
Mitochondrial DNA Phylogeography and the Conservation of Endangered Lesser Antillean *Icterus* Orioles

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Abstract: *Recent natural and anthropogenic disturbances have endangered two of the three oriole species endemic to single islands in the Lesser Antilles. The ongoing eruption of the Soufriere Hills volcano may have doomed the Montserrat Oriole (Icterus oberi), whereas high levels of nest parasitism by a cowbird threaten the Martinique Oriole (I. bonana). These orioles and related Antillean and Central American forms have been considered part of the Icterus dominicensis superspecies complex, but the taxonomic status of the different Antillean island populations has been long debated. To investigate levels of evolutionary differentiation among threatened Lesser Antillean orioles, we analyzed 2507 nucleotides of protein-coding mitochondrial DNA (mtDNA) sequence from orioles on Martinique, Montserrat, St. Lucia (I. laudabilis), Puerto Rico (I. dominicensis dominicensis), Mexico (I. d. prothemelas), and three Icterus outgroup species. Phylogenetic analyses of the mtDNA data supported the monophyly of Antillean members of the I. dominicensis complex and identified a star-like pattern of relationship among them. Mitochondrial distances between the Antillean populations were large (4.5–5.8% nucleotide divergence) and suggested that the Lesser Antillean orioles have been isolated evolutionarily from one another since the late Pliocene. The oriole taxa on Montserrat, Martinique, and St. Lucia meet species criteria under the phylogenetic species concept and represent evolutionarily significant units. The impending extinction of the phylogenetically unique Montserrat oriole highlights the vulnerability of island endemics to habitat degradation followed by rare and unpredictable natural catastrophes.*

Filogeografía Basada en ADN Mitocondrial y la Conservación de Orioles Antillanos del Género *Icterus*

Resumen: *Recientes disturbios antropogénicos y naturales han puesto en peligro de extinción a dos de los tres orioles endémicos en islas de las Antillas Menores. La erupción del volcán "Soufrière Hills" pudo haber sentenciado al Oriol de Montserrat (Icterus oberi) y los altos niveles de parasitismo de nidos amenazan al Oriol de Martinica (I. bonana). Estos orioles antillanos y formas relacionadas de Centroamérica, han sido considerados parte del complejo supraespecífico I. dominicensis, pero el estado taxonómico de las diferentes poblaciones de las islas antillanas ha sido tema de largos debates. Para indagar los niveles de diferenciación evolutiva entre los orioles amenazados en las Antillas Menores, analizamos 2507 nucleótidos de los códigos proteínicos del ADN mitocondrial (ADNmt) secuenciados de orioles de Martinica, Montserrat, Sta. Lucía (I. laudabilis), Puerto Rico (I. dominicensis dominicensis), México (I. d. prothemelas) y tres grupos de especies de Icterus lejanos. Los análisis filogenéticos de las secuencias de ADNmt indican que los miembros antillanos del complejo de I. dominicensis forman un grupo monofilético, pero las relaciones entre ellas no están resueltas. Las distancias mitocondriales entre las poblaciones antillanas fueron grandes (4.5–5.8% de divergencia nucleótida) y sugieren que los orioles de las Antillas Menores han estado evolutivamente aislados los unos a los otros desde al menos el Plioceno. Los taxa de orioles en Montserrat, Martinica y Sta. Lucía se pueden calificar como especies de acuerdo al concepto filogenético de especie y representan unidades evolutivamente significativas. La inminente extinción del filogenéticamente único Oriol de Montserrat recalca la vulnerabilidad de especies endémicas en islas, las cuales son afectadas por la degradación de su hábitat y por raras e impredecibles catástrofes naturales.*

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Introduction

Three species of *Icterus* orioles are endemic to single islands in the Lesser Antilles: the Montserrat Oriole (*I. oberi*), the Martinique Oriole (*I. bonana*), and the St. Lucia Oriole (*I. laudabilis*). Although these closely related taxa have played a prominent role in several models of West Indian biogeography (Bond 1956, 1963; Lack 1976), little is known about evolutionary differentiation among them. Two of these island populations have become endangered recently, highlighting the need for quantitative information about their evolutionary distinctiveness.

