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Comparative Genetic Diversity of Wild and Captive Populations of the Bare-Faced Curassow (*Crax fasciolata*) Based on Cross-Species Microsatellite Markers: Implications for Conservation and Management

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Abstract The bare-faced curassow (*Crax fasciolata*) is a large Neotropical bird that suffers anthropogenic pressure across much of its range. A captive population is maintained for conservation management, although there has been no genetic screening of stocks. Based on the six microsatellite markers developed for *Crax globulosa*, the genetic variability of *C. fasciolata* and possible differences between a wild and a captive population were investigated. Only three loci were polymorphic, with a total of 27 alleles. More than half of these alleles were private to the wild ($n = 8$) or captive ($n = 7$) populations. Significant deviations from Hardy–Weinberg equilibrium were restricted to the captive population. Despite the number of private alleles, genetic drift has probably promoted differentiation between populations. Our results indicate that wild *C. fasciolata* populations are genetically impoverished and structured, but species-specific microsatellite markers will be necessary for a more reliable assessment of the species' genetic diversity.

Keywords Bare-faced curassow · *Crax fasciolata* · Population genetics · Microsatellites · Conservation

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Introduction

The curassows (Cracinae) are large-bodied (adult body weight up to 4.3 kg), turkey-like birds distributed throughout the tropical and subtropical forests of South and Central America. As frugivores and seed dispersers, they play an important ecological role in Neotropical forests (Silva and Strahl 1991), but because of their body size, they are frequently targeted by hunters.

The bare-faced curassow (*Crax fasciolata*) inhabits moist lowland or dry forests in eastern-central and southern Brazil, Paraguay, eastern Bolivia, and the northeastern extreme of Argentina (Sick 1997). This range includes part of the southern Amazon basin, the Pantanal swamps, and the Cerrado savannas of the central Brazilian plateau. Although the species is not considered threatened at the present time (IUCN 2008), primarily because of its ample distribution, hunting pressure and habitat loss have contributed to its disappearance from many areas in which it was once abundant (Sick 1997). At least one subspecies, the Amazonian endemic *C. f. pinima*, is considered endangered in Brazil (Silveira 2008).

The Crax Foundation (*Fundação Crax*), based in the Brazilian state of Minas Gerais, has established a captive population of *C. fasciolata* as a source of individuals for reintroductions in areas that have been affected by hunting in the past. The species has been reared successfully in captivity for many years (Nogueira Neto 1973; Delacour and Amadon 2004), although there has been no genetic screening or management of captive populations, despite their small size, which is potentially problematic (see Gilpin and Soulé 1986; Frankham et al. 2002). Inbreeding in small populations may lead to a rapid loss of genetic variability and thence heterozygosity, and a putative increase of juvenile mortality (Allendorf and Leary 1986; Ralls and Ballou 1983; Ralls et al. 1988).

Little is known of the genetic characteristics of most species, however, and even less of the effects of anthropogenic impacts on the variability of wild populations. Such knowledge can be vital to the administration of captive populations and is fundamental to the success of metapopulation management strategies, including the reintroduction of captive-bred individuals. It seems likely that reintroduction will be more successful when the genetic variation of the captive population is similar to that found in natural populations (Hedrick et al. 1986; Frankham et al. 2002), as found in both plants (Martins and Jain 1979) and fish (Leberg 1990).

With these potential problems in mind, the genetic variability of *C. fasciolata* was investigated based on the six microsatellite markers developed for the endangered wattled curassow (*Crax globulosa*) by Hughes and Larson (2000). In addition to the characterization of the genetic variability of the species, possible differences between two populations (one wild and one captive) were assayed with the aim of developing genetic management protocols.

Materials and Methods

Two *Crax fasciolata* populations were sampled here. One is a wild population from the Porto Primavera Reservoir (22°25′S, 52°55′W) on the Paraná River between São

Paulo and Mato Grosso do Sul in southeastern Brazil. This reservoir was flooded in 1999, when blood samples were collected from 23 rescued *C. fasciolata* individuals (Pereira and Wajntal 2001). The original population of the area probably numbered in the thousands (Pereira and Wajntal 2001). The second population is in captivity at the Crax Foundation in Contagem, Minas Gerais, Brazil. Blood samples were collected from 30 individuals in 2003, representing about half the population. These individuals were donated to the institution from zoos, breeding centers, and confiscations; although their origins were unknown, they were almost certainly derived from a number of different populations. In both cases, the samples were maintained in absolute ethanol and DNA extraction followed the standard protocol of Sambrook et al. (1989).

