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FEATHERS FROM MUSEUM BIRD SKINS—A GOOD SOURCE OF DNA FOR PHYLOGENETIC STUDIES[1]

PETER LEETON AND LESLIE CHRISTIDIS
Department of Ornithology, Museum of Victoria, 71 Victoria Crescent Abbotsford, Victoria 3175, Australia

MICHAEL WESTERMAN
Department of Genetics and Human Variation, Latrobe University, Bundoora, Victoria 3083, Australia

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Molecular techniques such as DNA–DNA hybridization, restriction endonuclease analysis of mitochondrial DNA and DNA sequencing provide valuable insights into the evolution of birds, and have often challenged conventional taxonomies (e.g., Sibley and Ahlquist 1990). Although these new methods provide useful phylogenetic information, they sometimes suffer in comparison to morphological studies in coverage of taxa. This is because high-quality tissue material needed for such studies is not as readily available for as great a range of species as is the case with museum skins and skeletons. Samples from rare and extinct species present a further problem because tissue collection may not be possible. Recent advances in polymerase chain reaction (PCR, Saiki et al. 1988) technology have alleviated many of these problems. DNA has been extracted and sequenced from various mammal species, including extinct forms, in which material has been obtained from small sections of skin removed from museum skins (Pääbo 1989, Thomas et al. 1989). This technique has also been used with some success from bird skins (Smith et al. 1991). However, the small size and fragile nature of the skins of most bird specimens, compared to mammals, means that skins risk damage. This is a problem with rare or extinct specimen material (see Graves and Braun 1992).

An alternative source of DNA from birds is feathers. Taberlet and Bouvet (1991) recently reported success in using growing feather tips of fledgling birds as a source of DNA for sequencing. Ellegren (1991) also reported use of feathers from museum skins in DNA fingerprinting studies, although the differential degradation of the DNA limits the reliability of such analyses. Here we report a protocol for extracting DNA which we have successfully used on single feather tips of museum bird skins. This procedure minimizes both damage to the skins as well as the risk of recovering contaminated DNA.

The protocol used for such extractions is derived from Higuchi's (1988) technique for isolation of DNA from hair root. A single feather was plucked from the wing of a 100 year-old Night Parrot specimen (*Geopsittacus occidentalis*) and from the wing of a live Budgerigar (*Melopsittacus undulatus*), using sterile forceps to avoid contamination. The wing region was chosen because interference with the shaft tip is minimal during museum preparation. Feathers from other regions have also rendered non-contaminated DNA. Depending upon feather size, some 2–5 mm of the shaft was retained along with the tip (or rachis), while the rest was discarded. The tip was washed several times in 70% ethanol and then in distilled water. A control containing no feather shaft was also used throughout this procedure. The tip was then placed in a 1.5 ml microcentrifuge tube with 0.5 ml of digestion buffer (0.05 M Tris.HCl; 0.01 M EDTA; 0.1 M NaCl; pH 8.0), 0.04 M DTT, 10% SDS and 100 μg Proteinase K. The tissues were then completely digested for 12–18 hours at 37°C. Following digestion, samples were twice extracted with an equal volume of phenol and chloroform, and once with n-butanol. Sterile distilled water was added to the resulting aqueous layer from the n-butanol spin, and centrifuged at 4,000 rpm in a Millipore Ultrafree-MC microcentrifuge tube. Salts were removed by three successive washes with distilled water, and the final centrifugation was continued until a 50 μl volume was achieved.

One μl of the concentrate was used in a polymerase chain reaction to amplify a region of the mitochondrial cytochrome *b* gene. The PCR buffer included 50 ng of both primer L14841 and H15149 (Kocher et al. 1989), 1 unit of Tth polymerase and the appropriate buffer (Toyobo). Following initial DNA denaturation at 92°C for four minutes, the PCR conditions were maintained at 92°C for 40 seconds, followed by 53°C for 60 seconds for primer annealing and 72°C for 60 seconds for primer extension. This three-part cycle was repeated 35 times, after which a sample of the solution was run on a 2% agarose gel (Fig. 1). Ancient and fresh DNA was found to amplify to similar degrees under these conditions.

We have also successfully amplified DNA using primers located within the mitochondrial 12s RNA gene and the nuclear 28s RNA gene. When utilizing DNA from the older specimens, a problem has sometimes been encountered when amplifying regions >800 nucleotide pairs in length. In these cases, internal prim-

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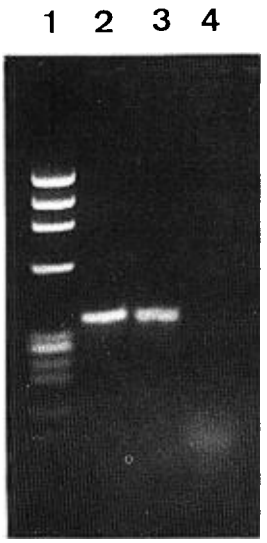

FIGURE 1. Double-strand PCR amplification of a 305 bp region of the cytochrome *b* gene. Lane 1: ϕx174 DNA digested with *Hae*III. Lane 2: Amplification of *Geopsittacus* DNA. Lane 3: Amplification of *Melopsittacus* DNA. Lane 4: Control sample containing no DNA except for the oligonucleotide primers. Lanes 2, 3, and 4 contain 5 μl of a 50 μl reaction.

ers are used to reduce the size of the region to be amplified (see Edwards et al. 1991). These amplification products can then be sequenced and used in phylogenetic analyses.

Museum specimens we have successfully used range in age from 40 to 120 years. No discernable differences have been found in the amount of DNA recovered from each specimen. Our current studies involve analyzing DNA sequences from within the parrots and the corvids, utilizing a large size range of feathers. We have also successfully amplified DNA from single feathers which were found in the field. As the feathers used were fully developed with no obvious pulp material left, enough cells must still be attached to the inner walls of the shaft tip to provide DNA for PCR amplification.

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