Of the three Lesser Antillean *Icterus* taxa, the Montserrat Oriole currently experiences the greatest risk of extinction. Since July 1995 the eruption of the Soufriere Hills volcano has severely damaged or destroyed the few forest patches that supported remnant populations of this species (Collar & Andrew 1988; Johnson 1988; Brosnan & Courtney 1997; Young et al. 1997). A population viability analysis (Brosnan & Courtney 1997) has suggested that the Montserrat Oriole faces a high probability of extinction even in the absence of additional volcanic disturbance. Recovery efforts that include the capture of Montserrat Orioles for captive breeding are being planned (D. B. Brosnan, personal communication; see also <http://www.sei.org/oriole.html>), but little information is available on the size of the remaining population. Even if some individuals have survived to the present, the prospect of continuing and potentially cataclysmic volcanic disturbance makes the species' near-term survival unlikely (Brosnan & Courtney 1997; Young et al. 1997).

Recent anthropogenic habitat alteration also has caused a severe reduction in the population size and habitat use of the endangered Martinique Oriole (Babbs et al. 1987; Collar et al. 1992). The decline of the Martinique Oriole has resulted primarily from intensive brood parasitism by the Shiny Cowbird (*Molothrus bonariensis*; Babbs et al. 1987), a species whose rapid spread throughout much of the West Indies in the past half-century has followed

forest clearing and land-use changes (Cruz et al. 1985). Icterids are favored Shiny Cowbird hosts, and island oriole populations are particularly vulnerable to Shiny Cowbird nest parasitism (Post & Wiley 1977; Cruz et al. 1985, 1989; Post et al. 1990).

The taxonomic status of the three Lesser Antillean oriole populations (Fig. 1) has long been debated. Most recent authorities (e.g., Sibley & Monroe 1990; American Ornithologists' Union 1998) have followed Bond (1956) in treating each island population as a full species within a superspecies complex that also includes the Black-cowled Orioles of the Greater Antilles (*I. d. dominicensis*) and Central America (*I. d. prothemelas*). Even Bond (1956) considered all Lesser Antillean orioles to be "representatives of *I. dominicensis*," reflecting his hypothesis that the Lesser Antillean populations were derived from Greater Antillean ancestors. Similarly, Blake (1968) noted that, "The Lesser Antillean orioles (*I. oberi*, *bonana*, and *laudabilis*) are derived from, and may be conspecific with, *I. dominicensis*." Lack (1976) formalized this hypothesis in a biogeographic model in which he suggested that the "*dominicensis*" orioles first colonized the Greater Antilles from Central America and that the Lesser Antilles were later colonized by Greater Antillean *I. d. dominicensis*.

We used mitochondrial DNA (mtDNA) sequence data to investigate the magnitude and pattern of evolutionary differentiation among the three endemic Lesser Antillean orioles and the more widespread Black-cowled Oriole of the Greater Antilles and Mexico. The mtDNA data permitted us to determine whether the three Lesser Antillean oriole populations have been evolutionarily isolated from one another by characterizing the genetic distinctiveness of each population. We also compared the magnitude of mitochondrial divergence among the Lesser Antillean oriole populations with genetic distances among other avian taxa. This comparative framework provided a context for determining for purposes of conservation whether each oriole population is an evolutionarily sig-

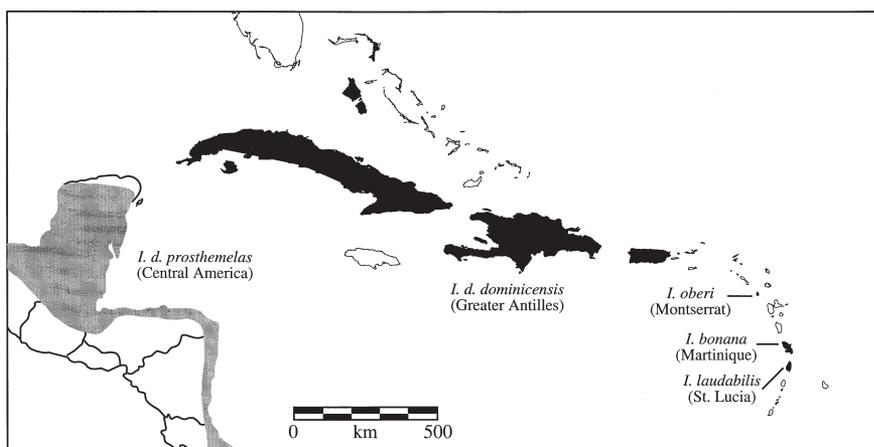


Figure 1. Distribution of taxa in the *Icterus* "dominicensis" superspecies complex.