Due to a lack of specific markers for *C. fasciolata*, polymerase chain reactions (PCR) were performed on the six microsatellite loci available for *C. globulosa* (Hughes and Larson 2000). The PCRs were performed in a volume of 20 µl containing 5–10 ng genomic DNA, 50 mM KCl, 1.5–2.5 mM $MgCl_2$, 10 mM Tris–HCl, 20 µM each dNTP, 0.5 µM each primer, and 1 U *Taq* DNA polymerase (Invitrogen). The reactions had the following protocol: 5 min at 94°C for denaturation, followed by 30 denaturation cycles of 30 s at 94°C, annealing for 30 s at 55–65°C (see Hughes and Larson 2000), extension for 1 min at 72°C, and a final cycle of 5 min at 72°C. Analysis of the amplified fragments was conducted in an ALFexpress II automatic DNA processor using Allelinks version 1.0 software (Amersham Biosciences) after 6% polyacrylamide gel runs.

Intrapopulational genetic diversity was quantified according to allele frequency, mean number of alleles per locus (*Na*), and observed (H_o) and expected (H_e) heterozygosity under Hardy–Weinberg equilibrium. These values were calculated using Popgene 32 (Yeh et al. 1997). Allelic richness (*Rs*) was estimated using Fstat version 2.9.3.2 (Goudet 2001). Private alleles (occurring in only one population) were identified by comparing populations. The significance of between-population differences in *Na*, *Rs*, and H_o was evaluated using Wilcoxon's signed-rank test. Unbiased estimators of exact significance probabilities were generated to evaluate deviations from Hardy–Weinberg expectations, using a Markov chain (5,000 dememorizations, 1,000 batches, and 10,000 iterations per batch) performed in Genepop software (Raymond and Rousset 1995). Significance was calculated per locus and per population.

The significance of differences between populations was examined using the exact test of population differentiation proposed in Genepop (Raymond and Rousset 1995), based on allele frequency variation. Considering allele size frequency in the two populations, we assumed a stepwise mutations model (Slatkin 1995) as being more conservative than an infinite alleles model (Kimura and Crow 1964). Given this, genetic differentiation between populations was also evaluated by calculating Slatkin's R_{st} (Slatkin 1995), an analog of Wright's (1969) F_{st}, adapted to microsatellite loci by assuming a high-rate stepwise mutation model. This analysis was conducted in the RST Calc package (Goodman 1997) with 1,000 iterations for both bootstrap and permutation tests.

Results

Amplification of the two loci CgAAT62 and CgAAT82 failed for all samples, even though both loci were polymorphic in *C. globulosa* (Hughes and Larson 2000). A third locus, CgAAT11, returned only a single allele (228 bps). Three other loci (CgAAT32, CgAAT85, and CgAAT190) were polymorphic and provide the basis for the analysis of genetic variation. A total of 27 different alleles were recorded, with 6–13 alleles per locus and 4–10 alleles recorded at a given locus per population. The two populations were very similar in terms of the number of alleles recorded at each locus, although more than half of the alleles (15) were private (Table 1).

Values of *Na*, *Rs,* and H_o were also relatively similar between populations (Table 2), and there were no significant differences between them ($P = 0.89$). No significant deviations from Hardy–Weinberg equilibrium were recorded in the wild population, either for individual loci or overall. In the captive population, by contrast, only one locus, CgAAT32, was in equilibrium, even after correction using the Bonferroni procedure (Rice 1989).

There is a clear pattern of differentiation between populations, based on the number of private alleles (Table 1), significant overall levels ($P < 0.001$) of population differentiation based on the variation in allele frequencies and significant overall genetic differentiation ($R_{st} = 0.16$, $P < 0.05$). In addition, two significant

Table 1 Allele frequencies for three polymorphic microsatellite loci in two Brazilian populations of *Crax fasciolata*

Locus								
CgAAT32			CgAAT190			CgAAT85		
Size (bp)	Relative frequency		Size (bp)	Relative frequency		Size (bp)	Relative frequency	
	Captive	Wild		Captive	Wild		Captive	Wild
140	**0.0500**		180		**0.1087**	187		**0.3913**
146	**0.1500**		186	**0.0333**		199	**0.1167**	
149	**0.0833**		192	0.0500	0.1304	202	0.2000	0.3478
152	0.0167	0.0217	195	0.1667	0.1087	205	0.6333	0.2174
155		**0.0652**	198	0.4167	0.5870	208	**0.0500**	
158	0.0167	0.4348	201	0.3333	0.0217	211		**0.0435**
161	0.0667	0.0217	207		**0.0217**			
164	0.3333	0.0870	216		**0.0217**			
167	0.1667	0.1304						
170	0.0667	0.1087						
173		**0.0435**						
176		**0.0870**						
185	**0.0500**							

Note: Values in bold type are for private alleles

Table 2 Intrapopulational variability in captive and wild Brazilian populations of *Crax fasciolata*

Population (sample size)	Locus	*Na*	*Rs*	H_o	H_e	HWE
Captive ($n = 30$)	CgAAT32	10	9.51	0.87	0.82	0.7807
	CgAAT190	5	4.94	0.77	0.68	0.0167
	CgAAT85	4	3.99	0.37	0.54	0.0016
	All[a]	6.33 (3.22)	6.15 (2.95)	0.67 (0.26)	0.68 (0.14)	0.0015
Wild ($n = 23$)	CgAAT32	9	9.00	0.78	0.76	0.5550
	CgAAT190	7	7.00	0.61	0.61	0.1741
	CgAAT85	4	4.00	0.52	0.68	0.5806
	All[a]	6.66 (2.52)	6.60 (2.52)	0.64 (0.13)	0.68 (0.07)	0.4504

