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

Christin L. Pruett, 1, 2 Daniel D. Gibson, 1 and Kevin Winker 1

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.

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 QiAmp 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'-CCAACCCCTACCCGATTCTTCG-3', and H 637cytb: 5'-AGATGCTTAGGGGTGTTTGTA 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-

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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).

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<thead>
<tr>
<th>Taxon Voucher a #/Location</th>
<th>Genbank #</th>
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<tr>
<td>1. C. a. occidentalis</td>
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<td>2. C. a. occidentalis</td>
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<td>6. C. a. ssp.?</td>
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<tr>
<td>7. C. a. ssp.?</td>
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<td>8. C. a. americanus</td>
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<td>9. C. a. americanus</td>
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<tr>
<td>10. C. a. americanus</td>
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<tr>
<td>11. C. erythropthalmus</td>
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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 ge-
netically 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 allohimy 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**

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**LITERATURE CITED**


