most important threats to this species (Scherer-Neto 1989, Martuscelli 1995). Its population was estimated as 3,500 to 4,500 birds in the 1980s and it declined to fewer than 2,000 individuals by 1991-1992 (Martuscelli and Scherer-Neto 1993). Recent estimates suggest that there are approximately 5,600 individuals, 3,600 in Paraná (P. S. N., pers. comm.) and 2,000 in São Paulo state (F. Schunck, pers. comm.), and that its population size is either stable or has suffered a small decline in the last years.

The extinction of small-sized populations becomes a matter of chance even if the causes of the original decline are prevented. There are five main factors of threat in small populations subject to stochastic variation: demographic fluctuation,

environmental variation, catastrophic events, genetic drift, and inbreeding depression (Mace and Lande 1991). There is a positive feed back among these factors leading small populations into an "extinction vortex" (the smaller the population gets, the more inbred it becomes, leading to further decline in numbers, and accelerating the extinction process) (Gilpin and Soulé 1986). Low genetic diversity may both affect individual fitness (Ralls and Ballou 1983, Templeton and Read 1983) and the evolutionary adaptation potential of populations (Frankel and Soulé 1981). Thus, information about the levels of genetic variability and inbreeding should be considered in the conservation of endangered species (Soulé 1980, Templeton *et al*. 1990, Hedrick and Miller 1992).

Despite a number of ecological and demographic studies (Scherer-Neto 1989, Martuscelli and Scherer-Neto 1993, Martuscelli 1995, Martuscelli 1997, Padua *et al*. 2001, Carrillo *et al*. 2002), there is no analysis of genetic diversity in red-tailed amazon populations.

The DNA fingerprinting technique has proven successful in measuring genetic diversity because it can provide a large amount of data per unit effort as it assays the variation across many loci simultaneously (Call *et al*. 1998). In addition, this technique can be easily applied for several species and is considered useful for biologists who seek to set management strategies for small wild populations (Rave 1995).

In the present study, we have assessed the levels of genetic variability both within and between *A. brasiliensis* from Ilha Comprida (southeastern São Paulo State) and Guaraqueçaba (northeastern Paraná) by using multilocus DNA fingerprinting to provide data on the genetic variability status of the extant population.

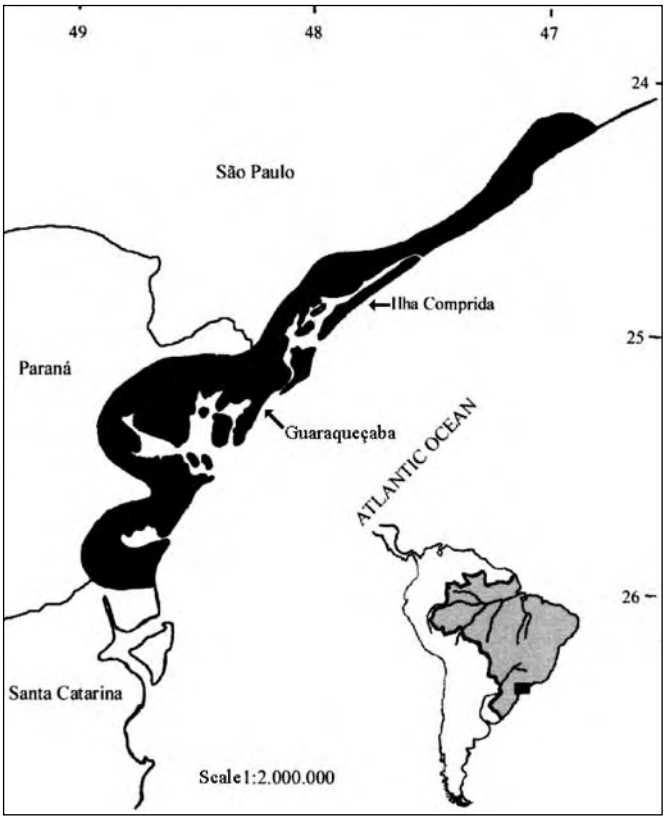

Figure 1. Distribution area of *Amazona brasiliensis* in southeastern Brazil (Stattersfield and Capper 2000). Localities where individuals were sampled are shown (Ilha Comprida and Guaraqueçaba).

MATERIAL AND METHODS

Blood samples (0.1 ml) were collected from the brachial vein of 21 individuals captured in the wild (but currently kept in captivity) in two localities that are around 100 km apart (Figure 1): 15 birds from Ilha Comprida (southeastern São Paulo State) and six from Guaraqueçaba (northeastern Paraná). The origin of the birds was confirmed by the personnel responsible for the captive breeding centers. The blood samples were stored at -20°C in 100% ethanol. Total DNA was isolated using proteinase K and phenol-chloroform-isoamilic alcohol (Bruford *et al*. 1992).

