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Estimates of the genetic variability in a natural population of Bare-faced Curassow *Crax fasciolata* (Aves, Galliformes, Cracidae)

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Summary

Most of Brazil's electricity is generated by hydroelectric power plants that require the flooding of huge areas and affect the stability of local ecosystems. The area of the Porto Primavera power dam was flooded in 1998 and a rescue programme was executed to save the fauna sheltering on treetops or emerging islands. Using DNA fingerprinting we estimated the genetic variability in a sample of 23 Bare-faced Curassow *Crax fasciolata* rescued in this area and found that the mean heterozygosity was $H = 0.89$. Although similar data on other natural populations of cracids is not available, the mean heterozygosity observed in the present sample is in the range found for free-living populations of two species of reintroduced cracids whose origin was captive breeding of a limited number of founders. We suggest that a collaborative captive breeding and reintroduction programme between the facilities holding the birds caught at Porto Primavera should start immediately to avoid the loss of genetic variability due to the small number of founders in captivity. Habitat protection, responsibility in fauna management and measures that prevent or correct the isolation of habitat fragments are needed to establish an equilibrium between progress and conservation in developing countries.

Introduction

The IUCN/SSC Cracid Specialist Group (Brooks and Strahl 2000) states that ''cracids may be the most important and most threatened family of birds in the Americas''. The importance of this family lies in its keystone role in regeneration of tropical forests through seed dispersal (by some guans of the genus *Penelope*), and control of plant density through seed predation (by some curassows of the genera *Crax* and *Mitu*). They also have a high sensitivity in relation to habitat quality, thus being significant tools in habitat regeneration and monitoring. Cracids also represent an important protein source for local populations of forest-dwelling humans. Due to habitat destruction and over-hunting, half of the 50 species are threatened (Collar *et al.* 1992).

Bare-faced Curassow *Crax fasciolata* is one of the most commonly kept curassows in zoological gardens around the world. There are several reports of successful captive breeding of the nominate form, from zoos and private breeding facilities in Germany, the U.K., Belgium and France (Delacour and Amadon 1973). In Brazil, captive breeding of this species has been achieved in several facilities, the first being in 1935 (Nogueira Neto 1973).

In the wild, this species inhabits riverine forests and woodland edges from the southern Amazon (from the Tapajós River, state of Pará to the State of Maranhão) across central Brazil to western São Paulo, Paraná, and Minas Gerais, as well as Paraguay, Argentina and Bolivia (Delacour and Amadon 1973, Sick 1993). Three races of Bare-faced Curassow are recognized and a fourth one suggested (Nardelli 1993). *C. f. fasciolata* inhabits Central Brazil, from south of the Amazon River to the western states of São Paulo and Minas Gerais, and in Paraguay and Argentina. The Amazonian form *C. f. pinima* is considered to be critically endangered (Brooks and Strahl 2000) and might be extinct in most of its former range, although reasonable numbers could be found in the forests of the Pindoré River in Maranhão in 1977 (Sick 1993). The third subspecies, *C. f. grayi*, inhabits the Bolivian part of the species's range. The existence of a fourth form, *C. f. xavieri* in Mato Grosso do Sul, Brazil (Nardelli 1993) has not been officially confirmed.

Although Bare-faced Curassow is considered an intermediate conservation priority, most of its range lies within an area of high agricultural activity, with high disturbance of riverine forest due to dam and hydroelectric power plant construction. In Brazil, 88% of electricity is generated from hydroelectric power plants (Federative Republic of Brazil 2000).

Construction of the Porto Primavera hydroelectric power plant began in the 1980s in the Paraná River (Figure 1), the most important tributary of the Prata Basin. After completion in the late 1990s, its total flooded area was 2,250 km^2, 80% of this area lying in Mato Grosso do Sul and 20% in São Paulo. During the flooding process a rescue programme was performed by the staff of CESP (Companhia Energética de São Paulo) and between December 1998 and October 1999 a total of 140 Bare-faced Curassows *C. f. fasciolata* were recovered from the remaining emerging islands and treetops. Excluding the area originally occupied by the watercourse, at the time of the rescue the flooded area was 1,450 km^2.

Sixty six (47%) of the rescued birds were released in nearby areas. The rest were transferred to zoological gardens or breeding facilities. Among the captured birds, seven were juveniles, 128 were adult birds and five birds were not aged. There were 75 males, 63 females, and the sex of two birds was not recorded.

