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GENETIC VARIABILITY ESTIMATES IN THE MARAJÓ YELLOW-CROWNED AMAZON (*AMAZONA OCHROCEPHALA XANTHOLAEMA*) (PSITTACIDAE)

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Resumo. O papagaio-campeiro da ilha de Marajó, *Amazona ochrocephala xantholaema*, é considerado uma subespécie endêmica do complexo *ochrocephala.* Devido à raridade desta subespécie, populações destas aves poderiam estar sujeitas a perda da variabilidade e a outros problemas genéticos originados em populações de tamanho efetivo reduzido. Estimamos os índices de similaridade entre indivíduos utilizando as sondas multilocais 33.6 e 33.15 que detectam regiões de minissatélite, através da análise de amostras de sangue de exemplares de duas regiões da ilha de Marajó e de uma região próxima, no Estado do Amapá. Os resultados, baseados na análise de pelo menos oito locos independentes, mostraram níveis de taxa de heterozigose superiores a 0.94. As diferenças entre os três grupos de amostras não foram estatisticamente significativos. Isso tudo sugere que ainda existe um grau apreciável de fluxo gênico entre as aves da ilha e do continente. A razão sexual da população estudada não diferiu de (1:1). Estudos relativos à anatomia, comportamento e vocalização dessas aves estão sendo realizados por membros de nosso grupo, visando a melhor caracterização deste taxon.

Abstract. Marajo Yellow-crowned Amazon is considered as an endemic subspecies, *xantholaema*, of the *Amazona ochrocephala* complex. It is rare and thus one would expect to detect problems related to small-sized populations, such as low genetic diversity. We evaluated the genetic diversity of this subspecies using the human minisatellite multilocus probes 33.6 and 33.15. Blood samples were collected from birds in two different localities in Marajó Island and from one locality nearby Amapá on the mainland. Our results based on data estimated from at least 8 independent *loci*, detected high heterozygozity levels (H>0.94). The differences observed between samples were not significant. Our results suggest that there is some amount of gene flow between birds from the island and those living on the mainland. The sex-ratio of the studied birds did not differ significantly from the expected ratio 1:1. More studies are being conducted in order to better assess the taxonomic status of *Amazona ochrocephala xantholaema.* *Accepted 4 June 2000.*

Key words: Marajó Yellow-crowned Amazon, Amazona ochrocephala xantholaema, *genetic variability, sex-ratio, DNA-fingerprinting.*

INTRODUCTION

The subspecies *Amazona ochrocephala xantholaema* (Berlepsch 1913) has been considered as endemic to the Marajó Island, northern Pará, Brazil. Data related to this bird are very scarce, since there exist only two pairs of museum skins available for study, at the Bern

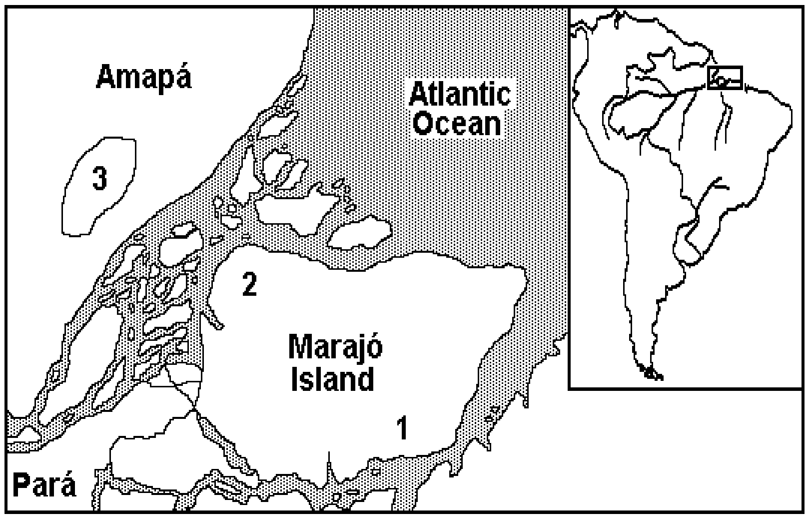

FIG. 1. Map of Northern Brazil showing the localities where the Marajó Yellow-crowned Amazon blood samples were collected. 1 = Eastern portion of Marajó Island, 2 = Northern portion of the island, 3 = Savanna regions from the state of Amapá.

