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SHORT NOTE

A DNA test for sex assignment in kiwi (*Apteryx* spp.).

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Recently developed molecular methods for the assignment of sex in avian species have facilitated studies in both ecology and conservation. In particular, recovery and breeding programmes of kiwi (*Apteryx* spp.) would benefit from the accurate identification of male and female birds. We report here the development of a robust DNA sex test capable of sexing all four species of kiwi.

Kiwi have become the focus of an intensive conservation programme in New Zealand. The numbers of kiwi have dropped considerably in the last 100 years, mainly due to predation by stoats, ferrets, dogs, and cats (McLennan *et al.* 1996). An important component of kiwi population management is to develop effective breeding and rearing strategies. A major problem with this work is the inability to differentiate between young males and females, as young kiwis (<1 yr) are morphologically and behaviourally very similar (Heather & Robertson 1996). After the first year of growth, sexes can be distinguished by differences in weight, call type, or the growth rate and length of bill (Colbourne & Kleinpaste 1983, 1984).

Avian sex chromosomes exist as ZZ (male) and ZW (female) genotypes. Various genetic sexing tests have now become available that rely on the amplification of W chromosome-linked DNA fragments (Fridolfsson & Ellegren 1999; Itoh *et al.* 2001). These tests require very little DNA and allow birds to be sexed quickly and at a very early age (Trefil *et al.* 1999). Bello & Sanchez (1999) used

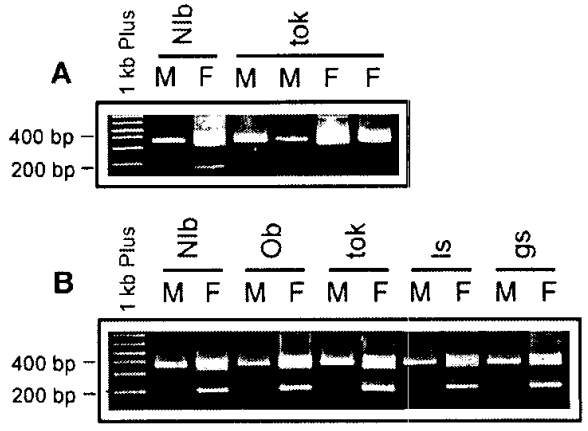

Fig. 1 Genetic sexing of kiwi feather DNA. **A.** DNA extracted from the feather pulp of male and female North Island brown (NIb) and tokoeka kiwi (tok) was subjected to PCR using the primers w1 and k7 (Huynen *et al.* 2001). A ~200bp DNA fragment amplified from female NIb kiwi DNA is not amplified from DNA extracted from female tok kiwi. **B.** Amplification of kiwi DNA using primers w5 and w7. DNA fragments of approximately 350 bp in length are present in all kiwi while only female kiwi have a ~200 bp fragment. Ob, ls, and gs refer to Okarito brown, little spotted, and great spotted kiwi respectively. 1 kb Plus DNA Ladder™ (Gibco BRL) was used to indicate DNA fragment sizes.

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Random Amplification of Polymorphic DNA (RAPD) analysis to isolate a sex-specific sequence from ostrich. Similarly, specific primers have also been developed for sexing emus (J. Halverson AgGen pers. comm.).

We have isolated a sex-specific DNA fragment from kiwis by RAPD analysis and have shown that this fragment is sex-specific across all species of ratite (Huynen *et al.* 2002). The sequence of several ratite sex-specific fragments have now been used to design highly efficient DNA amplification primers that enable us to sex kiwi using the genetic material from a single feather. However, we have recently found that these primers do not give a clear result for tokoeka kiwi (*Apteryx australis*) from Haast. To overcome this problem we have designed a further set of primers capable of distinguishing males from females for all kiwi species.

Feathers were received of brown kiwi (*Apteryx mantelli*) from the North Island (n=17) and Okarito (n=2), of tokoeka kiwi from Haast (n=5), of little spotted kiwi (*Apteryx owenii*) from Kapiti Island (n=9), and of great spotted kiwi (*Apteryx haastii*) from Canterbury (n=2). All feathers were supplied from known sex kiwi (sexed according to morphology and behaviour) without accompanying field notes that might have affected the interpretation of the DNA sexing results. DNA was isolated from the pulp of 1-4 feather(s) by incubation at 55°C overnight with 200 µl of SET buffer (100 mM NaCl, 10 mM EDTA, 50 mM Tris-Cl pH 8.0), 100 µg Proteinase K, 0.5% SDS, and 20 mM dithiothreitol (Kiatipattanasakul-Banlunara *et al.* 2002). The mixture was then extracted with an equal volume of phenol:chloroform and the DNA precipitated with 0.5 volumes of 7.5 M ammonium acetate and 3 volumes of ethanol. The DNA was resuspended in 30 µl of water and stored at 4°C. One µl of DNA was then added to a 10 µl mixture containing 10 mM Tris pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 100 µM of each dNTP, 1 µg/µl BSA, 20 ng of each primer; w5 (5′-AATCACCCTTTAA ACAAGCTGTTAAAGCAA-3′) and w7 (5′-CCTTTCTCAAATCTCTCTTTTGTTCTAGA-CAC-3′), and 0.3 U of AmpliTaq® (Perkin Elmer). The mixture was overlaid with mineral oil and subjected to the polymerase chain reaction (PCR) using a Hybaid OmniGene thermocycler with the following cycling profile; 94°C for 30 s and then 10 cycles of 94°C for 10 s, 55°C for 10 s, and 72°C for 20 s, followed by 30 cycles of 94°C for 10 s, 50°C for 10 s, and 72°C for 20 s. PCR products were separated by electrophoresis in 1.2%MS, 1%LE agarose (Boehringer Mannheim) in TBE buffer, stained with ethidium bromide and visualised over UV light (Sambrook *et al.* 1989).

