Short Communications

Wilson Bull., 113(2), 2001, pp. 228–231

Molecular "Cuckoo Clock" Suggests Listing of Western Yellow-billed Cuckoos May Be Warranted

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ABSTRACT.—The western subspecies of the Yellow-billed Cuckoo (*Coccyzus americanus occidentalis*) has undergone severe population declines during recent years. The current status of this subspecies has been disputed, however, because it cannot be easily separated from *C. a. americanus* using morphological characteristics. We sequenced most of the cytochrome b gene in five western U.S., three eastern U.S., and two Mexican Yellow-billed Cuckoos, and one Black-billed Cuckoo (*C. erythropthalmus*) to determine if the subspecies could be diagnosed genotypically. The haplotypes of the eastern and western subspecies differed by four fixed base changes, suggesting that they diverged approximately 205,000–465,000 yr ago. Two of these fixed differences cause amino acid coding changes. Our findings support continued separation of the two subspecies and recognition of the western subspecies as an evolutionarily significant unit. *Received 21 Sep. 2000, accepted 22 Aug. 2001.*

Based on morphological characteristics, two subspecies of the Yellow-billed Cuckoo (*Coccyzus americanus*) have been described, one in the east (*C. a. americanus*) and another in the west (*C. a. occidentalis*; American Ornithologists' Union 1957). However, there is disagreement over whether these two subspecies are sufficiently distinct to warrant recognition (Todd and Carriker 1922, Van Tyne and Sutton 1937, Mees 1970). Recent attempts to identify the subspecies based on morphology have been contradictory. Using primarily the same data set, Banks (1988, 1990) and Franzreb and Laymon (1993) came to opposite conclusions about the diagnosability of the two subspecies. This taxonomic question has become more important due to population declines of the western subspecies in many parts of its range (Gaines and Laymon 1984, Laymon and Halterman 1987, Hughes 1999). The U.S. Fish and Wildlife Service recently announced a review of evidence for the possible listing of the western subspecies as endangered (U.S. Fish and Wildlife Service 2000). We used a molecular genetic approach to aid resolution of this conservation question.

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METHODS

To limit sampling error due to small sample sizes, we analyzed birds from several geographic areas, including two Yellow-billed Cuckoos from southeast Alaska, three from New Mexico, two from Minnesota, one from Vermont, two from Veracruz, Mexico, and one Black-billed Cuckoo (*C. erythropthalmus*; Table 1). Because the two Alaska birds were vagrants, we measured wing, tail, and bill lengths and bill depth (following Banks 1988 and Baldwin et al. 1931) then used Franzreb and Laymon's (1993) discriminant function to verify that these birds are morphologically *C. a. occidentalis*. These analyses confirmed Gibson and Kessel's (1997) conclusion that these Alaska birds are the western form. We also measured the two Mexican specimens, which were taken during migration outside of the breeding range of either subspecies. These birds were of intermediate size and not readily identifiable as either subspecies. The rest of the samples were obtained within the breeding ranges of the respective subspecies during the breeding season.

Whole genomic DNA was extracted from muscle tissue samples of these birds using a QiaAmp DNA Extraction Kit (Qiagen Inc.). A 978 base pair portion of the mtDNA cytochrome b gene was amplified using standard polymerase chain reaction protocols (Palumbi 1996) and the highly conserved external primers L14841 (Kocher et al. 1989) and H16065 (Helm-Bychowski and Cracraft 1993). Amplified fragments were cycle sequenced (Hillis et al. 1996) on a Perkin Elmer 4800 thermal cycler and sequenced on an automated sequencer (ABI 373A). Both external primers and two internal primers developed in our lab (L519cytb: 5′-CCAACCCTTACCCGATTCTTCG-3′, and H 637cytb: 5′-AGATGCCTAGGGGGTTGTTTGA 3′) were used to sequence each individual in both directions to ensure that each base was correctly identified. Sequences were aligned, data critically examined, and protein coding verified using Sequencher (ver. 3.0, Gene Codes Corp., Ann Arbor, MI). A GenBank search showed that Yellow-billed and Black-billed cuckoo sequences were the best match to our data. To avoid the problem of acci-

TABLE 1. Uncorrected (below diagonal) and corrected (above diagonal) percent pairwise sequence divergence among ten Yellow-billed Cuckoos (*Coccyzus americanus*) and one Black-billed Cuckoo (*C. erythropthalmus*). The Tamura-Nei genetic distance model was used to determine corrected sequence divergences (Tamura and Nei 1993).

