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MITOCHONDRIAL-DNA POLYMORPHISM IN THE OILBIRD (*STEATORNIS CARIPENSIS*, STEATORNITHIDAE) IN VENEZUELA

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ABSTRACT.—Mitochondrial-DNA (mtDNA) polymorphisms were studied in the Oilbird (*Steatornis caripensis*). Twelve closely related (P = 0.06 to 0.35%) mtDNA haplotypes were found in Oilbird colonies studied in northeastern and northwestern Venezuela. Eleven of the mtDNA clones are related to the ancestral one by one or two mutational steps. Female-mediated gene flow is high ($Nm > 1$) among the colonies studied. As a consequence of the high female-mediated gene flow, no phylogeographic structuring among the mtDNA composite haplotypes was observed. Evidence from mtDNA analysis suggests that Oilbird populations in Venezuela have gone through a bottleneck. Results also seem to indicate that the annual postbreeding migrations of Oilbirds from the Guacharo Cave to the caves in the Mata de Mango area involve mostly the breeding adults, whereas juveniles disperse from the Guacharo Cave to the Mata de Mango cave system for longer periods. *Received 3 August 1993, accepted 15 November 1993.*

OILBIRDS (*Steatornis caripensis*) are cave-dwelling, nocturnal, and gregarious frugivores. They are restricted to the cave habitats of northern South America and Trinidad, and occur in Venezuela, Colombia, Guyana, Ecuador, Peru, Bolivia, northern Brazil, and Panama (Bosque 1986). Oilbird colonies in Venezuela are found primarily in the northeast and northwest (Fig. 1), with the exception of two colonies in the Andes and six colonies near the Brazilian border (Bosque 1986).

In Venezuela, Oilbirds breed once a year (Roca 1994). Reproduction within a colony is moderately synchronized and the reproductive period extends from March to September. At the end of the breeding season, most Oilbirds from the Guacharo Cave migrate southeast to an area known as Mata de Mango. Mata de Mango is a limestone mountain range covered by pristine tropical forest; deep canyons, rivers, waterfalls, caverns, and sinkholes are common in this area. Oilbirds begin to leave the Guacharo Cave shortly after nestlings fledge and there is no bias for age class. Snow (1979) and Roca (1994) indicated that postbreeding migrations are incomplete; between 5 and 27% of the colony remains at the Guacharo Cave during the nonbreeding season. Roca (1994) postulated that lack of food triggers migration and that those birds that stay behind are probably too old or weak for the journey. Postbreeding migrations occur in other Venezuelan colonies, in Ecuador and Colombia (Bosque and Ramirez 1988). Return of Oilbirds to the Guacharo Cave in March coincides with the onset of the breeding season, and most adults return to mate, build, or rebuild the same nests (Snow 1961, Bosque and Ramirez 1988, Roca 1994).

Demographic characteristics that influence the genetic architecture of Oilbirds are: colonies of limited size; breeding philopatry to particular caves; and life-long monogamy (Snow 1961). Their distribution in caves that are distant from one another suggests that the colonies may be genetically isolated from one another. However, postbreeding migrations may facilitate gene flow among different colonies. Mitochondrial-DNA (mtDNA) analysis can provide a measure of the degree of genetic separation of colonies as a function of isolation. It is expected that maximum divergence occurs between those colonies that have been isolated for the longest time. Conversely, if there is female-mediated gene flow between colonies, their genetic similarity will be proportional to the rate of female dispersal. I used restriction-fragment-length polymorphisms (RFLPs) in mtDNA to examine population structure in Venezuelan Oilbirds, and determine whether females disperse from their natal colony, or whether philopatry leads to genetic structure.

