Research Article

Genetic evaluation of the mating system in the blue-and-yellow macaw (*Ara ararauna*, Aves, Psittacidae) by DNA fingerprinting

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Abstract

More than 90% of birds are socially monogamous, although genetic studies indicate that many are often not sexually monogamous. In the present study, DNA fingerprinting was used to estimate the genetic relationships between nestlings belonging to the same broods to evaluate the mating system in the socially monogamous macaw, *Ara ararauna*. We found that in 10 of 11 broods investigated, the nestlings showed genetic similarity levels congruent with values expected among full-sibs, suggesting that they shared the same parents. However, in one brood, the low genetic similarity observed between nestlings could be a result of intraspecific brood parasitism, intraspecific nest competition or extra-pair paternity. These results, along with available behavioral and life-history data, imply that the blue-and-yellow macaw is not only socially, but also genetically monogamous. However, the occurrence of eventual cases of extra-pair paternity cannot be excluded.

Key words: Ara ararauna, breeding behavior, DNA fingerprinting, monogamy, Psittacidae.

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Introduction

Even though more than 90% of birds are socially monogamous (Lack, 1968), genetic studies showed that extra-pair paternity (EPP) is found in approximately 90% of species studied (Griffith *et al.*, 2002). Even among socially monogamous species, over 11% of the offspring are the result of EPP (Griffith *et al.*, 2002). The occurrence of EPP is so widespread that recent research has tried to identify factors explaining its apparent absence in some species (Arnold and Owens, 2002; Griffith *et al.*, 2002).

Although differences in life history and contemporary ecological factors have been proposed to account for interspecific variation in the level of EPP, there has been relatively little success in identifying robust biological correlates of this interspecific variation (Birkhead and Møller, 1996; Bennett and Owens, 2002). Phylogenetic analysis of EPP data has shown that more than 50% of interspecific variation can be explained by phylogeny (Arnold and Owens, 2002; Bennett and Owens, 2002; Griffith *et al.*, 2002), thereby implying that differences in EPP rates are likely to have been determined in the ancient evolutionary history of avian lineages (Griffith *et al.*, 2002). However, the relative importance of phylogenetic constraints on EPP incidence remains largely unknown (Kingma *et al.*, 2009).

The degree of paternal care appears to be a key predictor of genetic monogamy (Mulder *et al.*, 1994; Birkhead and Møller, 1996; Gowaty, 1996). Females that can rear offspring with little or no paternal care are likely to seek extra-pair copulations, whereas, whenever paternal care is essential, EPP rates tend to be low (Griffith *et al.*, 2002).

Another ecological factor that has been suggested to explain interspecific variation in the rate of EPP is the adult mortality rate (Mauck *et al.*, 1999; Wink and Dyrcz, 1999). According to Mauck *et al.* (1999), abandonment of a reproductive event can be nonadaptive in species with short reproductive lifespans, even in the face of extreme uncertainty of paternity. This is possibly so because a new reproductive event may be very unlikely to occur. It seems that longevity explains 25% of the variation in EPP rates (Arnold and Owens, 2002; Griffith *et al.*, 2002).

In some non-passeriform groups of birds that display male parental care and long reproductive lifespans, such as Sphenisciformes (*e.g.* Moreno *et al.*, 2000), Procellariiformes (Quillfeldt *et al.*, 2001), Strigiformes and Falconiformes (Muller *et al.*, 2001), EPP seems to occur

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infrequently or may even be entirely absent. These studies indicated that the species studied are both socially and genetically monogamous.

Psittaciformes (parrots and cockatoos) comprise another group of long-lived birds with high levels of parental investment, wherein most species are considered to be socially monogamous (Forshaw, 1989; Sick, 1997). Data from genetic analysis of the few so far studied, indicate that all those with this characteristic appear to be genetically monogamous (Caparroz *et al.*, 2001; Masello *et al.*, 2002; Ekstrom *et al.*, 2007; Taylor and Parkin, 2009). Thus, further analysis of other socially monogamous parrot species could contribute towards a better understanding, not only of the general genetic mating system in these non-passerine birds, but also the contribution of evolutionary and ecological factors determining EPP rates.

In the present work we used DNA fingerprinting to estimate the genetic relationship between nestlings in the same brood, in order to gather evidence of EPP in a socially monogamous species this being the blue-and-yellow macaw (*Ara ararauna*).

