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CONTRASTING PHYLOGEOGRAPHIC PATTERNS IN MITOCHONDRIAL DNA AND MICROSATELLITES: EVIDENCE OF FEMALE PHILOPATRY AND MALE-BIASED GENE FLOW AMONG REGIONAL POPULATIONS OF THE BLUE-AND-YELLOW MACAW (PSITTACIFORMES: *ARA ARARAUNA*) IN BRAZIL

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ABSTRACT.—Comparing the patterns of population differentiation among genetic markers with different modes of inheritance can provide insights into patterns of sex-biased dispersal and gene flow. The Blue-and-yellow Macaw (*Ara ararauna*) is a Neotropical parrot with a broad geographic distribution in South America. However, little is known about the natural history and current status of remaining wild populations, including levels of genetic variability. The progressive decline and possible fragmentation of populations may endanger this species in the near future. We analyzed mitochondrial DNA (mtDNA) control-region sequences and six microsatellite loci of Blue-and-yellow Macaws sampled throughout their geographic range in Brazil to describe population genetic structure, to make inferences about historical demography and dispersal behavior, and to provide insight for conservation efforts. Analyses of population genetic structure based on mtDNA showed evidence of two major populations in western and eastern Brazil that share a few low-frequency haplotypes. This phylogeographic pattern seems to have originated by the historical isolation of Blue-and-yellow Macaw populations ~374,000 years ago and has been maintained by restricted gene flow and female philopatry. By contrast, variation in biparentally inherited microsatellites was not structured geographically. Male-biased dispersal and female philopatry best explain the different patterns observed in these two markers. Because females disperse less than males, the two regional populations with well-differentiated mtDNA haplogroups should be considered two different management units for conservation purposes. *Received 4 November 2007, accepted 10 December 2008.*

Key words: *Ara ararauna*, Blue-and-yellow Macaw, microsatellite, mitochondrial DNA, sex-biased dispersal.

Patrones Filogeográficos Contrastantes en ADN Mitocondrial y Microsatélites: Evidencia de Filopatría de las Hembras y Flujo Genético Sesgado hacia los Machos entre Poblaciones Regionales de *Ara ararauna* (Psittaciformes) en Brasil

RESUMEN.—Comparar los patrones de diferenciación poblacional entre marcadores genéticos con diferentes modos de herencia puede brindar información acerca de patrones de dispersión y flujo genético sesgados de acuerdo al sexo. *Ara ararauna* es un psitácido neotropical con una distribución amplia en Sur América. Sin embargo, se conoce poco acerca de la historia natural y el estado actual de las poblaciones silvestres remanentes, incluyendo sus niveles de variabilidad genética. La disminución progresiva y la posible fragmentación de las poblaciones podrían amenazar a esta especie en el futuro cercano. Analizamos secuencias de la región de control del ADN mitocondrial (ADNmt) y seis loci microsatélites de individuos muestreados a través de la distribución de la especie en Brasil para describir la estructura genética poblacional, para hacer inferencias sobre la demografía histórica y el comportamiento de dispersión, y para proveer información para los esfuerzos de conservación. Los análisis de la estructura genética basados en ADNmt mostraron evidencia de la existencia de dos poblaciones principales en el oeste y este de Brasil que comparten algunos haplotipos de baja frecuencia. Este patrón filogeográfico parece haberse originado tras el aislamiento geográfico de poblaciones de *A. ararauna* hace aproximadamente 374,000 años y ha sido mantenido por el flujo genético restringido y la filopatría de las hembras. En contraste, la

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variación en los microsatélites (marcadores heredados biparentalmente) no estuvo estructurada geográficamente. La dispersión sesgada hacia los machos y la filopatría de las hembras son las mejores explicaciones de los patrones diferentes observados entre marcadores. Debido a que las hembras se dispersan menos que los machos, las dos poblaciones regionales con haplogrupos de ADNmt bien diferenciados deberían considerarse como dos unidades de manejo diferentes para propósitos de conservación.

Characterizing population structure in a species requires an integration of evolutionary (i.e., phylogeographic) and contemporary (e.g., behavioral) components (Avise 1994). Interpretation of the relative influence of historical and contemporary factors solely on the basis of genetic data can be complicated by a host of factors, including sex-biased dispersal and philopatry (Scribner et al. 2001). Dispersal is a life-history characteristic that has relevant effects on both the dynamics and the genetics of species (Clobert et al. 2001). In many species of birds and mammals, there is considerable sexual variation in the extent of natal philopatry, the distance moved by an individual from its birth site to its breeding ground. In mammals, females tend to remain in close proximity to the natal area, whereas males disperse. Conversely, in birds, the reverse pattern is generally found (e.g., females disperse and males are philopatric; Greenwood 1980, Clarke et al. 1997).

In a species with sex-biased dispersal, the simultaneous use of markers with different modes of inheritance (i.e., uni- or biparental) can reveal the contrasting patterns of spatial distribution of the species' genetic variation. For example, Melnick and Hoelzer (1992) compared allozyme and mitochondrial DNA (mtDNA) data in macaque monkeys (*Macaca mulatta*). Stronger population structure was detected with the mtDNA than with nuclear DNA. This was in accordance with behavioral observations of males leaving the natal troop before sexual maturity and females staying with the troop for life. Similar patterns were observed in a variety of other species (Karl et al. 1992, FitzSimmons et al. 1997, Ishibashi et al. 1997), including birds (Gutierrez 1994, Gibbs et al. 2000). By contrast, in Willow Ptarmigan (*Lagopus lagopus*), behavioral studies indicated female-biased dispersal, but lower population differentiation was observed in mtDNA data than in microsatellite data, even though female-biased gene flow should homogenize both nuclear and mitochondrial variation simultaneously (Piertney et al. 1998, 2000).

The relevance of genetic information, including data on population genetic structure, in species conservation has long been recognized (e.g., Lande and Barrowclough 1987, Simberloff 1988). The rationale for this is based on the theoretical understanding of the relationship between genetic diversity and fitness in response to selection: loss of genetic diversity increases autozygosity and reduces the ability of a population to adapt to future selective pressures (Fisher 1930, Chesser et al. 1980, Lacy 1987). Thus, to devise adequate conservation and management strategies for any species, it is important to incorporate an understanding of its genetic structure.

The Blue-and-yellow Macaw (*Ara ararauna*) is a Neotropical parrot with a broad geographic distribution from eastern Panama south through Colombia to Bolivia, Paraguay, and Brazil (Forshaw 1989, Collar 1997). This species inhabits seasonally flooded forests and gallery forests, and in the northwest it occurs in deciduous forest (Collar 1997). The global population size of the Blue-and-yellow Macaw is unknown, but the species is described as "common" in parts of its range (Collar 1997) and is not considered threatened. However, it is a very popular pet, and collecting pressures combined with habitat fragmentation have resulted in the extirpation of the species in Trinidad and its extirpation or decline in many areas of Brazil, Ecuador, and Colombia (Collar 1997). In Brazil, this species is considered critically endangered in the State of São Paulo (São Paulo State Government 1998), nearly extinct in the State of Rio de Janeiro (Bergallo et al. 1999), and vulnerable in the State of Minas Gerais (Machado et al. 1998). Under this scenario of progressive decline and possible fragmentation of populations, it is important to have a conservation plan for this species.

At present, little is known about the natural history and current status of the remaining Blue-and-yellow Macaw populations. Consequently, data on the genetic variability of wild populations are practically nonexistent. There is one DNA fingerprinting study of wild Blue-and-yellow Macaws from the southwestern State of Goiás in Brazil (Caparroz et al. 2001), in which the population analyzed showed a high mean genetic similarity index, similar to those found in species considered vulnerable or endangered.