Taxon	Voucher[a] #/Location	Genbank #	Taxon 1	2	3	4	5	6	7	8	9	10	11
1. *C. a. occidentalis*	UAM6953/Alaska	AF249268	—	0.10	0.21	0.21	0.10	0.41	0.51	0.82	0.62	0.51	8.98
2. *C. a. occidentalis*	UAM7059/Alaska	AF249269	0.10	—	0.10	0.10	0.21	0.31	0.41	0.72	0.51	0.41	8.86
3. *C. a. occidentalis*	MSB22170/New Mexico	AY46905	0.20	0.10	—	0.21	0.31	0.41	0.51	0.82	0.62	0.51	8.98
4. *C. a. occidentalis*	MSB21695/New Mexico	AY46906	0.20	0.10	0.20	—	0.31	0.41	0.51	0.82	0.62	0.51	8.98
5. *C. a. occidentalis*	MSB21007/New Mexico	AY46907	0.10	0.20	0.31	0.31	—	0.51	0.62	0.93	0.72	0.62	8.86
6. *C. a.* ssp.?	UAM10354/Mexico	AY46908	0.41	0.31	0.41	0.41	0.51	—	0.10	1.03	0.82	0.72	8.98
7. *C. a.* ssp.?	CNAV23931/Mexico	AY46909	0.51	0.41	0.51	0.51	0.61	0.10	—	1.13	0.93	0.82	8.86
8. *C. a. americanus*	UAM10130/Minnesota	AF249270	0.61	0.51	0.61	0.61	0.72	0.82	0.92	—	0.21	0.10	8.86
9. *C. a. americanus*	UAM10131/Minnesota	AF249271	0.82	0.72	0.82	0.82	0.92	1.02	1.13	0.20	—	0.31	9.21
10. *C. a. americanus*	UAM13376/Vermont	AY46910	0.51	0.41	0.51	0.51	0.61	0.72	0.82	0.31	0.10	—	8.38
11. *C. erythropthalmus*	UAM10132/Minnesota	AF249272	8.28	8.18	8.28	8.28	8.18	8.28	8.18	8.49	8.49	8.38	—

[a] UAM - University of Alaska Museum; MSB - Museum of Southwestern Biology; CNAV - Coleccion Nacional de Aves de Mexico.

dental amplification of nuclear copies of mitochondrial genes (Sorenson and Quinn 1998), we used only muscle tissue for extractions, amplified large fragments, examined electropherograms for co-amplified peaks, and verified protein coding. All sequences were deposited in GenBank (Table 1).

Both corrected and uncorrected percent sequence divergences were determined using PAUP* 4.0b (Swofford 1999). We accepted estimates of divergence rates as being approximately 2% per million years based on mtDNA molecular clocks developed independently for Canada Goose (*Branta canadensis*; Shields and Wilson 1987) and honeycreepers (genus *Hemignathus*; Tarr and Fleischer 1993). We use these dates only as rough estimates of actual divergence, however, because the rate of divergence in this molecular ‘‘cuckoo clock’’ has not been determined.

