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Molecular markers contribute to a breeding programme of the extinct-in-the-wild Alagoas Curassow *Mitu mitu* and confirm the validity of the species

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Summary

Alagoas Curassow *Mitu mitu* is considered extinct in the wild, having previously inhabited a small area in north-eastern Brazil that has since been replaced by sugar cane farms. Around 50 birds possessing morphological features of this species are still alive in captivity in Brazil, all being descendants from a breeding programme started in 1979, using offspring from a single male and two females captured from the wild. However, this captive population also includes some hybrids with the congeneric Razor-billed Curassow *M. tuberosa* and their descendants. Furthermore, the validity of Alagoas Curassow as a species is questionable. We used two molecular markers to study the validity of this taxon as a species, to detect potential hybrids present in the stock, and to estimate genetic variability among the remnant specimens. The analysis of 760 base pairs from mitochondrial cytochrome *b* and control region sequences revealed that at least three of the 20 birds analysed had sequences identical to those of Razor-billed Curassow. The other 17 birds presented sequences that diverged 2.6% from Razor-billed Curassow. Moreover, a sample from an Alagoas Curassow museum skin collected from the wild in 1951 had cytochrome *b* sequences identical to those of the 17 birds. These results confirm the Alagoas Curassow as a valid species. DNA fingerprinting profiles of the 20 descendants from the Alagoas Curassow breeding population showed that this group of birds is depauperate in genetic variability. There was an increase in genetic variability of birds born after 1990, attributed to the hybrid mating of Alagoas Curassow with Razor-billed Curassow. We suggest that birds born before 1990 should be handled separately from the others. However, if a decrease in chick harvest among birds of this group is detected due to inbreeding depression or ageing, cross-breeding between this group and the group of birds most closely related to it should be considered in order to enrich the progeny with the Alagoas Curassow genome.

Introduction

Most cracids are endemic to the Neotropics, and are important bioindicators of the health of their habitat (Brooks and Strahl 2000). The family encompasses 11 genera and approximately 50 species. Among these latter, 19 species are listed as Vulnerable, Endangered or Critically Endangered, mainly due to excessive hunting and habitat destruction (Brooks and Strahl 2000, BirdLife International 2000). The most threatened species of this family is Alagoas Curassow *Mitu mitu*.

Its former habitat was the Atlantic Forest in the state of Alagoas, north-eastern Brazil, but the last large forest remnants were replaced by sugar cane in the 1970s and 1980s. Its population size was estimated at less than 60 individuals in the late 1970s (Teixeira 1986) but now this species is considered extinct in the wild (Collar *et al.* 1992, BirdLife International 2000, L. F. Silveira unpublished data).

Linnaeus described the Alagoas Curassow in 1766, naming it *Crax mitu*, based on texts and illustrations published in 1648 by the German naturalist J. Marcgrave, in his famous ''Historia Rerum Naturalium Brasiliae'' (Teixeira 1992). For about three centuries, the species was not recorded in the wild, until Olivério Pinto captured a single female in 1951. This specimen is now housed at the Museu de Zoologia da Universidade de São Paulo, (MZUSP n° 37.188) in São Paulo, Brazil. Despite being considered a monotypic species (Collar *et al.* 1992, Nardelli 1993, del Hoyo *et al.* 1994, Sick 1997), many ornithologists have questioned its validity as a species, mainly because of its rarity and morphological similarity to Razor-billed Curassow *M. tuberosa*, an inhabitant of the Amazon Forest (Pinto 1952, Coimbra-Filho 1970, Sick 1980). Silveira (unpublished data) compared the single adult female specimen of Alagoas Curassow, collected in 1951, with nine females of the closely related Razor-billed Curassow and confirmed the validity of the former as a full species based on diagnostic characters of a bi-coloured bill, a bare auricular patch and a tail with buff rather than white tips and nearly all-black central rectrices.

In 1979, five birds were collected from the wild in the municipality of Barra de São Miguel (09°50'S, 35°54'W), Alagoas, Brazil, to be used in a captive breeding programme (Nardelli 1993). At the time of writing, all surviving birds are descendants of this breeding programme developed by Nardelli (1993) who successfully bred two females with a single male. In 1990, after the death of this single male, Nardelli began to cross some Alagoas Curassows with Razor-billed Curassows. After a deplorable misunderstanding between the breeder and official authorities, the original records of the breeding data became unavailable. Thus, only the birds that were born before 1990 can be considered as ''pure'' Alagoas Curassow. In 2000, the whole population of surviving birds (44 birds) was split between two different breeders. We had the opportunity to collect blood samples from the 20 birds held by one breeder. This group consisted of three birds born before 1990 and 17 born after.

