MOLECULAR PHYLOGENETICS AND CONSERVATION OF CARIBBEAN BIRDS

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AT THE 1997 MEETING OF THE Society of Caribbean Ornithology on Aruba, one of us (RER) presented a talk on the application of new techniques in DNA sequence analysis to understanding the evolutionary relationships among birds on West Indian islands. This was followed on the next day by a panel discussion, organized by Dr. Nedra Klein of Lewis and Clark University, Portland, Oregon, addressing many of the same issues in more detail. I was asked to prepare for the SCO bulletin *El Pitirre* a brief overview of molecular approaches to evolutionary relationships among species and populations and to summarize the main points and some examples from this talk. I am pleased to do this with my collaborator, Dr. Eldredge Bermingham. We have organized this summary to answer five basic questions: What is DNA sequence analysis? What kinds of information does it provide? What does this approach show about the distinctiveness of West Indian birds? How can this information be used in management and conservation? How can this information be accessed for particular needs? We will begin by providing some background about our own project.

We are interested in the regional history and biogeography of birds in the West Indies, which provide an ideal laboratory for studying processes of evolution and ecology. We would like to understand how colonization and extinction influence the avifauna of a particular island; we would also like to know how the ecological relationships of birds change over time. To pursue these goals, we needed to estimate the ages of individual island populations and determine their relationships to other island populations within the archipelago. As we shall explain below, this can be accomplished by measuring the amount of genetic change in independently evolving lineages of birds. These may be distinct lineages within a given population, populations of the same species on different islands, or different species.

We began to plan this study in 1989 and initiated field work in 1991. Several individuals have been closely involved in the work: we would like to mention especially Dr. Gilles Seutin, now at McGill University in Montreal, who was instrumental in establishing the field and laboratory programs for this project, Mr. Irby Lovette, at the University of Pennsylvania in Philadelphia, and Mr. Jeffrey Hunt at the Smithsonian Tropical Research Institute. Our first priority has been to collect samples of blood and tissues from island birds. We have now conducted field work on 14 islands and several continental localities[1], sponsored by the National Geographic Society. Our own samples have been supplemented by tissue specimens generously provided by several museums in the United States, particularly the Academy of Natural Sciences of Philadelphia, the Field Museum of Natural History (Chicago), the Louisiana State University Museum of Zoology, and the National Museum of Natural History (Washington, D.C.). As of this date, our tissue collection of West Indian birds represents over 3,000 individuals, including virtually all the species of small land birds. Our collection also includes representative geographic and phylogenetic outgroup taxa collected from continental locations in the Neotropics. We have sampled the majority of our specimens non-destructively (taking only tissue biopsies and blood samples) in accordance with our permits from the various island nations in the West Indies. We capture birds in mist nets, take samples of blood and breast muscle, and then release all individuals after processing. Blood and tissue samples are preserved in buffer solutions in small vials and returned to EB's laboratory in Panama, where the DNA work begins. The mortality rate resulting from our work has been about 2%. We are able to use this non-destructive sampling method because the birds of the West Indies are completely known and are readily identified in the hand. Additionally, we have tissue samples matched by voucher specimens in museum collections for many West Indian birds, against which we can check our DNA sequences.[2]

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[1]These are the following: Trinidad, Barbados, Grenada, St. Vincent, St. Lucia, Martinique, Dominica, Guadeloupe, Montserrat, Puerto Rico, Dominican Republic, Jamaica, New Providence, Abaco, and continental localities in Venezuela, Panama, and Honduras. In addition, we have obtained material from some other localities from various museum collections, and hope to visit Cuba in the near future.

[2]Although we have not collected museum specimens of birds in our work, we would like to emphasize that there are many types of studies involving the relationship between genetics, morphology, and taxonomy for which collecting is necessary. We have seen in our work that genetic variation between island populations does not always correspond to subspecific or other taxonomic distinctions, in which case a more thorough appraisal of morphological and genetic variation may be required to ascertain the distinctiveness of, and relationships among, island populations. Dr. Nedra Klein's study of the Yellow Warbler has demonstrated mixing of highly divergent lineages on several islands in the Lesser Antilles. In such cases, it is important to ascertain whether genetic variation is accompanied by recognizable morphological markers, which can only be accomplished with collected specimens.