RESULTS

We found four fixed base pair differences between the haplotypes of the two subspecies, excluding the Mexican birds because their subspecific affinity is equivocal. This represents a sequence divergence of 0.41–0.92% (approximately 205,000 to 465,000 yr divergence; Table 1). Within each subspecies, haplotype divergence was 0.10–0.31% for both subspecies. Thus, there is genetic diversity within each subspecies, and this preliminary evidence suggests that it includes at least 10.9–75.6% of the level of diversity found between the two subspecies. Two of the four fixed base pair differences between subspecies cause differences in amino acid coding. The two Mexican birds, taken on spring migration outside the breeding range of the species, share the same fixed differences with birds from the west. However, they differ from both eastern and western birds by possessing two unique fixed differences. Haplotype variation between Yellow-billed and Black-billed cuckoos showed 8.2–9.2% sequence divergence, suggesting that they diverged approximately 4.5 million years ago (Table 1). Divergence between the subspecies of Yellow-billed Cuckoos thus seems to be about 5–10% of the divergence between this species and its closest relative.

DISCUSSION

Morphological characteristics do not allow 100% diagnosability of the two subspecies of Yellow-billed Cuckoos, which is common among avian subspecies. However, our findings suggest that these two subspecies are genetically distinct. Multiple fixed base change differences in mtDNA suggest that the eastern and western subspecies have not shared a common ancestor for hundreds of thousands of years. Therefore, our data support the separation of the Yellow-billed Cuckoo into two subspecies and recognition of *C. a. occidentalis* as an evolutionarily significant unit (Moritz 1994) fully warranting management status independent from its eastern relative. Further genetic research throughout the species' range focusing on the few zones of contact between the subspecies (Banks 1988) would enable determination of whether or not introgression is occurring.

We think it is noteworthy that the sequence variation between the two subspecies includes two fixed amino acid differences in a gene that codes for a protein important in cell respiration. We do not know whether these amino acid differences affect the resulting protein in a selectively nonneutral manner, but the differences between the two subspecies are not immediately attributable to neutral genetic variation.

The strong genetic affinity between the two spring migrants from Veracruz and geographically and morphologically unequivocal *C. a. occidentalis* may shed light on the largely unknown migration route of *occidentalis*, but at least one other western bird has been recorded during migration on the Atlantic coast of Mexico during spring (Friedmann et al. 1950). Finally, in consideration of the apparently long independent evolutionary history of this lineage, the propensity for the development of allohiemy among migratory bird lineages through time (Salomonsen 1955), and profound habitat changes occurring in the Neotropics during the past half century, efforts must be made to delineate the seemingly unknown wintering grounds of this subspecies (Peters 1940, AOU 1957).

Yellow-billed Cuckoos seem to be another of many avian species that show patterns of biological differentiation that are not resolvable using phenotype alone (Ball and Avise 1992, Rising and Avise 1993, Zink 1996). This study re-emphasizes the utility of genetic markers for distinguishing evolutionarily significant units.

ACKNOWLEDGMENTS

R. M. Zink and J. T. Klicka of the Bell Museum of Natural History provided salvaged cuckoos from Minnesota, R. W. Dickerman of the Museum of Southwestern Biology provided New Mexico tissues, and B. P. Escalante of the Coleccion Nacional de Aves de Mexico provided tissues from Mexico.G. M. Spellman, J. A. Cook, T. C. Glenn, S. G. Sealy, and D. B. McNair provided helpful comments on the manuscript.

LITERATURE CITED

AMERICAN ORNITHOLOGISTS' UNION. 1957. Check-list of North American birds, 5th ed. Lord Baltimore Press, Baltimore, Maryland.

BALDWIN, S. P., H. C. OBERHOLSER, AND L. G. WORLEY. 1931. Measurements of birds. Sci. Publ. Cleveland Mus. Nat. Hist. 2:1–165.

BALL, R. M., JR., AND J. C. AVISE. 1992. MtDNA phylogeographic differentiation among avian populations, and the evolutionary significance of subspecies. Auk 109:626–636.

BANKS, R. C. 1988. Geographic variation in the Yellow-billed Cuckoo. Condor 90:473–477.