We characterized the entire mtDNA control region of the Blue-and-yellow Macaw and analyzed the variation of a large fragment from this region and six microsatellite loci of individuals sampled throughout the species' geographic range in Brazil. We describe the population genetic structure and population history of this species to make inferences regarding historical demography and dispersal behavior and to provide data for conservation efforts.

Methods

Sample collection and DNA extraction.—Approximately 0.1 mL of blood was collected from the brachial vein of 50 Blue-and-yellow Macaws (37 chicks found in natural nests and 13 adult captive birds caught in the vicinity) at six locations in Brazil (Table 1 and Fig. 1). All the samples were stored in absolute ethanol at –20°C and deposited in the tissue collection of the Laboratório de Genética e Evolução Molecular de Aves (LGEMA) at the University of São Paulo. Total genomic DNA was extracted using a standard proteinase K/SDS and phenol-chloroform protocol (Bruford et al. 1992).

Amplification and DNA sequencing.—A primer walking strategy was used to obtain new primers to sequence the entire mtDNA control region. The initial 1.6-Kb fragment was amplified for 10 individuals by polymerase chain reaction (PCR) with primers $GluL_{16737}$ (Eberhard et al. 2001; Table 2) and CRH_{1248} (Tavares et al. 2004; Table 2), and this fragment was sequenced using primer $GluL_{16737}$. A new forward primer (Table 2) was then designed in a conserved region ~500 base pairs (bp) from the 5′ end of the sequence, and this new primer was used with CRH_{1248} to amplify a shorter fragment. Subsequent use of this procedure with sequential and partially overlapping PCR products using new forward primers not only helped sequence the entire mtDNA control region but also helped to confirm sequence alignment.

We used this primer set (Table 2) to amplify and sequence the entire mtDNA control region of 28 Blue-and-yellow Macaws.

TABLE 1. Sampling locations of Blue-and-yellow Macaw in Brazil, sample sizes, and numbers of mtDNA control-region haplotypes (*H*).

Population	Latitude, longitude	Sample size: Wild	Sample size: Captive	*H*
Corginho, central Mato Grosso do Sul (CMS)	19°S, 54°W	7	2	6
Chapadão do céu, southwestern Goiás (SG)	18°S, 52°W	16	1	8
Chapada Gaúcha, northwestern Minas Gerais (NMG)	15°S, 45°W	2	2	3
Cavalcanti, northeastern Goiás (NG)	13°S, 47°W	7	3	6
Peixe, southeastern Tocantins (ST)	12°S, 48°W	3	2	3
Ilha do Marajó, northeastern Pará (wild samples) and Redenção, southern Pará (captive samples) (PA)	01°N, 49°W, 08°S, 50°W	2	3	4
Total		37	13	

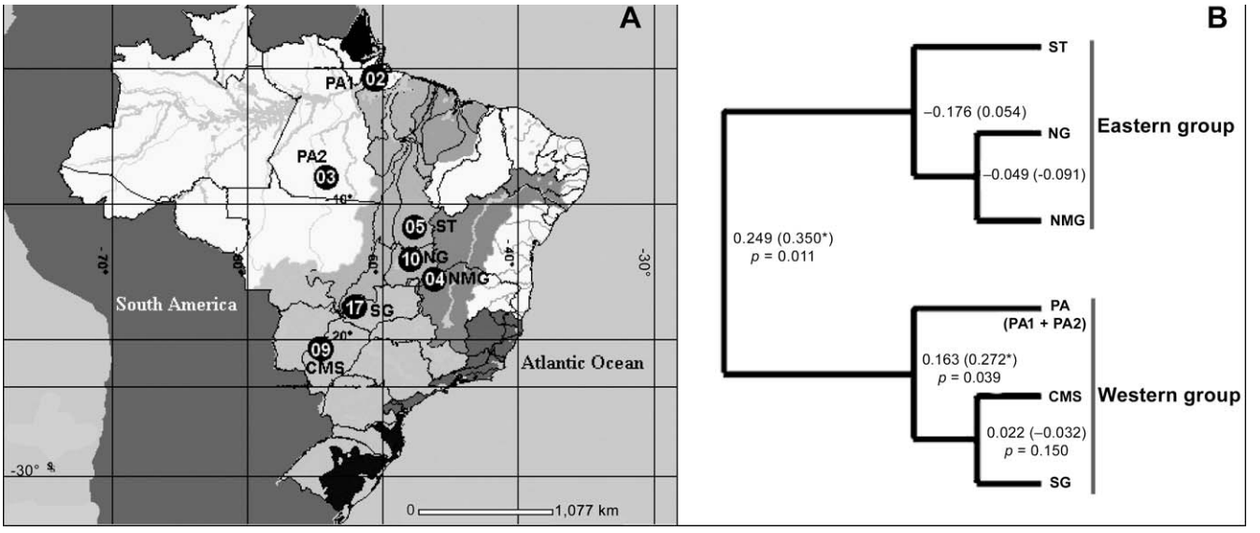

FIG. 1. (A) Regions in Brazil where we sampled Blue-and-yellow Macaws (for abbreviations, see Table 1; adapted from a map by Instituto Brasileiro de Geografia e Estatística). Numbers indicate sample size at each sampling site. Differently shaded regions depict the main hydrographic basins in Brazil. (B) Genetic relationships among the six populations sampled, as inferred from hierarchical analysis of nucleotide diversity; the values of g_{ST} that are shown represent the proportion of the total genetic diversity within a cluster. The probability of observing higher values of g_{ST} by chance is also shown. Numbers in parentheses indicate the Φ_{ST} values estimated by AMOVA. Significant Φ_{ST} values ($P < 0.05$) are indicated with an asterisk.

TABLE 2. Primer pairs used to amplify and sequence the entire mtDNA control region of Blue-and-yellow Macaw. Primer names indicate the position of the primer in the flanking or control region.

Forward primer	Sequence (5′→3′)	Reverse primer	Sequence (5′→3′)	Fragment size (bp)
$Lglu_{16737}$[a]	TCTTGGCAKCTTCAGTACCATGCTTT	H_{522}[a]	TGGCCCTGACYTAGGAACCAG	650
Lcr_{456}[b]	CACGAGAGATCAYCAACCCGGTGT	H_{1028}[b]	GTGTAAAACAAAGTGCATCAGGGT	650
Lcr_{840}[c]	TCATTTTMRCACTGATGCTTG	Hcr_{1322}[c]	GAGATAGTTGAGGCATAAGTGATTAA	400
Lcr_{1209}[c]	CACTTACAAACACYYACAAACAAAC	Hcr_{1248}[a]	TCTTGGCAKCTTCAGTACCATGCTTT	350

[a] Eberhard et al. 2001.
[b] Tavares et al. 2004.
[c] Present study.

Amplifications were performed in 10 µL with 20–50 ng of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.8 mM dNTPs, 1 µm each primer, and 0.5 U *Taq* polymerase (Pharmacia). Each reaction consisted of an initial step of 95°C for 5 min followed by 30 cycles of 95°C (1 min), 53°C (40 s), and 72°C (40 s), and a final extension of 72°C for 5 min. The PCR products were loaded on 0.8% agarose gels to check their concentration and the presence of a unique band. The products were purified by digestion with 5 U of exonuclease I (USB) and 0.5 U of shrimp alkaline phosphatase (USB) at 37°C (1 h) and then at 80°C (20 min). Both DNA strands were sequenced using the Big Dye terminator sequencing kit (Perkin-Elmer, Waltham, Massachusetts), following the manufacturer's instructions. Sequencing products were loaded in 5% polyacrylamide denaturing gels on an ABI 377 automated sequencer (Applied Biosystems, Foster City, California). The sequences were aligned using the computer program SEQUENCE NAVIGATOR (Applied Biosystems) and were deposited in GenBank (accession nos. EF446619–EF446668).