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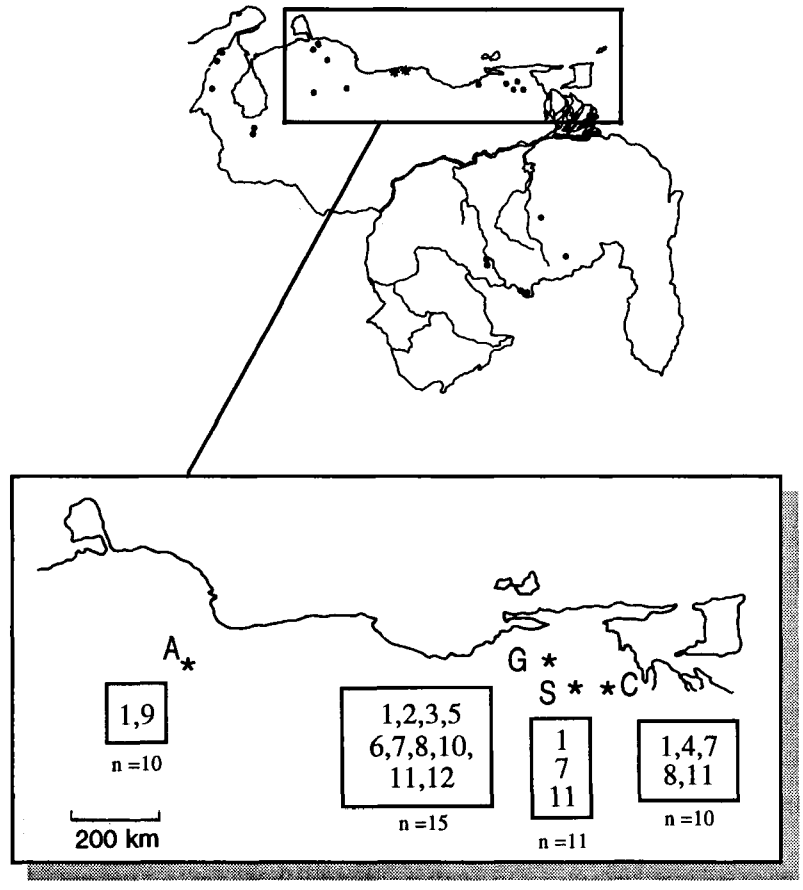

Fig. 1. Distribution of Oilbird in Venezuela. Asterisk indicates extinct colony, and dot an active colony. Enlarged area shows geographic distribution of mtDNA haplotypes numbered 1–12. Localities indicated: (G) Guacharo Cave; (S) Sucia Cave; (C) Clara Cave; and (A) Los Anadidos Cave.

MATERIALS AND METHODS

Nestlings that fell from their nests were collected from four localities in northeastern and northwestern Venezuela. In the northeast (State of Monagas), samples were collected from Guacharo Cave (colony size ca. 10,000 individuals, $n = 47$), Sucia Cave (ca. 1,000 individuals, $n = 26$), and Clara Cave (ca. 1,500 individuals, $n = 17$). Sucia and Clara caves are about 5 km apart and located in the Mata de Mango area, and these two caves are approximately 45 km from Guacharo Cave. In northwestern Venezuela (State of Yaracuy), nestlings were collected from Los Anadidos Cave (ca. 1,200 individuals, $n = 10$), which is approximately 500 km from the northeastern group of caves (Fig. 1).

The method of Lansman et al. (1981) for the isolation of mtDNA was modified in several ways because it would not render intact mtDNA from Oilbird heart and liver tissues. The concentration of EDTA in the homogenizing buffer was increased to 200 mM. Centrifugation speeds and time were increased to 754 $\times$ *g* for 10 min to reduce nuclear contamination of the mitochondrial pellet, and to 17,369 $\times$ *g* for 40 min to enhance mitochondrial yield. The pellet was washed several times (until no fat was visible in the supernatant).

