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DNA FINGERPRINTING AND PARENTAGE IN MASKED (*SULA DACTYLATRA*) AND BROWN (*S. LEUCOGASTER*) BOOBIES

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Resumo. – A técnica de "DNA fingerprinting" no estudo de filiação de Atobá-mascarado (*Sula dactylatra*) e o Atobá-marron (*S. leucogaster*). – O Atobá-mascarado (*Sula dactylatra*) e o Atobá-marrom (*S. leucogaster*) são aves marinhas de distribuição pantropical. Estas espécies são monogâmicas nas quais os dois membros do casal investem bastante no cuidado parental. Neste trabalho, observações de campo e a técnica de identificação individual através do DNA ("DNA fingerprinting") foram utilizadas para o estudo do sistema de cruzamentos das duas espécies. Amostras de sangue foram coletadas de indivíduos provenientes de duas colônias reprodutivas da costa brasileira. Treze famílias biológicas do Atobá-mascarado, da colônia reprodutiva do Arquipélago de Abrolhos (Bahia), e vinte e oito famílias do Atobá-marrom, da colônia reprodutiva das Ilhas Moleques do Sul (Santa Catarina), foram estudadas. Não foi encontrada qualquer evidência molecular de fertilizações extra-par ou parasitismo de ninho nas seis famílias de Atobá mascarado analisadas. Tais comportamentos não puderam ser avaliados no Atobá marrom devido à baixa variabilidade genética encontrada.

Abstract. – Masked Booby (*Sula dactylatra*) and Brown Booby (*S. leucogaster*) are pantropical seabirds. These species are socially monogamous. Thus, males and females have a high investment in parental care. In this work, field observations and DNA fingerprinting were applied to investigate the breeding system of both species. Two breeding colonies located on the Brazilian coast were visited. Thirteen Masked Booby biological families, from the breeding colony of Abrolhos Archipelago (Bahia State), and twenty eight Brown Booby biological families, from the breeding colony of Moleques do Sul Islands (Santa Catarina State) were studied. Blood samples were taken for DNA fingerprinting analyses. We did not find any molecular evidence of extra-pair fertilization nor brood parasitism in six Masked Booby families. However, it was not possible to evaluate the occurrence of such behavior in Brown Boobies since the band patterns obtained presented an unexpectedly low level of genetic diversity. *Accepted 4 April 2001.*

Key words: DNA fingerprinting, genetic variability, parentage, *Sula dactylatra, S. leucogaster*.

INTRODUCTION

Monogamy is the most common mating system recorded in birds, being almost universal among seabirds (Lack 1968). This can be due to the biparental care upon the chicks that is essential for the reproductive success. However, a large number of ecological, behavioral and genetic studies show the occurrence of extra-pair fertilizations. The investigation of parentage in monogamous species may be mistaken by the occurrence of intraspecific brood parasitism. Extra-pair copulations have been observed in at least 115 species of monogamous birds (Ford 1983). Thus, this strategy is widespread among birds previously thought to be truly monogamous (Austin & Parkin 1996). Many factors such as high density at breeding sites, genetic variation and sexual conflicts determine the costs and benefits for males and females to engage in extra-pair copulations (Petrie & Kempenaers 1998).

Møller & Birkhead (1991) presented the Paternity Assurance Hypothesis (PAH) which suggests that males may try to ensure the paternity of the offspring in their nest through physically guarding their mate or copulating frequently. Determination of the level of extra-pair paternity is central to the understanding of mating systems and to study other related phenomena such as sexual selection.

The Sulidae are considered as a socially monogamous family with biparental care. These species are long-lived and highly colonial and exhibit fidelity to their natal sites and to their mates. The pairs often remain together but some extra-pair relationships have been reported (Nelson 1978). The Masked Booby (*S. dactylatra*) and the Brown Booby (*Sula leucogaster*) usually produce clutches of two eggs, but rarely rear more than one chick (Anderson 1990).

In the present work, some field observations were made in breeding colonies of Masked and Brown boobies in Brazil and we also applied DNA fingerprinting (Jeffreys *et al.* 1985a, 1985b, 1985c) to study their mating systems. This technique has been used extensively in behavioral ecology to assess paternity and maternity (Gilbert *et al.* 1998, Graves *et al.* 1992) and to estimate genetic variability (Prodöhl *et al.* 1997, Nader *et al.* 1999, Pereira & Wajntal 1999). It is especially useful in species with no other molecular markers available.