For population genetic analyses, we sequenced only the first 1,290 bp of the 5′ portion of the control region (first three primer pairs in Table 2) and increased the sample size to 50 Blue-and-yellow Macaws. Homologous portions from one Blue-throated Macaw (*A. glaucogularis*; GenBank accession no. EU038111) and one Red-bellied Macaw (*Orthopsittaca manilata*; GenBank accession no. EU038110) were sequenced and used as outgroups in the phylogenetic analysis. The first species is sister to *A. ararauna* (Oliveira-Marques 2006), and the latter is closely related to *A. ararauna* (Tavares et al. 2006).

Population genetic analyses based on mtDNA.—We used the D^* and F^* tests (Fu and Li 1993) in DNASP, version 4.10.9 (Rozas et al. 2003), to test the hypothesis that the mutations detected in the control-region sequences in each population were selectively neutral. Statistical significance of D^* and F^* values were determined by comparing the observed values with the critical values described in Fu and Li (1993).

We used two approaches to study the population genetic structure of Blue-and-yellow Macaws. First, we performed a hierarchical analysis of nucleotide diversity (Holsinger and Mason-Gamer 1996). The statistical significance of support values at each node is tested by creating a null distribution of values generated by random resampling and comparing the observed value with this null distribution. The most valuable feature of this approach is that it allows any pattern to emerge from the data rather than using an *a-priori* hypothesis of the population structure (Holsinger and Mason-Gamer 1996).

The second method we used was hierarchical analysis of molecular variance (AMOVA) using Φ_{ST} (Excoffier et al. 1992) as implemented in ARLEQUIN, version 2.0 (Schneider et al. 2000). The Φ_{ST} analyses were performed using a matrix with Tamura (1992) distances between sequences. In contrast to the previous method, this approach requires an *a-priori* hypothesis of sets of populations at different hierarchical levels. Using the results of the Holsinger and Mason-Gamer (1996) analysis, we tested the hypothesis that two populations are present: western (SG+CMS+PA) and eastern (ST+NG+NMG) (Fig. 1).

Relationships among haplotypes were obtained by computing a median-joining network using the algorithm described by Bandelt et al. (1999) and implemented in NETWORK, version 4.1.1.0 (Fluxus Technology, Clare, United Kingdom). Additionally, haplotype trees were inferred by maximum parsimony (MP) and maximum likelihood (ML) using PAUP*, version 4b8 (Swofford 1999). These trees were rooted with two outgroups (*A. glaucogularis* and *O. manilata*). The MP tree was obtained by branch-and-bound search. Starting trees were obtained via stepwise addition with random addition of sequences. A hierarchical likelihood-ratio test performed in MODELTEST, version 3.7 (Posada and Crandall 1998), indicated that the best-fit evolutionary model was the Hasegawa-Kishino-Yano (HKY; Hasegawa et al. 1985) with a gamma distribution ($\alpha = 0.0156$) and transition:transversion ratio of 5:32. This model was used in the ML analysis that was performed using a heuristic tree search with starting trees obtained via stepwise addition with 10 random-addition sequence replicates. Statistical support of tree nodes was obtained by nonparametric bootstrap (1,000 replicates).

Microsatellite genotyping.—Five dinucleotide microsatellite loci (UnaCT21, 32, 43, 74, and UnaGT55) isolated from the Blue-and-yellow Macaw (Caparroz et al. 2003) and one (AgGT21) isolated from the St. Vincent Amazon (*Amazona guildingii*; Russello et al. 2001) were used to genotype all samples. The forward primer from each pair was synthesized with a 5′ M13 tail that allowed use of the universal dye-labeling method described by Boutin-Ganache et al. (2001). The PCR amplifications were carried out in a final volume of 12.5 µL containing 20–50 ng of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 80 µm M13 fluorescently labeled primer (TET or HEX; Applied Biosystems), 160 µm forward primer, 80 µm reverse primer, and 0.5 U *Taq* polymerase (Pharmacia). Each reaction consisted of an initial step of 95°C for 10 min followed by 35 cycles at 95°C for 60 s, 50 to 60°C for 40 s and 72°C for 40 s, and a final step at 72°C for 10 min. The PCR products were electrophoresed in 5% denaturing acrylamide gels and analyzed in an ABI 377 DNA Sequencer with GENESCAN and GENOTYPER, version 2.1 (Perkin-Elmer).

Statistical analyses of microsatellites.—Observed (H_O) and expected (H_E) heterozygosities were calculated for each locus and for each population using ARLEQUIN. Each locus was tested for deviation from Hardy-Weinberg equilibrium expectation using an exact test based on the Markov-chain method of Guo and Thompson (1992), as implemented in GENEPOP, version 3.3 (1,000 dememorization, 1,000 batches, 1,000 iterations; Raymond and Rousset 1995). Linkage disequilibrium between all pairs of loci among populations and within each population was calculated with Fisher's exact test using the same program (1,000 dememorization, 300 batches, 1,000 iterations). Type I error rates for tests of linkage disequilibrium and departure from Hardy-Weinberg expectations were corrected for multiple comparisons using the sequential Bonferroni procedure (Rice 1989).

Analysis of gene and genotype frequency differentiation among all pairs of populations was done with a contingency table test as implemented in GENEPOP. Probability values (P) for each pairwise comparison were estimated by a Markov-chain method using the same program (1,000 dememorization, 100 batches, 1,000 iterations). P values were adjusted using sequential Bonferroni correction.

We also quantified differentiation between pairs of populations by calculating the fixation indices (F_{ST} and R_{ST}). F_{ST} values were calculated assuming Kimura and Crow's (1964) infinite

alleles model (IAM) of mutations. However, because microsatellites may evolve in a stepwise fashion via DNA polymerase slippage and the gain or loss of one or a few repeat units, statistics that assume Ohta and Kimura's (1973) stepwise mutation model (SMM) may be more appropriate for microsatellites. Therefore, we present subpopulation fixation indices based on both the IAM (F_{ST}; estimated as described by Weir and Cockerham [1984]) and the SMM (R_{ST}; estimated as described by Slatkin [1995]). We used FSTAT (Goudet 2001) to calculate F_{ST} and RSTCALC (Goodman 1997) to calculate R_{ST}, and permutation procedures in both programs to test whether particular values were significantly different from zero. Table-wide critical values were adjusted using the sequential Bonferroni correction.

To test for population structure based on the shared ancestry of microsatellite alleles, we used STRUCTURE, version 2.1 (Pritchard et al. 2000). Following the suggestions of those authors, we ran the entire data set (without any prior information) to determine the most likely number of clusters. Using 10^5 estimation steps after 30,000 burn-in steps, we ran five replicates of each value of K (number of clusters) from 1 to 6.

Demographic history.—The hypothesis of demographic stability was tested for the eastern and western groups by comparing the observed pairwise nucleotide site differences in mtDNA (mismatch distribution) and the expected values for a stable population using DNASP. We also estimated Ramos-Onsins and Rozas's (2002) $R2$ statistic and the confidence interval (assuming a large constant population size; Hudson 1990) to distinguish population growth from constant population size using the same program.