We usually obtained about 10 ng of DNA per feather pulp and approximately 1 ng was used in an amplification reaction (Fig. 1). We have been able to extract DNA suitable for PCR from feathers that had been stored at room temperature for more than 6 years.

Initial PCR analysis, using primers w1 and k7 (Huynen *et al.* 2002) indicated that although North Island brown kiwi (NIb) could be reliably sexed, the same was not true for tokoeka kiwi from Haast. Using these primers, female NIb kiwi can be distinguished from males by the presence of several amplified products of ~350 bp as well as a single product of ~200 bp (Fig. 1a). Amplification of DNA from kiwi males results in a single ~350 bp product only. However, for tokoeka kiwi, no amplification of the ~200 bp female specific fragment could be detected, perhaps as a result of sequence differences at the primer binding site (Fig. 1a). For this reason new primers were designed (w5 and w7) that allow clear discrimination between males and females of all kiwi species. These primers differ from those used previously in that they bind to a different site of the W specific locus and are capable of amplifying the ~200 bp fragment from females of all species of kiwi including those of tokoeka kiwi (Fig. 1b).

As avian sex determination relies on a ZW (female) ZZ (male) configuration, the smaller fragment is likely to be W-chromosome linked. The larger (~350 bp) PCR product from both males and females may be Z-linked or autosomal (Huynen *et al.* 2002). The amplification of additional PCR products of about 350 bp in length in the female, suggests that this locus may be present in the female kiwi genome as multiple copies.

To date we have sexed 39 of 40 kiwi accurately The single mis-sexed bird (tested as male but known to be female) may be due to PCR contamination, or the preferential amplification of non-W linked loci at low DNA concentrations (Cagneux *et al.* 1997). Alternatively, the mis-sexed kiwi may have resulted from misidentification of the feather in the field (these nocturnal birds are often caught at night and feathers can adhere to the nets used to catch them or to clothing).

In summary, our new genetic test for sexing kiwi is quick, efficient, and requires the genetic material from only a single feather. Early identification of kiwi sex may aid in the study of sex-related behaviour, dispersal, and survival in kiwi. Results from our work has recently been used to assist in the establishment of an effective captive holding and breeding programme for little spotted kiwi.

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SHORT NOTE

An old record of banded dotterel *Charadrius bicinctus* from Vanuatu

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The nominate subspecies of the banded dotterel *Charadrius bicinctus* is a partial migrant. Many birds migrate from their breeding grounds in New Zealand to Australia in February-April and usually return by August-September (Lane & Davies 1987; Pierce 1999). Small numbers are recorded most years on Norfolk and Lord Howe Islands as either passage migrants or overwintering birds (e.g. Hermes *et al.* 1986; Moore 1999; Hutton 1991). Records of this species from islands north and north-east of New Zealand are more irregular. Small numbers have been seen at the Kermadec Islands, Fiji and New Caledonia (Heather and Robertson 1996; Smart 1973; Skinner 1983; Garrett & Garrett 1975; Barré & Dutson 2000). However its status in Vanuatu is less certain.

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The banded dotterel was first reported for Vanuatu (then New Hebrides) by Oliver (1951), who noted there was a single record, but gave no details. The first checklist of New Zealand birds (Fleming 1953) referred to "Rothschild Collection, American Museum of Natural History" (=AMNH), presumably indicating a specimen. This has generally been overlooked; for example, Bregulla (1992) wrote that undated reports of the species from Vanuatu were noted in a wader field guide (Marchant *et al.* 1986). This was evidently insufficient evidence for Bregulla to include the banded dotterel as part of the Vanuatu avifauna, as this species did not have its own account in his book. Marchant & Higgins (1993) also considered that reports of the banded dotterel from Vanuatu were 'unconfirmed'. Several other accounts have referred to Vanuatu as being within the species' range but