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Sex Identification of South American Parrots (Psittacidae, Aves) Using the Human Minisatellite Probe 33.15

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Many species of South American parrots are endangered, and captive breeding has become a standard procedure for species conservation. Because most South American parrots are not sexually dimorphic, an efficient means of determining the sex of individuals is an important tool in establishing and maintaining a viable breeding population in captivity.

DNA fingerprinting (Jeffreys et al. 1985) has been applied in a variety of wild species, including birds (Burke and Bruford 1987, Wetton et al. 1987). It was used to monitor genetic variability in captive Puerto Rican Parrots (*Amazona vittata*; Brock and White 1992) and to establish paternity in endangered species (Mathé et al. 1993). Recently, we used DNA fingerprinting to identify the sex of Peach-fronted (*Aratinga aurea*) and Golden (*Guaruba* [*Aratinga*] *guarouba*) parakeets (Miyaki et al. 1992) and suggested that fingerprinting also could be used to determine sex in other psittacines (Miyaki et al. 1993, 1995). Here, we present results on sex determination using the human minisatellite probe 33.15 (Jeffreys et al.

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TABLE 1. Pattern of intense bands in previously sexed and unsexed parrots. F and M are number of females and males; N and Nb are number of individuals and number with intense bands, respectively.

Species	Sexed				Unsexed		Total
	F	M	Method[a]	Bands[b]	N	Nb	
Amazona aestiva aestiva	5	8	k	−	10	0	23
Amazona aestiva xanthopteryx	1	3	k	−	0	0	4
Amazona amazonica	2	2	k	−	2	0	6
Amazona autumnalis diadema	1	0	k	−	1	0	2
Amazona brasiliensis	2	2	k, l	−	7	0	11
Amazona dufresniana rhodocorytha	0	0			4	0	4
Amazona farinosa	0	2	k	−	3	0	5
Amazona festiva	0	2	k	−	4	0	6
Amazona ochrocephala xantholaema	1	1	b	−	5	0	7
Amazona pretrei	3	3	d	−	4	0	10
Amazona vinacea	2	4	k, b	−	6	0	12
Amazona xanthops	1	0	k	−	4	0	5
Anodorhynchus hyacinthinus	3	5	k	+	55	32	63
Anodorhynchus leari	1	1	k, l	+	1	1	3
Ara auricollis	2	1	k	+	1	1	4
Ara ararauna	3	2	k, b	+	12	8	17
Ara chloroptera	2	5	k, b	+	15	4	22
Ara macao	2	5	k	+	9	4	16
Ara manilata	2	1	k	+	7	4	10
Ara maracana	4	1	k	+	2	0	7
Ara nobilis	2	4	k	+	11	3	17
Aratinga acuticaudatta	1	1	k	+	0	−	2
Aratinga aurea	2	2	k	+	32	14	36
Aratinga leucophthalmus	1	1	b	+	10	5	12
Aratinga mitrata	0	0			2	1	2
Aratinga solstitialis auricapilla	0	0			8	2	8
Aratinga solstitialis jandaya	0	0			4	4	4
Cyanopsitta spixii	3	4	k	+	0	−	7
Deroptyus accipitrinus	1	1	b	−	0	−	2
Guaruba guarouba	3	3	k, b	+	19	9	25
Nandayus nenday	2	0	b	+	21	10	23
Pionites leucogaster	1	1	k	−	4	0	6
Pionopsitta pileata	1	1	d	−	6	0	8
Pionus menstruus	2	2	b	−	7	0	11
Pyrrhura egregia	1	0	b	−	1	0	2
Pyrrhura frontalis	1	1	b	−	2	0	4
Pyrrhura picta	1	1	b	−	3	0	5
Triclaria malachitacea	1	1	d	−	0	−	2

[a] k, karyotype analysis; l, laparoscopy; b, breeding behavior; d, sexual dimorphism.
[b] Female-specific bands: −, absent; +, present.

1985) in 36 species belonging to 13 genera of South American parrots.

Methods.—Blood samples were collected from birds belonging to aviculturists and official establishments in Brazil. For some individuals, sex was determined by karyotyping, laparoscopy, or breeding behavior. Whenever possible, growing feathers (ca. 25 days old) were collected from birds whose sex was unknown and processed for karyotype analysis. Chromosome preparation and analysis followed Duarte and Caparroz (1995). The species and number of individuals studied are shown in Table 1.

The protocols used to obtain multilocus fingerprints followed Bruford et al. (1992). For each bird, 5 μg of genomic DNA were digested with the restriction enzyme *Mbo*I (for *Amazona*) or *Hae*III (for the other genera). Fragments were separated by electrophoresis through a 30-cm long 1% horizontal agarose gel. Electrophoresis was stopped when the 2-kilobase (kb) marker band had migrated to the bottom of the gel. The fractionated DNA fragments were transferred onto a nylon membrane by standard capillary Southern blotting (Sambrook et al. 1989).