We used the program IM (Hey and Nielsen 2004) to distinguish the contributions of isolation and migration to the observed patterns of genetic divergence between western and eastern groups (see below) on the basis of mtDNA data alone, because structuring of female lineages would be obscured by possible male-biased gene flow in microsatellites in a combined analysis. IM employs integrated likelihood to simultaneously estimate posterior probabilities for the following population parameters that are scaled by the neutral mutation rate, following the notation in Hey (2005). θ_{west}, θ_{east}, and θ_A are the standardized mutation rates for the western, eastern, and ancestral populations, respectively; m_{west} and m_{east} are the migration rates into the western and eastern populations, respectively; u is the neutral mutation rate for the entire sequence; $t = tu$ is the number of mutations since the populations split; Ne_{west}, Ne_{east}, and Ne_A are the effective number of individuals at the time of population divergence in the western group, eastern group, and ancestral population, respectively; $Ne_{(f)}m_{west}$ and $Ne_{(f)}m_{east}$ are the number of female migrants per generation into western and eastern populations, respectively; and t is the time of population splitting in millions of years and the expected time to the most recent common ancestor (TRMCA) for each pair of populations being compared. We used a burn-in period of 200,000 steps, when the chain became independent of the starting point, and then monitored the parameter values of the simulation for 7 million updates. We used a procedure for swapping among multiple heated chains to ensure that the distributions we obtained reflected the stationary distributions. Metropolis-coupling runs used 10 coupled chains that varied over a range of heating values ($g_1 = 0.7$ and $g_2 = 0.85$). The peaks of the resulting distributions were taken as estimates of the parameters. Credibility intervals were assessed for each parameter as the 90% highest posterior density (HPD) intervals, which are the boundaries of the shortest span that includes 90% of the probability density of a parameter. We used a mutation rate of $\mu = 5.77 \times 10^{-5}$ substitutions locus^{-1} generation^{-1} and a generation time of 9 years to convert parameter estimates to more easily interpreted units. The estimate of the control-region mutation rate was based on the divergence time between *A. ararauna* and *O. manilata* of 14 mya (Tavares et al. 2006) divided by the maximum pairwise Tamura-Nei distance between these species (90 substitutions lineage^{-1}).

For the microsatellite data, we examined the signature of population expansion on genetic variability by estimating the imbalance index (β) derived by Kimmel et al. (1998) for each population. To obtain a mean estimate and standard error of β for each locality, we used locus-specific values of Θ_v and Θ_{Po} to calculate β for each locus and then averaged these values across loci. Gene flow between the western and eastern populations was estimated using the maximum-likelihood method in MIGRATE (Beerli and Felsenstein 1999, 2001). This method does not assume the equilibrium island model in which gene flow is symmetrical among populations (gene flow can be asymmetrical). Search parameters were as follows: 10 short chains totaling 500,000 trees, of which 10,000 were recorded, followed by three long chains totaling 2,500,000 trees, of which 50,000 were recorded. We used adaptive heating with initial relative temperatures of 1, 1.5, 3.0, and 1,000 to avoid fatal attraction to $M = 0$. Nucleotide frequencies were estimated from the data, and initial estimates of theta and gene flow were obtained using F_{ST}. The analysis was run four times with different random numbers, and the results were averaged.

RESULTS

Control-region features.—Nuclear copies of mitochondrial genes are known to occur in birds (Quinn 1997). We considered that all the sequences analyzed in the present study should be of mitochondrial origin for the following reasons: (1) no contaminating products were detected after PCR; (2) there were no double peaks in sequence electropherograms; (3) there were perfectly matching sequences in overlapping PCR products; (4) there was variation among individuals in sequence without any evidence of variation within individuals (see [2] above); and (5) several conserved sequence blocks were present that are characteristic of the avian control region (details below).

The Blue-and-yellow Macaw control region has 1,484 bp and falls within the size range of those of many other bird species (1,000–1,500 bp; Baker and Marshall 1997, Ruokonen and Kvist 2002). We identified many of the conserved features typically found in the avian control region: C-, D-, and F-boxes, conserved sequence blocks (CSB 1 and 2), and termination-associated sequences (TAS-TATAT and TACAT) (data not shown). The average base composition found in the Blue-and-yellow Macaw control region was A = 25.7%, C = 27.5%, G = 15.0%, and T = 31.8%. The lower guanine frequency observed in the control region of this species was described as a characteristic of the L-strand of vertebrate mtDNA (Baker and Marshall 1997).

Control-region variation.—When outgroups were included, 209 (16.2%) polymorphic sites were observed within the 1,290 bp sequences. When only the 50 Blue-and-yellow Macaws were

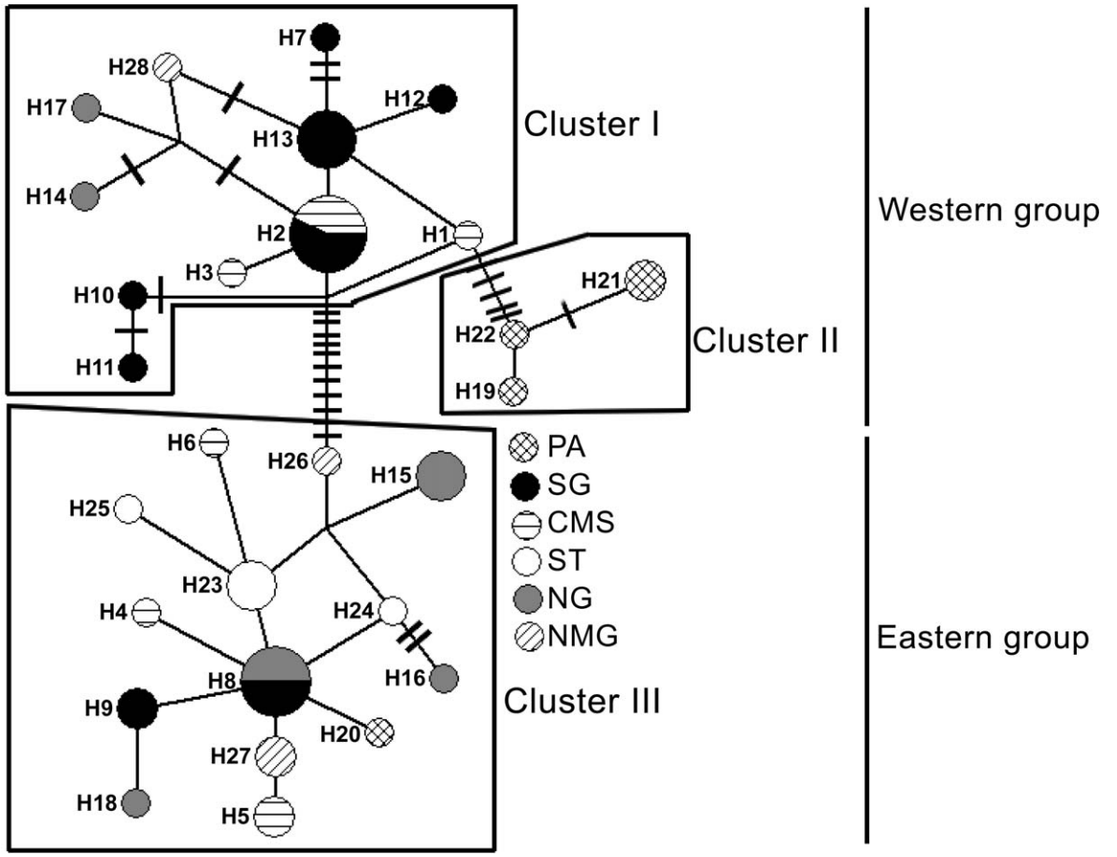

Fig. 2. Median-joining network showing relationships among the Blue-and-yellow Macaw mtDNA haplotypes (1,290 bp of control region). Sizes of circles are proportional to the number of individuals carrying each haplotype. Lines connecting haplotypes represent single-mutation steps. If more than one substitution separates two haplotypes, the number is represented by cross bars on the line. Populations are abbreviated as in Table 1.

considered, 39 polymorphic sites (3.0%) were found and only one indel of 1 bp (site 1,281) was observed in the alignment, whereas the remaining polymorphic sites had two or more nucleotide substitutions. The average transversion:transition ratio varied from 1:4 to 1:13, depending on the inclusion or exclusion of outgroup sequences, respectively. Values for D^* and F^* computed across all combined populations were −1.18 and −0.72, respectively. Both values were not significantly different from zero ($P > 0.1$). Therefore, we assume that the variation observed in the control region of Blue-and-yellow Macaw is selectively neutral.