Harsher conditions were used during lysis to rid the mtDNA of lipids and proteins. Mitochondria were resuspended in 5 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), lysed with 0.30 ml of 20% sodium dodecyl sulfate (SDS), and incubated at 65°C for 30 min. The lysate was cooled on ice and 0.175 ml/ml of lysate, of a high salt solution (60 ml 5 M K acetate, 11.5 ml glacial acetic acid, 28.5 ml H_2O) was added. After 2 h on ice, centrifugation at 34,858 $\times$ *g* for 5 min precipitated most of the SDS-protein complex. I extracted mtDNA in the supernatant at least five times with phenol-chloroform-isoamylalcohol (24:24:1) and twice with chloroform; the mtDNA was dialyzed overnight and precipitated.

This extraction-purification protocol did not render an mtDNA preparation completely free of nuclear contamination. Therefore, mtDNA preparations were

digested with *Pst*I, which produced two large fragments that were cut out of the agarose gel and purified (Maniatis et al. 1982). This purified mtDNA was then digested with *Ava*I, *Ava*II, *Hin*dIII, *Msp*I, *Sau*96I, and *Spe*I. Digestions were carried out in 10 μl reaction mixtures containing mtDNA and at least three units of enzyme. Digestions were extended for 16 h at 37°C to ensure complete digestion, and stopped by freezing.

Restriction fragments were end-labeled with ^{32}P using rapid end-labelling of DNA with the Klenow fragment of *E. coli* DNA polymerase I (Maniatis et al. 1982). Vertical polyacrylamide gel electrophoresis was used to separate mtDNA fragments of a 100 to 1,000 base pairs (4% gels), and fragments of 80 to 500 bp (5% gels; Maniatis et al. 1982). Autoradiography was used to visualize the fragments (Maniatis et al. 1982).

Presence or absence of restriction fragments was used to estimate nucleotide sequence divergence between mtDNA haplotypes using the index of the relative genetic similarity (*P*) of Nei and Li (1979). A *G*-test of significance for the differences in the haplotype frequencies was used (Sokal and Rohlf 1969).

The mtDNA haplotypes were clustered using the unweighted pair-group method with arithmetic averages (UPGMA) of Sneath and Sokal (1973). The fragment presence/absence matrix was subjected to a phylogenetic analysis using parsimony (PAUP; Swofford and Olsen 1990).

Gene flow between the different colonies was estimated by three different methods. The private-allele approach of Slatkin (1985) and Slatkin and Barton (1989) uses those alleles that are "rare" (i.e. unique haplotypes). The genetic-identity approach of Takahata and Palumbi (1985) takes into account the genetic similarity of alleles within and between demes. This approach uses an estimator of Wright's (1951) F_{ST}-statistic (G_{ST}) to estimate *Nm* and is based on identity probabilities, the probability that two sampled homologous DNA segments will be identical.

The cladistic method of Slatkin and Maddison (1989) is based on the minimum number of migration events necessary to explain the observed geographic distribution of mtDNA haplotypes. The method provides an estimate of *Nm*, where *N* is the size of each local population and *m* is the migration rate. When the phylogeny of mtDNA haplotypes is known, the geographic location of each population is treated as a multistate character with one state for each geographic location. A parsimony criterion is used on a tree of the phylogeny to determine the minimum number of migration events consistent with the tree. Slatkin and Maddison (1989) provided confidence intervals for *Nm*.

Results

The size of the mtDNA genome of the Oilbird is 16,250 ± SE of 150 base pairs (bp), typical of the size reported for many birds (Avise and Zink 1988, Shields and Helm-Bychowski 1988). The seven restriction endonucleases used produced between 65 and 69 mtDNA restriction fragments in each individual assayed. In total, 61 fragments were identical and 13 were polymorphic. This represents 355 bp of information or 2.21% of the mtDNA genome analyzed per individual. A 10-bp size polymorphism was observed in the restriction-fragment profiles of *Sau*96I. The two size classes were always scored as the same fragment.

