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SHORT NOTE

DNA sexing of weka (*Gallirallus australis*)

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Sexing morphometric birds is problematic, yet it is necessary for the conservation management of many such species, including the endangered weka (*Gallirallus australis*), a large flightless rail endemic to New Zealand. Weka are visually monomorphic and until now have only been sexed using behavioural and morphometric techniques (Beauchamp *et al.* 1999). Behavioural sexing is complicated as both male and female weka share the responsibility of nest building, egg incubation, and chick rearing. Likewise polygamy has been reported in weka (Guthrie-Smith 1914; Beauchamp 1986) as have same sex pair bonding in captivity (D. Emmerson, pers. comm.) and also in wild populations (A.J. Beauchamp, pers. comm.).

The accuracy of morphometric sexing of weka is compromised by variation in size and weight between island and mainland weka populations, between territorial and non-territorial weka, between seasons, and between subspecies. Although a useful sexing technique when sexual variation is sufficient to produce 2 morphological classes, weka do not have distinct sexual morphological classes. They cannot be sexed reliably by any single morphological variable and a combination of variables needs to be employed (Beauchamp *et al.* 1999). Even then, population-specific discriminant functions are necessary; using a discriminant function developed from another population, even within the same taxon, may still result in incorrect sexing (Beauchamp *et al.* 1999). Generally, discriminant functions are more reliable when sample sizes are large and measurements are carefully and accurately taken, but accurate measuring can increase handling of the birds and impart additional stress (Maho *et al*. 1992).

We investigated molecular techniques for sexing of weka. Molecular methods for sexing birds have been developed recently and have facilitated studies in both ecology and conservation (Millar *et al.* 1992; Griffiths *et al.* 1996; Griffiths *et al.* 1998; Fridolfsson & Ellegren 1999; Huynen *et al.* 2002; Huynen *et al*. 2003). Female birds are heterogametic (ZW) and males are homogametic (ZZ), therefore sexing can be made by the detection of the W-chromosome or W-chromosome sequences in a sample of unknown sex (Ansari *et al.* 1988). Various genetic sexing tests have now become available that rely on the amplification of W-chromosome-linked DNA fragments (Griffiths *et al.* 1996; Griffiths *et al.* 1998; Fridolfsson & Ellegren 1999; Itoh *et al*. 2001).

Molecular sexing methods are advantageous in that the tests require very little DNA and allow birds to be sexed quickly and at a very early age (Trefil *et al.* 1999). There is a negligible failure rate regardless of the condition or age of the bird and molecular sexing is applicable to a wide range of tissue and preservation types. Thus, blood can be stored in a freezer or in ethanol and used for sexing at a later date. However, molecular sexing can be expensive and time consuming and the tissue sampling is an intrusive process causing a moderate to high amount of stress to the birds (Maho *et al*. 1992).

The aim of our study was to trial the use of CHD/ restriction enzymes sexing methods in order to obtain a 100% accurate method of sexing weka. Blood samples were obtained from 30 *Gallirallus australis greyi* held temporarily in captivity at Rainbow Springs, Rotorua, from Mokoia Island, in Aug 1997. The blood was extracted from the brachial vein near the ventral surface of the elbow joint.

DNA was isolated by SDS/proteinase K digestion and phenol-chloroform extraction (Sambrook *et al.* 1989). Amplification of the DNA using PCR was carried out in 25µl volumes consisting of 50-100 ng of whole genomic DNA, PCR buffer II (PerkinElmer: 1 × 10 mM Tris-HCl, pH 8.3, 50 mM KCl), 1 .5 mM MgCl2 (25 mM), 0. 125 µl of dNTPs (20 mM of each deoxynucleotide), P2 (5′ -TCT GCA TCG CTA AAT CCT TT - 3′) and P3 (5′ - AGA TAT TCC GGA TCT GAT AGT GA - 3′) primers (Griffith *et al.* 1996) and 0.5 units of Taq DNA polymerase (*Amplitaq*: Perkin Elmer). The thermal profile was 95°C for 2 min followed by

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35 cycles of 94°C for 45 s, 50°C for 1 min and 72°C for 1 min, with a final cycle of 72°C for 10 min. Eight microlitres of each PCR reaction was size fractionated on 2% *NuSieve*/1% agarose gels in Tris acetic acid pH 8.0 buffer containing ethidium bromide (0.5 μg ml^{-1}) and run at 10 V cm^{-1} for 30 min, and visualised under ultraviolet light (312 nm). Negative template controls were run with all experiments. Eight microlitres of the remaining reaction was digested with *Hae* III (5 units: Gibco Life Technologies) in 1 × Gibco Life Technologies enzyme buffer 2. BSA (100 ng ml^{-1}) and spermidine (4 mM final concentration) to a total volume of 10 μl. Samples were incubated at 37°C for 2 h. The digested samples were then size fractionated and visualised again under UV light (312 nm). Male weka had 2 bands at 45 and 65 base pairs and females have 1 band of 110 base pairs.

