by Paul Hess

Identification by "Barcodes"

Headline writers went overboard in February 2007, announcing the revelation of 15 new North American bird species. That is not so, but the accurate story is eye-opening enough. The headlines refer to a 2007 publication in *Molecular Ecology Notes* <doi:10.1111/j.1471-8286.2007.01670.x> by Kevin C. R. Kerr and five coauthors, who have catalogued 643 North American bird species by "DNA barcodes". The principle is that a species can almost always be identified reliably—although somewhat controversially—by a particular segment of its mitochondrial DNA (mtDNA).

All but 42 of the 643 species thus far analyzed have unique mtDNA sequences in this segment that are different from those in any other species, according to the authors. The distinct sequences match those in taxa already classified as species, a point that Kerr and his colleagues suggest affirms barcoding's validity. Distinctive barcodes also correspond to 20 of 23 splits adopted by the American Ornithologists' Union during the past quarter-century. For the list, see Table 1 at the WebExtra accompanying this article <aba.org/pubs/birding/archives/vol39no4p35w1.pdf>.

Fifteen of the 643 species arouse the most lively ornithological stir. These contain two distinct barcode clusters in samples from different portions of the range (although not indicated by the authors as to subspecies): Northern Fulmar, Solitary Sandpiper, Western Screech-Owl, Warbling Vireo, Mexican Jay, Western Scrub-Jay, Common Raven, Mountain Chickadee, Bushtit, Winter Wren, Marsh Wren, Bewick's Wren, Hermit Thrush, Curve-billed Thrasher, and Eastern Meadowlark. Noting that taxonomists have previously suggested splits in nearly all of those, the Kerr team refers to its findings as "provisional" and "hypothetical" species—unrecognized, cryptic taxa. The authors emphasize that they are not actually identifying new species but, rather, are pointing to currently recognized species that deserve further taxonomic research.

A contrasting result has largely missed the headlines. Overlapping barcode clusters appear in 17 groups among 42 recognized species, perhaps implying that species within some of the groups should be merged. Examples are Snow Goose and Ross's Goose, eight "large white-headed gulls", and Golden-crowned Sparrow and White-crowned Sparrow, confirming earlier proposals regarding close genetic relationships within these groups. For the complete list, see Table 2 at the WebExtra <aba.org/pubs/birding/archives/vol39no4p35w1.pdf>. Most unexpected is an overlap of King Eider and Common Eider barcodes. Despite obvious differences between adult males, the two species are reported to hybridize extensively (Eugene M. McCarthy, *Handbook of Avian Hybrids of the World*, Oxford University Press, 2006); the barcode study provides additional evidence for a close evolutionary relationship between them. Kerr and his colleagues do not say that any of these groups necessarily represent single species; the overlapping clusters could represent recently diverged taxa that have not accumulated mtDNA sequence differences, or their component species could share mtDNA as a result of introgression by hybridization. Such unanswered questions can keep taxonomists busy for decades.

An analytical method called "DNA barcoding" shows genetic differentiation suggesting that 15 currently recognized North American bird species may actually consist of more than one species. **Solitary Sandpiper** is among the 15. *York County, Maine; September 2004. © Garth McElroy.*

Barcoding is explained in several publications available online. In a seminal paper in 2003, Paul D. N. Hebert and three coauthors analogized their method to the Universal Product Code that identifies retail products (*Proceedings of the Royal Society of London–B* 270:313–321). In 2004, Hebert and three other authors reported barcoding of 260 North American bird species, suggesting that splits of Solitary Sandpiper, Warbling Vireo, Marsh Wren, and Eastern Meadowlark might be appropriate, as well as mergers of American and Black Oystercatchers and American and

Northwestern Crows (*PLoS Biology* 2:1657–1663). The latter paper quickly aroused an extensive cautionary response by Craig Moritz and Carla Cicero, who discuss the method's "promise and pitfalls" (*PLoS Biology* 2:1529–1531). Problems of reconciling the DNA approach with classical taxonomic methods are also examined in a 2005 commentary by Rob DeSalle, Mary G. Egan, and Mark Siddall (*Philosophical Transactions of the Royal Society–B* 360:1905–1916).

For additional perspective, see the Consortium for the Barcode of Life <barcoding.si.edu> and "Management and Analysis" at the Barcode of Life Data Systems website <barcodinglife.org>. By 1 March 2007, the Data Systems had 7,417 barcoded specimens of 1,703 bird species from 84 countries, and the list is growing fast. "Our work on the North American birds is far from complete. While we have few missing species left to attack, we have others in need of a boost in sample size" (K. C. R. Kerr, personal communication).

Northern Bobwhite Decline

Northern Bobwhite populations in nearly every portion of the range have been declining severely for many decades. Government agencies and private wildlife researchers are working to restore critical habitat required by this species. *Osceola County, Florida; February 2006. © Brian E. Small.*

In a Texas Quail Council conservation report, Ernest Angelo, Charles Elliott, and Leonard Brennan asked poignantly in 2005: "Do you remember the 'good old days'? Do you remember when quail were a common sight? You could hear bobwhites whistling in the spring. You and your dogs could move 30 coveys a day. Do you remember when we had quail...where now we have concrete?"