METHODS

Two breeding colonies on the Brazilian coast were visited in 1996 and 1997. Thirteen nests of Masked Booby were monitored at the Abrolhos Arquipelago (18°10'S, 39°20'W). Twenty eight Brown Booby nests were studied in Moleques do Sul Island (27°51'S, 48°26'W). The birds were captured with hoop nets and banded. The rings were provided by Centro de Pesquisas para a Conservação das Aves Silvestres (CEMAVE). Blood samples from biological families (putative parents and chick) were collected in both colonies.

The protocols used to obtain multilocus fingerprints have been described in detail elsewhere (Bruford *et al.* 1992). Briefly, 5 μg of genomic DNA from each bird were digested with the restriction enzyme *Hae* III. The fragments were separated by electrophoresis through a horizontal 30 cm long 1% agarose gel at 40V for about 65–72 h. The fractionated DNA fragments were transferred onto a nylon membrane by capillary Southern blotting (Sambrook *et al.*, 1989). The membrane was hybridized with $\propto$ - ^{32}P labeled human multilocus minisatellite probes 33.6 and then with probe 33.15 (Jeffreys *et al.* 1985a, 1985b).

Even though we produced DNA patterns for all individuals, we only analyzed one membrane (hybridized with two minisatellite probes) for each species. The scorable bands on the autoradiographs were analyzed and bands in different individuals were considered to be the same if their migration distances were less than 0.5 mm apart and their intensities were similar (Bruford *et al.* 1992).

Genetic similarity (S) was evaluated using the band-sharing coefficient between two individuals, calculated from the formula: $S = 2N_{ab} / (N_a + N_b)$, where N_{ab} is the number of shared bands, and N_a and N_b are the number of bands in individuals "a" and "b", respectively (Wetton *et al.* 1987). Diversity within species was estimated using the S between adults, which were assumed as non relatives.

In addition, six Masked Booby chicks' profiles were analyzed looking for other puta-

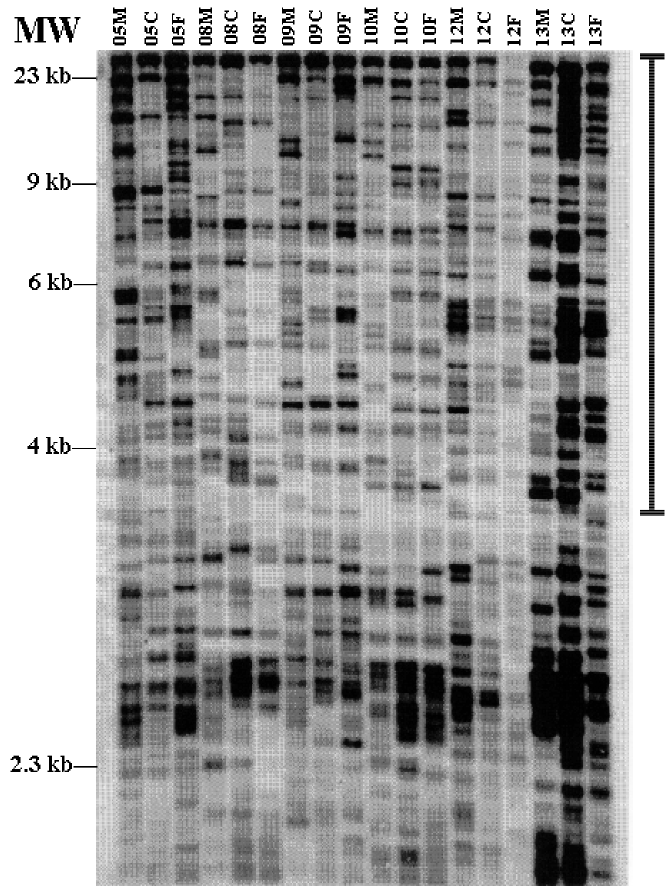


FIG. 1. Multilocus DNA fingerprints of six biological families (5, 8, 9, 10, 12, 13) of Masked Boobies from Abrolhos Archipelago breeding colony obtained with minisatellite multilocus probe 33.6. The bar on the right side indicates the region analyzed . M = putative father, C = chick, F = putative mother.

tive fathers and mothers. This was done assuming that one of the adults was the biological parent and comparing the chick's bands not present in that adult with the band patterns of all the other adults from the opposite sex.

