

# Development of genetic survey methodologies for cassowaries

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## Executive Summary

The Southern Cassowary, *Casuarius casuarius*, is a large, cryptic forest dwelling bird that is listed as an endangered species under the Australian Government's *Environment Protection and Biodiversity Conservation Act (1999)*, the State of Queensland's *Nature Conservation (Wildlife) Regulation (1994)*, and is listed on the IUCN Red List of Threatened Species. Despite long standing and widespread concern over the status of the species and its resilience in the face of increasing human pressures in Australia, there is considerable uncertainty over cassowary population sizes and trends. This uncertainty has persisted because of the difficulties associated with working with cassowaries. In complex and rugged environments such as tropical rainforest, large, cryptic and timid species become difficult to detect and almost impossible to monitor or study using the standard technologies available to ecologists and conservation biologists. The consequence of this has been that cassowary conservation and management has by necessity been done without the benefit of a detailed understanding of the species population dynamics and ecology.

This is an unsatisfactory situation and the aim of this project was to develop new technologies that would help overcome these difficulties and that would provide the means by which we could improve our understanding of the species and its management. Because animals pass some of their own DNA in their dung, DNA fingerprinting based on faecal DNA material has the potential to allow individual identification as well as estimation of relatedness between individuals and populations. This allows for population size estimation, determination of social structure, and patterns of gene flow within populations and species. Such a methodology developed for cassowaries would act as the crucial technology in monitoring and managing cassowary populations at all spatial scales.

In this work we set out to develop methods for extracting cassowary DNA from dung samples, to develop up a microsatellite library for DNA fingerprinting and individual discrimination, and to identify the survey and analysis approaches that would allow population estimation and monitoring. The overarching goal was to test the feasibility and utility of such a method for field censuses of cassowaries.

To demonstrate the feasibility of identifying cassowaries using molecular markers we successfully extracted genomic DNA from blood, organ tissue, feathers, and faecal material; developed and optimised primer pairs for amplification of microsatellite loci from the various sample types listed above; tested and optimised primer pairs specifically used in avian sexing; and evaluated primer pairs in terms of reliability of amplification and genetic variability using DNA from various sample types.

The project has met its objectives and we now have

- i) developed workable genetic fingerprinting techniques for application with cassowaries,
- ii) developed DNA extraction methodologies that work with all tissue types available to us but most importantly for our purposes, that effectively extract DNA from faecal samples of varying age and which have been stored in variety of ways, and,
- iii) identified appropriate survey structures and analysis approaches. Taken together these results translate into a viable non-invasive means of conducting censuses of cassowaries, of monitoring population trends, and of documenting population genetic structure and relationships within and between populations. We believe that this achievement represents the beginning of a new phase in the understanding of cassowary populations and ecology that will lead to more effective data-based management of the species.