Natural History Museum in Switzerland and at the Emílio Goeldi Museum in Brazil (Pinto 1938, Lepperhoff 1996).

Marajó Island, with an area of 49,602 km^2, is surrounded by the Atlantic Ocean and the rivers Amazonas and Tocantins and has about 250,000 inhabitants; its most important cities are located on the northeastern side. It can be divided in a western portion, with a dense tropical forest, and an eastern portion consisting of thick forests surrounded by open fields, commonly flooded during the rainy season.

Forshaw (1989) describes *xantholaema* as similar to nominotypical *ochrocephala*, except for having cheeks and ear coverts yellow, a green frontal band, the yellow of the head extending over nape, yellow thighs and a larger size. Few breeders have this parrot, but at least one (Luis Maluf, São Paulo, Brazil) was able to breed it in captivity. Goldhammer & Diefenbach (1988) note that the validity of this subspecies is questioned by some authors.

Goldhammer and Diefenbach (1988) state that this bird is not rare and feeds on nuts and fruits as do other Amazona parrots, the Babaçu and the Inaja palms being important in their diet. These authors believe that the behavior of this subspecies is very similar to the one observed in nominate forms.

The taxon is considered to be endemic and rare, being possibly subjected to phenomena related to small-sized populations, such as loss of genetic diversity due to genetic drift. Small populations are also known to be more sensitive to demographic and environmental fluctuations such as variation in death/birth rates, natural catastrophes, competition and disease (Gilpin & Soulé 1986, Primack 1993).

Low genetic variability is usually associated with harmful effects such as low survival and birth rates. Empirical evidence shows that heterozygosity is related to fitness in natural populations, and several studies have shown the association of heterozygosity with survival, disease resistance, higher growth and developmental rates, and developmental stability (Allendorf & Leary 1986, Westemeier *et*

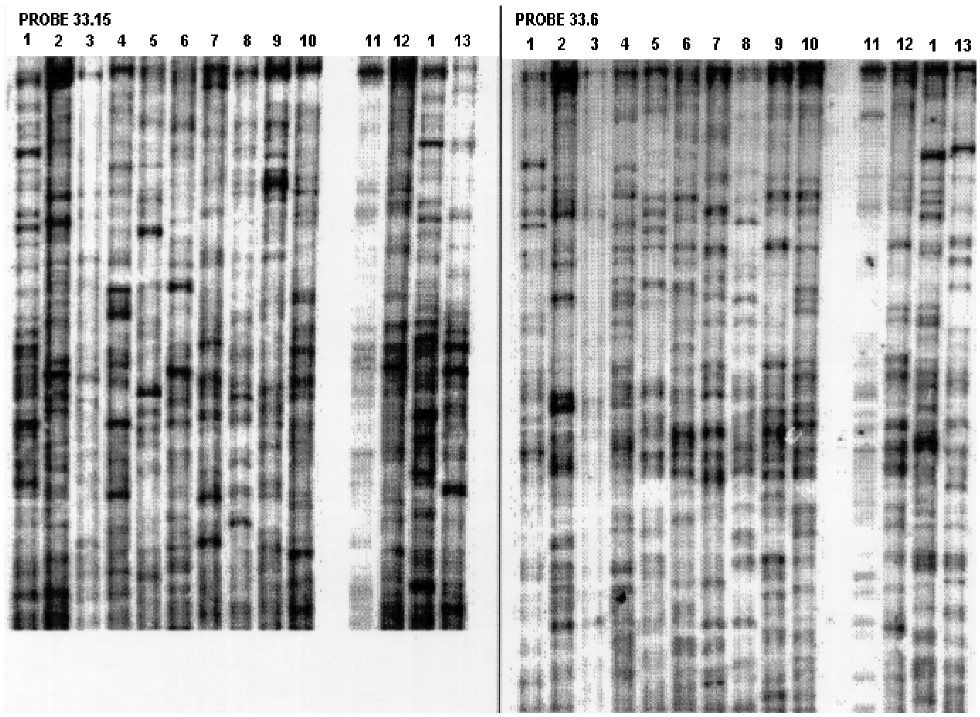

FIG. 2. Autoradiographs of the same membrane with samples collected on Marajó Island hybridized with probes 33.15 and 33.6.