RESULTS

The DNA patterns obtained for both species are shown in Figures 1 and 2. The visual analysis of chicks' profiles showed that all their bands were detected in the putative parents. Since the DNA patterns obtained from the Brown Booby families showed strikingly lower variability (Fig. 2), it was not possible to evaluate the paternity nor the maternity in this species.

The mean number of bands and the mean similarity coefficient (S) between unrelated individuals obtained with probes 33.6 and 33.15 are presented in Table 1. The band sharing coefficients obtained between unrelated and related Masked boobies show a

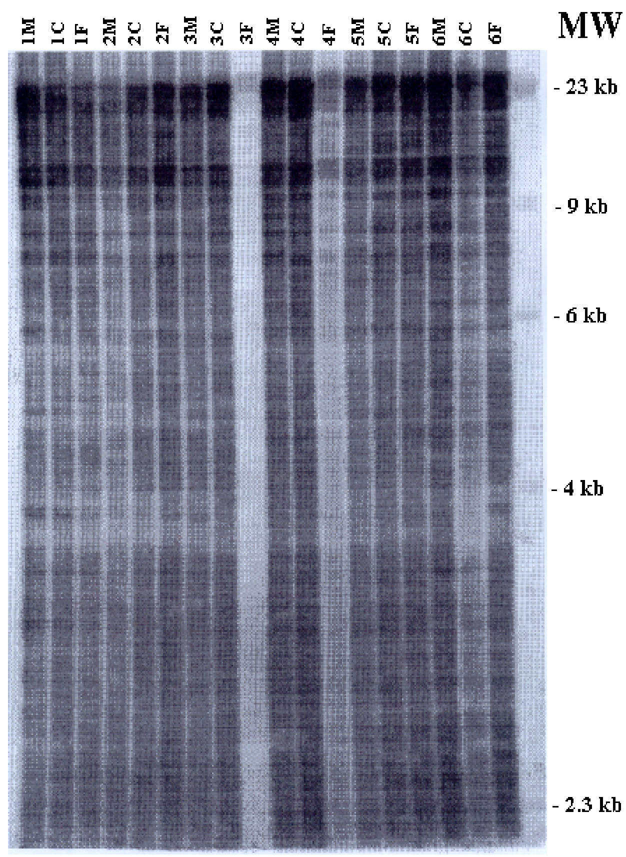


FIG. 2. Multilocous DNA fingerprints of six biological families (1-6) of Brown Boobies from Moleques do Sul breeding colony with minisatellite multilocus probe 33.15 showing the extremely low variable pattern. M = putative father, C = chick, F = putative mother.

broad overlap (Fig. 3).

Assuming that the adult Masked Booby female (or male) from each nest was the biological mother (or father), the band patterns indicate that adult males (or females) sampled in different nests could not have fathered (or mothered) the analyzed chick when the results of both probes are taken into account (Table 2).

DISCUSSION

Masked and Brown boobies usually lay two eggs in each nest (Nelson 1978). However, three eggs were observed in a Brown Booby nest in Moleques do Sul Island. After a short period of time, the female that was incubating took one egg away. We do not know if the female was able to recognize the eggs she had laid, but this behavior can be suggestive of nest parasitism. Unfortunately, it was not possible to collect these three eggs and a sample from the incubating female for our analysis. Nelson (1978) also noted the rarity of finding marked Brown Booby eggs in other nests as well as that of Masked Booby nests with three

TABLE 1. Mean number of bands and mean similarity coefficient (S) between unrelated individuals. Data obtained by the analyses of the results with probes 33.6 and 33.15 in Masked and Brown boobies.

Species	Probe	N[1]	n[2] ± SD	S[3] (mean) ± SD
Masked Booby	33.6	15	31.83 ± 3.92	0.49 ± 0.09
	33.15	15	20.06 ± 3.06	0.48 ± 0.13
Brown Booby	33.6	15	16.83 ± 2.14	0.55 ± 0.14
	33.15	15	29.33 ± 2.07	0.58 ± 0.13

[1]N = number of pairwise comparisons.
[2]n = mean number of bands.
[3]S = Band sharing coefficients between unrelated birds.