First, we briefly review some background information in genetics. Each cell in our bodies contains all the genetic information needed to direct our growth and development and regulate our biological processes. This information is contained in long chain-like molecules of DNA (**D**eoxyribo**N**ucleic **A**cid). Each DNA molecule consists of a long string of four different types of subunits, called nucleotides. The nucleotides are named after their principal structural components: adenine, thymine, cytosine, and guanine, or A, T, C, and G. Thus, the nucleotide sequence of any particular part of a DNA molecule might be written as AATCGGTTACCG, etc. This sequence is read three nucleotides at a time when proteins are made. Each nucleotide triplet specifies which of 20 different amino acids is placed in each position in the structural proteins and enzymes that are built on the genetic template. In this example, AAT = leucine, CGG = alanine, TTA = asparagine, CCG = glycine.

Many of the differences we observe between individuals in populations, and between different populations and species, are due to differences in the DNA sequences that encode particular proteins. Changes in the DNA sequence come about through mutations, which result in part from errors in copying the DNA as new cells are formed, including the sex cells that create the next generation. Changes also result from damage caused by environmental factors such as ultraviolet radiation, toxic chemicals of various sorts, and highly reactive products of our own metabolism. If these errors are not corrected, they are then transmitted as mutations from generation to generation.

Some mutations affect the structure and functioning of the organism, either beneficially or, more commonly, to the individual's detriment. In either case, if a mutation affects the reproductive rate of the individual that bears it, its frequency in the population might be increased or decreased accordingly, and the genetic composition of the population changes over time. This is, of course, what we refer to as evolution, which is responsible for most of the visible differences between species and accounts for the adaptation of species to their particular environments.

Other mutations have no visible effect on the organism. Some of these occur in parts of the DNA which are not translated into proteins (there are many of these regions in the DNA which sometimes represent old gene sequences no longer used). Others result in changes in proteins that have no functional consequence or more typically cause changes in the DNA sequence that have no effect on the amino acid sequence in the protein. For example, the three-nucleotide sequences CAA, CAC, CAG, and CAT all code for valine; thus, a change in the third position in this triplet has no effect on the amino acid sequence of the protein. Such mutations are generally considered to be unaffected by natural selection and are referred to as "neutral" mutations. The rate at which they appear in populations is determined only by the process of mutation, which is thought to occur at a more or less constant rate for a particular part of the genetic sequence. Thus, neutral mutations allow us to estimate the time that has passed since the divergence of two lineages by the number of nucleotide differences that have accumulated between them.

Most studies of the genetic relationships between lineages of birds are based on a special type of DNA found in the mitochondria of cells. Mitochondria are organelles responsible for much of the oxidative metabolism of the cell. They originated more than a billion years ago as symbiotic bacteria in cells of the organisms that were the ancestors of all present-day animals, plants, and fungi. Some of the DNA of the original bacterial symbionts is retained in our mitochondria. It is a single circular string of about 16-17,000 nucleotides in birds. Mitochondrial DNA (mtDNA) is transmitted only through the female line, and thus there is no mixing of maternal and paternal genes in mtDNA. There are several advantages to using mtDNA. Because of maternal inheritance, mtDNA is passed from generation to generation as a single unit and each mutation gives rise to a new, distinctive lineage which cannot mix with other lineages. Thus, ancestry is unambiguous. Furthermore, mtDNA in birds has a mutation rate that is several times higher than that of nuclear DNA (probably due to a less efficient DNA repair enzyme). This rate has been estimated as one change per 100 million (10^{-8}) nucleotides per generation, as a ball-park figure. Multiplied by 17,000 nucleotides, this is 0.17 mutations somewhere in the mtDNA molecule per 1,000 individuals per generation. By extrapolation, in a population having 6,000 females, about 1 mutation would appear per generation. This high rate of mutation creates a high diversity of mtDNA lineages within populations and causes relatively rapid divergence in the sequences of lineages between populations, as we shall see.