Besides concrete, the Northern Bobwhite faces other barriers including modern farming and forestry. "High bobwhite densities (or even stable populations) are no longer an accidental byproduct of the way we use the land. Bobwhite habitat now must be intentionally created," says L. Wes Burger Jr., an expert in bobwhite ecology at Mississippi State University (personal communication).

Birders as well as hunters and gamebird gourmets speak sadly about hearing and seeing fewer bobwhites year by year. The North American Breeding Bird Survey (BBS) shows an overall average decline of 3 percent annually from 1966 to 2005, amounting to a 70-percent decrease. Losses average nearly 80 percent in the eastern part of the range, and some individual states fare even worse. The species has been extirpated from much of its former northern limits in Michigan, Ohio, Pennsylvania, New York, New Jersey, and southern New England.

BBS data analyzed by Joseph A. Veech in 2006 show a high correlation between landscapes and bobwhite trends throughout the range (*Journal of Wildlife Management* 70:922–930). Among 539 BBS routes surveyed from 1966 to 1999, bobwhites decreased significantly on 468, disappeared entirely from 28, and increased significantly on only 43. Veech reports that bobwhites declined in relatively forested, artificial, and barren lands and vanished from routes surrounded by the highest proportion of artificial and barren lands (primarily urban/suburban areas and unreclaimed surface-mine sites). Increases were in areas with relatively more cropland, pastureland, and rangeland.

Even in those beneficial landscapes, bobwhites require special help because intensely cultivated and overgrazed areas are not appropriate habitats. To address this crucial need, government and private wildlife professionals in the Southeast Quail Study Group proposed the nation's first landscape-scale habitat restoration and bobwhite recovery plan in 2002. The U.S. Department of Agriculture heeded the call in 2004 with a new feature in its Conservation Reserve Program (CRP): economic incentives for farmers to create "buffers" of native vegetation around cultivated fields. Landowners in 35 states reserved more than 136,000 acres by November 2006, and enrollment continues until the end of 2007.

Such attention to microhabitat is vital, according to researchers at Mississippi State. Samuel K. Riffell and Burger told the U.S. Farm Service Agency in 2006 that higher numbers of bobwhites and other grassland birds are overwhelmingly associated with CRP habitats in seven U.S. Bird Conservation Regions. "But all CRP habitat is not equal," Burger says (personal communication). Bobwhite numbers correlate most highly with recently planted habitat containing a diverse mixture of annual weeds, native grasses, and bare ground. In another study, Rick Hamrick, Burger, and Dave Godwin found that bobwhites in Mississippi in 2006 were twice as abundant in native-grass buffers planted around croplands as at farms without buffers. As concrete spreads and modern farms become inhospitable, CRP funding, intensive habitat management, and conservation-minded farmers are increasingly essential to the Northern Bobwhite's future.

Can Birds Count?

The answer was clear to French game ranger Charles Georges Leroy in 1802. He said of crows, "They count, that is certain." Leroy made his case in a charming treatise, *The Intelligence and Perfectibility of Animals from a Philosophic Point of View*, based on observations of hunters tricking crows to come close enough to shoot. Two men walked into a hut near a nest, then one walked away. The crows were not fooled. Three men walked in, then two walked away. Again, the crows were not fooled. Aware that one man was still inside, the birds kept their distance. They were not tricked until five or six hunters entered, then all but one left—which Leroy interpreted as reaching crows' arithmetical limit.

Northern Mockingbird is the latest bird species to offer evidence of counting ability—or, at least, an ability to distinguish between relatively large and small quantities. Foraging efficiency may be a strong evolutionary incentive for the development of numerical competence. *Belknap County, New Hampshire; May 2003. © Garth McElroy.*

Modern researchers sought more evidence. In the 1930s and 1940s, German ethologist Otto Koehler pioneered experiments with birds' numerical competence. He demonstrated that Eurasian Jackdaws, Gray Parrots, Budgerigars, ravens, magpies, and pigeons can judge relative differences between very small quantities (although they do not necessarily "count" absolute numbers). Koehler found that various species did not falter until differences in quantity were between five and six or between six and seven.

Add Northern Mockingbird to the list, according to George L. Farnsworth and Jennifer L. Smolinski in 2006 (*Condor* 108:953–957; video at <staff.xu.edu/~farnsworth>). They designed a feeder to release food when a mockingbird pulls sticks from either of two ends. Testing five wild birds, the authors placed different numbers of sticks at each end. For optimal foraging, the birds should choose the end with fewer sticks to obtain food faster and with less effort. Indeed, when the alternatives were one vs. six sticks and two vs. five sticks, the birds chose to use the fewer sticks significantly more often. But when the choice was three vs. four sticks, no bird showed a significant preference. Does this mean that the birds could not discriminate when the difference was only one stick, or that the saving in time and effort did not matter to them? To find out, Farnsworth and Smolinski suggest experiments with longer delays between feeding trials, when the minor difference might be important to hungrier birds.