A total of 28 haplotypes was found among the 50 Blue-and-yellow Macaw samples. Pairwise distances calculated as the proportion of different sites between two haplotypes averaged 0.78% (range: 0.08–1.55%). Most haplotypes were restricted to only one population, and only haplotypes H2 and H8 were found in more than one population (Fig. 2). Differentiation among haplotypes as depicted in a median-joining network was moderate to large, with haplotypes being separated by 1–20 substitutions (Fig. 2). There were three main haplotype clusters in the network: cluster I, comprising most (85%) of the haplotypes found in the two western sampling sites (SG and CMS); cluster II, composed exclusively of haplotypes in the western sampling site (PA); and cluster III, comprising 65% of haplotypes found in most eastern sampling sites (ST, NG, and NMG). Haplotype clusters I and II were separated by 5 nucleotide substitutions, whereas haplotype clusters I + II were separated by ≥11 nucleotide substitutions from cluster III. Thus, haplotypes from clusters I and II tended to occur in populations in western Brazil (hereafter, "western haplogroup"), and those from cluster III in populations of eastern Brazil ("eastern haplogroup"). However, low-frequency haplotypes H14, H17, and H28 in haplotype cluster I of western populations were sampled in the eastern populations, and haplotypes H4, H5, H6, H9, and H20 from haplotype cluster III of eastern populations were sampled at low frequencies in western populations.

Phylogenetic relationships among Blue-and-yellow Macaw haplotypes estimated by MP and ML (trees not shown) were similar. However, they showed the same relationships as the unrooted network (Fig. 2), and outgroups rooted the network between the western and eastern haplogroups. These results confirmed the presence of two main haplogroups and that the PA haplotypes, excluding H20, were in the western haplogroup, as in the network. The bootstrap support values for the divergence of the two major

haplogroups were 100% in both MP and ML trees, and each haplogroup was also well supported. Within the western haplogroup, the three PA haplotypes found in northwestern Brazil formed a distinct sub-clade (bootstrap values >82% in both analyses).

Microsatellite variability.—Genetic variation at the six microsatellite loci analyzed in the six sampled populations is described in the Appendix. Three to 11 alleles locus^{-1} were observed. The observed heterozygosity locus^{-1} ranged from 0.23 (AgGT21) to 0.88 (UnaCT43). No significant derivation from Hardy-Weinberg equilibrium was detected in any locus. Furthermore, there was no evidence of nonrandom association of genotypes ($P > 0.05$) in any of the 15 tests for linkage disequilibrium performed on all possible pairwise comparisons of the loci analyzed.

Population structure.—Although many of the mitochondrial haplotypes detected in our samples are represented by a single individual and were found in only one locality (Fig. 2), geographic patterns of haplotype distribution were observed. Hierarchical analysis of nucleotide diversity detected the same groups described above. Approximately 25% of the total genetic diversity in the samples was apportioned between western and eastern populations. Additionally, within the western population a significant component of variance (16%) was identified between PA and the two southwestern localities (SG and CMS).

Conversely, variation in microsatellite loci was not structured geographically. No significant P values ($P > 0.05$) were observed in pairwise comparisons among populations across all loci in either genic or genotypic compositions, which indicates that the allelic and genotypic distributions were similar across localities. In addition, the overall F_{ST} value estimated with AMOVA was not statistically significantly different from zero ($F_{ST} = 0.00069$; $P > 0.05$), and all individuals were assigned to one cluster using STRUCTURE.

Demographic patterns.—The observed mismatch distribution for western and eastern populations was bimodal and, thus, not congruent with a model of population expansion. Distribution is usually multimodal in demographically stable populations but is expected to be unimodal in populations that experienced a recent demographic expansion (Slatkin and Hudson 1991, Rogers and Harpending 1992). The $R2$ values were 0.4 and 0.12 for western and eastern haplogroups, respectively. In both cases, the $R2$ values were within the associated confidence interval, which suggests that the two regional populations have not undergone a recent demographic expansion and can be considered to be in demographic equilibrium.

The coalescent analysis of the control-region sequences of Blue-and-yellow Macaws in IM produced parameter estimates that had unimodal posterior distributions (not shown). Similar estimates of effective population size for all populations (ancestral, western, and eastern) were consistent with stable population size since the split from the ancestral population (Table 3). Estimates of demographic parameters with their 90% highest posterior density (HPD) intervals indicate that the western and eastern populations split ~0.37 mya (HPD = 0.19–0.86) and that female gene flow between them is low to moderate (Table 3). Posterior probabilities of TMRCA peaked at 7.94 (HPD = 4.53–14.67), which indicates that haplotypes coalesced to a common ancestor ~1.24 mya (HPD = 0.71–2.29).

The mean values of β for each of the six sampled populations based on microsatellite data ranged from 0.58 ± 0.42 (PA) to 1.41 ± 1.84 (ST) (data not shown). All population-specific values have 95% confidence intervals that overlap with an equilibrium value of 1.0. This supports the idea that these populations are close to or at demographic equilibrium. Estimates of gene flow based on microsatellite data using the coalescent approach in MIGRATE were symmetric between western and eastern populations (western to eastern $Nm = 1.01$, eastern to western $Nm = 1.04$ immigrants per generation). This level of gene flow is sufficient, in theory, to prevent significant differentiation in neutral microsatellites by genetic drift in large populations (Mills and Allendorf 1996).

Discussion

Analyses of population genetic structure in Blue-and-yellow Macaws based on the mtDNA control region showed evidence of two major regional populations: SG+CMS+PA (western haplogroup) and NG+ST+NMG (eastern haplogroup). However, these regional populations also showed evidence of low to moderate gene flow between them, given that they shared few haplotypes and that some closely related haplotypes are found in the two geographic regions (Fig. 2). This pattern can be attributed to low to moderate levels of female gene flow (Table 3) between historically isolated populations. Although the relatively small sample sizes and high diversity of mtDNA haplotypes make it somewhat difficult to draw many conclusions, it seems that much of the movement of mtDNA lineages between regions has occurred over a long period, as evidenced by the lack of sharing between the geographic groups of specific mtDNA haplotypes other than two common ones. Our analysis with IM also indicated that female gene flow occurred

Table 3. Coalescent estimates (MLE) and the 90% highest posterior density (HPD) intervals of demographic parameters based on mtDNA, assuming the splitting of western and eastern groups from an ancestral population. Population parameters that are scaled by the neutral mutation rate (θ_{west}, θ_{east}, and θ_A) are the standardized mutation rates for western, eastern, and ancestral populations, respectively; m_{west} and m_{east} are the migration rates into the western and eastern populations, respectively; $t = tu$ is the number of mutations since the populations split, where u is the annual locus-mutation rate; Ne_{west}, Ne_{east}, and Ne_A are the effective number of individuals in the western, eastern, and ancestral populations at the time of population divergence; $Ne_{(f)}m_{west}$ and $Ne_{(f)}m_{east}$ are the number of female immigrants per generation into western and eastern populations, respectively; and t is the time of the population split in millions of years.