In April 2000 we received blood samples from 14 males and nine females that were rescued between December 1998 and January 1999 and transferred to the breeding facilities at CESP Paraibuna, state of São Paulo, in March 1999. The Paraibuna staff had previously been involved with captive breeding and reintroduction of other cracid species into reforested areas (Pereira and Wajntal 1999). To our knowledge the present work is the first estimate of the genetic variability of a natural cracid population.

Material and methods

Blood samples were taken from 23 wild (14 males and nine females) Bare-faced Curassows rescued from Porto Primavera. The samples were kept in absolute ethanol at room temperature. Multilocus DNA fingerprints were obtained according to the detailed protocol reported in Bruford *et al.* (1992) and applied before in cracids (e.g. Pereira *et al.* 1996). Briefly, 4 μg of genomic DNA from each bird was completely digested with 15 units of *Mbo*I for a period of 16–18 hours. The fragments were electrophorized through a 30-cm-long horizontal 1%

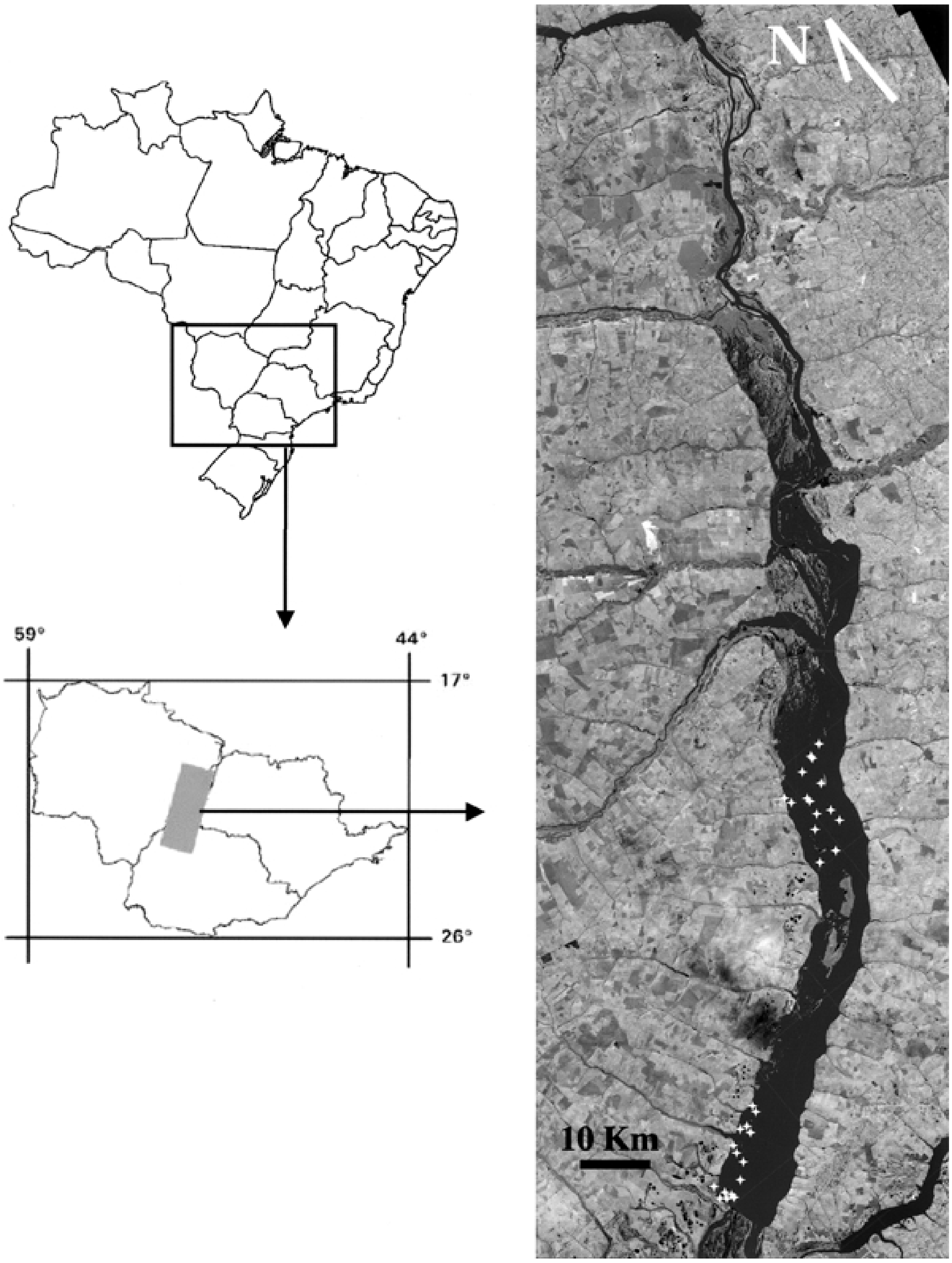

Figure 1. Location of the study area and satellite image of the Porto Primavera Dam, Brazil (Landsat 7/ETM+5/TM images(WRS 223/074-03.12.2000; WRS 223/075–08.29.1999; WRS 223/076–06.03.1999) at the first stage of flooding. The GPS coordinates, available for some of the rescued birds, were plotted on the image(stars).

agarose gel, until the 2.3 kilobase (kb) λ-*Hind*III molecular marker loaded in the first lane had migrated to the bottom of the gel. One of the cracid DNA samples was loaded in the second and in the last lane of the gel to estimate band migration distortion. 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 probes 33.6 and 33.15 (Jeffreys *et al.* 1985a) were 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 33.6 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 minutes each. Autoradiographs were obtained after one to three days of exposure at –80 °C using X-ray film and two intensifying screens. The membrane was dehybridized with 0.25M NaOH for 10 minutes at 45 °C, and with 0.1%SSC/1% SDS for 30 minutes at 45 °C, and a new hybridization in identical conditions was performed with probe 33.15.

Bands were marked on acetate overlays according to Westneat (1990). The band-sharing coefficient, 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 is therefore given by 1 minus the band-sharing coefficient. Assuming that the bands scored are independent markers, the mean probability that all *n* bands in an individual's fingerprint are present in a second random unrelated individual can be conservatively estimated as $< X_n$ (Jeffreys *et al.* 1985b, Bruford *et al.* 1992). Mean heterozygosity (H) was estimated as $H = 2q(1–q)/(2q–q^2)$ (Sundt *et al.* 1994), where q is the mean allelic frequency of bands estimated from the similarity index as $x = 2q–q^2$ (Jeffreys *et al.* 1995b).