At this point, we should mention laboratory techniques briefly because DNA sequence analysis is an intensive laboratory procedure. Three major steps are required to go from a tissue sample to a genetic sequence: extraction of DNA, amplification of DNA, and determination of the DNA sequence. Extraction begins by breaking up the cells in the tissue using detergents and other chemicals. The DNA is then isolated by alternately dissolving and precipitating the DNA so that it can separated by centrifugation from other cell components. Next, some specific region of the more-or-less purified whole DNA (including both nuclear and mitochondrial types) is amplified by Polymerase Chain Reaction (PCR). In this step, the amount of a specific short sequence is increased many million-fold to obtain a large number of copies of the same DNA region. This is done by borrowing the DNA replication machinery (polymerase enzyme) from a type of bacterium found in hot springs. The process is referred to as thermocycling. We can specify which part of the DNA sequence we amplify by supplying a specific sequence of DNA assembled in the laboratory, often about 20 nucleotides long, that attaches to a particular unique point in

the extracted DNA molecule and causes the replication to take place only at that point. This short DNA sequence is called a primer. Amplification proceeds by using two of these primers, typically displaced from one another by 1,000-2,000 nucleotides, which create overlapping strands of DNA (or PCR products) between the two primer points. In the laboratory, the temperature of the reaction solution containing the DNA, enzyme, and primers is then changed in a way that causes repeated cycles of DNA replication.

In our research, we work primarily with primers that permit the amplification of mtDNA genes such as ATP synthase, cytochrome b, cytochrome oxidase, and NADH dehydrogenase. After a particular region of the mtDNA has been amplified, the nucleotide sequence of these products is determined by a rather complex procedure which, fortunately, has been more or less fully automated. We will not delve into the specifics. What we obtain is a sequence of DNA for each individual bird, that is, AATCGGTTACCG and so on, up to 1,000 or 2,000 nucleotides in length for a particular primer pair.

What information does DNA sequencing provide?

The primary data are the nucleotide sequences. Secondary data are the number of nucleotide differences between sequences, which we can quantify as percent sequence divergence ([number of differences/total number of nucleotides examined]$\times$ 100). Suppose we find the following sequences of 20 nucleotides in three individuals:

individual 1:	ATC CAT TCC AGG TAC ATT GA ...
individual 2:	ATC CAT CCC AGA TAC ATT GA ...
individual 3:	ATA CGT CCC AGG TAC ATG GA ...

The bold letters indicate positions at which there have been changes in the nucleotides (often called nucleotide substitutions). Individuals 1 and 2 differ at 2 positions out of 20 and so they exhibit a 10% sequence divergence. Between individual 3 and either individual 1 or 2 there are 4 differences and thus 20% divergence. This information suggests that individual 1 and individual 2 shared a common female ancestor more recently that either did with individual 3. The interpretation of this information depends on where individuals 1, 2, and 3 were sampled. If they lived together on the same island, the genetic differences between the sequences provide a measure of the genetic diversity of the island population. If they lived on different islands, and if the differences between the islands were much greater than differences within the islands, we could conclude that the island populations of individuals 1 and 2 are more closely related to each other than either are to that of individual 3. Alternatively, we may say that population 3 is genetically more distinctive, and has had a longer independent evolutionary history, than have populations 1 and 2.

Sequence divergence among individuals and populations also tell us something of the history of a taxon within a region. If a population has a very low genetic diversity—perhaps all the individuals have the same nucleotide sequence for a particular mtDNA region—we can infer that the mtDNA of all the individuals was recently descended from that of a single female. This is most likely to happen when an island is colonized by a small number of individuals, the so-called founder effect. Because each individual carries only one identical set of mtDNA genomes in its cells, colonization of an island by a single pair of birds (that is, by only one mother) results in there being only a single mtDNA sequence in the descendants of the pair. Even when there are several females in the founding population, these do not carry all the genetic diversity of the parental population from which the new one is derived. In addition, some of the DNA sequences might be lost just by chance when a particular female carrying a unique sequence dies.

An older, established population might also have low genetic diversity if it has been reduced to small size—a population bottleneck—sometime in the recent past. Often this can be distinguished from a colonization event by the fact that the mtDNA sequences present on the island, no matter how few in number, are highly diverged from those of other islands or continental areas from which the population might have been derived.