Increasingly, neuroscientists are comparing avian numerical ability with that of mammals, including humans. The bird known to the widest public in this research is Alex, the Gray Parrot made famous by Irene M. Pepperberg in her book *The Alex Studies* (Harvard University Press 1999). One of Alex's achievements is an apparent ability to judge absolute numbers—in other words, to truly count. Such evidence for actual enumeration in birds is rare. Other recent studies of birds are reviewed by Jacky Emmerton in the cyberbook *Avian Visual Cognition*, edited and published by Robert G. Cook in 2001 (<pigeon.psy.tufts.edu/avc>). A review by Andreas Nieder in 2006 covers the neurobiology of numerical competence in birds and mammals (*Nature Reviews/Neuroscience* 6:177–190; <nature.com/nrn/journal/v6/n3/index.html>). Both publications show how far knowledge of birds' powers and limitations has advanced since Charles Georges Leroy's assurance two centuries ago.

Yellow-rumped Warbler Taxonomy

Twenty-four named species disappeared from the American Ornithologists' Union *Check-list* in 1973, all reclassified as subspecies (*Auk* 90:411–419). Among them were Myrtle

Warbler and Audubon's Warbler, merged as Yellow-rumped Warbler based on John L. Hubbard's seminal study of their hybridization in 1969 (*Auk* 86:393–432). He found that they "interbreed and backcross freely" where their ranges meet in western Canada, as shown by intergradation in throat color, auricular color, supraloral spot, postocular line, wing pattern, and tail pattern.

Hubbard lamented that he could not obtain direct evidence by measuring gene flow. George F. Barrowclough produced the first such evidence in 1980, analyzing samples taken from Hubbard's geographic areas (*Auk* 97:655–668). Barrowclough used the newly advanced technique of gel electrophoresis to determine genetic relationships from characteristics in blood proteins. Using mathematical models, he also judged whether selective forces are driving Myrtle and the Audubon's in separate evolutionary directions. Both methods led him to support the merger: "[T]here is no more reason to treat these two taxa as species than there is for any other case of geographical variation in plumage characters."

A new analysis of mitochondrial DNA in **Yellow-rumped Warbler** samples supports the current treatment of North American "Audubon's" and "Myrtle" taxa as a single species. Shown here is an individual of the "Audubon's" subspecies group. *Riverside County, California; April 2006. © Brian E. Small.*

A new genetic analysis by Borja Milá, Thomas B. Smith, and Robert K. Wayne in 2007 sheds further light on the close Myrtle/Audubon's relationship (*Molecular Ecology* 16:159–173). Sequences in three regions of mitochondrial DNA (mtDNA) show a consistent pattern of shared haplotypes between the Myrtle subspecies-group and the North American portion of the Audubon's subspecies-group, indicating very recent divergence from a common ancestor. The authors estimate that these two groups separated less than 18,000 years ago, just after North America's last glacial maximum—a remarkably brief evolutionary period, considering the multiple plumage differences that now distinguish them.

In the same study, Milá, Smith, and Wayne recommend elevating two nonmigratory Yellow-rumped Warbler populations to species rank. These are subspecies known as Black-fronted Warbler (*nigrifrons*) of northwestern Mexico and Goldman's Warbler (*goldmani*) of Guatemala and southernmost Mexico. The two taxa are well differentiated genetically and are reciprocally monophyletic—i.e., reflecting mutually exclusive evolutionary lineages. The authors estimate that *nigrifrons* and *goldmani* separated from each other during the middle Pleistocene, and that the migratory Myrtle/Audubon's lineage and the sedentary *nigrifrons/goldmani* lineage diverged from a common ancestor during the early Pleistocene. In this scheme, the Myrtle/Audubon's group is a "sister clade" to *nigrifrons* and *goldmani*, closely related to them but an independent evolutionary unit.

Unexpectedly, these genetic findings are contrary to the traditional placement of *nigrifrons* and *goldmani* with *auduboni* in an "Audubon's Warbler group"—a union based upon shared plumage characters. Milá views this as the study's "most striking" result, an indication of just how rapidly plumage can evolve (personal communication): "The sedentary forms had always been classified as subspecies of Audubon's because they all have a yellow throat, yet the genetic data show that using plumage color to classify forms is misleading in this case. Even though Myrtle and Audubon's look very different, they are actually the most closely related pair."

The research team speculates that Myrtle and Audubon's are in an early stage of speciation. This view harkens back to Hubbard's original opinion that the two groups should be classified as "semispecies"—evolutionary biologist Ernst Mayr's term for "populations that have acquired some, but not yet all, attributes of species rank; borderline cases between subspecies and species". Milá and his colleagues emphasize that analyzing nuclear genes will be necessary to understand the Myrtle/Audubon's relationship more fully and to confirm the patterns of gene flow shown by mtDNA among all of the Yellow-rumped Warbler groups.