To evaluate the status of Alagoas Curassow as a species and to detect hybrid birds, we sequenced fragments of the mitochondrial control region and cytochrome *b* genes of 20 birds possessing morphological features diagnostic of Alagoas Curassow and compared their sequences to those of other valid congeners. Similar procedures based on mtDNA sequences have been used by other researchers to ascertain specific status of birds and mammals (Zink and Blackwell-Rago 2000, Bradley and Baker 2001). In cracids, this approach has been applied before among several species of curassow, to check the validation of *Crax viridirostris* and *C. estudilloi* described solely on the basis of single specimens. In this case, the sequences of *C. viridirostris* and *C. estudilloi* were found to be identical to those obtained for Blue-billed Curassow *C. alberti* and Yellow-knobbed Curassow *C. daubentoni* substantially diminishing the possibility that they are valid species (Joseph *et al.* 1999).

We also sequenced 166 bp of the cytochrome *b* from a skin fragment extracted from a museum specimen of Alagoas Curassow collected from the wild in 1951, to evaluate if the sequences obtained for most of the studied birds represented the sequence from this species.

The genetic variability of the 20 Alagoas Curassows was estimated utilizing DNA fingerprinting techniques, which have proved useful in estimating overall genetic variability in many groups of birds, including gallinaceous birds (Hanotte *et al.* 1992) and Cracidae (Pereira *et al.* 1996, Pereira and Wajntal 1999, 2001a,b). Based on the results obtained in this study, we present management recommendations in order to ameliorate further degradation of genomic variability in the captive population of Alagoas Curassow.

Materials and methods

Taxa

Approximately 0.1 ml of blood was taken by venipuncture from 20 birds belonging to Nardelli's original stock. All these birds possessed phenotypic characteristics of Alagoas Curassow. Blood samples were also taken from three Razor-billed Curassows and one Crestless Curassow, *M. tomentosa* and kept in 0.5 ml of absolute ethanol at room temperature. All birds sampled were from private aviaries. A skin sample of Alagoas Curassow was obtained from the Museu de Zoologia da Universidade de São Paulo (MZUSP n° 37.188) in São Paulo, Brazil. All specimens of Alagoas Curassow found in other museums and private collections have been donated by Nardelli from his captive breeding programme, and were not considered in this study.

DNA sequencing from blood samples

DNA was extracted from the 24 blood samples following standard protocols (Sambrook *et al.* 1989). Two mitochondrial DNA fragments were isolated by polymerase chain reaction (PCR) with primers Dloop L (5'-TTG TTC TCA ACT ACG GGA AC-3') and Dloop H (5'-GTG AGG TGG ACG ATC AAT AAA T-3') for the first domain of the control region (G. Rowe, pers. comm.) and primers reverse cyt *b* L (Kornegay *et al.* 1993) and CBH15764 (Miyaki *et al.* 1998) for the cytochrome *b*. DNA amplification was performed in 10.0 µl of 2.0 mM of each dNTP, 10.0 µM of each primer, 1–50 ng of genomic DNA, 0.5 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech) and 1.0 µl of buffer 10X supplied with *Taq* DNA polymerase. The reaction was performed for 5 min at 95 °C for denaturation, followed by 30 cycles of 95 °C for 40 s, 54 °C for 30 s, and 72 °C for 30 s, and a final extension of 10 min at 72 °C. Products were purified with 15 U of exonuclease I (USB) and 1.5 U of shrimp alkaline phosphatase (USB) for 60 min at 37 °C followed by 10 min at 80 °C. Sequencing reactions and purifications were performed according to the manufacturer's recommended protocol (Big Dye terminator cycle sequencing kit; Applied Biosystems) and loaded in an automated DNA sequencer ABI Prism 377 (Applied Biosystems). Both strands of each DNA fragment were sequenced.