BANKS, R. C. 1990. Geographic variation in the Yellow-billed Cuckoo: corrections and comments. Condor 92:538.

FRANZREB, K. E. AND S. A. LAYMON. 1993. A reassessment of the taxonomic status of the Yellow-billed Cuckoo. West. Birds 24:17–28.

FRIEDMANN, H., L. GRISCOM, AND R. T. MOORE. 1950. Distributional check-list of the birds of Mexico, part I. Cooper Ornithological Club, Berkeley, California.

GAINES, D. AND S. A. LAYMON. 1984. Decline, status, and preservation of the Yellow-billed Cuckoo in California. West. Birds 15:49–80.

GIBSON, D. D. AND B. KESSEL. 1997. Inventory of the species and subspecies of Alaska birds. West. Birds 28:45–95.

HELM-BYCHOWSKI, K. AND J. CRACRAFT. 1993. Recovering phylogenetic signal from DNA sequences: relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome b gene. Mol. Biol. Evol. 10:1196–1214.

HILLIS, D. M., B. K. MABLE, A. LARSON, S. K. DAVIS, AND E. A. ZIMMER. 1996. Nucleic acids IV: sequencing and cloning. Pp. 321–381 *in* Molecular systematics (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.). Sinauer, Sunderland, Massachusetts.

HUGHES, K. M. 1999. Yellow-billed Cuckoo (*Coccyzus americanus*). No. 418 *in* The birds of North America (A. Poole and F. Gill, Eds.). The Birds of North America, Inc., Philadelphia, Pennsylvania.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. 86:6196–6200.

LAYMON, S. A. AND M. D. HALTERMAN. 1987. Can the western subspecies of the Yellow-billed Cuckoo be saved from extinction? West. Birds 18:19–25.

MEES, G. F. 1970. On some birds from southern Mexico. Zool. Mededelingen 44:237–245.

MORITZ, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends Ecol. Evol. 9:373–375.

PALUMBI, S. R. 1996. Nucleic acids II: the polymerase chain reaction. Pp. 205–247 *in* Molecular systematics (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.). Sinauer, Sunderland, Massachusetts.

PETERS, J. L. 1940. Checklist of the birds of the world, vol. IV. Harvard Univ. Press, Cambridge, Massachusetts.

RISING, J. D. AND J. C. AVISE. 1993. Application of genealogical-concordance principles to the taxonomy and evolutionary history of the Sharp-tailed Sparrow (*Ammodramus caudacutus*). Auk 110:844–856.

SALOMONSEN, F. 1955. The evolutionary significance of bird migration. Dan. Biol. Medd. 22:1–62.

SHIELDS, G. F. AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. J. Mol. Evol. 24:212–217.

SORENSON, M. D. AND T. W. QUINN. 1998. Numts: a challenge for avian systematics and population biology. Auk 115:214–221.

SWOFFORD, D. L. 1999. PAUP*: phylogenetic analysis using parsimony, ver. 4.0b. Sinauer, Sunderland, Massachusetts.

TAMURA, K. AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 18:387–404.

TARR, C. L. AND R. C. FLEISCHER. 1993. Mitochondrial-DNA variation and evolutionary relationships in the Amakihi complex. Auk 110:825–831.

TODD, W. E. C. AND M. A. CARRIKER, JR. 1922. The birds of the Santa Marta region of Colombia: a study in altitudinal distribution. Annal. Carnegie Mus. 14:1–611.

U.S. FISH AND WILDLIFE SERVICE. 2000. Endangered and threatened wildlife and plants: notice of 90-day finding for a petition to list the Yellow-billed Cuckoo as endangered and commencement of a status review. Fed. Regist. 65:8104–8107.

VAN TYNE, J. AND G. M. SUTTON. 1937. The birds of Brewster County, Texas. Misc. Publ. Mus. Zool. Univ. Michigan. 37:1–115.

ZINK, R. M. 1996. Comparative phylogeography in North American birds. Evolution 50:308–317.

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