When populations of a species on two different islands have high genetic diversity but share a substantial proportion of genetic sequences, population geneticists interpret the pattern to represent occasional (or frequent) movement of individuals between islands. Movement of one bird (a female in the case of mtDNA) per generation is thought to be sufficient to keep two island populations from diverging from each other. Data from Bananaquits (*Coereba flaveola*) in the Lesser Antilles illustrate some of the properties of genetic variation within island populations:

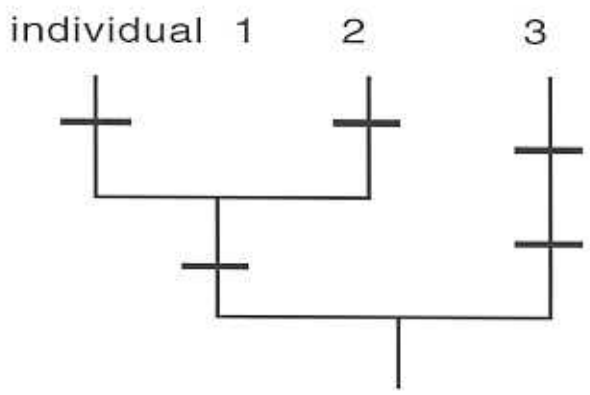
mtDNA sequence type	Island						
	MO	GU	DO	MA	SL	SV	GR
1	5	6	8	3	9		
2			1		2		
3			4	2	1		
unique group A			6 (4)	3 (3)	6 (6)		
4						5	2
5						3	11
unique group B						7 (5)	4 (1)
Individuals (sequences)	5 (1)	6 (1)	19 (7)	8 (5)	18 (9)	15 (7)	17 (3)

NOTE: The unique groups include sequences found only on a single island; i.e., on Dominica (DO) 6 individuals each had one of four sequences found on no other island. Sequences in group A were similar to other sequences (1-3) more widely distributed in the northern Lesser Antilles; those in group B were more similar to sequences (4 and 5) shared between St. Vincent (SV) and Grenada (GR).

As you can see, Dominica (DO), Martinique (MA), and St. Lucia (SL) share several mtDNA sequences, which differ completely from sequences shared by populations on St. Vincent (SV) and Grenada (GR). Unique sequences within in each of these groups of islands are most similar to the shared sequences within each group, indicating recent common ancestry. We interpret this pattern to represent either sufficient continuing movement of Bananaquits between the first three islands to prevent genetic divergence, or recent colonization events within each of the island groups. We consider the sequence divergence that has accumulated between St. Vincent and Grenada, on one hand, and St. Lucia, Martinique, and Dominica, on the other, to indicate a complete barrier to the movement of Bananaquits between the two groups of islands. The presence of unique sequences on each of the islands may be due to inadequate sampling of individuals or to mutations that were not carried between the islands by movement of individuals. The Bananaquits examined in populations on Guadeloupe (GU) and Montserrat (MO) carried a single sequence type, which happens to be the commonest of the Dominican sequences, suggesting that these populations were established recently by a small number of founders from Dominica. We should add that the mtDNA sequences in Bananaquit populations on Puerto Rico to the north and Venezuela to the south are highly divergent from those of the two Lesser Antillean groups presented here.

WHAT DOES THIS APPROACH SHOW ABOUT THE DISTINCTIVENESS OF WEST INDIAN BIRDS?

Genetic divergence between island populations provides information from which we can construct hypotheses or scenarios for the evolutionary relationships among them. These hypotheses usually take the form of a phylogenetic tree, in which the most ancestral gene sequence occupies the trunk position and each branch point in the tree represents a mutational step that separates one lineage into two different daughter lineages. One must always remember that, in the absence of a fossil record, our knowledge is based only on present-day genetic information, which is represented at the tips of the smallest branches of the phylogenetic tree. Rather sophisticated computer techniques are available for reconstructing phylogenetic trees and assigning a degree of confidence to each of the branch points. The three hypothetical sequences described above provide a simple case, as shown in the following diagram, where the heavy horizontal bars represent nucleotide substitutions:



Turning to a couple of real cases, one from the Greater Antilles and one from the Lesser Antilles, we begin to see the power of the molecular phylogenetic approach. We emphasize that these are only preliminary versions of the molecular phylogenies for these groups. The first example is that of the

todies (genus *Todus*) which comprise an endemic family (Todidae) presently restricted to the Greater Antilles. There are five recognized species, one each on Cuba, Jamaica, and Puerto Rico, and two on Hispaniola. The phylogenetic tree shown in Figure 1 portrays several aspects of the evolutionary histories of todies quite clearly, and also has a few surprises. First, the sequences we have used in this analysis do not provide enough resolution to pinpoint the closest relative of the todies or to estimate the age of the group. Fossil remains from the Oligocene (> 24 million years ago) of Wyoming and Europe have been assigned to the family, but there appears to be too little sequence divergence between todies and several groups of coraciiform birds (kingfishers, jacamars, motmots) for the Todidae to be of such age. Alternatively, todies may have evolved uniquely in the West Indies from a kingfisher-like colonist. What we can say, however, is that the present species of todies probably formed about 6-7 million years ago when independent lineages were established on the major islands from an ancestor that clearly was a tody, much like its descendants.

How can we estimate the age of a branch point between lineages? If we take as a rule of thumb that mutations occur at the rate of one in 100 million per nucleotide per generation, this is about 1% per million years. (This value is likely to vary widely depending on the particular DNA sequence and group of organisms considered, and should be taken only as a coarse approximation.) Population genetics theory tells us that for neutral mutations, the rate of replacement of nucleotides in the population is equal to the mutation rate. Thus, we might suppose that a particular sequence changes at about 1% of its nucleotide positions per million years, and that two sequences diverge from each other at a rate of 2% per million years. This gives us an approximate meter stick for estimating age.

The tody data are not sufficiently well resolved to show with certainty the order in which populations on the Greater Antilles were established. However, the Narrow-billed Tody (*Todus angustirostris*), one of the two species on Hispaniola, is more closely related to the Puerto Rican Tody (*Todus mexicanus*) than to the second Hispaniolan species (*T. subulatus*), suggesting either a secondary colonization of Hispaniola from Puerto Rico, or that the Puerto Rican Tody was derived from one of two differentiated Hispaniolan todies. Apropos of the second hypothesis, we have discovered that populations of Narrow-billed Todies on the northern and southern mountain ranges of Hispaniola are highly divergent genetically, perhaps having been evolutionarily independent for 2 million years. From the standpoint of conservation, these two populations probably should be considered as different species. The Hispaniolan todies raise the possibility that other species exhibit similar divergence between populations in the northern and southern ranges. We should emphasize that these results are preliminary and that additional sequence for other DNA regions might be helpful