The membrane was hybridized with minisatellite 33.15 probe (Jeffreys et al. 1985), which was labeled with [α-^{32}P] dCTP or [α-^{32}P] dATP. Pre-hybridization in 0.263 M Na_2HPO_4, 1 mM EDTA, 7% SDS, and 1% BSA at 65°C lasted for 4 h, and the probe was added and left overnight at 65°C. The membrane was washed in 0.25 M Na_2HPO_4, 1% SDS, 2×SSC, 0.1%

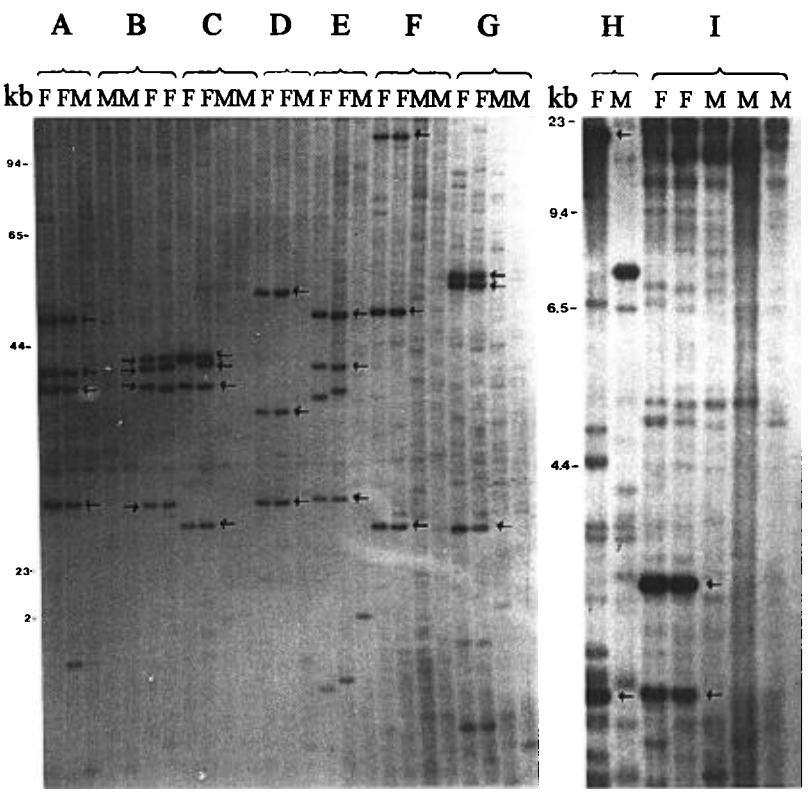

FIG. 1. Parrot DNA hybridized with human minisatellite probe 33.15. Female-specific bands are shown with arrows. (A) *Ara auricollis*, (B) *Ara chloroptera*, (C) *Ara macao*, (D) *Ara manilata*, (E) *Ara maracana*, (F) *Ara nobilis*, (G) *Anodorhynchus hyacinthinus*, (H) *Anodorhynchus leari*, (I) *Cyanopsitta spixii*. F = female, M = male.

TABLE 2. Molecular size of the sex-linked bands in South American parrots.

Species	Number of bands	Molecular size (kb)
Anodorhynchus hyacinthinus	3	3.1; 5.1; 5.2
Anodorhynchus leari	2	3.1; 18.5
Ara ararauna	2	1.9; 2.5
Ara auricollis	4	2.7; 3.7; 3.9; 4.8
Ara chloroptera	4	2.7; 3.7; 4.0; 4.1
Ara macao	4	2.5; 4.0; 4.1; 4.3
Ara manilata	3	2.7; 3.5; 4.0
Ara maracana	3	2.7; 3.9; 4.6
Ara nobilis	3	2.5; 4.7; 11.5
Aratinga aurea	2	3.7; 7.6
Aratinga acuticaudatta	3	2.9; 3.6; 4.6
Aratinga leucophthalmus	2	3; 7.6
Aratinga mitrata	3	2.9; 3; 10.5
Aratinga solstitialis auricapilla	3	4.2; 5.0; 6.0
Aratinga solstitialis jandaya	3	4.1; 5.2; 5.8
Cyanopsitta spixii	2	3.1; 3.5
Guaruba guarouba	2	2.8; 5.7
Nandayus nenday	3	3.9; 4.8; 6.0

SDS and in 1×SSC, 0.1% SDS at 65°C. The filter was then autoradiographed at −70°C using Kodak RX film with one or two intensifying screens.