nificant unit (ESU; Ryder 1986; Moritz 1994). Furthermore, the phylogenetic hypothesis established for Lesser Antilles orioles identified sister-group relationships potentially useful for oriole rescue programs. For example, identifying the taxon most closely related to each endangered population could aid captive breeding programs that employ foster hosts of eggs or nestlings or that involve interspecific hybridization (Avise & Nelson 1989). Alternatively, if an endangered population does become extinct, a closely related taxon might be introduced to re-create the pre-extinction structure of an island's avian community. Finally, we used the genetic information to draw attention to a valuable heuristic example involving the Montserrat Oriole, a taxon for which anthropogenic habitat alteration followed by cataclysmic natural disturbance has resulted in probable extinction. Although *I. oberi* appears to be the only Montserrat species with a high degree of phylogenetic differentiation, many other Antillean birds are now found only in patchy lowland habitats or in high-elevation forests. Habitat fragmentation and range restriction are likely to render these species particularly susceptible to the large-scale natural disturbances that occur frequently in the Lesser Antilles.

Methods

Most samples (all except those representing *I. leucopteryx* and *I. d. prosthmelas*) consisted of small pectoral muscle biopsies preserved in a DMSO/EDTA/NaCl solution (Seutin et al. 1991, 1993). Biopsy samples were taken from mist-netted individuals, which were subsequently released. This technique allowed us to obtain high-quality tissues for mtDNA analysis without sacrificing the individuals studied (Baker 1981). Because only one oriole species each occurs on Montserrat, St. Lucia, and Martinique, species-level identifications were straightforward. We obtained tissue biopsies from oriole populations on Montserrat (*I. oberi*; $n = 2$; 28 May–2 June 1993), Martinique (*I. bonana*; $n = 2$; 6 August 1991), St. Lucia (*I. laudabilis*; $n = 4$; 21–24 July 1991), and Puerto Rico (*I. dominicensis*; $n = 1$; 7 June 1994). For use in outgroup rooting, we also obtained biopsy samples from Yellow Orioles from Trinidad (*I. nigrogularis*; $n = 2$; 18 April 1995) and a Yellow-tailed Oriole (*I. mesomelas*; $n = 1$; 25 November 1994) from central Panama. Montserrat Oriole tissues were obtained prior to the advent of volcanic activity on that island, and few samples were obtained from any island owing to the difficulty of capturing these largely arboreal birds in mist nets. All biopsy specimens were collected, transported, and archived under appropriate permits. Biopsy samples and associated DNA extracts are permanently archived in the genetic resources collection at the Smithsonian Tropical Research Institute.

Frozen tissue samples representing the Jamaican Oriole (*I. leucopteryx*, $n = 2$) were loaned by S. Lanyon and the Field Museum of Natural History (FMNH). Tissues from the Central American subspecies of the Black-cowled Oriole (*I. d. prosthmelas*, $n = 2$) were loaned by the University of Kansas Museum of Natural History (UKMNH). Skin vouchers for these *I. leucopteryx* and *I. d. prosthmelas* tissues are archived at the FMNH (specimens 331149 and 331145), UKMNH (specimen MBR 4510), and Universidad Autonoma de Mexico (specimen GES 261).

Muscle tissue is a preferred source of mtDNA for avian genetic studies because mtDNA exists in much lower copy number in alternative sources such as plucked feathers. Blood samples, although easily obtained, also represent a potentially problematic source of avian mtDNA; because avian erythrocytes are nucleated, DNA extracted from blood is comparatively nuclear-genome enriched. The DNA was extracted from each biopsy specimen following the phenol-chloroform extraction protocol of Seutin et al. (1993), except that the final suspension was purified by dialysis rather than ethanol precipitation. We then used the protocols of Kessing et al. (1989) and Lovette et al. (1998) to amplify three regions of the mitochondrial genome via the polymerase chain reaction (PCR; information on PCR and sequencing primers and reaction conditions is available from the authors). Target sequences obtained from each individual included the entire ATP-synthase 8 and 6 coding sequence (ATPase 6 and 8), the entire NADH subunit 2 (ND2) coding sequence, and 624 nucleotides of the cytochrome oxidase subunit I (COI) gene. Each PCR product was used to seed a DyeDeoxy Terminator Cycle Sequencing reaction (Applied Biosystems Division of Perkin Elmer, Inc., Foster City, CA) which was conducted following the manufacturer's protocols and then sequenced via electrophoresis in an Applied Biosystems model 377 automated DNA sequencer.

All mitochondrial genes are fully linked. Examination of our sequences indicated that the ATPase 6, ATPase 8, ND2, and COI coding regions had similar base compositions. The combinability test of Farris et al. (1995), as implemented in Paup* 4.0b1 (Swofford 1998), identified no significant difference between phylogenetic trees generated using each gene region independently (1000 heuristic replications; $p > 0.872$). We therefore conducted all distance calculations and phylogenetic analyses using the combined ATPase 6, ATPase 8, ND2, and COI nucleotide sequences (2507 base pairs) from each individual.