Every individual was assigned a composite haplotype of six letters, each corresponding to the restriction-fragment pattern observed for each restriction endonuclease. The common haplotype was designated by C, and variants were designated by A, B, D, E, F, and G. The observed frequencies and geographic distributions of the 12 haplotypes observed are shown in Table 1. A *G*-test with a Williams' correction for small sample size (Sokal and Rohlf 1969) indicated that haplotype frequencies among different colonies were significantly different ($P < 0.05$). Eleven of the mtDNA haplotypes are related, by one or two mutational steps, to the ancestral one (haplotype 1). It is considered to

TABLE 1. Composite haplotypes of 46 Oilbirds from four colonies with haplotype labels (1–12) and frequencies in each colony. Positions 1 to 6 of composite haplotype contain letters designating different fragment patterns produced by restriction enzymes *Ava*I, *Ava*II, *Hin*dIII, *Msp*I, *Sau*96I, and *Spe*I.

Label	Composite haplotype	Colony	No. birds
1	CCCCCC	Guacharo	6
		Sucia	5
		Clara	3
		Anadidos	9
2	CCCCFC	Guacharo	1
3	CCCCAC	Guacharo	1
4	CCCBCC	Clara	1
5	CECCCC	Guacharo	1
6	CCCECC	Guacharo	1
7	CCCCDC	Guacharo	1
		Sucia	5
		Clara	4
8	CCCBDC	Guacharo	1
		Clara	1
9	CCCCGC	Anadidos	1
10	CDCCEC	Guacharo	1
11	CDCCCC	Guacharo	1
		Sucia	1
		Clara	1
12	CECCBC	Guacharo	1

TABLE 2. *Nm* values (lower left) and G_{ST} values (upper right) estimated by Takahata and Palumbi (1985) method.

	Guacharo	Sucia	Clara	Anadidos
Guacharo	—	0.129	0.133	0.296
Sucia	3.37	—	0.0076	0.333
Clara	3.25	131.0	—	0.329
Anadidos	1.18	2.50	2.53	—

be ancestral because it is present in 50% of the birds sampled, it is found in all colonies, and it is related to all others (Avise et al. 1987). Values of *P* among Oilbird haplotypes ranged from 0.0006 to 0.0035 ($\bar{x}$ = 0.0018), indicating that haplotypes are closely related.

The geographic distribution of the composite mtDNA haplotypes (Fig. 1) shows that only 4 of 12 haplotypes are widespread. The PAUP parsimony analysis generated 16 most-parsimonious trees and the consensus tree showed a polychotomy (uncertain resolution) of haplotypes 2 through 9. The UPGMA phenogram showed no obvious geographic clustering of mtDNA haplotypes.

Gene flow (*Nm*) was estimated by three different methods. The private-allele approach gave an estimate for *Nm* of 0.76. The genetic-identity approach gave *Nm* values above 1 (Table 2). The cladistic method estimated *Nm* to be 1.81 and 1.23 based on UPGMA and PAUP trees, respectively. The respective 95% confidence intervals were 0.17 and 30.69.

DISCUSSION

These *P* values (0.06 to 0.35%) for Oilbirds are similar to those obtained for other conspecific comparisons of avian mtDNA (Kessler and Avise 1985, Ovenden et al. 1987, Shields and Wilson 1987, Tegelstrom 1987, Ball et al. 1988, Hare and Shields 1992). In avian species where females can disperse over large areas, closely related mtDNA haplotypes can be found in geographically distant localities, and considerably divergent ones can be present in the same locality (Tegelstrom 1987). Thus, mtDNA haplotypes will not show geographic structure. Birds are highly mobile; therefore, phylogeographic differentiation in mtDNA haplotypes is expected to be minor (Ball et al. 1988). Oilbirds are highly mobile; during the breeding season their home ranges can expand over an area of up to 96.3 km^2, with the maximum distance between feeding localities of about 150 km. During their postbreeding dispersal they are capable of flying 240 km in one night in search for food (Roca 1994). Therefore, the limited mtDNA phylogeographic population structure is probably attributable to a high level of dispersal and gene flow.

