

# Simplified polymerase chain reaction (PCR)-based sexing assists conservation of an endangered owl, the Norfolk Island Boobook *Ninox novaeseelandiae undulata*

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## Summary

In 1986 a single Norfolk Island Boobook Owl *Ninox novaeseelandiae undulata* remained. As part of a re-establishment programme, two male New Zealand Moreporks *N. n. novaeseelandiae* were introduced, one of which survived to pair with the female in the wild and breed successfully. By 1995 the population numbered 12 or 13 individuals of which seven were second generation (F<sub>2</sub>). However, there were only two breeding pairs. As the 11 hybrids could not be sexed using morphometrics we developed a molecular method based on a recently described avian polymerase chain reaction (PCR)-based sexing technique. The population was found to contain six females and five males. A scarcity of mature males was established as the main factor slowing the recovery effort.

Recent progress in molecular biology has revolutionized several fields, not least conservation biology (e.g. Griffiths and Tiwari 1995, Avise 1994, 1996). Among other advances, relatively simple techniques now allow accurate sexing of individuals, sometimes without the risks associated with handling (Griffiths and Tiwari 1993). Often such knowledge is critical to conservation efforts.

The Norfolk Island Boobook *Ninox novaeseelandiae undulata* is confined to the small, isolated Norfolk Island Group, an Australian territory. It is one of about 26 of the world's owl taxa listed as endangered and currently the only endangered owl taxon in Australia (Clarke *et al.* 1978, Garnett 1992). On plumage characteristics, morphological and biogeographical grounds, the owl is classified as a large, distinctive subspecies of the New Zealand Morepork *N. n. novaeseelandiae* (Olsen 1996). Recent molecular analyses confirm that the New Zealand owl is the closest relative (L. Christidis and J. Norman pers. comm., National Museum, Victoria, Australia). In 1986 only one Norfolk Island Owl, a female, was extant (Olsen *et al.* 1989). As part of a re-establishment programme, two male New Zealand Moreporks were introduced, one of which survived to pair with the female and breed successfully (Olsen 1996). By late 1995, the population numbered 12 or 13 individuals of which seven were second generation (F<sub>2</sub>). However, there were only two breeding pairs.

Norfolk Island owls are significantly larger than New Zealand Moreporks. Because both are sexually dimorphic, with females the larger sex, New Zealand females approach Norfolk Island males in size and weight (Olsen *et al.* 1989, Olsen 1996). This has created difficulties in determining the sex of the hybrid

offspring using morphometrics. Surgical sexing is an unacceptable risk when numbers are so low.

A lack of knowledge of the sex ratio of the population has hampered management of the owls. Assessment of early progress suggested an apparent shortage of mature males to form breeding pairs (Olsen 1996). Without knowledge of the sex of the younger non-breeding individuals, an informed appraisal of a proposal to introduce more males from New Zealand was difficult.

The recovery effort is carried out *in situ* with minimal intervention. However, when nestlings are banded, a small blood sample is taken routinely. DNA extracted from these blood samples was used to sex the 11 hybrid offspring. The sexing method was based on techniques described by Griffiths and Tiwari (1995) and Griffiths *et al.* (1996). Polymerase Chain Reaction (PCR) primers P8 (R. Griffiths and R.J.G. Dawson, unpubl. data) and P2 (Griffiths and Tiwari 1995) amplify two regions, one within the CHD-W gene and another within an homologous gene, CHD-NW (Griffiths *et al.* 1996). In birds, CHD-W is linked to the female-specific W chromosome whereas CHD-NW is situated within either the Z chromosome or an autosome (Griffiths *et al.* 1996). Thus DNA samples from females are expected to produce two PCR products whereas samples from males produce one.

PCR reactions were carried out in an FTS-960 Thermal Sequencer (Corbett Research) following a profile of: one cycle of 3 minutes at 94 °C, 1 minute at 57 °C and 1 minute at 72 °C; followed by 29 cycles of 30 seconds at 94 °C, 30 seconds at 57 °C and 30 seconds at 72 °C. Each 25 µl PCR reaction included 1.25 units *Taq* polymerase (Pharmacia), manufacturer's PCR buffer (final concentration: 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl, pH 9.0; Pharmacia), 0.2 mM of each nucleotide (Promega), 0.2 µM of each primer and ~120 ng genomic DNA. Initially, products were visualized on a 1% agarose gel stained with ethidium bromide.

