Appendices.

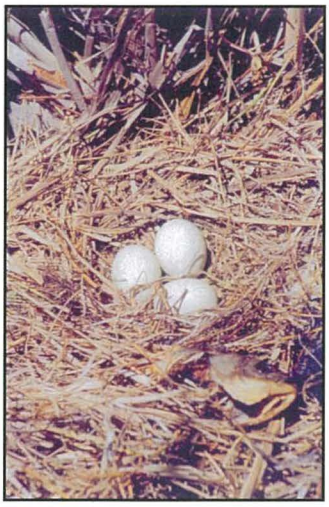
Appendix 1.1 Department of Conservation National Banding Scheme band recoveries collected over 15-years (1984-1999) for the Australasian Harrier.

	Juvenile Male	Juvenile Female	Adult Male	Adult Female	Total
< 50 km	3	10	7	2	*22*
50-100 km	2	1	2	1	*6*
100-200 km	0	2	0	3	*5*
200-400 km	0	1	0	2	*3*
400-800 km	1	0	1	1	*3*
800 + km	0	0	1	0	*1*
Total	*6*	*14*	*11*	*9*	***40***

Appendix 1.2 Nest and chick growth of a breeding pair of Australasian Harriers over eight weeks at Pukepuke Lagoon (December - January 2001). *(A)* Nest site of TX 10 (female) & TX 16 (male) in flax and raupo swamp at Pukepuke Lagoon. *(B)* Clutch, nest material and prey remains (Carp, *Carassus auratus*) eaten by female while incubating. *(C)* 2 week-old chick. *(D)* 3 week-old chick. *(C)* 4 week-old chick. *(D)* 5 week-old chick. *(E)* 6 week-old chick.

(A)

(B)



(C)

(D)

(E)

(F)

(G)

Appendix 2.1 Manuscript submitted to *Pacific Conservation Biology.*

RESEARCH NOTE

A DNA test for sex assignment in Australasian Harrier

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Abstract: Recently developed molecular methods for the assignment of sex in avian species have facilitated more accurate studies in both ecology and conservation. In particular, gender spacing and dispersal studies of Australasian Harrier (*Circus approximans*) would benefit from the accurate identification of male and female birds. We report here the application of a sex test capable of sexing harriers using DNA extracted from feather bases. The test relies on the genetic amplification and detection of W chromosome nucleotide sequences.

Keywords: Avian sexing, W chromosome, Australasian Harrier, *Circus approximans*

Introduction

Adult raptors show considerable sexual dimorphism, more correctly termed reversed sexual dimorphism (RSD) because, in contrast to most birds, the female is larger than the male (Olsen 1990; Dijkstra *et al.* 1998). However, as juveniles and young adults, male and female Australasian Harriers *(Circus approximans)* are difficult to distinguish as they share similar size, plumage coloration, and behavioural traits (Baker-Gabb 1978). Consequently, sexing methods based on morphometrics often produce gender misclassifications.

In recent years molecular techniques have been used to successfully sex a wide range of avian species (Griffiths *et al.* 1996; Bradbury *et al.* 1997; Lessells *et al.* 1998; Fridolfsson and Ellegren, 1999; Nesje and Roed, 2000) including the genus *Circus* (Griffiths *et al.* 1998), although a technique has never been developed for the Australasian Harrier. Molecular sexing is used here as a method to accurately assign gender to radio-tagged birds in a wider study of harrier ecology with specific emphasis on sex-related spacing and habitat use (Wong 2002).

Avian sex chromosomes consist of a ZZ (male) or ZW (female) genotype. Various genetic sexing tests have now become available that rely on the amplification of different-sized DNA fragments from the Z and W chromosome. These tests require very little DNA and allow birds to be sexed quickly and at a very early age (Griffiths *et al.* 1998; Trefil *et al.* 1999). We have previously isolated a sex-specific marker for Kiwi (Huynen *et al.* 2002) and show here that DNA primers designed to amplify this marker can also be used to sex Australasian Harriers.

Methods

DNA was isolated from 1-4 Australasian Harrier (*Circus approximans*) feather bases by Proteinase K/SDS digestion and phenol/chloroform extraction (Sambrook *et al.* 1989). The DNA was resuspended in 50 μl of water and stored at 4°C. 1 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; k7 (5'-

TCTCTTTTGTTCTAGACACCCT-3') and w1 (5'-CCTTTAAACAAGCTGTTAAAGCA-3'), and 0.2 U of AmpliTaq® (Perkin Elmer). The mixture was overlaid with mineral oil and subjected to the polymerase chain reaction (PCR) using the following cycles; 94°C for 120 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).