DNA sequencing from skin sample

All procedures for DNA extraction and sequencing of the skin fragment from the museum specimen were done in a laboratory where bird samples had not been manipulated, to avoid contamination. DNA was extracted using QIAamp DNA Mini Kit as recommended by the manufacturer (Qiagen Inc.). We performed two concomitant extractions, one containing fragments of the skin sample and the other performed without any DNA source, to verify that there was no possible contamination. New reagents were used in these isolations. A fragment of the cytochrome *b* was amplified by PCR, with primers CBH15764 (Miyaki *et al.* 1998) and CBL15562 (5′-TAT TTC TCC YTA AAA GAC CTG TTA GGG TT-3′, C. Y. Miyaki pers. comm.) in the same conditions as described above for DNA extracted from blood samples. Following this, a second amplification was performed in the same conditions using 2 µl from the first reaction, for both the previous reactions containing the museum skin and the one without any DNA source. Sequencing reaction and purification were performed as described for blood samples and loaded in an automated DNA sequencer ABI Prism 377 (Applied Biosystems).

Sequences analysis

Both strands of the DNA fragments of each bird were visually aligned and corrected for ambiguities in Sequence Navigator (Applied Biosystems). The control region and cyt *b* sequences of each bird were concatenated before further analysis. The cyt *b* sequence from the skin sample was visually aligned with the corresponding cyt *b* sequences obtained from the other 24 birds. Uncorrected *p*-distances were estimated in PAUP 4.0b8 (Swofford 2001). Sequences obtained in this study have been deposited in GenBank under accession numbers AY098533–AY098580.

DNA fingerprinting

Multilocus DNA fingerprints were performed as described in Bruford *et al.* (1992). Briefly, 4 µg of genomic DNA from each of the 20 captive Alagoas Curassows was completely digested with 15 units of *Mbo*I for a period of 16–18 hrs. The fragments were electrophorized through a 30-cm long horizontal 1% agarose gel, until the γ-*Hind*III molecular marker of 2.3 kb loaded in the first lane had migrated to the anodal end of the gel. Digested DNA sample of the same bird was loaded in the second and in the last lane of the gel to estimate band migration distortion and provide a better scoring of the bands. The fractionated DNA fragments were transferred onto a nylon membrane (Hybond Nfp, Amersham) by capillary Southern blotting (Sambrook *et al.* 1989).

The human multilocus minisatellite probe 33.15 (Jeffreys *et al.* 1985a) was radiolabelled by the random priming method with [α-^{32}P] dCTP. The membrane was pre-hybridized for one hour at 65 °C in a solution containing only 0.263M Na_2PO_4 and 7% SDS. The probe was added to this solution and left overnight at the same temperature. The membrane was then washed in 2 × SSC, 0.1% SDS and in 1 × SSC, 0.1% SDS at 65 °C, for 20 min each. Autoradiographs were

Table 1. Variable sites found in the 24 curassows sampled. Numbers are written vertically and correspond to position in the mtDNA sequence analysed. Dots and a dash represent, respectively, the same base and a gap in the sequence related to the most frequent Alagoas Curassow haplotype shown on the top.

Species	Number of birds	Control region haplotype	Cyt *b* haplotype
		00011122222333333	00011122233333
		15835902258111223	18806923812245
		37666187983456745 1	11344149113674
Alagoas Curassow	16	TGGTCTCCCCCAGCTTAC	GTATCTCCGTCCAT
Alagoas Curassow	1	. . . . . T	. . . . .
Alagoas Curassow	3	. . . C . CAT . TTGA . . . GT	ACGC . CTTACT . . .
Razor-billed Curassow	3	. . . C . CAT . TTGA . . . GT	ACGC . CTTACT . . .
Crestless Curassow	1	CA - CT . . TTTT . . TCC . .	A . GCTCTT . C . TGC

obtained after one to three days of exposure at –80 °C using X-ray film and two intensifying screens.

Bands in the range of 3.8 to 15.5 kb were marked on acetate overlays according to Westneat (1990). The band-sharing coefficient (BSC), or index of similarity, between individuals was calculated as: $x = 2N_{AB}/(N_A + N_B)$ where N_{AB} is the number of bands shared by *A* and *B*, N_A and N_B are the number of bands present in birds *A* and *B*, respectively (Wetton *et al.* 1987, Bruford *et al.* 1992). The genetic variability was therefore the reciprocal of the index of similarity. Assuming that the bands scored were independent markers, the mean probability that all *n* bands in an individual's fingerprint were present by chance in a second random unrelated individual was conservatively estimated as $< X^n$ (Jeffreys *et al.* 1985b, Bruford *et al.* 1992). Mean heterozygosity (H) was given by $H = 2q(1–q)/(2q–q^2)$ (Sundt *et al*. 1994), where q, the mean allelic frequency of bands, is obtained from $x = 2q–q^2$ (Jeffreys *et al.* 1995b).