We used Paup* 4.0b1 (Swofford 1998) to estimate genetic distances between individuals using the Kimura 2-parameter (K2; Kimura 1980) distance metric. We then used Paup* to reconstruct the evolutionary relationships among all unique mtDNA haplotypes via neighbor-joining (NJ) and maximum likelihood (ML) analyses. Neighbor-joining analyses were based on K2 distances, and 1000

bootstrap replicates were performed by the NJ bootstrap method implemented in Paup*. Maximum-likelihood (ML) reconstructions were conducted by means of the quartet-puzzling search algorithm of Strimmer and von Haeseler (1996), and we compensated for rate variation among sites with an eight-parameter gamma distribution. The ML searches were run for 1000 puzzling steps with the Hasagawa-Kishino-Yano substitution model (Hasagawa et al. 1985). Nucleotide frequencies were determined empirically from the sequence data matrix. The ratio of ML transition to transversion and gamma parameter were estimated by conducting a preliminary quartet-puzzling search on a subset of the sequence matrix that included one individual from each of the eight species included in this study.

Results

We obtained 2507 nucleotides of protein-coding mtDNA sequence from two Montserrat Orioles, four St. Lucia Orioles, two Martinique Orioles, one Puerto Rican Black-cowled Oriole, two Mexican Black-cowled Orioles, one Yellow-tailed Oriole, two Jamaican Orioles, and two Yellow Orioles. Each sequence included the entire coding region (842 bp) of the ATPase 6 and ATPase 8 genes (which overlap by 10 bp), the entire coding region (1041 bp) of the ND2 gene, and 624 bp of the COI gene. All sequence variants have been deposited in GenBank (accession numbers AF109410–AF109457 inclusive). A total of 535 nucleotide sites varied across all individuals, 323 sites varied among taxa in the *I. dominicensis* su-

perspecies complex, and 230 sites varied among haplotypes from the four eastern Caribbean populations from Puerto Rico through St. Lucia.

Several recent studies (Arctander 1995; Sorenson & Quinn 1998) have reported the existence of nuclear copies of avian mitochondrial genes (termed “numts” or “pseudogenes”) that may be preferentially amplified in PCR reactions, thereby potentially confounding analyses of genetic divergence and phylogenetic relationship. Several lines of evidence suggest that the oriole sequences reported here are mitochondrial in origin. We observed a single amplification product in our PCR reactions and found no instances of “double peaks” in our automated sequencer chromatographs. No insertions, deletions, or stop codons were present within any oriole sequence. Base frequencies and substitution patterns (including a high proportion of synonymous substitutions) were typical of avian mtDNA evolution. Finally, we obtained similar patterns of nucleotide variation and gene trees from three different regions of the mitochondrial genome that were each amplified and sequenced independently. These gene regions span more than 4600 nucleotides, and a nuclear transposition of a mitochondrial sequence of this length is unlikely (but see Lopez et al. 1996).

All phylogenetic reconstructions (Fig. 2) placed the mainland taxon *I. d. prosthemelas* basal to a cluster of Antillean lineages that included Puerto Rican *I. d. dominicensis* and the three Lesser Antillean populations. The internode separating *I. d. prosthemelas* from this Antillean group was long and universally well supported (Fig. 2). Despite the large number of variable nucleotide

