

**MOLECULAR APPROACHES USED TO INFER EVOLUTIONARY
HISTORY, TAXONOMY, POPULATION STRUCTURE, AND
ILLEGAL TRADE OF WHITE-TAILED BLACK-COCKATOOS
(*CALYPTORHYNCHUS* SPP.) IN AUSTRALIA**

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary education institution.

Nicole E. White

ABSTRACT

Cockatoos are charismatic, iconic Australasian birds, yet this family (Cacatuidae) is inadequately understood from a genetic perspective. Herein, this thesis describes a detailed genetic study of endangered white-tailed black-cockatoos (WTBCs, *Calyptorhynchus* spp.). The species is endemic to Western Australia and has undergone a demographic decline of ~50% since European arrival. Causes of the decline are complex, but likely involve a combination of anthropogenic habitat loss, poaching and shooting. Through extensive sequencing of nuclear (nu) and mitochondrial (mt) genes (10 complete mt genomes, 138 mtDNA sequences, and 89 nuDNA sequences), in addition to microsatellite profiling 840 cockatoos, the primary aim of this research was to resolve the evolutionary history, taxonomy, and population structure of WTBCs. The data presented here will facilitate conservation and management of WTBCs, and assist in policing the illegal trade and harvest from the wild.

With the use of a comprehensive nuDNA and mtDNA dataset (inclusive of five novel cacatuid mt genomes), fossil calibrations and Bayesian modeling, the evolutionary history of cockatoos was investigated (chapter two). The divergence of Cacatuidae from Psittacidae was estimated to have occurred during the Eocene (95% CI 51.6-30.3 Ma). A cockatoo multi-gene phylogeny for 16 of 21 species shows a most recent common ancestor in the Oligocene (95% CI 38.1-18.3 Ma). This analysis enabled, for the first time, an accurate phylogenetic placement for numerous cacatuids that have been historically difficult to resolve. From this investigation, insights into the diversification and radiation of the subgenus *Zanda* (white- and yellow-tailed black-cockatoos) show they shared a common ancestor ~1.3 million years ago. The lack of any clear species-specific sequence differences between the two forms of WTBC (*C. baudinii* and *C. latirostris*) prompted a more in-depth study using microsatellites (chapter three). Twenty highly polymorphic microsatellite loci were developed and used to generate a database containing 660 contemporary and 24 historical samples. Genotyping 156 cockatoos from seven other genera demonstrated the utility of the microsatellite markers beyond WTBCs.

The DNA sequence and microsatellite data immediately brought to the forefront inconsistency with the current taxonomic recognition of WTBCs, namely that negligible genetic differentiation, a high degree of gene flow and genetic admixtures for the long- (*C. baudinii*) and short-billed (*C. latirostris*) forms was evident. A detailed investigation (chapter

four) into genetics, morphology, vocal dialect, diet and reproductive behaviour resulted in the decision to synonymise the two forms to *C. baudinii* Lear 1832. The reclassification advocated here will result in reprioritisation of conservation management and recovery plans, increase the protection of an endangered species, and facilitate the accurate species identification in wildlife forensic casework.

To examine the effects of severe native vegetation clearing on WTBCs, contemporary and historical samples were used with a spatial and temporal landscape approach to examine; population structure, genetic diversity, and gene flow (chapter five) in severely modified habitats. Despite overall low genetic substructure ($K=2$) and population divergence ($F_{ST} = 0.013$), a possible hypothesis for this substructure is the result of the clearing of the wheatbelt, where only 7% of the native vegetation remains in an area 95,800 km², and could act as a major boundary separating WTBC of the western and eastern regions. Importantly, the genetic data support the vegetation corridor of the deep-south as the primary remaining migration passageway facilitating gene flow between the regions. The genotypes of the historical individuals and western region birds suggest WTBC of the eastern region have recently suffered a loss of genetic variation and fitness effects of genetic drift. Overall, despite a dramatic decline in census size the historic and contemporary samples show genetic diversity was not affected in the same way, with no significant reduction detected ($H_E \sim 0.70$).

Lastly this thesis set out to apply this newly developed molecular toolkit in a wildlife forensic context. Three cases involving suspected poaching and shooting of black-cockatoos are presented (chapter six). The established DNA databases enabled high-resolution kinship and identity testing to be conducted ($P_{ID} 2.6E-20$; $P_{IDsibs} 2.4E-8$). In one case, a cockatoo poached from a tree hollow was matched to DNA from eggshell recovered from the nest. The second case showcased the utility of the WTBC provenance (nest sites) database, as the kinship and geographic origin of a poached WTBC was determined. The third case identified the number of WTBCs allegedly shot at a fruit orchard from body part remains. The cockatoo population databases are the first of their kind in Australia, and demonstrate the efficacy and probative value of molecular approaches to inform wildlife enforcement agencies responsible for the protection of Australian fauna.

NOTE

The Appendix of this thesis (section 8.0) consists of published journal articles resulting from this doctoral research and has been removed from the electronic version of this thesis. Citations for the journal articles are listed below.

JOURNAL ARTICLE CITATIONS

White NE, Phillips MJ, Gilbert MTP, Alfaro-Núñez A, Willerslev E, Mawson PR, Spencer PBS, Bunce M (2011) The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). *Molecular Phylogenetics and Evolution* **59**, 615-622.

White NE, Dawson R, Coghlan ML, Tridico SR, Mawson PR, Haile J, Bunce M (2011) Application of STR markers in wildlife forensic casework involving Australian black-cockatoos (*Calyptorhynchus* spp.). *Forensic Science International: Genetics*, doi:10.1016/j.fsigen.2011.10.003.

White NE, Mawson PR, Dawson R, Bunce MA, Spencer PBS (2009) Characterization and cross-species utility of 20 microsatellite markers for population and forensic applications in the endangered Carnaby's Black-cockatoo, *Calyptorhynchus latirostris*. *Conservation Genetics Resources* **1**, 341-345.

Coghlan ML, **White NE**, Parkinson L, Haile J, Spencer PBS, Bunce M (2011) Egg forensics: an appraisal of DNA sequencing to assist in species identification of illegally smuggled eggs. *Forensic Science International: Genetics*, doi:10.1016/j.fsigen.2011.1006.1006.

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