Results

DNA sequencing

Amplification of each mitochondrial DNA region resulted in single PCR products for each bird. From these PCR products we obtained unambiguous sequences with similarity to corresponding sequences of other birds deposited in GenBank. The reading frame for cytochrome *b* sequences did not show any stop codon or changes in amino acid sequence that could indicate that they were of nuclear instead of mitochondrial origin. Contamination did not occur as no amplification was observed in the negative control reactions.

The concatenated sequences obtained for each of the 24 birds were 760 bp long (390 bp for cytochrome *b* and 370 bp for control region). Thirty-two variable sites were found (Table 1). The sequences from the Razor-billed and the Crestless Curassows differed by 2.8% (21/760). Three different haplotypes were found in the 20 birds presumed to be Alagoas Curassow on the basis of phenotypic characteristics. Three individuals among 20 individuals of Alagoas Curassow had sequences identical to those of Razor-billed Curassow. Among the other 17 individuals, 16 possessed identical haplotypes, including two birds born before

1990, and a single male born in 1984 differed by a single transition nucleotide substitution in the control region. Uncorrected *p*-distance between the haplotypes of Alagoas and Razor-billed Curassows was 2.6% (20/760) and between Crestless and Alagoas Curassows was 3.0% (23/760).

The cytochrome *b* fragment obtained from the skin sample had a size of 202 bp, producing an analysable sequence of 166 bp that was found to be identical to those 17 birds presenting the most common haplotype. This sequence differed by 2.4% (4/166) from Razor-billed Curassow and by 3.0% (5/166) from Crestless Curassow sequences.

DNA fingerprinting

The mean number of bands detected by the human multilocus minisatellite probe 33.15 for the 20 captive-kept Alagoas Curassows was 14.4 ± 3.2 (range from 9 to 16 bands). Among these an average of 5.2 ± 2.1 fragments were shared, providing an average BSC of 0.358 ± 0.12. Considering only the birds born before 1990 (prior to the inclusion of Razor-billed Curassow in the breeding programme) the mean BSC was 0.501 ± 0.17. After Razor-billed Curassows were introduced in the breeding programme, the mean BSC decreased to 0.354 ± 0.12. Excluding the three Alagoas Curassows whose mitochondrial DNA sequences were identical to that of Razor-billed Curassow (see above), the mean BSC was 0.327 ± 0.11. The probability of two unrelated birds sharing identical profiles by chance was extremely remote and had a probability in the order of 1 in 10^{-5} to 10^{-7}. Mean heterozygosity was lower for birds born before 1990 (H = 0.82) compared with those born after this date (H = 0.89).

Discussion

Is the Alagoas Curassow Mitu mitu *a valid species?*

The status of Alagoas Curassow as a species has been questioned in the last three centuries mostly because of its rarity and morphological similarity to Razor-billed Curassow (Pinto 1952, Coimbra-Filho 1970, Sick 1980). Our research addressed this question, which is of extreme importance for the management of this highly endangered taxon, by comparing the distances of mitochondrial genes between three different but closely-related lineages of the genus *Mitu*: Alagoas Curassow, Razor-billed Curassow and Crestless Curassow. The estimated distance of 2.6% between Alagoas and Razor-billed Curassow is almost the same as that between Alagoas and Crestless Curassow (3.0%), as well as between Razor-billed and Crestless Curassow (2.8%). Similar divergence for these same two mitochondrial DNA regions has been found between other recognizable congeneric species of the family Cracidae: 1.3% between Bare-faced Curassow *Crax fasciolata* and Black Curassow *C. alector*; 2.6% between Bare-faced Curassow and Red-billed Curassow *C. blumenbachii*; and 1.4% between Chestnut-bellied Guan *Penelope ochrogaster* and Dusky-legged Guan *P. obscura* (E. T. Grau unpubl. data). Distances equivalent or higher than the distance between valid species should help to validate questionable congeneric species (Zink and Blackwell-Rago 2000, Bradley and Baker

2001). Thus, as Crestless Curassow is clearly a distinct species from the other two and the distances between the three species are almost equivalent, our data favour the hypothesis that Alagoas Curassow is indeed a distinct species from Razor-billed Curassow. Moreover, the findings presented here are concordant with sequence data collected from other mitochondrial genomic regions that show the uniqueness of the Alagoas Curassow when compared with 13 other recognizable curassow species (S. L. Pereira and A. J. Baker subm.).