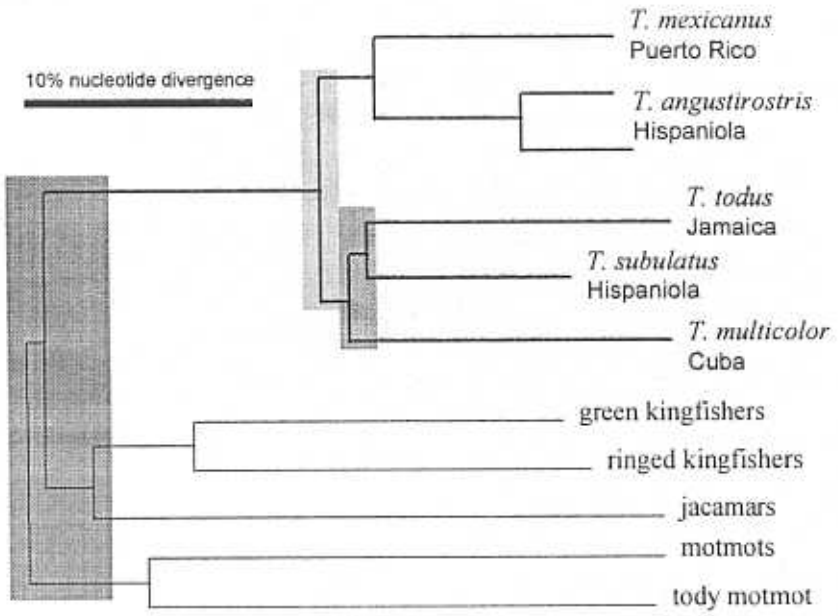


Figure 1. Phylogenetic relationships of the todies (Todidae) based on ATPase sequences of the mitochondrial DNA. Gray shading indicates poorly resolved branch points in the phylogenetic tree. The solid bar represents 10% nucleotide divergence or approximately 5 million years of separate evolution. The closest sister taxon of the todies cannot presently be resolved among kingfishers, motmots, and other groups of coraciiform birds living in tropical America. DNA sequences prepared in collaboration with Lowell Overton, who is a Ph.D. student in Biology at the University of Arkansas.

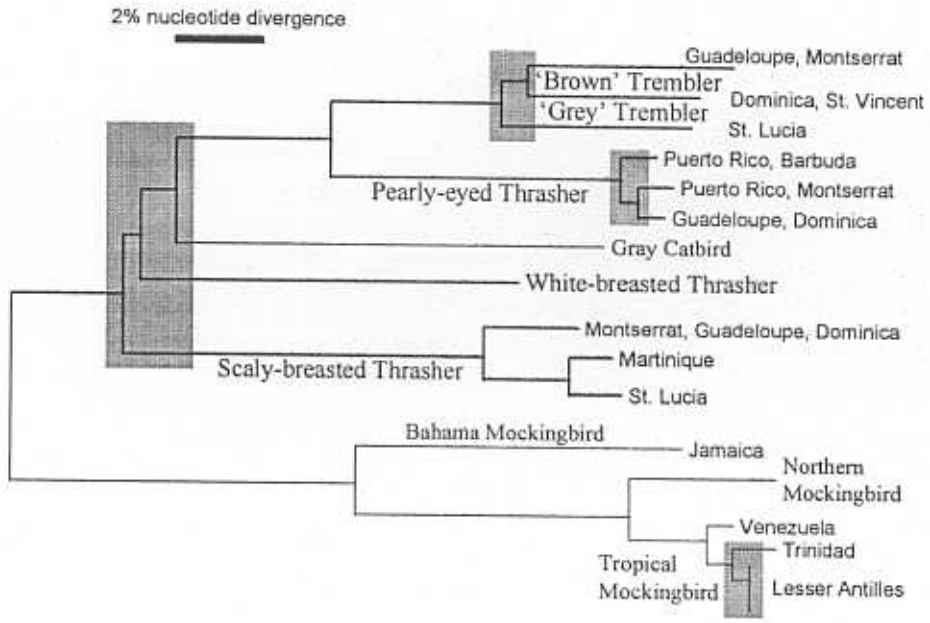


Figure 2. Phylogenetic tree featuring the distinctive Lesser Antillean mimids based on ATPase sequences of mitochondrial DNA. The scale of nucleotide substitution suggests that the initial radiation of the Lesser Antillean clade occurred about 5 million years ago. Gray shading indicates poorly resolved branch points in the phylogenetic tree. The solid bar represents 2% nucleotide divergence or approximately 1 million years of separate evolution. The DNA sequences were prepared by Mr. Jeffrey Hunt at the Smithsonian Tropical Research Institute.

in resolving the relationships among the Cuban, Jamaican, and Hispaniolan Broad-billed Todies.

Another group of distinctive West Indian birds is made up of the endemic mimids of the Lesser Antilles and Puerto Rico. These include the Pearly-eyed Thrasher (*Margarops fuscatus*), Scaly-breasted Thrasher (*Margarops fuscus*), White-breasted Thrasher (*Ramphocinclus brachyurus*), and trembler (*Cinclocerthia ruficauda* species group). The phylogenetic tree in Figure 2 shows that these form a single set of lineages, or clade, along with the Gray Catbird (*Dumetella carolinensis*) of North America. Again, the resolution at the base of this phylogenetic tree is not yet good enough to work out where the Gray Catbird fits into the branching order, but it is possible that this species originated from a West Indian ancestor. Among the clearer points to be made from this analysis is that the Scaly-breasted and White-breasted Thrashers are genetically the most distinctive of the taxa, their lineages originating from the base of the Antillean endemics. The Pearly-eyed and Scaly-breasted Thrashers, which are now both placed in the genus *Margarops* are not closely related (the Pearly-eyed Thrasher is more closely allied with the trembler); perhaps the latter should be given back its old generic name, *Allenia*. Finally, the tremblers appear to comprise three distinct lineages rather than two (Gray and Brown Trembler) as previously suspected.