Material and Methods

Blood samples (0.1 mL) were collected from the brachial vein of 22 nestlings that had been found in 11 natural nests, thus two chicks per nest, at three localities in central Brazil (Table 1). Unfortunately, it was not possible to sample their putative parents, through the extreme difficulty and stress involved in capturing adults. The samples were stored at -20 °C in 100% ethanol. Total genomic DNA was extracted from blood incubated overnight at 37 °C in a solution containing 0.1% SDS, 100 mM Tris-HCl (pH 8.0), 10 mM NaCl, 10 mM EDTA and 10 mg/mL proteinase K, and subsequently purified using the standard phenol-chloroform-isoamyl alcohol method (Bruford *et al.*, 1992).

Table 1 - Sampling localities in Brazil and band sharing coefficients (x) based on multilocus minisatellite data between nestlings of blue-and-yellow macaw of the same brood (two nestlings per brood).

Locality (abbreviation) latitude/longitude	Nest	x
Peixe, southeastern Tocantins (ST)	P01	0.59
12° S, 48° W	P04	0.73
	P06	0.61
Cavalcante, northeastern Goiás (NG)	C10	0.48
13° S, 47° W	C10B	0.68
	C13	0.57
	C19	0.61
Corginho, central Mato Grosso do Sul (CMS)	A06	0.87
19° S, 54° W	A08	0.22
	A11	0.61
	A13	0.62

Approximately 6 μg of genomic DNA from each nestling were digested overnight with the restriction enzyme *Hae*III at 37 °C. DNA fragments were electrophoresed and then transferred onto nylon membranes by Southern blotting (Sambrook *et al.*, 1989). Each membrane was hybridized with the human multilocus minisatellite probe 33.6 (Jeffreys *et al.*, 1985a), and then 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 autoradiographed at -70 °C, using Kodak RX film with two intensifying screens. Only bands between 4.0 and 23.0 kb were analyzed, as described by Westneat (1990). Bands sharing coefficient between individuals (index of similarity) were estimated using the formula: $x = 2N_{AB}/(N_A+N_B)$, where N_{AB} is the number of bands shared between individuals A and B, and N_A and N_B the number of bands present in these same two individuals, respectively (Wetton *et al.*, 1987; Bruford *et al.*, 1992). Only bands with the same electrophoretic mobility (migration distance of band centers within 0.5 mm) between two individuals were considered to be one and the same allele. The frequency (q) of each scorable band was estimated as: $q = 1 - (1-x)^{1/2}$ (Jeffreys *et al.*, 1985b). Assuming the absence of mutations and linkage, the mean expected similarity index between full-sibs was estimated by: $x_i = (4+5q-6q^2+q^3)/[4(2-q)]$ (Jeffreys *et al.*, 1985c). The mean expected similarity index between half-sibs was considered as half of x_i, *i.e.* $x_h = x_i/2$. The statistical significance of the difference in similarity indexes between nestlings of the same brood versus other broods was calculated by using the Mann-Whitney U test as implemented in Statistica 7.1 (StatSoft).

Results

The restriction enzyme/minisatellite probe combination used here produced band patterns that were exclusive to each individual (data not shown). The mean number of bands scored per individual in the three localities ranged from 19.40 ± 5.18 to 23.83 ± 2.79 (Table 2). Band sharing coefficients between nestlings of the same brood varied from 0.48 to 0.88 (Table 1). These coefficients were similar to those expected for full-sibs, and statistically higher than those observed among nestlings from different broods (considered non-related, p < 0.01; Mann-Whitney U test; Table 2). The only exception was a nestling-pair found in nest A08 with a coefficient of 0.22. This value was similar to those observed among nestlings from different broods (considered non-related), and to those expected among half-sibs (Table 2).

Discussion

We found that in 10 of the 11 broods of *Ara ararauna* investigated, nestlings showed genetic similarity levels congruent with those expected between full-sibs, thereby implying that the majority of nestlings of the same brood

Table 2 - Number of nests of *Ara ararauna* (Nnests) analyzed per locality, mean number of bands scored (n), mean band sharing coefficients estimated between nestlings of the same brood (xs) and between nestlings of different brood (xd), the frequency of each scorable band (q) and expected coefficient between full sibs (xi) and half-sibs (xh).