TABLE 1. Similarity index (SI), mean allelic frequency (q) and heterozygosity (H) estimated by hybridization of samples collected from different localities with probe 33.6.

Locality	N	SI ± sd	q	H
Eastern Marajó	21	0.11 ± 0.09	0.0566	0.9708
Northern Marajó	5	0.19 ± 0.09	0.1000	0.9473
Amapá	14	0.20 ± 0.06	0.1055	0.9443

al. 1998, Saccheri *et al.* 1998).

Very little is known about the demography, ecology and behavior of Marajó Yellow-Crowned Amazon. Field studies were performed in order to fill this gap. Further data involving the different subspecies of *Amazona ochrocephala* that occur in Brazil will be published elsewhere (Martuscelli *et al.*, in prep.).

The aim of the present work was to estimate the genetic variability present in *Amazona ochrocephala xantholaema* in samples collected from characteristic savanna vegetation in Marajó Island and surrounding areas (Fig. 1).

We also studied a family (parents and their chicks) of *A. o. xantholaema* from an aviculturist and estimated the minimum number of independent *loci* detected in this taxon by

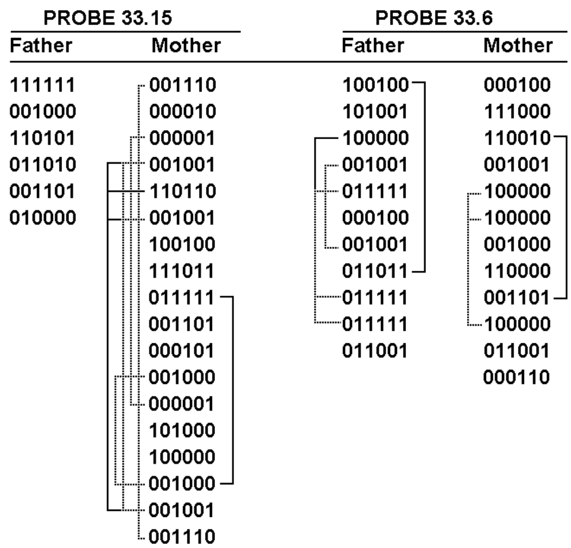

FIG. 3. Band segregation analysis. The dotted lines represents the co-segregating fragments and the continuous lines represent the allelic fragments. The analysis was performed in both parents and their 6 chicks.

the probes used in the present work.

METHODS

We used 100 µl blood samples obtained by venipuncture from each one of 60 specimens of *Amazona ochrocephala*. Samples were immediately transferred to vials containing 500 µl of 100% ethanol. Twenty-six of the blood samples were collected at the eastern portion of the Marajó Island, 22 birds belonging to local residents that provided the information on the site of the original nests and four being captured from the wild; five samples were collected at the northern part of the island and 21 in savanna regions from the state of Amapá on the nearby mainland. The remaining birds belonged to an aviculturist (parents and 6 nestlings born in captivity). According to the information obtained, all birds, except these 6 nestlings, had been captured in the wild.

DNA was extracted from blood samples as described by Bruford *et al.* (1992). An aliquot from this DNA was digested with *Mbo*I 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. Four gels were loaded, two with the 26 samples from Eastern Marajó, half in each gel, chosen at random, one with birds of each of the 3 sampled localities and another with the 6 nestlings and their parents. The first three gels were used for analysis of genetic variability, the fourth for analysis of band segregation (Bruford *et al.* 1992). The fractionated DNA fragments were transferred onto nylon membranes (Hybond, Nfp, Amersham) by capillary Southern blotting (Sambrook *et al.* 1989). The DNA of the same bird was loaded at both extremities of the agarose gel in order to detect any distortions occurring during electrophoresis.