	θ_{west}	θ_{east}	θ_A	m_{west}	m_{east}	$t = tu$	Ne_{west}	Ne_{east}	Ne_A	$Ne_{(f)}m_{west\ to\ east}$	$Ne_{(f)}m_{east\ to\ west}$	t
MLE	32.0	33.2	27.1	0.58	0.001	2.40	138,648	143,848	117,418	0.01	4.64	0.374
Lower HPD	15.2	16.9	6.4	0.17	0.000	1.22	65,858	73,224	27,730	0.00	0.65	0.190
Upper HPD	71.6	73.3	117.2	2.70	0.092	5.50	310,226	317,591	507,799	1.68	48.33	0.858

asymmetrically and mainly from the eastern to the western haplogroup (Table 3). Although direct studies of dispersal (e.g., radiotelemetry) are not available for Blue-and-yellow Macaw, the species is known to fly long distances and is potentially able to disperse over broad areas (Forshaw 1989).

In contrast with these results based on mtDNA control-region sequences, the two haplogroups showed no significant genetic differentiation based on microsatellite loci. There are three possible reasons for the differences in phylogeographic patterns observed between these two DNA markers: (1) the haplogroups are not in demographic equilibrium, and the high similarity in microsatellite loci is mainly attributable to recent sharing of ancestral polymorphisms rather than to gene flow; (2) differences in the effective population size and mutation rate of each type of marker result in mtDNA showing inherently higher levels of differentiation; or (3) male-biased gene flow and large effective population sizes have prevented microsatellite, but not female-specific mtDNA, differentiation between eastern and western haplogroups. We consider each of these possibilities below.

Hypothesis of common ancestry.—A high level of similarity among populations based on microsatellite data can result from a high level of retained ancestral polymorphism within populations (Gibbs et al. 2000). However, microsatellite data showed that in all populations the confidence interval for the imbalance index included the expected equilibrium value of 1. This suggests that the populations sampled are close to or at equilibrium (for details, see Kimmel et al. 1998). Additionally, the IM-estimated divergence time of the western and eastern haplogroups seems to be sufficiently old for the sorting of ancestral polymorphisms by genetic drift. Thus, recent common ancestry is an unlikely explanation for lack of differentiation observed on the basis of microsatellite data.

Hypothesis of genetic characteristics of markers.—Comparison of levels of variation between mtDNA and microsatellite data is not easy, because one is based on DNA sequence and the other on allele frequencies, which requires different measures of differentiation. As noted by Seielstad et al. (1998), comparisons of levels of differentiation among different classes of genetic makers need to take into account genetic characteristics that could bias such comparisons. Specifically, the fourfold difference in effective population size between organelle and autosomal markers (Birky et al. 1989) means that mtDNA may inherently show higher levels of differentiation at equilibrium than microsatellites, even though the microsatellite mutation rate is roughly an order of magnitude higher. However, the faster rate of mutation in microsatellites should have been sufficient to produce detectable allele frequency differences between eastern and western populations since the time of population divergence of females estimated with mtDNA.

Hypothesis of sex-biased dispersal.—Mitochondrial DNA is maternally inherited in birds; if females are more philopatric than males, this alone could result in greater population structure than that obtained by our analysis of data from biparentally inherited microsatellites. We found that female gene flow is asymmetric and low to moderate between western and eastern haplogroups, resulting in immigrant mtDNA haplotypes occurring at low frequency in both populations. Estimates of gene flow using microsatellites in MIGRATE were symmetric and showed about two migrants generation^{-1} between the two populations, which would theoretically prevent significant differentiation in allele frequencies, given selective neutrality and the large effective population sizes (see Mills and Allendorf 1996). Thus, the results suggest that males disperse over longer distances than females and that male-biased dispersal is at a rate high enough to explain the lack of population structure in nuclear DNA. Alternative explanations are different selective sweeps on mtDNA (Ballard and Whitlock 2004) in both populations or a random phylogeographic division without any clear geographic barrier to dispersal (Irwin 2002). However, these hypotheses are not supported by neutrality tests or by the exchange and persistence of immigrant haplotypes in western and eastern haplogroups.

Discrepancies in population structure obtained by markers with different inheritance patterns were observed in several organisms, including mammals (Melnick and Hoelzer 1992, Ishibashi et al. 1997), turtles (FitzSimmons et al. 1997), and birds (Scribner et al. 2001, Gay et al. 2004). In all cases, these discrepancies are concordant with the expected dispersal behavior of the sexes in these species. Male-biased dispersal in the Blue-and-yellow Macaw differs from the general pattern observed in birds of natal and breeding dispersal as either female-biased or equal with no differences between the sexes (Greenwood 1980, Clarke et al. 1997). However, most bird species for which dispersal data are available are passerines, whereas ~73% of the nonpasserines showed evidence of male-biased dispersal (Clarke et al. 1997).

Numerous hypotheses have related sex biases in dispersal to differences in the advantages that philopatry conveys to males and females in competition for mates or breeding resources, or to the influence of male–female differences on the fecundity cost of dispersal (Greenwood 1980, Dobson 1982, Johnson 1986, Pusey 1987, Wolff and Plissner 1998, Perrin and Mazalov 2000). However, predictions based on these different hypotheses depend on the mating system of the species considered. Greenwood (1980) hypothesized that female-biased dispersal in birds is associated with monogamy, resource defense by males, and local competition for mates among females, whereas mammalian male-biased dispersal is associated with polygyny, resource defense by females, and male defense of mates. Considering that competition for mates may be the most important factor in the evolution of dispersal patterns, male-biased dispersal observed in the Blue-and-yellow Macaw may be related to local competition for mates among males. However, little is known about the natural history of the Blue-and-yellow Macaw, so an explanation of sex-related differences in dispersal must await future ecological and genetic studies.

Demographic history.—The phylogeographic pattern observed in mtDNA sequences of the Blue-and-yellow Macaw can be interpreted as a relatively ancient isolation of individuals in western and eastern populations ~374,000 years ago, strong female philopatry, and male-biased dispersal among these populations. The paleogeological events that may have contributed to this contrasting phylogeographic pattern could possibly be related to the origin and formation of the main hydrographic basins in Brazil. The three haplotype clusters (Figs. 1 and 2) are geographically distributed in three different basins: cluster I in the Prata river basin, cluster II in the Amazonas river basin, and cluster III in the Araguaia–Tocantins river basin. Blue-and-yellow Macaws nest in holes in dead palm trees mainly of the genus *Mauritia* (Forshaw 1989, Collar 1997). These palm species occur in seasonally flooded

nascent and gallery forests that border rivers and streams, and the origin and formation of the watercourses could have shaped the geographic distribution of this species.

Implications for conservation.—The microsatellite survey indicates no population structure among the Blue-and-yellow Macaws we studied, which suggests that they should be considered a panmictic population; yet mtDNA data indicate two partially isolated groups. However, given that females disperse less than males, these two groups should be considered two different management units (Moritz 1994b) for conservation purposes. Particularly, if females do not move much, local populations could be extirpated by anything that increased female mortality or reduced their reproductive success. However, this result should be viewed with caution, because the Blue-and-gold Macaw has a continental geographic distribution, occurring throughout most of South America, and the results presented here involved few sampled localities and few individuals. Thus, more extensive sampling is required to better understand the historical and current processes of population structuring in this species. In addition, study and monitoring of local populations are important to better understand the population dynamics and to establish conservation strategies for this species.

