CHROMOSOME ANALYSIS AND SEX IDENTIFICATION OF YELLOWEYED PENGUINS *MEGADYPTES ANTIPODES*

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Yelloweyed Penguins *Megadyptes antipodes* are not obviously sexually dimorphic, making it impossible to sex birds visually with any degree of accuracy. In general males are larger and heavier than females, however, there exists a considerable overlap in body masses throughout the year (Richdale 1951). Even premoult masses do not exhibit reliable sexual differences. Richdale (1951) described 11 criteria for sexing Yelloweyed Penguins: two depend on general body size, three on body mass, and six on behaviour. All these criteria rely on frequent and extended observations, and familiarity with marked birds. The efficacy of the more intrusive methods of anoscopy and laporoscopy may be dependent upon the state of the cloacal papillae and seasonal gonadal regresion.

We measured total skull length, from the back of the foramen magnum to the tip of the bill, for a sample of 15 banded Yelloweyed Penguin pairs. The sex of birds was determined by observing behaviour during the pre-egg and incubation phases (Seddon & Darby 1990). There was a significant difference in total skull length between males and females (paired t-test, t = 11.9, df = 14, $P<0.001$). Males had skull lengths of not less than 141 mm (mean skull length = 145 mm, SD = 2.6, range 141-149), whereas female skull lengths were less than or equal to 140 mm (mean skull length = 137, SD = 1.7, range 134-140). Whereas these differences are small, they are sufficient to distinguish the sex of known pairs. As a field identification for lone birds, those individuals with skull lengths greater than or equal to 145 mm may be assumed to be males, whereas those with skull lengths less than 137 mm may be asumed to be females. Because overlap in skull length may exist between males and females, birds with skull lengths between 137 and 145 mm should be sexed in conjunction with other methods. The most reliable field measurement for sexing lone Yelloweyed Penguins is a combination of skull length and foot length (from the back of the heel to the tip of the pad of the mid toe) (Darby & Seddon 1990). Even with this combined measure, however, there is a 13.6% overlap of the distributions of the two sexes as determined by dissection (Darby & Seddon 1990). What is clearly required is a single, non-lethal technique of sex identification, that does not rely on observation at a particular time of year. We report here on a procedure to sex Yelloweyed Penguins by chromosome analysis.

Heteromorphic sex chromosomes have been found in all birds to date, with the possible exception of ratites. In all cases the male is the homogametic sex, designated ZZ, and the female the heterogametic ZW (Shields 1987). The Z chromosome is generally the fourth or fifth longest in the entire chromosome set, with the centromere

usually centrally placed (Ohno 1970). The W chromosome is usually much smaller. The great difference in morphology, in particular the length difference between the Z and W chromosomes makes diagnosis of sex both accurate and straightforward (Parker *et al.* 1991).

It is possible to sex birds from gross karyotypic data obtained from short term blood cultures (Hungerford *et al.* 1966). In this technique, the sex chromosomes are identified by constructing a karyotype, or karyogram, in which the chromosomes are arranged in pairs of presumed homologues according to the visual similarity of their external morphology, including overall size, centromere position, and pattern of banding. The chromosomes are arranged in pairs and where one pair contains elements that are not identical, or exhibits heteromorphism in size or centromere position, these are the sex chromosomes of the female (Shields 1987).

One-ml samples of blood were collected into pre-heparinized syringes from the brachial arteries of banded Yelloweyed Penguins. Samples were transferred to heparinized containers and kept at body temperature while in the field. Whole blood was inoculated into vials containing Hams F10 + P.H.A. culture medium. Vials were incubated at 37°C for 72 h. Cultures were then treated with 0.075 m KCL solution, fixed in three changes of methanol/acetic acid (3:1), spread on to slides and air dried. Slides were incubated at 60°C overnight, then treated with dilute trypsin solution to induce G-bands, and finally stained with Leishmans. Slides were scanned at 100x magnification, and metaphases analysed at 1000x magnification. Photographs (35 mm) were taken of suitable metaphases and karyotypes were produced.

Cultures were inevitably grossly contaminated. To counter contamination Penicillin and Streptomycin, Gentamycin and Fungazone were added to the culture medium. Contamination persisted, so incubation times were reduced to 48 h. It is not known whether the contaminants were present in the blood of the penguin, or on the surface of the skin. Contamination persisted even when the skin surface was swabbed with alcohol before the blood sample was drawn.

The metaphases obtained exhibited two primary classes of chromosome (Fig. 1a), as found in nearly all avian species to date (Shields 1982). These consisted of a relatively low number of large chromosomes (macrochromosomes), and numerous very small chromosomes (microchromosomes), difficult to study with conventional microscopy. The sex chromosomes are macrochromosomes. The karyotype for female 6074 and her mate male 5706 are presented (Fig. 1b). The eight pairs of macrochromosomes are shown.

Sixteen blood samples were collected, of which seven were cultured successfully. Three samples did not have clear metaphase cells leaving only four samples with analysable metaphases. From these the sexes of four birds were identified, two males and two females, with an additional three birds being sexed by the identification of breeding pairs over two breeding seasons.

Chromosome analysis may be performed using blood feather pulp obtained from a single growing feather. Feather pulp preparations may yield better quality chromosome preparations than those obtained from peripheral blood samples, and may also be preferable where it is important to minimize the disturbance to individuals or breeding colonies (Parker *et al.* 1991).

Although time-consuming, chromosomal analysis will be of most value in reliably assigning sex to long-lived birds such as penguins, particularly those which are the subject of long-term banding studies.