With regard to the tremblers, we found one distinct lineage on Guadeloupe and Montserrat, a second on St. Lucia, and a third on Dominica and St. Vincent. This creates a geographical puzzle: the St. Lucian lineage, placed geographically between Dominica and St. Vincent, is interposed between island populations of one of the Brown Trembler lineages. We occasionally see geographically complex relationships of this kind, which might arise from our failure to sample lineages from particular islands, or from the extinction of lineages on intermediate islands. There is also a possibility of what is called lineage sorting, which results in the haphazard disappearance of lineages shared by an ancestral population on different islands. These processes can only be resolved by additional sampling of individuals and genetic sequences, including nuclear genes. Geographical anomalies do, however, raise red flags which show us where more work has to be done. To date, such geographic anomalies have turned out to be rather uncommon in the West Indies.

Much more could be said about inferences from genetic data, but the examples from Bananaquits, todies, and mimids presented above give a general idea of what is possible.

How can this information be used in management and conservation?

(1) *Identification of genetically unique taxa and island populations.* Most importantly, genetic data can provide information on the distinctiveness of island populations and their relationships among each other. Knowing that Narrow-billed Todies of Hispaniola comprise two distinct populations that have been separated for perhaps 2 million years (4% sequence divergence) provides a much stronger incentive for management because neither population can stand in for the

other. These probably should be considered as two different species, each of which potentially would have special management considerations. Similarly, knowing that the Brown Trembler of Guadeloupe is distinct genetically from that of neighboring Dominica multiplies the danger of losing either one of these endemic populations from the standpoint of preserving genetic diversity within the West Indies. In another typical example, populations of Adelaide's Warbler (*Dendroica adelaidae*) on Barbuda and St. Lucia differ by more than 2% nucleotide divergence from each other and by more than 4% from the population of Adelaide's Warbler on Puerto Rico. These are all called the same species, but they clearly have had independent evolutionary histories for a million years or so and are genetically very distinctive. Mitochondrial DNA sequence data show that orioles of the *Icterus dominicensis* group diverged 2-4 million years ago, with populations on Montserrat (*I. oberi*), Martinique (*I. bonana*), and St. Lucia (*I. laudabilis*) all being highly distinctive from their relatives in the Greater Antilles. These have been called different species, but an appreciation of their ages drives home the long, intimate, and unique association of each of these populations with its island home. Each taxon will have its own story, of course, but molecular phylogenetic data provide the best hope of making quantitative assessments of the distinctiveness of island populations and their relative genetic value for conservation.

(2) *Identification of critical habitats for conservation and management.* If we can determine the genetic uniqueness of the populations on a given island, this information can be combined with the habitat distributions of the species to establish conservation values for different types of habitats. This would allow managers to defend the preservation of tracts of critical habitat to maintain an island landscape that can support the maximum biological diversity. Our genetic analyses suggest that highly endemic species of birds are most often found in environments that differ most from lowland forests, which were undoubtedly the habitats of their colonizing ancestors. Thus, montane forests, cloud forests, arid scrub, and wetlands are the most critical habitats for preservation of avian diversity on islands. Of course, different groups of animals have different habitat requirements, and so a knowledge of local natural history has to be used to judge the generality of results obtained for land birds. Similar molecular phylogenetic analyses are also underway with groups of reptiles and insects, and undoubtedly some other taxa will be included as these approaches gain adherents and the West Indies become more widely recognized as an important opportunity for evolutionary studies and management applications of genetic approaches to conservation.

