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FULL PAPER

Are the Australian crows synonymous? Molecular analysis leads to the Australian White-eyed Crow, *Corvus indistinctus*

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Corvidae crows form a world-wide family of songbirds (*Passeriformes*) of which the taxonomic relationships are often unclear. In particular, we closely examine the relationships of the five species of native corvids of Australia (Australian Raven, *Corvus coronoides*, Little Raven, *C. mellori*, Forest Raven, *C. tasmanicus*, Torresian Crow, *C. orru* and Little Crow, *C. bennetti*) using DNA-DNA hybridisation and powerful computing methods to produce a molecular phylogenetic analysis that conclusively shows that these five species are in fact synonymous and we suggest the name Australian White-eyed Crow *Corvus indistinctus*. The low genetic differentiation and lack of substantial physical differential characteristics support that these five species be reduced to eight subspecies of *C. indistinctus*.

Introduction

Recent work suggests that the songbirds (*Passerifomes*) originated in Australasia 50-45 MYO,[1] radiating outwards to produce the oscine and non-oscine familes known to us today.[2] Among the birds in that initial radiation was the common ancestor of the corvids, currawongs, riflebirds, etc.[3] Later descendants of this proto-corvid then re-radiated back to Australasia to introduce true corvids to the region.[1,4] It is known from the low number of species in Australasia that they are relatively recent arrivals to this part of the world with differentiation of the present species being recent in areas as far apart as Australia, Papua New Guniea, the Bismarck Archipelago and New Caledonia.[5] In each of these areas, the resident crow species are superficially very similar and it is this similarity that has prompted us to investigate the relationship of these species in order to establish the time period since the arrival of the first ancestor of these species and the degree of speciation that has occurred since that event. Previous work by Rowley[4] and Vaurie[5] suggested that there were five species of corvid present in Australia with one, the Forest Raven, consisting of two subspecies, although since that time, several other species have been identified as having subspecies, such as Australian Raven, consisting of two populations, *C. coronoides coronoides* in the east and *C. c. perplexus* in the west with a limited intermediate zone in the Gawler Ranges, Eyre Peninsula and Lake Eyre, as noted by Higgins.[6]

It is noted in the literature and in field guides that resolving members of each species when mixed flocks occur, such as those in south-western NSW, is non-trivial and requires extensive photographic and sonographic measurement if in-hand inspection is not available. Given the recent arrival of corvids to Australia, the difficulty of reliably differentiating species and the unclear subspecific assignment of several of the five species, we decided to investigate the relationships between these birds, primarily in order to determine the date of radiation across Australia but also to determine the extent to which speciation has taken place.

Methodology

Liver and muscle samples stored in ethanol, skin samples from foot pads of museum specimens, as well as feathers were used for DNA extraction. Sequences of 179 individuals from ten widely-spaced areas in Australia were included (Appendix A). DNA from feathers and museum material was extracted by incubation of tissues in a 10% Chelex (Bio-Rad) solution containing proteinase K (0.5 mg/ml) for 4 h at 56 °C (with agitation). Subsequently extractions were heated to 95 °C for 5 min and centrifuged for 1 min. The supernatant was purified using the QIAquick PCR Purification Kit (Qiagen) with a final volume of 30–70 µl elution buffer. DNA from muscle samples was extracted by overnight incubation at 50 °C in extraction buffer (10 mM Tris–HCl, pH 8.0, 10 mM EDTA, 50 mM NaCl, 40 mM dithiothreitol, 1% SDS, 0.5 mg/ml proteinase K). DNA was purified by two PCI (phenol/chloroform/isoamylalcohol, 25:24:1) and one CI (chloroform/isoamylalcohol, 1:1) extractions followed by precipitation with 1/10 vol. 3 M NaAc, 3× vol. EtOH. The following primers were used for the amplification of the control region (CR): CR-Cor+ (ACCCTTCAAGTGCGTAGCAG) and Phe-Cor−(TTGACATCTTCAGTGTCATGC). These primers amplify a partial sequence of the CR (positions 693–1308 of the reference sequence of *C. moneduloides*, AJ458536) as well as 21 bp of the adjacent *tRNA-Phe* gene (length of PCR fragment ≈ 680 bp). To obtain the CR sequence from old samples with bad DNA quality overlapping PCR fragments were amplified using various primers: For the 5′-fragment CR-Cor3− (TAAAAATTGTTGTTTATTTTG) or CR-Cor6− (GATGAT TTGGACAATCTAGG) in combination with CR-Cor+, and for