A pilot study of the CHD primers was initially performed on six weka of known sex (3♂♂, 3♀♀); all 6 were sexed correctly. The remaining 24 samples were sexed using the CHD/restriction enzyme method and compared to the sex designation they had been assigned. In total, 27 of the 30 weka had a concordant sex assignment result. Three individuals were involved in discrepancies and were analysed twice to remove the possibility of laboratory error. They involved 2 males being mistaken for a female and 1 female being mistaken for a male. It is possible that additional food through captive feeding, inaccurate morphometric sampling measurements, and age class designations could explain these incorrect morphometric sex designations.

Molecular sexing overcomes these issues, and in theory is able to give a 100% accurate sexing designation. Although molecular sexing is often invasive in that it requires tissue sampling, it does have the advantage of being able to sex weka of all ages, weights, and subspecies. Fortunately, sexing using CHD has recently been shown to work using non-invasive tissue sampling e.g., faeces (Robertson *et al*. 1999), urine (Nota & Takenata 1999), and feathers (Grant 2001; Huynen *et al*. 2003).

Being able to give correct sex assignment is extremely important in the management of this threatened species. Weka form strong pair bonds from a very early age and it is important to ensure that mistakes such as same sex pair bonding are not made when attempting to breed them captivity. Captive-bred weka, and weka from viable populations, are used to supplement existing wild populations. It is also important that biased sex ratios are not developed in the populations. The avoidance of a male-biased sex ratio is particularly important when establishing new wild populations, especially using small numbers of weka.

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LITERATURE CITED

Ansari, H.; Kagagi, N.; Sasaki, M. 1988. Morphological differentiation of sex chromosomes of sex chromosomes in three species of ratite birds. *Cytogenetics and cell genetics 47*: 185-188.

Beauchamp, A. J. 1986. A case of co-operative rearing in wekas. *Notornis 33*: 51-52.

Beauchamp, A. J.: Butler, D.J.; King, D. 1999. Weka, (*Gallirallus australis*) recovery plan 1999-2009. *Threatened species recovery plan 29*. Wellington, New Zealand Department of Conservation.

Fridolfsson, A. K.; Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *Journal of avian biology 30*: 1 16-21.

Fridolfsson, A. K.; Cheng, H.; Copeland, N.G.; Jenkins, N.A.; Raudsepp, N.C.; Woodage, T.; Chowdhary, B.; Halverston, J.; Ellegren, H. 1998. Evolution of the avian sex chromosomes from an ancestral pair of autosomes. *Proceedings of the National Academy of Sciences, USA 95*: 8147-8151.

Grant, A. 2001. DNA sexing of the brown kiwi (*Apteryx mantelli*) from feather samples. *DoC Science internal series 13*. Wellington New Zealand Department of Conservation,.

Griffiths, R., Double, M.C.; On, K.; Dawson, R.J.G. 1998. A DNA test to ask most birds. *Molecular ecology 7*: 107 1-1075.

Griffiths, R., Dann, S.; Dijkstra, C. 1996. Sex identification in birds using two CHD genes. *Proceedings of the Royal Society, London B. Biological Sciences 263*: 1251-1256.

Guthrie-Smith, H. 1914. Muttonbirds and other birds. Whitcombe and Tombs Ltd. Christchurch.

Huynen, L.; Miller, C. D.; Lambert, D. M. 2002. A DNA test to sex ratite birds. *Molecular ecology 11*: 851-856.

Huynen, L.; Lambert, D. M.; McLennan, J. A.; Rickard, C.; Robertson, H. A. 2003. A DNA test for sex assignment in kiwi (*Apteryx* spp.). *Notornis 50*: 231-233.

Itoh, Y.; Suzuki, M.; Ogawa, A.; Munechika, I.; Murata, K.; Mizuno, S. 2001. Identification of the sex of a wide range of Carinatae birds by PCR using primer sets selected from chicken EE0.6 and its related sequences. *Journal of heredity 92*: 315-321.

Maho, Y. L.: Karmann, H.; Bliot, D.; Handrian, Y.; Robin, J.P.; Mioskowski, E.; Cherel, Y.; Farni, J. 1992. Stress in birds due to routine handling and a technique to avoid it. *American journal of physiology R*: 775-781.

Millar, C. D.; Lambert, D.M.; Bellamy, A.R.; Stapleton, P.M.; Young, E.C. 1992. Sex specific restriction fragments and sex ratios revealed by DNA fingerprinting in the brown skuas. *Journal of heredity 83*: 350-355.

Nota, Y.; Takenata, O. 1999. DNA extraction from urine and sex identification of birds. *Molecular ecology 8*: 1235-1238.

Robertson, B. C.; Minot, E.O.; Lambert, D.M. 1999. Molecular sexing of individual kakapo, *Strigops habroptilus*, from faeces. *Molecular eEcology 8*: 1347-1350.

Sambrook, J.; Fritsch, E. F.; Maniatis, T. 1989. *Molecular cloning; a laboratory manual. 2nd ed.* New York, Cold Spring. Harbour Laboratory Press.

Trefi, P.; Bruno, M.M.; Mikus, T.; Thoravel, P. 1999. Sexing of chicken feather follicle, blastodermal and blood cells. *Folia Biol* (*Praha*) *45*: 253-256.

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