(3) *Introductions of individuals between islands.* Transport of individuals from one island to another may be a suitable management approach for a threatened population, as an emergency action to bolster a declining population and perhaps infuse new genetic variation. It is essential in such cases that the evolutionary relationships between island populations be known so that widely different genetic lineages are not mixed. From the example of the Bananaquit in the Lesser Antilles (where populations are hardly endangered), it would be appropriate to introduce individuals to St. Lucia from Martinique but not from St. Vincent, whose Bananaquit populations have had a long period of independent evolution. Populations with low genetic variability, such as those of the Bananaquit on Guadeloupe and the northern Lesser Antilles, might be helped if there were a need to do so by infusion of genetic diversity from closely related populations on Dominica, where there are many related genetic lineages.

(4) *Assessment of the history of population size.* Large samples of the genetic diversity of island populations can be used to infer the history of population change. We have seen how colonization events can greatly reduce the genetic diversity of an island population, such as that of the Bananaquit on Guadeloupe. When island populations are old and have had time to accumulate equilibrium levels of genetic diversity, as indicated by their divergence from sister populations on other islands, low diversity can reveal recent bottlenecks in population size. Todies and Bananaquits on Puerto Rico provide an interesting comparison in this regard. The genetic diversity among the todies in our Puerto Rican sample is about what one would expect of a population of 1-2 birds per hectare (0.9-1.8 million on the entire island), whereas the genetic diversity among Bananaquits (with a population of about 10 per hectare, or 9 million total) is far too low. These data suggest that Bananaquit populations have fluctuated greatly in the past, but that todies have maintained a more constant level. Of course, two species are not enough for a generalization, but the work of Joe Wunderle, of the U.S. Forest Service in Puerto Rico, and others, suggests that frugivorous and nectarivorous birds such as the Bananaquit are more vulnerable than such insectivorous birds as todies to such disturbances as hurricanes and droughts. It is possible that additional population-level analyses of diversity might allow us to predict the vulnerability of populations to catastrophic disturbances, judged from past population performance. This might also permit a closer focusing of management efforts.

(5) *Lessons from historical extinctions of island populations.* One pattern that seems quite consistent is that older island populations, that is, populations most distinct genetically from sister populations on other islands, have had the highest rates of extinction from anthropogenic causes, whether habitat destruction, hunting, or introduction of diseases and predators. This pattern seems to hold true for the Galapagos and Hawaiian Islands, as well as for the West Indies. Such is clearly the case for the trembler on Martinique, and for the House Wren (*Troglodytes aedon*) on Martinique and Guadeloupe. Regardless of the particular causes of the extinctions of these populations, these represented old, genetically distinctive lineages within the Lesser Antilles. It is also clear that the risk of extinction is higher on smaller islands than on larger islands and, by implication, it is higher in habitats with smaller areas than in habitats with larger areas

on a particular island. Again, this knowledge should help to identify potentially vulnerable populations and the habitats that support them.

How can this information be accessed for particular needs?

Analysis of DNA sequences from tissue samples is time-consuming and expensive. Thus, although we now have a relatively complete sample of island populations of small West Indian land birds, analysis of this material will take many years, and the resulting publications will not be easily accessible or interpretable by many individuals working on conservation issues on particular islands. In addition, although tissue collections are extensive, they are not complete. Some taxa, such as swifts and swallows are difficult to capture in nets without special efforts. Others, especially taxa of great conservation concern, are often missed in spite of efforts to catch them, because of their rareness and local distribution. For example, in the Lesser Antilles, we failed to capture the Forest Thrush (*Cichlherminia lherminieri*) on St. Lucia, the White-breasted Thrasher on Martinique, and the Pearly-eyed Thrasher on St. Lucia and Martinique.

To make molecular phylogenetic results available to individuals interested in conservation and management of local populations, we shall respond to direct requests for such information. If we have sequences on hand that would provide answers to your questions, we can provide an explanation of the data and interpretation of the results. If we have suitable unprocessed samples and the information from these can be used in the context of our own studies, we can give these a high priority. In each case, we could provide suitable documentation of specimens, techniques, and results to make a clear assessment of the status of a particular island population. When the data contain ambiguities, these would also be explained. Our goals are to provide genetic results, on an island-by-island basis, that are directly applicable to conservation issues within the West Indies, perhaps even leading to a regional comprehensive assessment of critical species, island populations, and habitats. This will take time, of course, but we have a good beginning. Meanwhile, we would like to hear your comments and will provide assistance where we can.

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