The DNA fingerprinting methodology used is described in detail by Bruford *et al.* (1992). Approximately six micrograms of genomic DNA from each bird were digested overnight with the restriction enzyme *Mbo* I at 37°C. Two agarose gels were made using different individuals from each locality. DNA fragments were transferred onto nylon membranes by Southern blotting (Sambrook *et al.* 1989). Each membrane was hybridized with either of the human multilocus minisatellite probes 33.6 and 33.15 (Jeffreys *et al.* 1985). The membranes were washed in 0.25 M Na_2HPO_4, 1% SDS, 2x SSC, 0.1% SDS, and 1x SSC, 0.1% SDS at 65°C. The filters were then

Table 1. Estimates of the genetic similarity obtained using the 33.6 and 33.15 human multilocus minisatellite probes in *Amazona brasiliensis* from two localities.

Locality	Probe	*N*	*n*±sd	*x*±sd	x^n
Ilha Comprida, SP	33.15	8	23.75±3.92	0.334±0.107	4.9×10^{-12}
	33.6	10	25.90±2.81	0.226±0.102	1.9×10^{-17}
Guaraqueçaba, PR	33.15	5	26.60±2.07	0.383±0.091	8.2×10^{-12}
	33.6	5	24.40±4.34	0.208±0.051	2.3×10^{-17}

N = number of individuals analyzed; *n*±sd = mean number of bands scored ± standard deviation; *x* = mean band sharing coefficient, x^n = probability of unrelated individuals sharing the same DNA band profile by chance.

autoradiographed at -70°C using Kodak RX film with two intensifying screens.

Only the bands between 4.0 and 23.0 kb were analyzed as described by Westneat (1990). The band sharing coefficient between the individuals (index of similarity, x) was estimated using the formula: $x = 2N_{AB}/(N_A+N_B)$; where N_{AB} is the number of bands shared between the individuals A and B, and N_A and N_B are the number of bands present in individuals A and B, respectively (Wetton *et al.* 1987, Bruford *et al.* 1992). The significance of the difference in band sharing coefficients was examined by the Mann-Whitney U-Test. Assuming that each band scored is an independent marker, the mean probability that all bands in an individual's profile are present in another unrelated individual chosen at random was estimated as x^n, where x corresponds to the mean band sharing coefficient and n the mean number of bands scored (Jeffreys *et al.* 1985, Bruford *et al.* 1992).

RESULTS

The mean number of bands detected, the mean band-sharing coefficients (x), and other data estimated with the minisatellite probes are shown in table 1. The values of x obtained with the probe 33.6 were lower than the ones obtained with the probe 33.15. For both probes, the mean band-sharing coefficients estimated among the individuals from Ilha Comprida and Guaraqueçaba were not statistically different ($\alpha < 0.05$, Mann-Whitney test). The mean band-sharing coefficients found between individuals from these two localities, 0.224±0.087 for probe 33.6 and 0.325±0.067 for probe 33.15, are very similar to those observed within each locality (Table 1). In addition, none of the DNA bands was exclusive to any of the two groups studied.

DISCUSSION

The analysis of the results suggests that the two amazon groups studied do not present significant genetic differences and could be considered as a single population. The mean band-sharing coefficients between all individuals were 0.223±0.091 and 0.335±0.088 for probes 33.6 and 33.15, respectively. These values are intermediate between those observed in non-endangered and in endangered bird species (Table 2). In general, the estimates of mean genetic similarity

Table 2. Mean band sharing coefficients (x) obtained using multilocus minisatellite probes in some bird species.

Species	IUCN	Probe	x	Reference
Branta sandvicensis	VU	M13/33.6/33.15*	0.63 to 0.77	Rave 1995
Cyanopsitta spixii	EW	33.6 33.15	0.64 0.62	Caparroz *et al*. 2001b
Anodorhynchus hyacinthinus	VU	33.6 33.15	0.45 0.46	C. Y. Miyaki and N. M. R. Guedes, unpublished
Coturnix coturnix japonica #	--	33.6	0.46	Burke and Bruford 1987
Amazona brasiliensis	VU	33.6 33.15	0.34 0.22	Present study
Propyrrhura maracana	NT	33.6 33.15	0.26 0.24	Craveiro and Miyaki 2000
Ara chloroptera	--	33.6 33.15	0.27 0.20	Caparroz *et al*. 2001a
Passer domesticus	--	33.6 33.15	0.28 0.17	Burke and Bruford 1987
Ficedula hypoleuca	LC	33.6 33.15	0.13 0.27	Burke and Bruford 1987
Branta leucopsis	LC	33.15	0.36	Tegelström and Sjöberg 1995
Branta canadensis maxima	LC	33.15	0.17	Tegelström and Sjöberg 1995