Note: *Na* number of alleles, *Rs* allelic richness rarefied to 23 individuals, H_o observed heterozygosity, H_e Nei's (1973) expected heterozygosity, *HWE P* value for Hardy–Weinberg equilibrium exact test

[a] Standard deviation in parentheses

linkage disequilibria ($P < 0.05$, following Bonferroni adjustment) were observed in the captive population.

Discussion

Genetic Diversity and Differentiation

Despite their other differences, the two populations were remarkably similar in their genetic diversity, although this appears to be largely coincidental, considering the great differences in the history of the two populations. Heterozygosity levels in both populations of *C. fasciolata* were also very similar to those recorded in a population of the endangered wattled curassow, *Crax globulosa* (Hughes and Larson 2000).

The present study further corroborates the findings of Pereira and Wajntal (2001), who analyzed the same wild population of *C. fasciolata* using DNA fingerprinting techniques and found levels of genetic diversity similar to those of two reintroduced guan (*Penelope* spp.) populations established from a small number of captive founders. This evidence suggests that the Porto Primavera population may have lost some of its original genetic variation as a result of habitat loss and population decline over the past few decades. Despite its varied origins, the low genetic diversity recorded in the captive population may reflect similar processes on a wider scale throughout Brazil.

The results of the present study certainly do not indicate that the samples were taken from a large panmictic population, as implied by the relatively ample geographic distribution and potentially large effective population size of the species. The significant differentiation between the two populations, in particular the number of private alleles, is probably related to factors such as habitat fragmentation and the breeding system of the species, which is considered to be site-faithful (Pereira and Wajntal 2001). Given this, genetic drift has probably played an important role in the loss of genetic diversity and in the differentiation between populations.

Unfortunately, with the exception of the study of Hughes and Larson (2000) for *C. globulosa*, no data are available on the intraspecific diversity of cracines based on microsatellite markers. In the present case, the number of alleles, which is a more sensitive measure of genetic diversity than heterozygosity, compares favorably with *C. globulosa*, at least in the loci CgAAT32 and CgAAT190, for which only six alleles were recorded for each locus in *C. globulosa*, in a sample of similar size ($n = 23$) (Table 2).

Hardy–Weinberg and Linkage Disequilibria

Deviations from Hardy–Weinberg equilibrium are often related to inbreeding in small and isolated populations, although they may also be related to factors such as the presence of null alleles (Callen et al. 1993) or the admixture of different populations or demes, the Wahlund effect (Wahlund 1928). Null alleles are relatively common in studies using heterologous markers, but the lack of markers specific to *C. fasciolata* hampers the detection of possible deficiencies in heterozygotes. However, considering that amplification was invariably successful in this study, and that Hardy–Weinberg disequilibrium was restricted to the captive population, it seems reasonable to conclude that null alleles were either extremely rare or, more probably, absent.

These results indicate that the captive population is derived from several geographically distinct and genetically impoverished populations, which is consistent with its history. The linkage disequilibria observed in the captive population appear to corroborate the admixture hypothesis. It seems unlikely that it would have been caused by physical linkage, given that no linkage disequilibrium was observed at any locus in the wild population.

Implications for Conservation

Good working knowledge of the genetic variability of a species is fundamental to the development of effective conservation strategies, as demonstrated by the golden lion tamarin (*Leontopithecus rosalia*) conservation program (Frankham et al. 2002), which relies on a large captive population with reduced inbreeding levels through careful genetic management (two-thirds of this population were derived from a single breeding pair) for reintroductions.

As proposed management of *C. fasciolata* also includes the reintroduction of captive-born individuals, similar knowledge of the genetic variability of both captive and wild populations may help guarantee the success of conservation programs. The relatively modest diversity and the large proportion of private alleles recorded here point to the existence of locally structured populations, which will need to be diagnosed adequately, given that gene flow may also constrain local adaptation (Slatkin 1987) and may cause a reduction in fitness related to outbreeding depression, through which coadapted gene complexes are lost or altered (Shields 1982; Templeton 1986). Given this, the analysis of additional, geographically diverse populations should be a main priority for future research.

Although the polymorphism observed in the loci CgAAT32, CgAAT85, and CgAAT190 is useful for the screening of individuals, our results also indicate the need for the development of species-specific microsatellite markers to ensure a more reliable assessment of genetic diversity in *C. fasciolata*. The analysis of additional markers may provide important alternative insights into the genetic and geographic diversity of the species. These data will be essential for the development of an effective captive breeding program and for the establishment of protocols for the translocation and reintroduction of individuals between populations.

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