Results and Discussion

DNA was extracted from feather bases and subjected to PCR using the primers k7 and w1. We usually obtained about 2 ng of DNA per feather base and approximately 4 ng was used in an amplification reaction (Fig 1A).

PCR analysis of DNA from feathers indicated the presence of two DNA fragments in females but only one in males (Fig 1A). As avian sex determination relies on a ZW (female) or ZZ (male) configuration, the larger fragment is most likely W-chromosome linked. Interestingly, more amplified product appears to be present in the harrier female, suggesting that this marker may be present in the female genome as multiple copies.

In summary, our new genetic test for sexing harriers is quick, efficient, and requires the genetic material from only a few feathers. Early identification of harrier sex may aid in the study of sex-related behaviour, dispersal, and survival in these birds.

Acknowledgements

This research was made possible by a grant (MAU702) from the Marsden Fund "Sexing the Lost Giants of New Zealand" and was also supported by the Ecology Group, Institute of Natural Resources Massey University, Ornithological Society of New Zealand, and Raptor Association of New Zealand.

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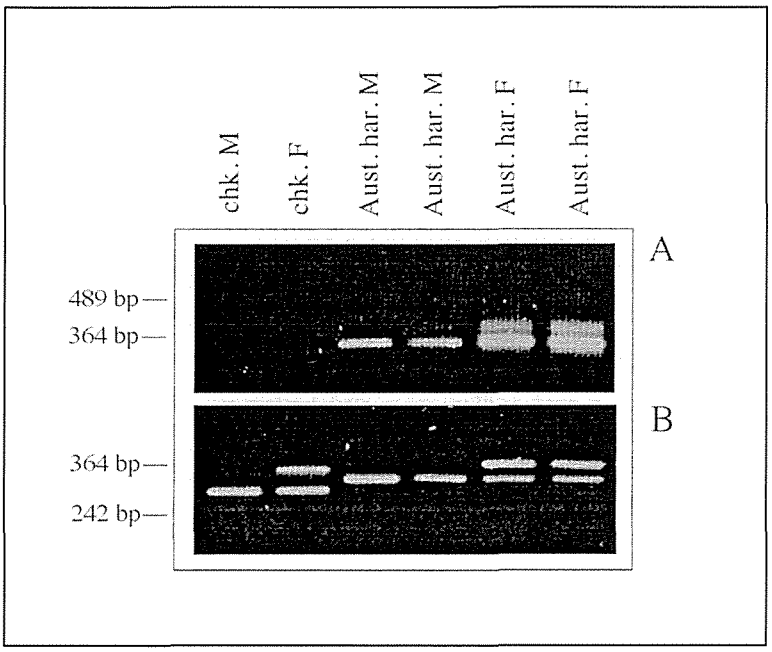
Figure legend

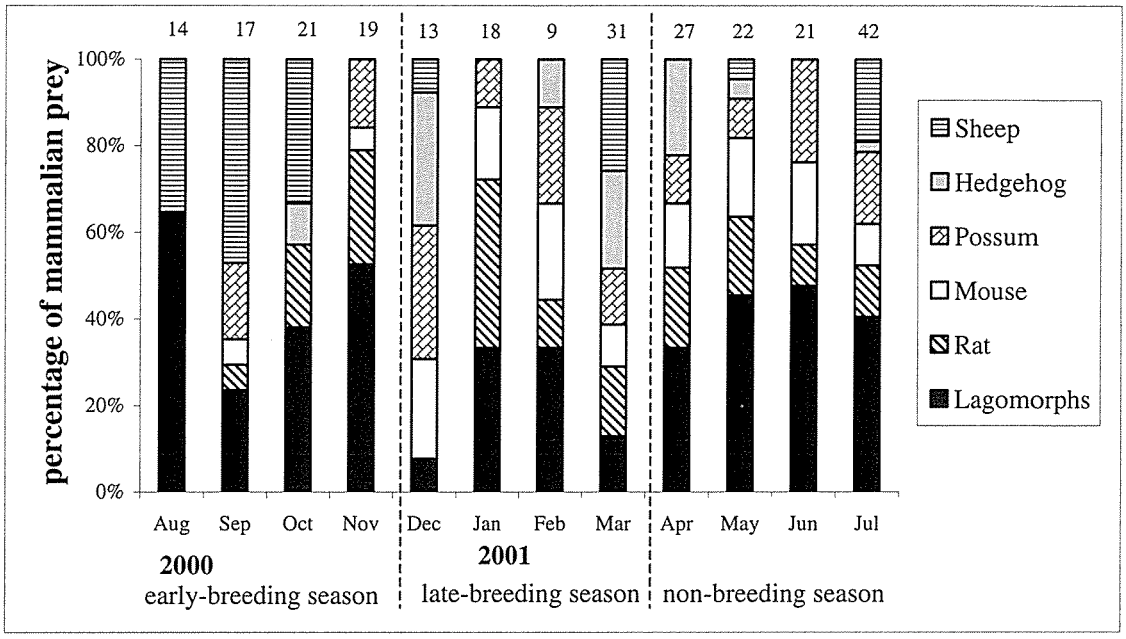
Figure 1. Genetic sexing of Australasian Harrier feather DNA. (**A**) DNA extracted from feather bases was subjected to PCR using the primers k7 and w1. DNA fragments of approximately 360 bp in length are present in all Harriers while female Harriers also have ~420 bp fragment. (**B**) Sexes were verified by using the avian sexing primers p2 and p8 (Griffiths *et al.* 1998) and then digestion of a Z-linked *Hae*III. site. Chk., Aust. har., M, and F refer to Chicken, Australasian Harrier, male, and female respectively. DNA markers are provided in base pairs (bp) by pBluescriptSK (Statagene) digested separately with *Rsa*I and *Msp*I.