Results.—A pattern of two to four intense sex-specific bands was detected with the 33.15 probe in all females studied in the genera *Ara* and *Aratinga* and also in females of *Anodorhynchus hyacinthinus*, *A. leari*, *Cyanopsitta spixii*, *Guaruba guarouba*, and *Nandayus nenday* (see Fig. 1). These intense bands also were present in some previously unsexed individuals of *Aratinga solstitialis auricapilla*, *A. s. jandaya*, and *A. mitrata* (Table 1). Each species showed unique patterns of female-linked bands according to their molecular size (Table 2); these species-specific profiles are a potential tool for species identification. Female-linked bands also were observed in long-tailed species even when other restriction enzymes were used (data not shown). Sex-linked bands were absent in known females of eight species of *Amazona* and of *Pionus menstruus*, *Deroptyus accipitrinus*, *Pionites leucogaster*, *Pionopsitta pileata*, *Pyrrhura egregia*, *P. frontalis*, *P. picta*, and *Triclaria malachitacea*. We used *Mbo*I for members of *Amazona* because this enzyme produced more polymorphic DNA fingerprint profiles; even when

we used *Hae*III, no sex-linked fragments were observed (data not shown).

Discussion.—Until recently, only three methods of sex identification were available for birds: fecal hormone assay, chromosome analysis, and laparoscopy. Hormone analyses vary with reproductive condition and thus are age and season dependent. Chromosome analysis is reliable but time consuming, and in many occasions appropriate metaphase cells cannot be found for analysis without repeated attempts. Birds have to be handled twice for karyotyping, once for removing a sample of feathers and a second time for collecting the growing feather pulp. Because laparoscopy is a surgical procedure, birds are exposed to anesthesia and to the risks of surgery.

Recently, several DNA techniques have been developed for sex identification in birds (de Kloet and de Kloet 1992, Griffiths and Tiwari 1993, May et al. 1993). Griffiths and Tiwari (1995) determined the sex of the last Spix's Macaw (*Cyanopsitta spixii*) from shed feathers that were collected in the wild and used as a source of DNA for PCR amplification. In our application of DNA fingerprinting in South American parrots, we identified female-specific intense bands in some species using the human minisatellite probe 33.15 (Miyaki et al. 1992, 1993). Other authors also have detected sex-specific bands in DNA fingerprinting of birds using probe 33.15 (Rabenold et al. 1991, Longmire et al. 1992) and probe 33.6 (Graves et al. 1993). Although probe 33.15 could not be used to identify the sex of all the species we studied, it was invaluable for sexing macaws (*Ara, Anodorhynchus,* and *Cyanopsitta*) and conures (*Aratinga* and *Nandayus*). These genera belong to the "long, point-tailed" group and are considered to be closely related. Our method could not be used to sex the short-tailed parrots in the genus *Amazona,* and our data suggest that it also was not adequate for sexing other short-tailed species such as *Pionus menstruus, Pionites leucogaster,* and *Pionopsitta pileata* (which has sexually dimorphic plumage), as well as the "long, wide-tailed" *Deroptyus accipitrinus* and *Triclaria malachitacea* (the latter has sexually dimorphic plumage). The absence of female-linked bands was not due to the fact that a different restriction enzyme was applied. No sex-specific pattern was detected in the three species of *Pyrrhura* we studied, each of which has a long, sharp-pointed tail.

In all cases where we performed karyotyping, we confirmed that the intense band pattern was present in all females and absent in males. Because of the difficulties inherent in chromosome analysis (i.e. lack of growing feathers and failure to obtain appropriate metaphase cells), it was impossible to determine the sex of many of the species that we studied with probe 33.15. However, the agreement between the DNA results and the cytogenetic data in all species for which both studies were performed suggests that the pattern of a few intense bands detected in some unsexed individuals was restricted to females.

Little is known about the phylogenetic relationships of psittacines. The available data are based on chromosomal evolution (Valentine 1990, Christidis et al. 1991), albumin (Sibley 1960), and the cytochrome-*b* gene (Birt et al. 1992, Leeton et al. 1994). Based on karyotype correlations, Valentine (1990) proposed an early separation of *Amazona* from other genera. Based on habitat exploration, Montón (1977) suggested that the short-tailed and the long-tailed birds have behavioral differences. The presence of w-chromosome-linked minisatellite sequences in most long- and sharp-tailed New World psittacines, and their absence in the short-tailed species, provide support for the separate evolution of these two groups. Preliminary analysis of mitochondrial gene (12S and 16S rDNAs and cytochrome-*b*) sequences of nine species of Brazilian parrots also supports the separation of the long- and short-tailed species (Miyaki 1996).

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