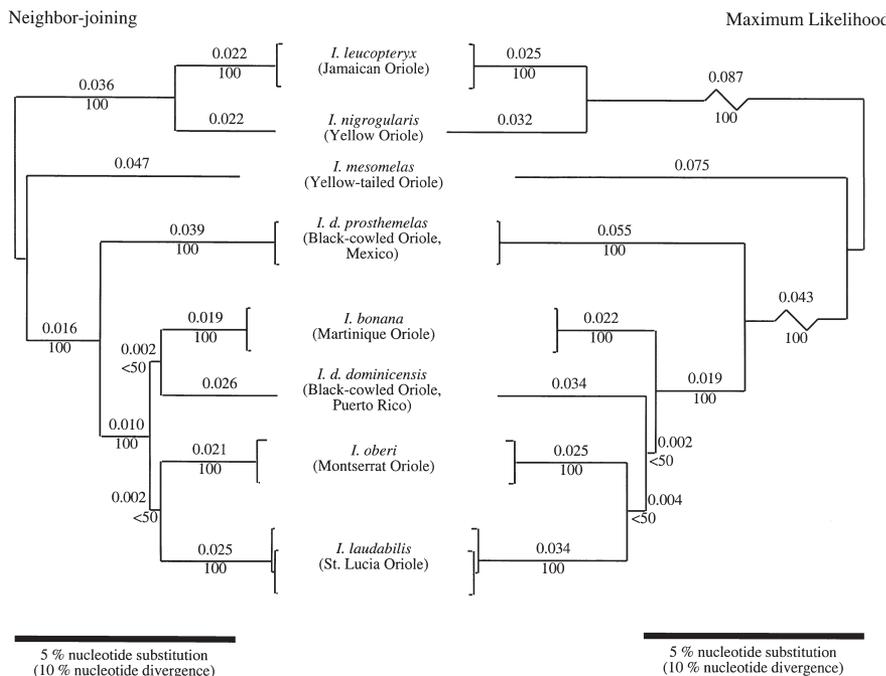


Figure 2. Neighbor-joining (NJ) and maximum-likelihood (ML) trees depicting phylogenetic relationships among oriole haplotypes. Trees are based on comparisons of 2507 nucleotides of protein-coding mtDNA sequence. Numbers above branches give branch lengths (K2 distances on NJ tree, Hasagawa-Kishino-Yano distances on ML tree). Numbers below branches indicate bootstrap proportions on NJ tree and reliability scores on ML tree.

sites in our data set, we found little evidence for hierarchical phylogenetic structure among the four Antillean lineages. The mtDNA divergence values were similar across all pairs of island populations, ranging between 4.5% and 5.8% (108.5–138.3 mean nucleotide substitutions; Table 1). Both ML and NJ analyses clustered the oriole lineages of Puerto Rico, Montserrat, Martinique, and St. Lucia into a star-like topology (Fig. 2). In these reconstructions, short basal internodes connected the long terminal branch leading to each population (Fig. 2). Reliability scores (ML) and bootstrap support (NJ) for these short basal internodes were invariably low (Fig. 2). Reconstructions based on maximum parsimony searches (not shown) resulted in a similar lack of structure; strict consensus trees of all equally short trees grouped these lineages in a four-way polytomy.

Although sample sizes were insufficient to characterize within-population mtDNA diversity, we found little divergence among haplotypes from any single population: 0.28% (7 nucleotide differences) between the two Montserrat individuals, 0.04% (1 nucleotide difference) between the two Martinique individuals, and 0–0.28% (0–7 nucleotide differences) between the four St. Lucia individuals. Two St. Lucia individuals shared identical 2507 base-pair haplotypes, as did the two *I. nigrogularis* outgroup samples. Only a single representative of these repeated haplotypes was included in our phylogenetic analyses. The presence of several closely related but distinct mtDNA haplotypes in each of the three Lesser Antillean oriole populations suggests that these small island populations have historically maintained modest levels of genetic diversity.

Discussion

Genetic Divergence between Eastern Caribbean Oriole Populations

The most striking results of our study are the consistently large mitochondrial divergences among the four eastern Caribbean *Icterus* populations (Table 1). These high divergences indicate that each island-specific lineage has been genetically isolated long enough to accumulate substantial numbers of mtDNA nucleotide substitutions. Comparisons with other studies show that each Lesser Antillean oriole population is as genetically distinct as many avian species. For example, the large mitochondrial differences among the four eastern Caribbean *Icterus* populations are similar to the mean divergence between 35 North American avian sister species (5.1%; Klicka & Zink 1997). Similarly, genetic divergences among these four oriole populations exceed those in one of the most intensively studied North American avian superspecies, the “Black-throated” *Dendroica* warblers (*D. nigrescens*, *D. occidentalis*, *D. townsendi*, and *D. vi-*

Table 1. Genetic distances between taxa in the *Icterus dominicensis* complex and several *Icterus* outgroup taxa based on comparisons of 2507 nucleotides of mitochondrial DNA.^a