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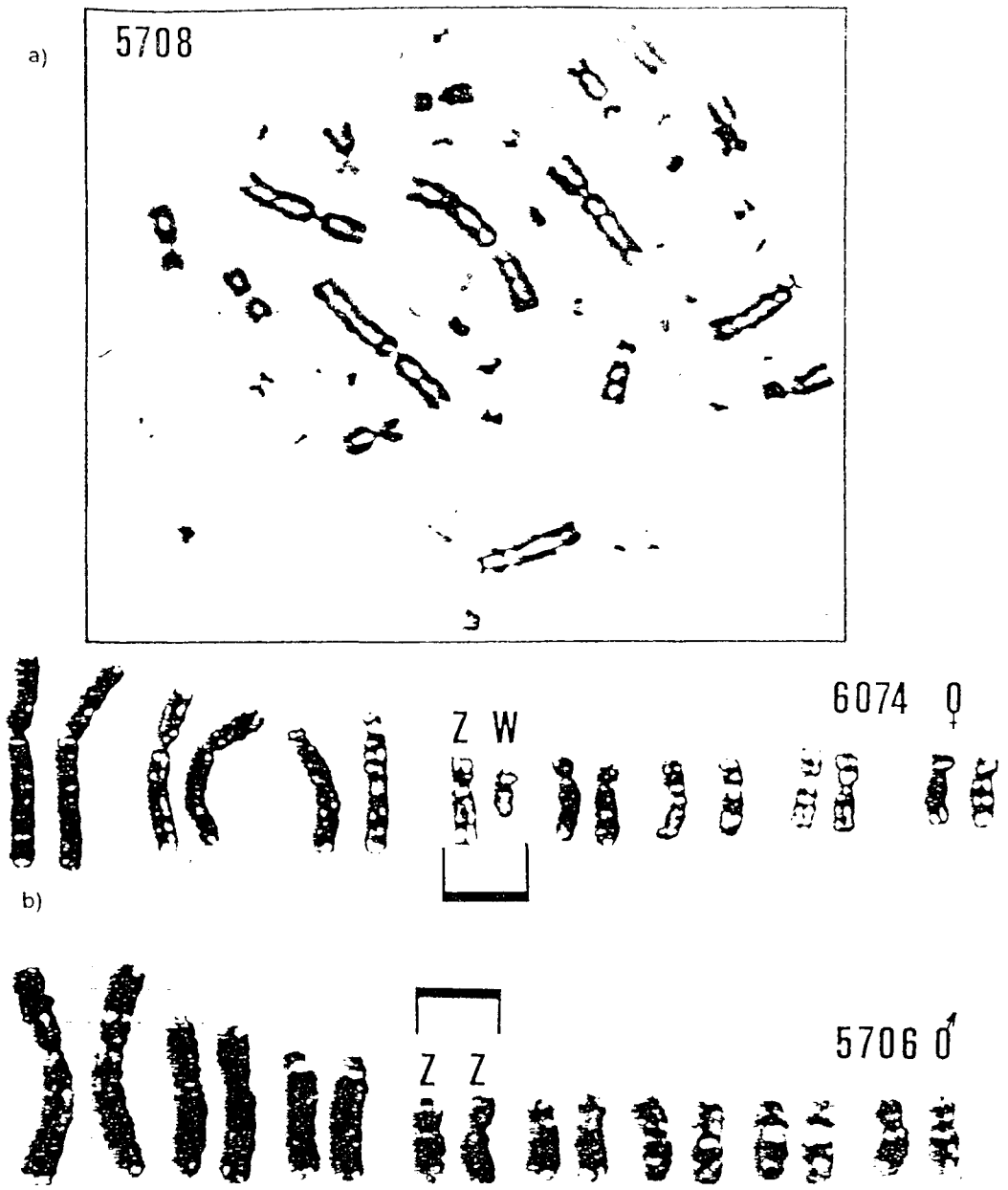

Figure 1

Chromosomes of the Yelloweyed Penguin *Megadyptes antipodes* a) Metaphase preparation showing 16 macrochromosomes and numerous microchromosomes, b) Karyotype for a pair of Yelloweyed Penguins showing the eight pairs of macrochromosomes, with the sex chromosomes (ZW and ZZ) occupying the fourth position.

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MIGRATION, DISPERSAL AND NOMADISM

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A symposium on the topic Migration, Dispersal and Nomadism will be held at Langebaan, western Cape, South Africa, from Sunday evening 12 September to Thursday 16 September 1993. The symposium will take place on the shores of Langebaan Lagoon, a RAMSAR site and one of southern Africa's prime wader (shorebird) localities in the West Coast National Park. At the time of the symposium the migrant waders will be arriving from their northern breeding grounds, and the spring flowers in the park will, weather permitting, be close to their best. Marine islands within the park support endemic seabirds such as the Jackass Penguin *Sphenicus demersus*, the Cape Gannet *Morus capensis* and the Bank Cormorant *Phalacrocorax neglectus*.

All persons interested in attending this symposium should inform Mr. TB Oatley, Avian Demography Unit, Department of Statistical Sciences, University of Cape Town, Rondebosch 7700, South Africa, in order to be put on the mailing list to receive a copy of the First Announcement which will be posted in late 1992.

Anyone wishing to present a paper or a poster should also inform Mr Oatley. Papers on all aspects of migration will be welcomed, but special attention will be devoted to the Palaearctic-African, intra-African, and Southern Ocean migration systems (to be convened by J. Cooper, Editor of *Marine Ornithology*) and to the conservation of migrant birds. The concepts dispersal and nomadism will be interpreted liberally, and will include, for example, topics such as the foraging movements of seabirds. Papers presented at the symposium will be considered for publication in special number of *Ostrich*, the journal of the Southern African Ornithological Society.