*Mean similarity coefficients obtained by combining the data for all probes; VU – Vulnerable; EW – Extinct in the wild; EN – Endangered; -- - Not threatened; NT – Near threatened; LC – Least concern; #Inbred laboratory population.

obtained by the same probes in non-endangered bird species are usually below 0.27 (Burke and Bruford 1987, Hanotte *et al.* 1992, Fleischer *et al.* 1994, Tegelström and Sjöberg 1995, Rave 1995, Caparroz *et al*. 2001a). However, the technique used in the present study has some shortcomings such as the impossibility of identification of specific alleles and also fragments of the same mobility may not represent necessarily the same allele (Hills 1987). Thus, our estimates, as well as data from other populations studied by the same technique, may represent an overestimate of the fraction of alleles shared between the individuals and the actual genetic variability might be higher than our data suggests.

In populations that present low effective size for a long time interval, which is usually the case of endangered species, the mean band-sharing coefficient is expected to increase with the increase of inbreeding (Lynch 1991). This correlation was inferred for the naked mole rat (*Heterocephalus glaber*, Faulkes *et al*. 1990; Reeve *et al*. 1990), in a small, endangered island population of gray wolves (*Canis lupus*, Wayne *et al*. 1991), and in populations of blue ducks (*Hymenolaimus malacorhynchos*) confined to single-river catchment areas (Triggs *et al*. 1992). In all these studies, the mean band-sharing coefficients were higher than 0.4. In an extreme case, some individuals of the California Channel Islands fox (*Urocyn littoralis*) found within the same islands present up to 100% of similarity at minisatellite loci, and show fixed band differences between the island populations (Gilbert *et al*. 1990).

Even though the number of samples available at this time is small, our results, together with those of other researchers who have been studying this species, are compatible with the following scenario for *A. brasiliensis*: (1) the current population size is a matter of preoccupation as small sized populations are sensitive to stochastic factors; (2) the immediate main threats on this species are habitat loss and pouching; (3) the genetic variability seems to be moderate and there are no evidences of increased levels of inbreeding; and (4) the birds sampled in two different localities, 100 km apart, probably constitute a single population.

Concluding, for conservation purposes, it seems that in short-term the *Amazona brasiliensis* population is more vulnerable to habitat loss (more intense in São Paulo state) and pouching than to inbreeding. Thus, efforts should be directed to lessen these current threats, preserving its present genetic variability.

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REFERENCES

Baillie, J. E. M., C. Hilton-Taylor and S. N. Stuart (2004) *2004 IUCN Red List of Threatened Species. A Global Species Assessment*. Switzerland and Cambridge: IUCN, Gland.

Bruford, M. W., O. Hanotte, J. F. Y. Brokfield and T. Burke (1992) Single-locus and multilocus DNA fingerprinting, p. 225-269. *In*: A. R. Hoelzel (ed.) Molecular genetic analysis of populations: a practical approach. New York: Oxford University Press.

Burke, T. and M. W. Bruford (1987) DNA fingerprinting in birds. *Nature* 327:149-152.

Call, D. R., J. G. Hallett, S. G. Mech and M. Evans (1998) Considerations for measuring genetic variation and population structure with multilocus fingerprinting. *Mol. Ecol.* 7:1337-1346.

Caparroz, R., N. M. R. Guedes, C. A. Bianchi and A. Wajntal (2001a) Analysis of the genetic variability and breeding behaviour of wild populations of two macaw species (Psittaciformes: Aves) by DNA fingerprinting. *Ararajuba* 9:43-49.

_____, C. Y. Miyaki, M. I. Bampi and A. Wajntal (2001b) Analysis of the genetic variability in a sample of the remaining group of Spix's Macaw (*Cyanopsitta spixii*, Psittaciformes: Aves) by DNA fingerprinting. *Biol. Cons.* 99:307-311.

Carrillo, A. C., E. A. B. Sipinski, M. L. Cavalheiro and K. L. Oliveira (2002) Conservação do papagaio-de-cara-roxa (*Amazona brasiliensis*) no estado do Paraná, p 193-213. *In*: M. Galetti and M. A. Pizo (eds.) Ecologia e conservação de psitacídeos no Brasil. Belo Horizonte: Melopsittacus Publicações Científicas.

Craveiro, R. B. and C. Y. Miyaki (2000) Analysis of the genetic variability of *Propyrrhura maracana* (Psittacidae, Aves) using DNA fingerprinting. *Ararajuba* 8:79-84.

Faulkes, C. G., D. H. Abbott and A. L. Mellor (1990) Investigation of genetic diversity in wild colonies of naked mole-rats (*Heterocephalus glaber*) by DNA fingerprinting. *J. Zool. Lond.* 221:87-97.