In a number of other avian species, a similar lack of phylogeographic differentiation has been attributed to female dispersal and gene flow. Tegelstrom (1987) investigated mtDNA variation in the Great Tit (*Parus major*) in three neighboring localities in Sweden, and found no obvious spatial structuring of mtDNA clones. Ball et al. (1988) conducted a continentwide survey of Red-winged Blackbirds (*Agelaius phoeniceus*) and found very little population structure. Tegelstrom et al. (1990) studied the Pied Flycatcher (*Ficedula hypoleuca*) in Sweden, and found no obvious geographic structuring in the very closely related mtDNA clones.

In Oilbirds, gene flow was inversely related to distance, the exception was the estimates between Sucia and Anadidos, and Clara and Anadidos. This is the result of Sucia, Clara, and Anadidos having fewer different haplotypes than Guacharo and Anadidos (Fig. 1) and, therefore, appearing more similar than Guacharo and Anadidos.

My mtDNA analysis has shown that the Oilbird colonies studied in Venezuela do not represent isolated populations. Rather, they are dynamically interconnected by migration of adults that have been shown to be philopatric (Snow 1961, Roca 1994) and by dispersal of juveniles from the Guacharo Cave to the Mata de Mango area. Nesting space is very limited in the caves where Oilbirds are found (pers. obs., Snow 1961, Roca 1994). When adults return to breed, they tend to occupy the same nest. A few young and inexperienced birds end up without a nest and, therefore, without a mate remain as floaters in the colony and cannot breed. I believe that most of the juveniles remain in the Mata de Mango area for some time, a few may return to the Guacharo Cave to breed, and others breed in the caves of the Mata de Mango.

The Oilbird is probably a relatively old species, given that the family Steatornithidae may have arisen about 50 mya near the base of the caprimulgiform radiation, possibly derived from the Coraciiformes (Olson 1987). Oilbirds apparently originated and differentiated in North

America, and had the same diet as modern representatives. The present-day species must be regarded as a relic in South America (Olson 1987).

Ancestral representatives of today's *Steatornis* may have colonized South America after the Central American land bridge was formed and when the Pleistocene climatic changes forced them to migrate south. Oilbirds were probably established in South America during the last glacial periods. Warm and humid conditions during interglacial periods may have caused the expansion of low montane forests on which oilbirds depended. This, in turn, may have facilitated migration and colonization of new habitats with subsequent establishment of new colonies. Less favorable conditions may have caused the reduction of some forest habitats, and the extinction of some colonies, and may have reduced gene flow among those surviving. These conditions could have produced a bottleneck (by founder effects and local extinction of lineages) in the population, whereby genetic variability would have been reduced. As climatic conditions became favorable again, subpopulations that were subdivided because of ecological conditions could have begun to recover from this bottleneck. New mutations appeared, and with the reestablishment of gene flow, they could have begun to spread through the subpopulations.

A reduction in Oilbird genetic diversity due to a bottleneck effect is also suggested from morphological and nuclear-genome data. Lentino (1990) assayed 37 protein loci from Oilbirds collected from five colonies in Venezuela and determined that all loci were monomorphic and fixed for the same allele. He also found no significant difference in 49 morphological characters studied. The reduced genetic divergence and low frequencies of most mtDNA variants suggests that these mtDNA mutations may have arisen in the population in the recent past. However, this homogeneity could also be a product of gene flow. Due to the great dispersal capabilities of Oilbirds and to the observed rates of female mediated gene flow, these mtDNA haplotypes have spread through the population.

The presence of unique mtDNA haplotypes in some colonies and not in others (i.e. haplotype 9 in Anadidos; haplotype 4 in Clara; haplotype 8 in Clara and Guacharo; haplotypes 2, 3, 5, 6, 10, 12 in Guacharo) may not be due completely to extinction of lineages as a result of more recent ecological disturbances or the appearance of new mutations, but could also reflect the fact that sample sizes were relatively small. A more exhaustive survey would probably show that rare composite mtDNA haplotypes are more widely distributed, confirming the lack of geographic structuring due to gene flow observed in this survey.

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Literature Cited

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18:489–522.