Results

DNA fingerprinting profiles obtained here with human multilocus minisatellite probes 33.6 and 33.15 for Bare-faced Curassow were polymorphic, and each bird could be easily identified by its unique pattern of bands. Only scorable fragments in the range of 3.0 to 11.0 kb for probe 33.6 and in the range of 2.8 to 12.5 kb for probe 33.15 were analysed. Band distortion was not larger than 0.2 mm as measured by the duplicate loads of one of the birds.

An average of 15 bands was observed for each probe. The mean band-sharing coefficient was 0.345±0.11 for probe 33.6 and 0.35±0.11 for probe 33.15. The probability of two unrelated birds having the same DNA fingerprint profile was 1.16×10^{-7} and 1.44×10^{-7}, for probes 33.6 and 33.15, respectively. Mean heterozygosity of the studied population was estimated as H = 0.89.

All juveniles rescued were not associated with any adult bird and were treated as independent individuals. Thus, the ratio of rescued males to females was not significantly different from 1:1 (63 females, 75 males, two birds of unreported sex). We considered the 140 rescued birds as 70 potential pairs occupying an area of 1,450 km^2, i.e. there was at least one pair of Bare-faced Curassow for every 20.7 km^2 prior to the flooding process. The real density could have been higher, but no data on how many birds left the area or died during the flooding process were available.

Discussion

Population densities for other Cracidae species given previously have included 1–6 Rusty-margined Guans *Penelope superciliaris* per 10-km transect in different fragments of the Atlantic forest in the western state of São Paulo, Brazil (Cullen *et al.* 2000) and 6–8 Great Curassows *Crax rubra* on a 45-km transect in Tikal, Guatemala (Donegan 2001). Moreover, Gonzalez (1999) observed cracids in the north-eastern Peruvian Amazon and estimated densities per square kilometre to be 5.22 Razor-billed Curassows *Mitu tuberosa*, 3.38 Blue-throated Piping-guans *Pipile cumanensis*, 2.47 Speckled Chachalacas *Ortalis guttata*, and 1.87 Spix's Guans *Penelope jacquacu*. A higher density for the latter species (8.2 birds/km^2) was found in Amazonian Ecuador by Johnson (1993), who also reported densities for Salvin's Curassows *Mitu salvini* of 3.8 and 1.6 birds/per km^2, in areas with low and high hunting pressures, respectively. Our estimates of occupation density for Bare-faced Curassow may not be too divergent from the mean area occupied per pair before the flooding process, considering that (1) most of the flooding occurred very quickly; (2) this species is considered to be site-faithful; and (3) it normally takes a considerable time to observe a pair, or detect them through their vocalizations during field observations.