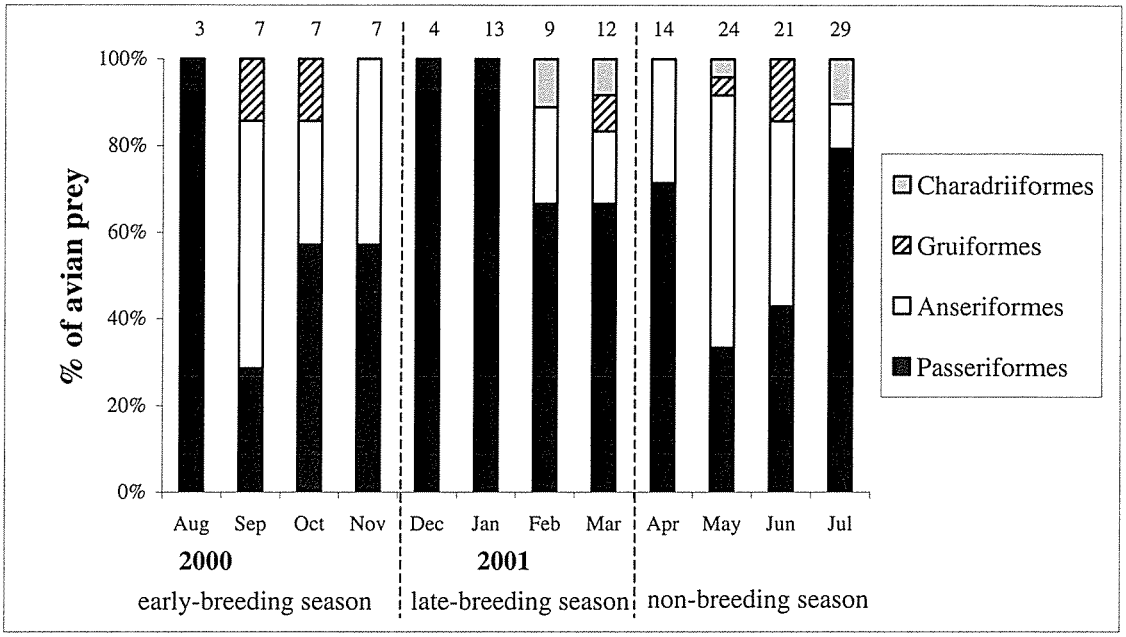
Appendix 3.1 Frequency and percentage of occurrences of prey items found in 312 pellets collected from a communal roost at Pukepuke Lagoon between August 2000 and July 2001.