Our results also highlight a potential problem in using mtDNA sequences alone to identify conservation units (e.g., Moritz 1994a, b; Holland and Hadfield 2002; Vieites et al. 2006). Several mitochondrial genome characteristics—such as its small size, ubiquity, maternal inheritance with no or rare recombination (Horai et al. 1995, Arnason et al. 1996), and faster evolutionary rate than nuclear DNA (Brown et al. 1982)—make this marker attractive for identifying units for conservation and designing management plans. However, caution should be used when defining units for conservation on the basis of any single character. As discussed above, there are some characteristics intrinsic to markers (effective population sizes) or to the biology of the species (male-biased gene flow) that can lead to misinterpretation of data. Thus, comparisons based solely on mtDNA may not represent the true genetic relations among populations. This does not mean that mtDNA data should be ignored in the identification of conservation units (e.g., Holland and Hadfield 2002); rather, we suggest that it is important to obtain corroborating evidence from nuclear markers to support conclusions from mtDNA. Finally, our study reinforces the observation that male-biased dispersal occurs in nonpasserine birds (Clarke et al. 1997) and that this can have a significant role in determining the genetic structure of bird populations.

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Literature Cited

Arnason, U., A. Gullberg, A. Janke, and X. XU. 1996. Pattern and timing of evolutionary divergences among hominoids based on analyses of complete mtDNAs. Journal of Molecular Evolution 46:650–661.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York.

Baker, A. J., and H. D. Marshall. 1997. Mitochondrial control region sequences as tools for understanding evolution. Pages 51–82 *in* Avian Molecular Evolution and Systematic (D. P. Mindell, Ed.). Academic Press, New York.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13:729–744.

Bandelt, H.-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers of two populations using a coalescent approach. Genetics 152:763–773.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences USA 98:4563–4568.

Bergallo, H. G., C. F. D. Rocha, M. V. Sluys, and M. A. S. Alves. 1999. A fauna ameaçada do Estado do Rio de Janeiro. Ciência Hoje 26:18–23.

Birky, C. W., Jr., P. Fuerst, and T. Maruyama. 1989. Organelle gene diversity under migration, mutation, and drift equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121:613–627.

Boutin-Ganache, I., M. Raposo, M. Raymond, and C. F. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:24–26.

Brown, W. M., E. M. Prager, A. Wang, and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. Journal of Molecular Evolution 18:225–239.

Bruford, M. W., O. Hanotte, J. F. Y. Brookfield, and T. Burke. 1992. Single-locus and multilocus DNA fingerprinting. Pages 225–269 *in* Molecular Genetic Analysis of Populations: A Practical Approach (A. R. Hoelzel, Ed.). IRL Press, Oxford, United Kingdom.

Caparroz, R., N. M. R. Guedes, C. A. Bianchi, and A. Wajntal. 2001. Analysis of the genetic variability and breeding behavior of wild populations of two macaw species (Psittaciformes, Aves) by DNA fingerprinting. Brazilian Journal of Ornithology 9:43–49.

Caparroz, R., C. Y. Miyaki, and A. J. Baker. 2003. Characterization of microsatellite loci in the Blue-and-gold Macaw, *Ara ararauna* (Psittaciformes: Aves). Molecular Ecology Notes 10:441–443.

Chesser, R. K., M. H. Smith, and I. L. Brisbin. 1980. Management and maintenance of genetic variability in endangered species. International Zoo Yearbook 20:146–154.

Clarke, A. L., B.-E. Sæther, and E. Røskaft. 1997. Sex biases in avian dispersal: A reappraisal. Oikos 79:429–438.

Clobert, J., J. D. Nichols, E. Danchin, and A. Dhondt, Eds. 2001. Dispersal. Oxford University Press, Oxford, United Kingdom.

Collar, N. J. 1997. Family Psittacidae (Parrots). Pages 280–477 *in* Handbook of the Birds of the World, vol. 4 (J. del Hoyo, A. Elliott, and J. Sargatal, Eds.). Lynx Edicions, Barcelona, Spain.

Dobson, F. S. 1982. Competition for mates and predominant juvenile male dispersal in mammals. Animal Behaviour 30:1183–1192.

Eberhard, J. R., T. F. Wright, and E. Bermingham. 2001. Duplication and concerted evolution of the mitochondrial control region in the parrot genus *Amazona*. Molecular Biology and Evolution 18:1330–1342.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford University Press, Oxford, United Kingdom.

FitzSimmons, N. N., C. Moritz, C. J. Limpus, L. Pope, and R. Prince. 1997. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics 147:1843–1854.

Forshaw, J. M. 1989. The Parrots of the World, 3rd ed. Lansdowne, Willoughby, Australia.

Fu, Y.-X., and W.-H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.

Gay, L., P. Defos du Rau, J.-Y. Mondain-Monval, and P.-A. Crochet. 2004. Phylogeography of a game species: The Red-crested Pochard (*Netta rufina*) and consequences for its management. Molecular Ecology 13:1035–1045.

Gibbs, H. L., R. J. G. Dawson, and K. A. Hobson. 2000. Limited differentiation in microsatellite DNA variation among northern populations of the Yellow Warbler: Evidence for male-biased gene flow? Molecular Ecology 9:2137–2147.

Goodman, S. J. 1997. R_{ST} Calc: A collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Molecular Ecology 6:881–885.

Goudet, J. 2001. FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices, version 2.9.3.2. [Online.] Available at www2.unil.ch/popgen/softwares/fstat.htm.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28:1140–1162.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Gutierrez, P. C. 1994. Mitochondrial-DNA polymorphism in the Oilbird (*Steatornis caripensis*, Steatornithidae) in Venezuela. Auk 111:573–578.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

Hey, J. 2005. On the number of New World founders: A population genetic portrait of the peopling of the Americas. PLoS Biology 3:e193.

Hey, J., and R. Nielsen. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics 167:747–760.

Holland, B. S., and M. G. Hadfield. 2002. Islands within an island: Phylogeography and conservation genetics of the endangered Hawaiian tree snail *Achatinella mustelina*. Molecular Ecology 11:365–375.

Holsinger, K. E., and R. J. Mason-Gamer. 1996. Hierarchical analysis of nucleotide diversity in geographically structured populations. Genetics 142:629–639.

Horai, S., K. Hayasaka, R. Kondo, K. Tsugane, and N. Takahata. 1995. Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proceedings of the National Academy of Sciences USA 92:532–536.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Pages 1–44 *in* Oxford Surveys in Evolutionary Biology, vol. 7 (D. J. Futuyma and J. D. Antonovics, Eds.). Oxford University Press, New York.

Irwin, D. E. 2002. Phylogeographic breaks without geographic barriers to gene flow. Evolution 56:2383–2394.

Ishibashi, Y., T. Saitoh. S. Abe, and M. C. Yoshida. 1997. Sex-related spatial kin structure in a spring population of grey-sided voles (*Chethrionomys rufocanus*) as revealed by mitochondrial and microsatellite DNA analyses. Molecular Ecology 6:63–71.

Johnson, C. N. 1986. Sex-biased philopatry and dispersal in mammals. Oecologia 69:626–627.

Karl, S. A., B. W. Bowen, and J. C. Avise. 1992. Global population genetic structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear loci. Genetics 131:163–173.