Fragment-specific restriction using *DdeI* or *HaeIII* (Griffiths and Tiwari 1995, Griffiths *et al.* 1996) was successful in only one (Crimson Rosella *Platyercus elegans*) of the six species we tested. However, in five of the six species (all except the owl) it was possible to resolve the two products amplified from females, without restriction, simply using a 3% agarose gel. Unfortunately, the products amplified from female Boobook Owls proved to be too similar in size to be resolved in agarose and a 6% denaturing polyacrylamide gel was required to provide adequate resolution (Figure 1). To visualize the PCR products in acrylamide, forward primers end-labelled with gamma <sup>32</sup>P-dATP (0.8 µCi/pmol of primer) were included in each PCR reaction mix. The polyacrylamide gel was run for 1.5 hours at 35 Watts, dried and exposed overnight. This gave a distinctive one- or two-band pattern for each of the 11 owls, indicating that the hybrid population was composed of six females and five males (Figure 1). The validity of the technique was confirmed by its correct assignment of sex to all known-sex individuals tested. These included six owls of known sex (included in Figure 1), based on their breeding histories, and several individuals of the five other species (*n* > 10 for both genders of each species; sexually monomorphic species sexed by dissection; representative individuals shown in Figure 1).

Thus the sex ratio of the owl population was almost unity and the supposed

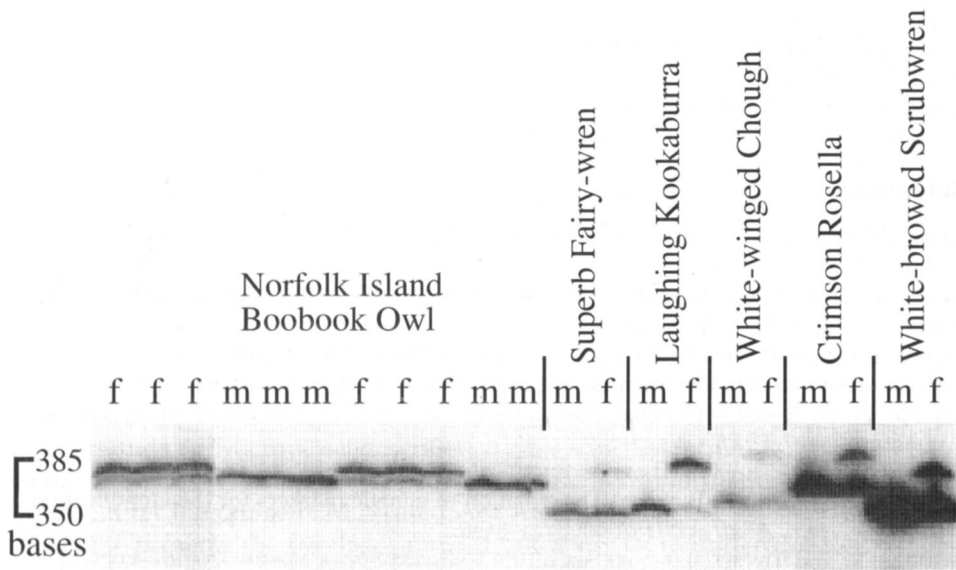


Figure 1. The distinctive one- (m, male) or two- (f, female) band pattern produced by PCR-amplification of DNA samples using the P2 and P8 primers. Sex-specific band patterns produced by the eleven individuals comprising the entire hybrid population of the Norfolk Island Boobook Owl in 1995 are shown. Examples of band patterns produced from known-sex individuals of five other species (Superb Fairy Wren *Malurus cyaneus*, Laughing Kookaburra *Dacelo novaeguineae*, Crimson Rosella *P. elegans*, White-winged Chough *Corcorax melanorhamphos*, White-browed Scrubwren *Sericornis frontalis*) illustrate the universality of the technique. All products were run on a 6% denaturing gel and visualized by end-labelling the P8 primer with 32P. Note: with the exception of the the Norfolk Island owl, PCR-products from all species shown can be resolved more simply, in a 3% agarose gel.

scarcity of mature males was established as the main factor slowing the recovery effort. Limited data suggest that males mature at 3 years. If this is so, there is the potential for another two males to join the existing two mature males in the 1996 breeding population (and two more in 1998). Early results from the 1996 breeding season indicate that at least one of the newly matured males has paired and produced chicks.

This simple PCR-based technique has been applied successfully to six taxonomically distant species and avoids the problem, discussed by Lessells and Mateman (1996), of finding a fragment-specific restriction site. It would have been useful at the commencement of the owl project, when uncertainty over the sex of the last extant individual of this little-known subspecies was a major concern. At this stage of the programme it is providing invaluable information for management.

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