	Frequency	% of Prey Group	% Total Prey
Mammalia			
Lagomorphs *(Lepus europaeus, Oryctolagus cuniculus)*	91	35.8	21.8
Rats *(Rattus norvegicus, Rattus rattus)*	39	15.4	9.2
Mouse *(Mus domesticus)*	29	11.4	6.7
Possum *(Trichosurus vulpecula)*	35	13.8	8.2
Hedgehog *(Erinaceus europaeus)*	22	8.7	5.1
Sheep *(Ovis sp.)*	38	15.0	9.0
Aves			
Passeriformes *(Acanthisittidae, Accentor, Alaudidae , Cracticidae, Emberizidae, Fringillidae, Hirundinidae, Muscicapidae, Ploceidae, Prunellidae, Sturnidae, Zosteropidae)*	94	62.6	22.5
Anseriformes *(Anatidae)*	43	28.7	10.1
Charadriiformes + Gruiformes *(Rallidae, Laridae, Recurvirostridae, Charadriidae)*	13	8.7	2.9
Other			
Insecta			
Coleoptera *(Carabidae, Scarabaeidae)*	2	8.3	0.2
Orthoptera *(Gryllidae)*	4	16.7	0.7
Unidentifiable insect	5	20.8	1.0
Osteichthyes *(Cyprinidae, Anguillidae, unidentifiable fish)*	4	16.7	0.7
Egg Shells	9	37.5	1.9
Total	415		

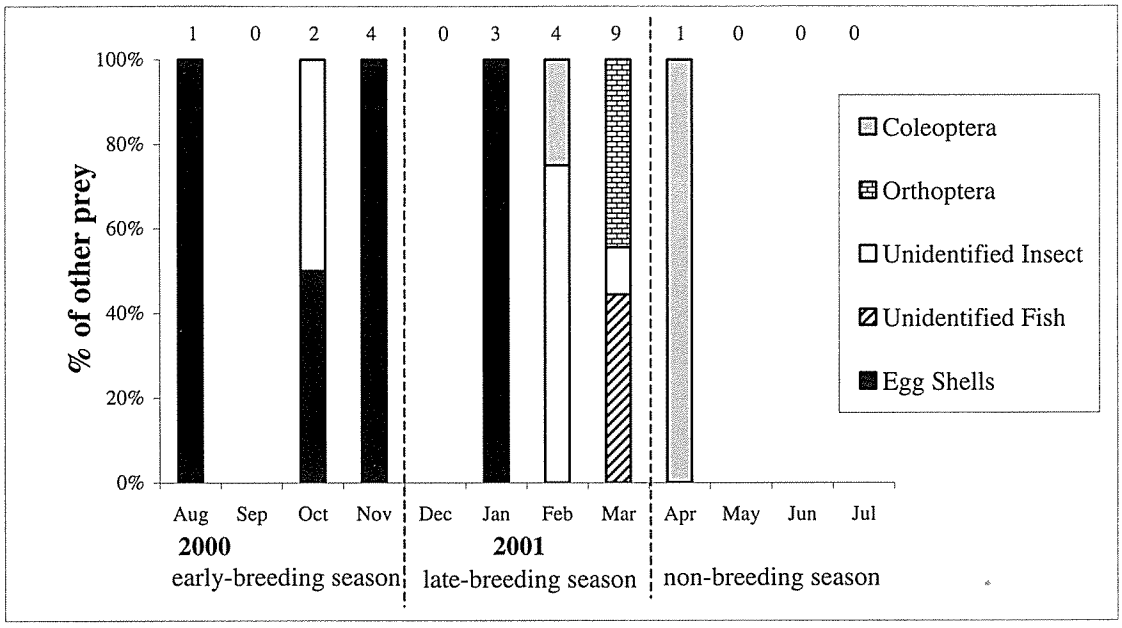
Appendix 3.2 Percentage of occurrences of mammals in the diet of the Australasian Harrier collected over 12 months from a communal roost at Pukepuke Lagoon. Months are separated (vertical dashed lines) into three biological seasons, early-breeding season, late-breeding season and non-breeding season. Pellets were collected from August 2000 to July 2001. Columns contain the proportion of prey remains from pellets collected during each month. The number of pellets with mammals present collected for each month is shown at the top of their respective column.