All membranes were pre-hybridized in 0.263mM Na_2HPO_4, 1mM EDTA, 7% SDS,

TABLE 2. Paternal (P) and maternal (M) *loci* segregating in six chicks, detected by probes 33.15 and 33.6.

Number of independent *loci* with probes	33.15	33.6
Paternal	6	6
Maternal	11	9
Average	8.5	7.5
Average number of bands	18.9 ± 4.9	21.5 ± 4.0
Bands shared between probes	6.8 ± 2.1	

and 1% BSA at 65° C, and after four h, probe 33.6 (Jeffreys *et al.*, 1985a) was added to the solution. After 15 h, the membrane was washed 3 times for 10 min with 3 solutions: 0.25M Na_2HPO_4/1% SDS, 2XSSC/0.1% SDS, and 1XSSC/0.1% SDS. The membranes were exposed with two intensifying screens to an X-ray film, stripped, and reprobed with minisatellite 33.15 (Jeffreys *et al.* 1985b) as described above.

The analysis of band segregation (Bruford *et al.* 1992) was performed in order to establish whether the number of independent *loci* is sufficient to be representative of the genetic variability of this taxon.

The analysis of the genetic variability was carried out in each autoradiograph using the similarity index (SI), also known as the band sharing coefficient or BSC (Wetton *et al.* 1987). The latter was calculated for each pair of individuals in the same autoradiograph with the formula $SI = 2N_{AB}/(N_A + N_B)$ where N_{AB} is the number of bands shared between individuals A and B and N_A and N_B are the total of bands present in each individual.

Using the SI estimated for each pair of individuals in the same autoradiograph, a mean SI was obtained separately for samples from different localities in Marajó Island as well as for the sample of *A. ochrocephala* obtained in Amapá.

The mean SI was used to obtain an approximate estimate of the mean heterozygosity (H) of a population after the usual formula $H = 2(1-q)/(2-q)$ (Sundt *et al.* 1994), where q (the mean allelic frequency) was directly estimated from $q = 1-\sqrt{1-SI}$ (Jeffreys *et al.* 1985b).

The sex ratio was estimated using a single set of primers amplified by PCR (Griffiths *et al.* 1998, Miyaki *et al.* 1998).

Pairwise comparisons between results obtained with two different probes were performed with the Mann-Whitney test; the similarity indexes of samples from three different localities were compared using the Kruskal-Wallis non-parametric ANOVA test.

RESULTS

Examples of the autoradiographs obtained with each probe are shown in Fig. 2. The mean similarity index estimate for 21 birds from the eastern part of Marajó Island was 0.12 ± 0.08 for probe 33.15 and 0.11 ± 0.08 for probe 33.6. The index of the birds from the northern part of the island was 0.19 ± 0.09 and the index of the birds from Amapá was 0.17 ± 0.04 (Table 1).

The results of both probes were compared with the non-parametric Mann-Whitney test, which provided non-significant differences. As the number of independent *loci* detected with each probe (Fig. 3, Table 2) was close to eight and 1/3 of the total of fragments detected (close to 20 for each probe) could be detected by both probes, the total amount of available information on genetic similarity between individuals did not

TABLE 3. Values of similarity index (SI) estimated by hybridization of samples collected from different localities with probe 33.6, compared in the same gel.

Locality	N	SI ± sd
Eastern Marajó	5	0.13 ± 0.08
Northern Marajó	5	0.19 ± 0.09
Amapá	7	0.20 ± 0.08

increase significantly with the use of a second probe. For this reason, different samples were compared using the results of only one probe (33.6). In order to perform all comparisons between samples in a single gel, a further gel was prepared containing 5 samples selected randomly from the population at the eastern part of the island, all the five individuals that were collected on the northern part and seven individuals from Amapá (Table 2). No significant differences were found between the full sample of eastern birds and the five individuals subsampled from this population. This indicates that the latter five can be used in comparisons with other populations on a single gel. On comparing the similarity indexes of the three samples representing eastern and northern Marajó and Amapá (Table 3), no significant differences were found with the Kruskal-Wallis nonparametric ANOVA test ($H = 3.551$; $P = 0.17$), indicating that the three sampled populations have similar heterozygosity levels (Table 1).