Locality	Nnests	n ± sd	xs ± sd	xd ± sd	q	xi	xh
ST	03	19.40 ± 5.18	0.642 ± 0.074	0.227 ± 0.106	0.121	0.601	0.300
NG	04	22.91 ± 3.91	0.585 ± 0.085	0.206 ± 0.092	0.109	0.591	0.296
CMS	04	23.83 ± 2.79	0.580 ± 0.268	0.229 ± 0.099	0.122	0.602	0.301

sd – standard deviation. Refer to Table 1 for locality abbreviations.

were produced by the same parents. As the actual social parents could not be sampled, it is not possible to rule out the possibility that both exceptional nestlings were the result of either intraspecific brood parasitism or intraspecific nest competition, or even full-brood EPP (with only one mother and one father involved). To further investigate this, it would be necessary to develop a methodology for sampling social parents without causing undue stress.

In contrast, the low genetic similarity observed between nestlings of one brood (nest A08, Table 1) suggests that they were not full-sibs. In this case, both social parents were probably not genetic parents of at least one of the chicks, possibly the result of either intraspecific brood parasitism or intraspecific nest competition, or even, as mentioned, EPP. As discussed previously, it would be necessary to obtain samples from the social parents to test these alternatives. Based on these results, at least 9% of the broods could not be attributed to both social parents. On considering three other previously analyzed broods (Caparroz *et al.*, 2001), this rate decreases to 7%. To date, there are only two reported cases of the presence of nonfull sibs in the same brood, in socially monogamous parrot species, one in the green-winged macaw (*Ara chloropterus*, Caparroz *et al.*, 2001), and the other in the burrowing parrot (*Cyanoliseus patagonus*, Masello *et al.*, 2002). In both cases, the authors suggested that intraspecific brood parasitism (or intraspecific nest competition) was the most likely explanation.

The blue-and-yellow macaw is a long-lived socially monogamous species, with male parental care. These biological traits are assumed to be correlated with low EPP rates (see Introduction). Once successfully fledged from the nest, macaws seem to have a high survival rate (Myers and Vaughan, 2004). Well-documented longevity records for macaws include a specimen of *A. ararauna* which was housed at the Copenhagen Zoo for 46 years (Brouwer *et al.*, 2000). Therefore, the offspring of a single breeding season most likely represents a small proportion of the potential lifetime reproductive production. Any reduction in adult survival of long-lived species by the investment in raising current offspring, exerts a larger influence on lifetime reproductive success than in short-lived bird species (Masello *et al.*, 2002). Thus, long-lived birds, such as macaws, are not expected to invest in broods of doubtful paternity (Mauck *et al.*, 1999). However, the parents in both hyacinth

(*Anodorhynchus hyacinthinus,* N.M.R. Guedes, personal communication) and scarlet (*Ara macao*, D.J. Brightsmith, personal communication) macaws accept nestlings translocated from other broods, *i.e.* the social parents are unable to identify chicks from other pairs. In fact, this behavior, as observed in these two macaws, suggests selective inability in recognizing EPP offspring, thereby implying that EPP could be rare or nonexistent in these species. As *A. macao* and *A. ararauna* are phylogenetically related, it would be expected that *A. ararauna* parents are also unable to distinguish their own biological chicks from unrelated ones that are eventually in their nest.

The low rate of EPP observed in *A. ararauna* may be due to effective male investment in avoiding EPP, as they are strongly committed to parental care. Two main paternity-guarding strategies have been proposed for birds, namely, mate-guarding and frequent within-pair copulation (Birkhead and Møller, 1992). Blue-and-yellow macaws have nearly always been observed flying in pairs or in family groups (Gilardi and Munn, 1998; R. Caparroz, personal observation), except when females are incubating or brooding. Furthermore, males are always observed hanging in close proximity to the nest during the egg-laying period, and seem to follow the female every time she leaves the nest (R. Caparroz, personal observation). This behavior is possibly related to mate-guarding by males.

Other contemporary ecological factors, such as breeding density and breeding synchrony have also been proposed as correlated with EPP rates (see Griffith *et al.*, 2002). However, there is insufficient information on breeding density and synchrony in the blue-and-yellow macaw to permit further analysis. Further investigation of the influence of these factors is required when the relevant data are available. Moreover, since there is possibly a large phylogenetic component in EPP frequency (see Introduction), evolutionary inertia may contribute to the low levels or absence of EPP in this macaw species.

Overall, our genetic results, along with available information on behavior and life history, testify to the blue-and-yellow macaw being socially and genetically monogamous. Nevertheless, the occurrence of full-brood EPP in some of the nests analyzed cannot be excluded. In at least 7% of the broods studied here, one of the nestlings could be a result of nest parasitism or EPP. Access to parent-samples

is essential to confirm our findings, as well as to accurately estimate EPP rates in this species.

Acknowledgments

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