Taxon	n ^b	<i>I. nigrogularis</i> (outgroup)	<i>I. leucopteryx</i> (outgroup)	<i>I. mesomelas</i> (outgroup)	<i>I. d. prothemelas</i> (Mexico)	<i>I. d. dominicensis</i> (Puerto Rico)	<i>I. oberi</i> (Montserrat)	<i>I. bonana</i> (Martinique)	<i>I. laudabilis</i> (St. Lucia)
<i>I. nigrogularis</i>	2	—	108.0	240.0	261.0	253.0	252.5	246.5	255.8
<i>I. leucopteryx</i>	2	0.045	—	236.0	253.0	255.0	262.5	247.5	260.3
<i>I. mesomelas</i>	1	0.106	0.104	—	234.5	227.0	219.8	218.5	231.8
<i>I. d. prothemelas</i>	2	0.114	0.111	0.104	—	184.5	184.0	166.0	181.3
<i>I. d. dominicensis</i>	1	0.110	0.112	0.100	0.080	—	117.0	108.5	138.3
<i>I. oberi</i>	2	0.111	0.115	0.096	0.079	0.049	—	110.0	119.3
<i>I. bonana</i>	2	0.111	0.108	0.096	0.070	0.045	0.046	—	120.3
<i>I. laudabilis</i>	4	0.112	0.114	0.102	0.077	0.058	0.049	0.050	—

^aValues below diagonal give mean Kimura two-parameter distances between taxa; values above diagonal indicate mean absolute number of nucleotide differences.

^bNumber of individuals.

rens; Mengel 1964; Mayr & Short 1970; Bermingham et al. 1992; American Ornithologists' Union 1998) in which interspecific mtDNA sequence divergence ranges between 0.9% and 4.2% (Lovette et al. 1999).

Divergence values may be used to determine the ages of lineages under the assumption of a mitochondrial "clock" (Hasagawa et al. 1985; Hillis et al. 1996). A number of independent calibrations have suggested that the descendants of a common avian ancestor diverge at a rate of approximately 2% nucleotide substitution per million years (Shields & Wilson 1987; Tarr & Fleischer 1993; Randi 1996; Nunn et al. 1996; Fleischer et al. 1998). Nonetheless, the extent to which mitochondrial DNA evolves in a clocklike manner is disputed (e.g., Ayala 1986; Gillespie 1986), and calibrations based on other avian taxa and different molecular techniques must be applied with caution. Application of the 2% per million years rate of divergence indicates that Lesser Antillean orioles separated 2.3–2.9 million years ago.

Phylogeography of Lesser Antillean Orioles

Our phylogenetic reconstructions suggest that three Lesser Antillean oriole species and the Puerto Rican population of *I. d. dominicensis* are more closely related to one another than they are to *I. d. prosthemelas* of the Central American mainland (Fig. 2). The phylogenetic grouping of these four eastern Caribbean populations is consistent with evolutionary and biogeographic scenarios suggesting that the Lesser Antillean populations were derived from Greater Antillean ancestors (Bond 1956; Lack 1976). Our phylogenetic hypothesis for orioles in the *I. dominicensis* superspecies group, however, is not consistent with the current taxonomy of these taxa (American Ornithologists' Union 1998), which assigns full species status to the Lesser Antillean populations but conspecific subspecies status to the Greater Antillean and Central American populations. A full consideration of the phylogenetic and taxonomic relationships of the *I. dominicensis* superspecies complex is not the focus of our investigation and would require more complete geographic sampling of Greater Antillean *I. d. dominicensis* and a broader representation of continental *Icterus* species. Thus, the remainder of our discussion focuses on the apparently monophyletic assemblage of eastern Caribbean oriole populations in order to provide a phylogenetic frame of reference for the endangered Montserrat Oriole and threatened Martinique Oriole.

Our phylogenetic reconstructions demonstrated an apparent lack of hierarchical structure among the four eastern Caribbean oriole populations (Fig. 2). In all reconstructions, the internodes connecting the four island lineages were short, and statistical support for a bifurcating topology was lacking. The star phylogeny exhibited by these trees represents either data limitations or the approximately contemporaneous separation of the four

lineages. A common limitation of mtDNA data is "saturation," a genetic divergence plateau reached when most variable nucleotide sites have undergone multiple changes along reconstructed branches. The star-like pattern of our oriole trees is unlikely to be an artifact of nucleotide saturation, however, because even the most variable type of substitution in our data set (synonymous transitions) does not approach saturation until pairwise divergence exceeds 10%. Insufficient data may also lead to star-like trees: as the time between branching events decreases, the number of nucleotide substitutions that occur between them also decreases. Hence, reconstructing branching orders when species originate over a short period of time can require large amounts of nucleotide sequence. But given the length of the mitochondrial sequences included in our analyses and the relatively high rate of mtDNA evolution, it is reasonable to infer an approximately contemporaneous divergence of the four eastern Caribbean *dominicensis* oriole lineages.