By sexing 17 individuals (11F:8M), we found no significant deviations from the expected 1:1 male to female ratio.

DISCUSSION

Our data on DNA fingerprinting in a sample of *Amazona ochrocephala xantholaema* from Marajó Island suggest that the population has high genetic variability levels, with an heterozygosity close to 0.95. The pattern of segregation among the fragments present in the DNA profiles of a family consisting of the parents and their chicks showed that around 8 independent *loci* are actually being detected by each probe, and that around 25% of the total fragments are detected by both probes. This explains the fact that our estimates of the mean heterozygosity values, based on eight independently inherited *loci,* are very similar using the probes singly or in combination.

The amount of heterozygosity here estimated is within the range of that observed in large sized, non-endangered populations (Burke & Bruford 1987, Hanotte *et al.* 1992). Field observations are not compatible with laboratory results as only few flocks composed by a small number of individuals could be observed by us (P. M. & C.Y.) on Marajó Island. The absence of this *taxon* from most museum collections as well as its rarity in aviculture have led to the view that *xantholaema* is rare.

Estimates of mean heterozygosity in natural populations of parrots or of captive birds with known origin (and which can be considered representative of a wild population) are scarce. Brock & White (1992) estimated the mean band sharing coefficients from a non-endangered and the endangered Puerto Rican Parrot *A. vittata*. The mean band sharing coefficient (SI) among unrelated individuals of the non-endangered species (Hispaniolan parrot) was 0.16, while this coefficient was 0.45 among the endangered Puerto Rican Parrots. Miyaki *et al.* (1998) studied a population of hyacinth macaws (*Anodorhynchus hyacinthinus*) from the Pantanal region (Mato Grosso do Sul, Brazil). The mean band sharing coefficients were between 0.30 and 0.40 for each of the probes used (33.15 and 33.6). The population size of these macaws is estimated as between 1,500 to 2,000 individuals. Similar results have been obtained for *Amazona brasiliensis* (Caparroz *et al.* 1998), an endemic species that inhabits the southeastern coast of

Brazil, with a population of about 3,600 birds (Martuscelli, 1995) and for *Amazona kawalli* (Caparroz *et al.* 1998), a species that has been described recently (Grantsau & Camargo 1989) and is endemic to the Tapajó River (Pará, Brazil) (Martuscelli & Yamashita 1997). In the natural populations of *Anodorhynchus hyacinthinus* and *Amazona kawalli*, the presence of population specific fragments, present in all individuals, was interpreted as evidence of allelic loss due to randon drift expected in small sized isolated populations. These patterns were not seen in the different samples studied in our work.

Our results on the genetic variability of *Amazona ochrocephala xantholaema* from Marajó Island are not those expected for a small-sized isolated population, resident in Marajó Island for at least 100 years. However, our data could be explained by occurrence of a large panmictic population or, alternatively, the high variability levels here detected could be maintained by at least one migrant from a large donor population per generation (Wright 1931, 1932, 1940; Mills & Allendorf 1996): in fact, it is known that with a small number of migrants per generation a substantial divergence in allele frequencies between donor and recipient populations is still expected, with resulting heterozigozity levels in the recipient population generally high. Henriques & Oren (1997), examined two specimens of *Amazona ochrocephala* from Marajó and stated that one of them was "consistent with Berlepsch's original description" but the other had "very little yellow on the head" (sic), thus suggesting that the migrant hypothesis might be responsible for the high variability detected in the present paper. However, preliminary observations by Martuscelli and Yamashita (in prep.) in birds from Amapá indicate that the population on the mainland is indistinguishable from the birds found on Marajó Island. There is also one mainland report of a bird indistinguishable from *xantholaema* of Marajó Island, seen in the vicinity of the Paru river in the state of Pará (Low 1986); this observation, however, needs confirmation.

The results of a comparative study involving the anatomy, ecology, behavior, vocalization records and distribution of *Amazona ochrocephala* are being analyzed and will be published elsewhere (Martuscelli and Yamashita, in prep.). These data will hopefully provide further evidence on the real status of the Marajo's Yellow-Crowned Amazon.

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