TABLE 2. Alternative Masked Booby fathers and mothers based on the band patterns.

Chick	Putative fathers/mothers	
	Probe 33.6	Probe 33.15
05C[1]		12M[2]
09C		13M
10C	09M	5M
12C	08M/13F[3]	

[1]C = chick number.
[2]M = male number.
[3]F = female number.

eggs and switching eggs between neighboring nests. He also observed Brown Booby nests with three eggs and suggested that most of them had suffered brood parasitism. Gilard (1992) observed that female Brown Boobies tended to forage farther from the shore than males. This behavior could increase the chances of extra-pair copulation (EPC) and nest parasitism.

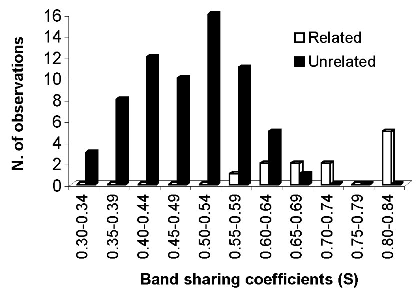
DNA fingerprinting has been used successfully in breeding behavioral studies of many species (Dixson *et al.* 1988; Austin *et al.* 1993; Dickinson & Akre 1998). However, a minimal level of variability is required to detect phenomena such as EPC. In populations that present low level of genetic variability, DNA fingerprinting can fail to reveal paternity (Bruford & Altmann 1993).

For both species, the genetic similarity between individuals was very high (Table 1). The similarity index values found in this work, specially for the Brown Booby, are comparable to those found in endangered species such as the Spix's macaw (*Cyanopsitta spixii*; mean S = 0.62 ± 0.11; Caparroz *et al.* 2001). The S indexes cannot be used to estimate relatedness due to the high overlap between unrelated and related individuals.

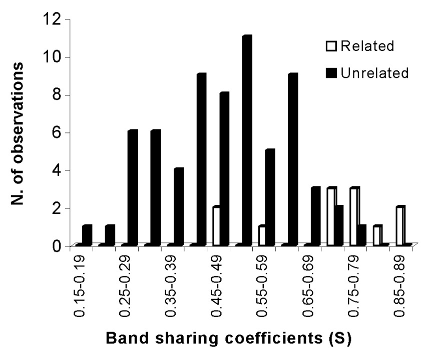
The analysis of six nests revealed that when the results of both minisatellite probes were considered, no other male (nor female) Masked Booby besides the one sampled in the nest could have fathered (or mothered) the respective chick. This result is consistent with Masked Booby having characteristics of a monogamous species: it is colonial and has biparental care of chicks (Nelson 1978).

Even though the molecular results did not allow us to determine whether extra-pair paternity and nest parasitism occur in the Brown Booby, this species also has the same characteristics of monogamous species, and so we believe that extra-pair fertilizations are not frequent. Besides, in our field observations we saw the maintenance of pairs between successive breeding seasons in the Brown Booby colony. It would be interesting to develop more variable markers to gather more data on the mating systems in such monogamous species.

Some hypotheses can be made to try to explain such low genetic variability: it is a



(a) 33.6



(b) 33.15

FIG. 3. Histograms showing the number of observations for each class of band sharing coefficient intervals comparing unrelated and related Masked Boobies from Abrolhos Archipelago using (a) probe 33.6 and (b) probe 33.15.

characteristic of the Sulid family, a bottleneck effect or even a founder effect. An estimate of the genetic variability in other breeding colonies of Brown Boobies along the Brazilian coast has also revealed low genetic variability (Baumgarten *et al.* in prep). An unpredictable low level of genetic variability using mitochondrial DNA sequences was found in Knot (*Calidris canutus*) populations around the world and a bottleneck event and recent population expansion were suggested as an explanation (Baker *et al.* 1994). This could be the best explanation for the lack of genetic variability in the boobies' populations that we studied. In addition, these boobies exhibit natal philopatry. This can result in inbreeding and could also help explain the elevated band sharing coefficient (S). Thus, other studies using this approach on other populations, or even on other Sulidae species, are necessary for a better understanding of the phenomenon.

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