Avise, J. C., and R. M. Zink. 1988. Molecular genetic divergence between avian sibling species: King and Clapper rails, Long-billed and Short-billed dowitchers, Boat-tailed and Great-tailed grackles, and Tufted and Black-crested titmice. Auk 105: 516–528.

Ball, R. M., Jr., S. Freeman, F. C. James, E. Bermingham, and J. C. Avise. 1988. Phylogeographic population structure of the Red-winged Blackbirds assessed by mitochondrial DNA. Proc. Natl. Acad. Sci. USA 85:1558–1562.

Bosque, C. 1986. Actualización de la distribución del Guácharo (*Steatornis caripensis*) en Venezuela. Bol. Soc. Venezolana Espel. 22:1–10.

Bosque, C., and R. Ramirez. 1988. Post-breeding migration of Oilbirds. Wilson Bull. 100:675–677.

Hare, M. P., and G. F. Shields. 1992. Mitochondrial DNA variation in the polytypic Alaska Song Sparrow. Auk 109:126–132.

Kessler, L. G., and J. C. Avise. 1985. A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. Mol. Biol. Evol. 2:109–125.

Lansman, R. A., R. O. Shade, J. F. Shapira, and J. C.

AVISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. 17:214-226.

LENTINO, M. 1990. Flujo génico y variabilidad morfológica en el Guácharo (*Steatornis caripensis*). M.S. thesis, Univ. Simón Bolívar, Caracas, Venezuela.

MANIATIS, T., E. F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory.

NEI, M., AND W-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.

OLSON, S. L. 1987. An early Eocene Oilbird from the Green River formation of Wyoming (Caprimulgiformes: Steatornithidae). Docum. Lab. Geol. Lyon 99:57-69.

OVENDEN, J. R., A. G. MACKINLAY, AND R. H. CROZIER. 1987. Systematics and mitochondrial genome evolution of Australian rosellas (Aves: Platycercidae). Mol. Biol. Evol. 4:526-543.

ROCA, R. 1994. Oilbirds of Venezuela: Ecology and conservation. Nuttall Ornithological Club, No. 24 (R. A. Paynter, Ed.). Cambridge, Massachusetts.

SHIELDS, G. F., AND A. C. WILSON. 1987. Subspecies of the Canada Goose (*Branta canadensis*) have distinct mitochondrial DNAs. Evolution 41:662-666.

SHIELDS, G. F., AND K. M. HELM-BYCHOWSKI. 1988. Mitochondrial DNA of birds. Curr. Ornithol. 5:273-295.

SLATKIN, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53-65.

SLATKIN, M., AND N. H. BARTON. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349-1368.

SLATKIN, M., AND W. P. MADDISON. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics 123:603-613.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman, San Francisco.

SNOW, B. K. 1979. The oilbirds of Los Tayos. Wilson Bull. 91:457-461.

SNOW, D. W. 1961. The natural history of the Oilbird, *Steatornis caripensis*, in Trinidad. W.I. Part 1, General behavior and breeding habits. Zoologica 46:26-48.

SOKAL, R. R., AND J. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco.

SWOFFORD, D. L., AND G. J. OLSEN. 1990. Phylogeny reconstruction. Pages 411-501 *in* Molecular systematics (D. M. Hillis and C. Moritz, Eds.). Sinauer Associates, Sunderland, Massachusetts.

TAKAHATA, N., AND S. R. PALUMBI. 1985. Extranuclear differentiation and gene flow in the finite island model. Genetics 109:441-457.

TEGELSTROM, H. 1987. Genetic variability in mitochondrial DNA in a regional population of the Great Tit (*Parus major*). Biochem. Genet. 25:95-110.

TEGELSTROM, H., H. P. GELTER, AND M. JAAROLA. 1990. Variation in Pied Flycatcher (*Ficedula hypoleuca*) mitochondrial DNA. Auk 107:730-736.

WRIGHT, S. 1951. The genetical structure of populations. Ann. Eugen. 15:323-354.