We observed no relationship between geographic and genetic distance in comparisons between the four eastern Caribbean oriole populations. The largest pairwise divergences occurred between the Martinique and St. Lucia populations (Table 1), which are on adjacent islands only 27 km apart. In contrast, the smallest interisland divergence was observed between the Puerto Rico and Martinique populations (Table 1), which are separated by 517 km.

Conservation Implications

The magnitude of sequence divergence between the Lesser Antillean *Icterus* populations indicates that they are distinct phylogenetic species that should each be considered ESUs for purposes of conservation. These populations also may be valid biological species (Mayr 1963), but information is lacking on their reproductive compatibility in sympatry. Nonetheless, each Lesser Antillean oriole population is as distinct with respect to its mtDNA sequence as are many sympatric avian species-level sister taxa.

In general, mitochondrial data alone may be insufficient for assessing ESU or phylogenetic species status because mtDNA is maternally inherited and therefore does not reflect male-mediated gene flow (Degnan 1993; Moritz 1994; Avise 1995). Patterns of mitochondrial variation are likely to be especially misleading if female dispersal is low and male dispersal is high (Avise 1995). Furthermore, even female-mediated gene flow could be difficult to identify by means of mtDNA markers if only a few individuals from each population are examined, especially if immigrant mtDNA haplotypes are present at low frequencies. In the present study, however, two additional lines of evidence suggest that each island population is genetically isolated. First, the disjunct oriole populations of the Lesser Antilles (*I. oberi* and *I. bo-*

nana; see Fig. 1) are separated by unoccupied islands that support apparently suitable habitat. This distribution pattern suggests that Lesser Antillean orioles are sedentary, an argument bolstered by the fact that there have been no reports of vagrant orioles on other Lesser Antillean islands. Second, unique morphological characters distinguish each population (Ridgway 1902; Bond 1956). Differences in plumage color, pattern, and sexual dimorphism are particularly notable in comparisons between the Martinique and St. Lucia Orioles, the populations with the least geographic separation (for illustrations of these taxa see Bond 1985). This morphological divergence probably reflects differentiation in nuclear-encoded genes, thereby demonstrating that population-specific differences are not restricted to the mtDNA genome.

The high degree of genetic differentiation among these orioles parallels patterns seen in other West Indian taxa with fragmented distributions. For example, highly disjunct populations of Adelaide's Warbler (*Dendroica adelaidae*) on Puerto Rico, Barbuda, and St. Lucia have much greater differentiation than conspecific populations of two other warblers (*D. plumbea* and *D. pityophila*) with restricted but more continuous ranges (Lovette et al. 1998). These comparisons suggest that other Lesser Antillean birds with conspicuous range disjunctions (e.g., *Cinclocerthia ruficauda*, *Myadestes genibarbis*, and *Ciclbherminia lherminieri*) may include two or more evolutionarily distinct lineages of conservation importance.

Conservation efforts aimed at severely endangered avian taxa often involve captive breeding or reintroduction programs, and a captive breeding effort is being planned for the Montserrat Oriole in the event that surviving individuals can be obtained (D. B. Brosnan, personal communication). The lack of phylogenetic structure among the four eastern Caribbean *Icterus* lineages has implications for the design of such programs. In the case of extreme demographic reduction followed by human attempts at population rescue, genetic data can provide an objective means of identifying closely related taxa for use as surrogate parents. In the unfortunate situation where an endangered population is represented by a very small number of surviving individuals, mitochondrial affinities might also identify candidate taxa for interspecific crosses aimed at maximizing the preservation of the "dying" species' genes (Avice & Nelson 1989). Finally, if an island oriole population goes extinct, restocking with a closely related form might help restore the ecological and phylogenetic structure of the island's avian community.

The lack of population-level hierarchical structure in our reconstructions precludes the identification of a single taxon that is most closely allied to each of the two endangered populations. Instead, our phylogenetic analysis suggests that the four eastern Caribbean populations

are all approximately equally divergent from one another. Although the collection of more genetic information might ultimately support a single bifurcating tree, the magnitude of evolutionary differentiation along the resulting population-level internodes is likely to be small. Thus, from a phylogenetic perspective, either nonthreatened Antillean taxon (*I. dominicensis* or *I. laudabilis*) would be appropriate for an oriole rescue plan utilizing sister taxa, and the choice between these species should probably be based on demographic or ecological criteria.