Fleischer, R. C., C. L. Tarr and T. K. Pratt (1994) Genetic structure and mating system in the palila, an endangered Hawaiian honeycreeper, as assessed by DNA fingerprinting. *Mol. Ecol.* 3:383-392.

Frankel, O. H. and M. E. Soulé (1981) *Conservation and evolution*, Cambridge University Press, Cambridge.

Gilbert, D. A., N. Lehman, S. J. O´Brien and R. K. Wayne (1990) Genetic fingerprinting reflects population differentiation in the California Channel island fox. *Nature* 344:764-766.

Gilpin, M. E. and M. E. Soulé (1986) Minimum viable populations: processes of species extinction, p. 19-34. *In*: M. E. Soulé (ed.) Conservation biology: the science of scarcity and diversity. Sunderland: Sinauer Associates.

Hanotte, O., M. W. Bruford and T. Burke (1992) Multilocus DNA fingerprints in gallinaceous birds: general approach and problems. *Heredity* 68:481-494.

Hedrick, P. W. and P. S. Miller (1992) Conservation genetics: techniques and fundamentals. *Ecol. Appl.* 2:30-46.

Hill, W. G. (1987) DNA fingerprints applied to animal and bird populations. *Nature* 327:98-99.

Jeffreys, A. J., J. F. Y. Brookfield and R. Semeonoff (1985) Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 317:818-817.

Lynch, M. (1991) Analysis of population genetic structure by DNA fingerprinting, p. 113-126. *In*: T. Burke, G. Dolf, A. J. Jeffreys and R. Wolff (eds.) DNA fingerprinting: approaches and applications. Switzerland: Birkhauser, Basel.

Mace, G. M. and R. Lande (1991) Assessing extinction threats: toward a re-evaluation of IUCN threatened species categories. *Cons. Biol.* 5:148-157.

Martuscelli, P. (1995) Ecology and conservation of the Red-tailed Amazon *Amazona brasiliensis* in south-eastern Brazil. *Bird Cons. Int.* 5:225-240.

_____ (1997) Nest predation on the Red-tailed Amazon *Amazona brasiliensis* in Southeastern Brazil. *Papageienkunde* 1:183-188.

_____ and P. Sherer-Neto (1993) Conservation of the Red-tailed Amazon (*Amazona brasiliensis*) in Southeastern Brazil. *Pitirre* 6:7-8.

Padua, S. M., S. Navas, K. Oliveira and A. C. Carrillio (2001) Saving the Red-tailed Amazon, flagship of the Atlantic rainforest in Brazil. *Cyanopsitta* 61:12-17.

Ralls, K. and J. Ballou (1983) Extinction: lessons from zoos, p. 164-184. *In*: C. M Schonewald-Cox, S. Chambers, B. MacBryde and W. L. Thomas (eds.) Genetics and Conservation. London: Benjamin/Cummings.

Rave, E. H. (1995) Genetic analyses of wild populations of Hawaiian geese using DNA fingerprinting. *Condor* 97:82-90.

Reeve, H. K., D. F. Westneat, W. A. Noon, P. W. Sherman and C. F. Aquadro (1990) DNA fingerprinting reveals high levels of inbreeding in colonies of the eusocial naked mole-rat. *Proc. Natl. Acad. Sci. USA* 87:2496-2500.

Sambrook, J., E. F. Fritsch and T. Maniats (1989) *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press.

Scherer-Neto, P. N. (1989) *Contribuição à biologia do papagaio-de-cara-roxa* Amazona brasiliensis. M.Sc. Dissertation, Curitiba: Universidade Federal do Paraná.

Soulé, M. E. (1980) Threasholds for survival: maintaining fitness and evolutionary potential, p. 151-170. *In*: M. E. Soulé and B. A. Wilcox (eds.) Conservation biology: an evolutionary-ecological perspective. Sunderland: Sinauer Associates.

Stattersfield, A. and D. Capper (2000) *Threatened birds of the World*. Barcelona: Lynx Edicions.

Tegelström, H. and G. Sjöberg (1995) Introduced Swedish Canada geese (*Branta canadensis*) have low levels of genetic variation as revealed by DNA fingerprinting. *J. Evol. Biol.* 8:195-207.

Templeton, A. R. and B. Read (1983) The elimination of inbreeding depression in a captive herd of Speke´s gazelle, p. 241-261. *In*: C. M Schonewald-Cox, S. M. Chambers, B MacBryde and L. Thomas (eds.) Genetics and Conservation. Menlo Park, CA: Benjamin/Cummings Publ.

_____, K. Shaw, E. Routman and S. K. Davis (1990) The genetic consequences of habitat fragmentation. *Ann. Missouri Bot. Gard.* 77:13-27.