Kimmel, M., R. Chakraborty, J. P. King, M. Bamshad, W. S. Watkins, and L. B. Jorde. 1998. Signatures of population expansion in microsatellite repeat data. Genetics 148:1921–1930.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725–738.

Lacy, R. C. 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1:143–158.

Lande, R., and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pages 87–123 *in* Viable Populations for Conservation (M. E. Soulé, Ed.). Cambridge University Press, Cambridge, United Kingdom.

Machado, A. B. M., G. A. B. Fonseca, R. B. Machado, L. M. S. Aguiar, and L. V. Lins. 1998. Livro Vermelho das Espécies Ameaçadas de Extinção da Fauna de Minas Gerais. Fundação Biodiversitas, Belo Horizonte.

Melnick, D. J., and G. A. Hoelzer. 1992. Differences in male and female macaque dispersal lead to contrasting distributions of nuclear and mitochondrial DNA variation. International Journal of Primatology 13:379–393.

Mills, L. S., and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509–1518.

Moritz, C. 1994a. Applications of mitochondrial DNA analysis in conservation: A critical review. Molecular Ecology 3:401–411.

Moritz, C. 1994b. Defining "Evolutionarily Significant Units" for conservation. Trends in Ecology and Evolution 9:373–375.

Ohta, T., and M. Kimura. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genetical Research 22:201–204.

Oliveira-Marques, A. R. 2006. Filogenia Molecular de Espécies do Gênero *Ara* (Psittaciformes, Aves). Msc. thesis, Universidade de São Paulo, São Paulo.

Perrin, N., and V. Mazalov. 2000. Local competition, inbreeding, and the evolution of sex-biased dispersal. American Naturalist 155:116–127.

Piertney, S. B., A. D. C. MacColl, P. J. Bacon, and J. F. Dallas. 1998. Local genetic structure in Red Grouse (*Lagopus lagopus scoticus*): Evidence from microsatellite DNA markers. Molecular Ecology 7:1645–1654.

Piertney, S. B., A. D. C. MacColl, P. J. Bacon, P. A. Racey, X. Lambin, and J. F. Dallas. 2000. Matrilineal genetic structure and female-mediated gene flow in Red Grouse (*Lagopus lagopus scoticus*): An analysis using mitochondrial DNA. Evolution 54:279–289.

Posada, D., and K. A. Crandall. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pusey, A. E. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. Trends in Ecology and Evolution 2:295–299.

Quinn, T. W. 1997. Molecular evolution of the mitochondrial genome. Pages 3–28 *in* Avian Molecular Evolution and Systematic (D. P. Mindell, Ed.). Academic Press, New York.

Ramos-Onsins, S. E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092–2100.

Raymond, M., and F. Rousset. 1995. GENEPOP: Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

Rozas, J., J. C. Sánchez-Delbarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

Ruokonen, M., and L. Kvist. 2002. Structure and evolution of the avian mitochondrial control region. Molecular Phylogenetics and Evolution 23:422–432.

Russello, M., D. Calcagnotto, R. DeSalle, and G. Amato. 2001. Characterization of microsatellite loci in the endangered St. Vincent Parrot, *Amazona guildingii*. Molecular Ecology Notes 1:162–164.

São Paulo State Government. 1998. Decreto no. 42.838. de 4 de fevereiro de 1998. Declaração das espécies da fauna silvestres ameaçadas de extinção e as provavelmente ameaçadas de extinção no Estado de São Paulo e dá providências correlatas. Diário Oficial. São Paulo 108(25), 5 fev.

Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN: A Software for Population Genetic Data Analysis. University of Geneva, Geneva, Switzerland. [Online.] Available at lgb.unige.ch/arlequin/.

Scribner, K. T., M. R. Petersen, R. L. Fields, S. L. Talbot, J. M. Pearce, and R. K. Chesser. 2001. Sex-biased gene flow in Spectacled Eiders (Anatidae): Inferences from molecular markers with contrasting modes of inheritance. Evolution 55:2105–2115.

Seielstad, M. T., E. Minch, and L. L. Cavalli-Sforza. 1998. Genetic evidence for a higher female migration rate in humans. Nature Genetics 20:278–280.

Simberloff, D. 1988. The contribution of population and community biology to conservation science. Annual Review of Ecology and Systematics 19:473–511.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Swofford, D. L. 1999. PAUP*: Phylogenetic Analysis using Parsimony (*and Other Methods), version 4.0b8. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition–transversion and G+C-content biases. Molecular Biology and Evolution 9:678–687.

Tavares, E. S., A. J., Baker, S. L. Pereira, and C. Y. Miyaki. 2006. Phylogenetic relationships and historical biogeography of Neotropical parrots (Psittaciformes: Psittacidae: *Arini*) inferred from mitochondrial and nuclear DNA sequences. Systematic Biology 55:454–470.

Tavares, E. S., C. Yamashita, and C. Y. Miyaki. 2004. Phylogenetic relationships among some Neotropical parrot genera (Psittacidae) based on mitochondrial sequences. Auk 121:230–242.

Vieites, D. R., Y. Chiari, M. Vences, F. Andreone, F. Rabemananjara, P. Bora, S. Nieto-Román, and A. Meyer. 2006. Mitochondrial evidence for distinct phylogeographic units in the endangered Malagasy poison frog *Mantella bernhardi*. Molecular Ecology 15:1617–1625.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wolff, J. O., and J. H. Plissner. 1998. Sex biases in avian natal dispersal: An extension of the mammalian model. Oikos 83:327–330.

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APPENDIX. Genetic variation at six microsatellite loci of Blue-and-yellow Macaws from six populations (n = number of individuals genotyped; range = size range of alleles in base pairs; H_E and H_O are expected and observed heterozygosities, respectively).

	Locus					
Locality[a]	UnaCT21	UnaCT32	UnaCT43	UnaCT74	UnaGT55	AgGT21
CMS						
n	8	8	8	8	8	8
Number of alleles	6	7	8	4	4	3
Range	247–257	258–276	199–219	232–246	184–192	308–312
H_E	0.867	0.800	0.908	0.533	0.592	0.342
H_O	0.750	0.750	1.000	0.375	0.750	0.375
SG						
n	17	17	17	17	17	17
Number of alleles	8	4	7	3	4	3
Range	247–261	262–272	199–217	240–246	184–192	308–312
H_E	0.866	0.618	0.829	0.520	0.403	0.171
H_O	0.882	0.647	0.882	0.412	0.353	0.118
NMG						
n	4	4	4	4	4	4
Number of alleles	4	3	5	3	2	1
Range	245–259	262–268	205–217	240–246	184–190	308
H_E	0.821	0.750	0.857	0.750	0.607	—
H_O	1.000	0.500	1.000	0.750	0.500	—
NG						
n	10	10	10	10	10	10
Number of alleles	8	2	7	4	4	3
Range	247–273	262–264	199–217	232–246	184–192	308–312
H_E	0.874	0.505	0.842	0.721	0.489	0.363
H_O	0.800	0.200	1.000	0.600	0.600	0.300
ST						
n	5	5	5	5	5	5
Number of alleles	4	2	4	3	2	2
Range	249–255	262–264	199–215	240–246	184–190	308–312
H_E	0.800	0.533	0.889	0.844	0.356	0.200
H_O	1.000	0.800	0.600	0.400	0.400	0.200
PA						
n	5	5	5	5	5	5
Number of alleles	6	6	6	4	3	2
Range	247–261	262–276	205–217	232–246	184–190	308–310
H_E	0.889	0.911	0.889	0.800	0.533	0.356
H_O	0.800	0.800	0.800	0.600	0.400	0.400

[a] Localities abbreviation as in Table 1.