The recent volcanic crisis on Montserrat, coupled with the high level of mtDNA differentiation observed between the Montserrat Oriole and its congeners, raises a general question regarding the genetic distinctiveness of other avian populations on Montserrat. Lesser Antillean birds show a wide variety of phylogeographic patterns (Klein & Brown 1994; Seutin et al. 1994; Bermingham et al. 1996; Lovette et al. 1998; Ricklefs & Bermingham 1998). Although *I. oberi* is the only avian species endemic to Montserrat, cryptic differentiation in other members of the Montserrat bird community might have gone unrecognized. A broader survey of genetic differentiation in Lesser Antillean birds suggests, however, that *I. oberi* is the only resident species on Montserrat that is phylogenetically differentiated from its closest relatives (Table 2). The lack of interisland differentiation observed in the

Table 2. Mitochondrial DNA genetic distances between the Montserrat Oriole and closest island relatives compared with those of 13 additional Montserrat bird species.

Species	Islands compared ^a	mtDNA divergence (%) ^b
<i>I. oberi/I. bonana</i>	MO, MA	6.06
<i>I. oberi/I. leucopteryx</i>	MO, SL	6.78
<i>I. oberi/I. d. dominicensis</i>	MO, PR	5.03
<i>Columbina passerina</i>	MO, GU	0.00
<i>Eulampis jugularis</i>	MO, GU	0.00
<i>Orthorhynchus cristatus</i>	MO, GU	0.12
<i>Eulampis holosericeus</i>	MO, GU	0.12
<i>Vireo altiloquus</i>	MO, AN	0.00
<i>Cinclocerthia ruficauda</i>	MO, GU	0.00
<i>Margarops fuscatus</i>	MO, GU	0.00
<i>Margarops fuscus</i>	MO, GU	0.12
<i>Ciclbherminia lherminieri</i>	MO, GU	0.00
<i>Quiscalus lugubris</i>	MO, GU	0.00
<i>Coereba flaveola</i>	MO, GU	0.12
<i>Loxigilla noctis</i>	MO, GU	0.00
<i>Tiaris bicolor</i>	MO, GU	0.00

^aIsland populations abbreviated: MO, Montserrat; GU, Guadeloupe; PR, Puerto Rico; MA, Martinique; SL, St. Lucia; AN, Antigua.

^bATPase-only distances involving the Montserrat Oriole are shown (first three values in column) to highlight the genetic distinctiveness of this taxon. Remaining values are mtDNA divergence values that indicate percent nucleotide differentiation in the complete ATPase 6 and ATPase 8 genes (842 nucleotides) among the most similar haplotypes sampled in each pairwise comparison across islands (E.B. & R.E.R., unpublished data).

14 Montserrat bird species (Table 2) provides evidence of gene flow (or recent colonization) between Montserrat and nearby islands and suggests that these species might reestablish populations via immigration in the event that they are locally extirpated on Montserrat by volcanic activity.

Catastrophic Extinction and the Montserrat Oriole

Severe natural disturbances are common in the Lesser Antilles. Hurricanes occur annually in the region, and Montserrat itself was devastated as recently as 1989 by hurricane Hugo. Major volcanic eruptions have occurred on four Lesser Antillean islands within the past century (Soufriere volcano, St. Vincent, 1902; Mt. Pelee volcano, Martinique, 1902; Soufriere volcano, Guadeloupe, 1976–1977; Soufriere Hills volcano, Montserrat, 1995–present). Despite the high frequency of these natural catastrophes over evolutionary time, levels of mitochondrial divergence suggest that the three Lesser Antillean orioles have persisted as independent evolutionary lineages for perhaps 2.5 million years. During this period they have weathered both long-term changes in Lesser Antillean climate (Pregill & Olson 1981) and short-term perturbations caused by hurricanes or volcanoes (Tanner et al. 1991; Waide 1991).

This historical setting makes the plight of the Montserrat Oriole particularly noteworthy. Within the past 50 years, anthropogenic habitat alteration restricted the Montserrat Oriole to a few forest remnants. These remaining populations appeared to be demographically stable (Collar & Andrew 1988; Johnson 1988), but since 1995 all remaining Montserrat Oriole habitat has been severely affected by volcanic disturbances ranging from repeated heavy ashfalls to complete devegetation (Brosnan & Courtney 1997; Young et al. 1997). The extinction of the Montserrat Oriole is likely in the near future if it has not already occurred. Conservation biologists have long recognized that populations suffering from human-mediated range restrictions or habitat fragmentation may experience a heightened risk of extinction from natural catastrophe. Nonetheless, supporting examples are uncommon owing to the rarity of severe catastrophes, and few are as dramatic as the case of the Montserrat Oriole, in which the links between changing land-use practices, natural catastrophe, and impending extinction are unusually direct.

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