Interspecific hybridization

Our DNA sequencing results showed that three birds of the breeding programme born after 1990 have mitochondrial sequences identical to Razor-billed Curassow. In addition, our DNA fingerprinting results showed an increase in the genetic variability among the birds born after 1990. Taken together, these results constitute strong evidence that hybridization has occurred between Alagoas and Razor-billed Curassows after 1990. Since mitochondrial DNA is maternally transmitted, our findings confirm that hybridization occurred between females of Razor-billed Curassow and males of Alagoas Curassow, and that at least one female Razor-billed Curassow was involved in the breeding. It would be useful to detect the ancestral male genome as well; however, the necessary tools are not available to us at present.

Management

Our DNA fingerprinting results showed that the birds born before 1990, which we are assuming to represent ''pure'' Alagoas Curassows, had a similarity index expected for first-degree relatives. This result is not surprising since all birds alive today are the outcome of only three birds captured from the wild (one male and two females). The increase in the genetic variability detected after 1990 to levels found for other non-threatened curassows (Pereira and Wajntal 1999, 2001a,b) can be attributed to the crosses of Alagoas Curassow with Razor-billed Curassow in captivity (Nardelli 1993). The best management programme for the conservation of Alagoas Curassow should include all surviving birds, especially those with a ''pure'' Alagoas Curassow background. The programme should aim to preserve the genetic constitution present in the ''pure'' surviving birds, but should also carefully consider the potential ill-effects that can arise due to inbreeding, such as decreased fertility and survival, as well as other possible effects due to the greater age of the three birds born before 1990. It is also possible that the surviving birds have already overcome the effects of inbreeding depression in a similar way as achieved experimentally in Speke's gazelle *Gazella spekei* as reported by Templeton and Read (1983). They demonstrated that after several rounds of consanguineous matings, there was a reduction in lethal equivalents in the outcome of inbred parents as compared with that of non-inbred parents. As a practical result, the reduction in inbreeding depression resulted in decreased mortality rates and increased birth weight of the offspring, demonstrating that animals can quickly adapt to even extreme inbreeding and that this adaptation can occur in relatively small populations.

However, as the breeding success among the ''pure'' Alagoas Curassows cannot be assured and the genetic constitution of the remaining birds is unknown, the best strategy available at this time is to detect the birds that, through the technology used in the present work, showed the higher similarity with the ''pure'' birds, with similarity indexes higher than 0.5. Analysing the data in Table 2, it is possible to identify a group of two males and three females that show high genetic similarity to the birds born before 1990 (BSC = 0.577 ± 0.03) as well as between males and females from this group (BSC = 0.624 ± 0.02). Mating between these selected birds has a higher chance of preserving most of the Alagoas Curassow genome than other matings and the outcome of these couples, or between them and the ''pure'' Alagoas Curassows, should be encouraged. It is interesting to point out that similarity indices between these selected birds and the birds that inherited a Razor-billed Curassow mitochondrial genome are much lower (BSC = 0.297 ± 0.05), as expected for birds that have an enriched Alagoas Curassow constitution when compared with birds with the Razor-billed Curassow contribution.

Three birds born after 1990 had DNA sequences and morphological features of Alagoas Curassow but a BSC > 0.5 with the three ''hybrid'' birds and a BSC of 0.283 ± 0.07 when compared with those three ''pure'' birds born before 1990.

Thus, among the 20 birds, three are ''pure'' Alagoas Curassow, five have a higher probability of also being pure or having a higher contribution of the Alagoas Curassow genome and 12 may be considered as being of mixed genomic constitution between Alagoas and Razor-billed Curassow genomes. The lower indices in comparison with the pure or selected group probably represent a higher contribution of the Razor-billed Curassow genome, but as those 12 birds have the typical morphological features of Alagoas Curassow, they certainly harbour some of the original Alagoas Curassow genome. Thus, they should not be excluded from the programme, as the main objectives are the preservation of most of the genetic constitution of this extinct-in-the-wild species as well as to try to establish a viable population in protected areas within its original distribution.