Genetic variability estimates using the same procedure as reported here have been performed in six species belonging to four different genera of cracids: Black-fronted Piping-guan *Pipile jacutinga* (Pereira *et al.* 1996), Dusky-legged Guan *Penelope obscura* and Rusty-margined Guan *P. superciliaris* (Pereira and Wajntal 1999), and Nocturnal Curassow *Nothocrax urumutum* and Red-billed Curassow *Crax blumenbachii* (Pereira and Wajntal 2001). Most of the previously reported results refer to captive-bred birds, with the exception that some birds came from the wild but were of unknown origin. The mean heterozygosity estimates for the different species varied, with H = 0.96 in Red-billed Curassow from one breeding facility to H = 0.75 in a second highly inbred stock of the same species. The highest variability was interpreted by the authors as indicative of variable origins of the wild-caught birds present in this first breeding facility. Red-billed Curassow is considered as endangered and is only rarely found in three different forest reserves in Brazil (Collar *et al.* 1992, Brooks and Strahl 2000), so remnant populations constitute true isolates that might have been brought together in captivity.

Our data on the population of Bare-faced Curassow rescued from Porto Primavera resulted in a mean similarity index of 0.35 for this population (H = 0.89), close to the values estimated for populations of two species of *Penelope* that have established in reforested areas after the reintroduction of birds born in captivity and originated from a small number of founders (H = 0.90; H = 0.89). Thus, it is probable that the population studied from Porto Primavera has lost part of its original variability during the process of population size reduction related to habitat reduction during the last few decades.

Although Bare-faced Curassow is not considered an endangered species, a captive-breeding programme for these birds is planned in order to provide birds to be released as soon as the area has been restored. This is part of the conservation plan undertaken by CESP, the State of São Paulo hydroelectric company responsible for the construction of the dam.

Cracids are known to have potential to breed in captivity (Nogueira-Neto 1973, Pereira *et al.* 1996, Pereira and Wajntal 1999) and thus the establishment of a programme of captive breeding for Bare-faced Curassow would probably achieve success. The success of this programme would also depend on other issues such as an education programme involving the human communities around the release areas, and recovery of the original habitat and monitoring of the released birds, being conducted together in a multidisciplinary way.

Caution should be taken in order to assure equal contribution of all founder couples to the planned reintroductions, in order to avoid loss of genetic variability typically found in wild-established populations of Red-billed Curassow, and Dusky-legged and Rusty-margined Guans. However, the reduced genetic variability did not seem to have a negative impact on the success of the reintroduction programmes for these species (Azeredo 1996, Pereira and Wajntal 1999).

It is well known that genetic drift and relaxed selection alter the allele frequencies in captive populations in a few generations due to the small number of founders, leading to a higher inbreeding coefficient. These changes in allele frequencies can eliminate alleles that provide better fitness for survival in the wild (Lacy 2000). To avoid this problem we strongly suggest two simple procedures to help maintain the genetic variability of Bare-faced Curassow as estimated in Porto Primavera: (a) the reintroduction must start immediately while the captive population held at CESP facilities harbours the genetic variability of the wild population from which it came; and (b) those birds collected in Porto Primavera that are now being held at zoos and private breeding facilities should also be used in an integrated conservation programme, by contributing offspring to be released in the area.

Our data suggest that the population of Bare-faced Curassow studied might be isolated from other populations of the same species and that other taxa that inhabit this area might be affected in a similar way. Habitat fragmentation in the study area has resulted from agricultural activities; many of what seem to be the largest forest fragments are in fact areas cultivated with exotic eucalyptus trees (Figure 1). The establishment of protective measures is therefore an urgent matter and establishment of appropriate contact zones between similar forest fragments should be a high priority. This may result in better prospects for all the faunal elements that remain in this highly disturbed ecosystem.

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