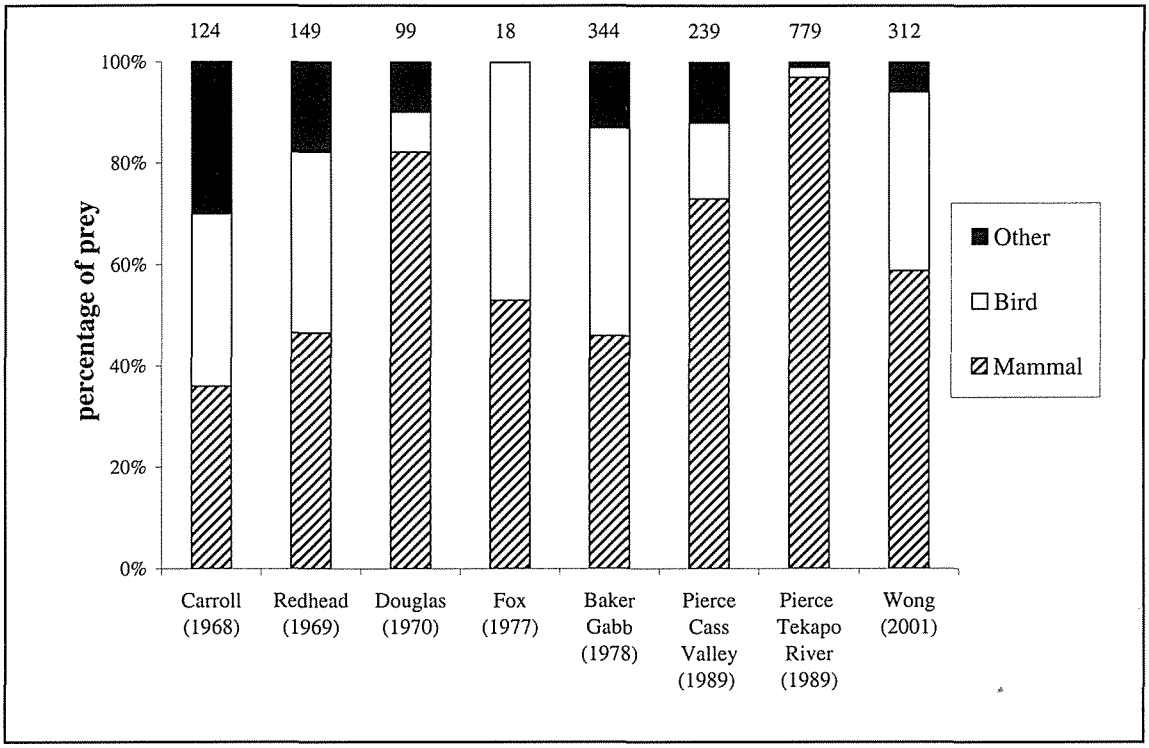
Appendix 3.3 Percentage of occurrences of birds in the diet of the Australasian Harrier collected over 12 months from a communal roost at Pukepuke Lagoon. Months are separated (vertical dashed lines) into three biological seasons, early-breeding season, late-breeding season and non-breeding season. Pellets were collected from August 2000 to July 2001. Columns contain the proportion of prey remains from pellets collected during each month. The number of pellets with birds present collected for each month is shown at the top of their respective column.



Appendix 3.4 Percentage of occurrences of other prey (not mammalian or avian) in the diet of the Australasian Harrier collected over 12 months from a communal roost at Pukepuke Lagoon. Months are separated (vertical dashed lines) into three biological seasons, early-breeding season, late-breeding season and non-breeding season. Pellets were collected from August 2000 to July 2001. Columns contain the proportion of prey remains from pellets collected during each month. The number of pellets with 'other prey' present collected for each month is shown at the top of their respective column.



Appendix 3.5 Comparison of six diet studies of the Australasian Harrier from different locations in New Zealand. Percentage of mammals, birds and other prey are represented for each respective study. The number of samples collected in each study is shown at the top of their respective columns.



Appendix 4.1 Activity, habitat and weather information codes used at each fix for the eight Australasian Harriers radio-tagged at Pukepuke Lagoon

ACTIVITY CODE
1 = perched/grounded
2 = flying
3 = feeding
4 = courtship
5 = incubating
6 = territorial defence

HABITAT CODE
1 = Pasture
2 = swamp
3 = sand dunes
4 = forest
5 = open water
6 = long grass

WEATHER CODE
1 = sunny no wind
2 = sunny light wind
3 = sunny med wind
4 = sunny strong wind

5 = overcast no wind
6 = overcast light wind
7 = overcast med wind
8 = overcast strong wind

9 = light rain no wind
10 = light rain light wind
11 = light rain med wind
12 = light rain strong wind

13 = med rain no wind
14 = med rain light wind
15 = med rain med wind
16 = med rain strong wind

17 = strong rain no wind
18 = strong rain light wind
19 = strong rain med wind
20 = strong rain strong wind

Appendix 4.2 Utilisation plots of combined seasonal range for eight radio-tagged Harriers at Pukepuke Lagoon showing percentage of fixes defining a core range of total range area. Marked decreases in the slope of the distribution curve indicates core range. Small ranges show no obvious slope discontinuity in the utilisation plot thus have similar home and core range size. Utilisation plots were also produced for all birds present in the breeding and non-breeding season.