We have previously shown that the lack of genetic variability was not a problem for the establishment of a wild population of two other cracid species, Dusky-legged Guan and Rusty-margined Guan *P. superciliaris* in reforested areas (Pereira and Wajntal 1999). However, the success of establishing a viable population in the wild by reintroducing captive-bred birds with low genetic variability is unpredictable (Rave *et al.* 1994, Rave 1995, Tegelstrom and Sjoberg 1995). These birds and their descendants would appear to be limited in their ability to adapt to habitat and climatic variations and moreover it seems unlikely that natural selection could improve this condition because of the genetic uniformity of this population. Thus, the progeny of the 12 birds with both variable contributions from the Alagoas Curassow genome and with a high genetic variability, might represent in the future an important contribution to the reintroduction programme, in the same way that the introgression of foreign genetic contribution seems to have been important for preserving the Florida panther genome (Roelke *et al.* 1993).

Table 2. Band-sharing coefficient for all possible pairs among the 20 birds studied obtained with human minisatellite probe 33.15. Males are in the first row and females in the first column. The last two numbers of each bird identification correspond to the year they were born and ZMN stands for Zoobotânica Mário Nardelli.

FEMALES\ MALES	03ZMN84 *	02ZMN89 *	09ZMN91 †	07ZMN91	02ZMN92 ‡	04ZMN93	01ZMN93	03ZMN94 †	01ZMN95 ‡	01ZMN98
02ZMN87*	0.466	0.687	0.642	0.384	0.482	0.400	0.344	0.592	0.206	0.347
08ZMN91	0.358	0.390	0.432	0.285	0.421	0.352	0.315	0.333	0.315	0.312
06ZMN92†	0.533	0.562	0.642	0.307	0.344	0.400	0.413	0.592	0.275	0.434
03ZMN92†	0.555	0.421	0.647	0.187	0.400	0.387	0.342	0.424	0.285	0.344
04ZMN92‡	0.296	0.413	0.320	0.173	0.384	0.272	0.153	0.250	0.307	0.500
02ZMN96	0.266	0.437	0.428	0.461	0.413	0.240	0.275	0.518	0.344	0.173
04ZMN96	0.333	0.321	0.285	0.461	0.758	0.320	0.344	0.296	0.551	0.434
07ZMN95	0.214	0.200	0.230	0.416	0.592	0.173	0.370	0.320	0.384	0.285
01ZMN97	0.193	0.303	0.206	0.296	0.400	0.384	0.466	0.357	0.466	0.250
04ZMN98†	0.275	0.580	0.444	0.320	0.285	0.166	0.285	0.615	0.214	0.272

* Birds born before 1990 (pure).
† Birds with band-sharing coefficients < 0.5 with the three pure birds.
‡ Birds with mtDNA sequence identical to the Razor-billed Curassow.

Habitat availability for reintroduction

During September and October 2001 one of us (LFS) and field ornithologist Fábio Olmos conducted surveys in forest fragments in Alagoas State. The objective was to assess the conservation status of the selected fragments, search for some north-eastern Atlantic Forest endemics and try to gather evidence of the survival of Alagoas Curassow in the wild.

The results (Silveira and Olmos unpublished data) showed that most remnants of forest are small and are restricted to steep valleys, which have proven to be unsuitable for sugar cane plantations, or have been preserved intentionally to keep water resources intact. However, intensive hunting and selective logging are common in these remaining forest fragments. Despite their efforts, no evidence of the existence of Alagoas Curassow was found.

However, some fragments of mature forest were discovered, the best conserved being that located at Fazenda Petrópolis in Usina Santo Antônio, a sugar cane plantation. This fragment has many freshwater creeks and fruit trees and would seem to be a suitable habitat that should be considered for a reintroduction programme for Alagoas Curassows.

A reintroduction programme, however, takes time to be effectively implemented. The owners of the selected areas (despite being private or government owned, e.g. state parks or reserves) must guarantee real protection, and an educational programme must be implemented involving the local population, before one can begin a reintroduction programme. The reintroduction of cracids in Brazil has been successful previously (Pereira and Wajntal 1999, R. Azeredo pers. comm.), and one can use these examples as an incentive for the re-establishment of a population of the Alagoas Curassow in its original habitat.

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