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A Rapid Procedure for Obtaining Chromosome Preparations from Birds

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Compared with most aspects of ornithological research, cytogenetic studies employing banding techniques have been largely neglected (Shields 1983). A major reason for this is the difficulty of obtaining chromosomal material suitable for C- or G-banding. Tissue culture (Stock and Bunch 1982, Shields 1983) is time consuming and requires extensive laboratory facilities, while blood cultures are not always reliable in providing good harvests of cells (de Boer 1980, de Boer and Belterman 1980). In addition, such *in vitro* techniques are impractical for field use and so are limited to captive birds. Although squash preparations of feather pulp (Shoffner et al. 1967) or embryonic material (Jensen 1975) can be used in the field, they do not provide material suitable for chromosome banding. The present report describes an *in vitro* technique using bird bone marrow that overcomes these problems. This technique represents a modification of the *in vivo* colchicine method (Lu 1969).

The tibia is removed and the nonpink fatty distal portion discarded. With a narrow-gauge syringe needle, the remaining marrow is flushed from the tibia with Eagles medium (C. S. L. Australia) into a 5-ml disposable tube (Falcon, Maryland, USA) and gently homogenized with a 23-gauge needle. To each 5 ml of media-cell suspension, 0.1 ml of a 0.001% colchicine solution is added. This is then incubated at 37°C for 37–42 min. Shorter incubation provides prometaphase cells suitable for G-banding, but these are unsatisfactory for standard or C-banded preparations. Under field conditions it is sufficient for cultures to be incubated close to one's own body for 40 min. After incubation, the culture is centrifuged (manual or battery-operated in the field) for 2 min (800–1,000 rpm) until a pellet forms in the bottom of the tube. Then the supernatant is replaced with a 0.75-M KCl solution. The resuspended pellet is incubated as above for another 20 min and then recentrifuged for 2 min. To conclude, the KCl solution is replaced by fixative (3:1 methanol : acetic acid) and the cell suspension stored indefinitely at 0°C or less. However, the fixative has to be replaced 3 times in the laboratory prior to making slide preparations.

The method presented has several advantages over previously published techniques. With *in vivo* colchicine techniques (Lu 1969) there is limited control over chromosome contraction because the effect of the treatment is influenced by the age, weight, and physiological state of the bird. These problems are eliminated through the *in vitro* procedure, with the result that the preparations are suitable for both unbanded and banded chromosomes (Fig. 1). The *in vitro* method is also readily applicable to field conditions where the majority of birds collected are shot, thereby ruling out *in vivo* techniques. Preparations obtained from freshly killed birds are suitable for G-banding, while standard and C-banded karyotypes can be obtained from birds that have been dead for

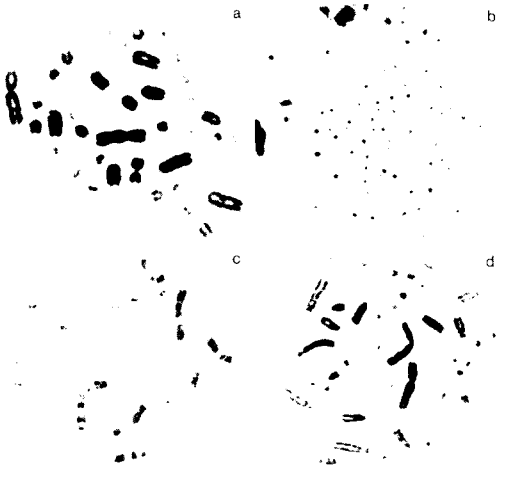

Fig. 1. (a) Giemsa-stained karyotype of *Podargus strigoides* (Caprimulgiformes). (b) C-banded karyotype of a male *Ninox novaesseelandiae* (Strigiformes). (c) G-banded karyotype of a female *Hirundapus caudacutus* (Apodiformes). (d) Giemsa-stained karyotype of a female *Grallina cyanoleuca* (Passeriformes); this bird had been dead for 3 h prior to removal of bone marrow.

up to 3 h (Fig. 1d). The simplicity and flexibility of this technique, combined with the high quality of its results, are singular advantages, and much needed in developing avian cytogenetic studies.

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The Effect of Allopreening on Tick Burdens of Molting Eudyptid Penguins

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Formerly, there was a tendency to neglect the effect of ectoparasites on social birds. It now is being realized that such parasites may precipitate colony desertion and so have powerful short-term effects on colonial seabirds (Feare 1976; King et al. 1977a, b; Duffy 1983), may influence nesting behavior (Houston 1979), and may maintain variability in populations subject to sexual selection (Hamilton and Zuk 1982).

Allopreening, the behavior whereby one bird preens another, is one means by which ectoparasite burdens could be reduced. Goodwin (1983) considered the function of allopreening to be the removal of ectoparasites but provided no substantiation. Other authors either implicitly (Welty 1982) or explicitly (Harrison 1965) doubted that allopreening was of value for plumage maintenance, and considered the behavior primarily served sexual or social purposes (Simmons 1967, Gaston 1977). Similar uncertainty surrounds the role of allogrooming by mammals (Bell and Clifford 1964, Sparks 1967, Jolly 1972, Broom 1981).

The degree of uncertainty surrounding the function of avian allopreening is due, in large part, to the lack of any demonstration that allopreening actually reduces the ectoparasite load of wild birds. Ticks are conspicuous ectoparasites of penguins. I compared parasite loads of paired penguins that are allopreened and unpaired penguins that are not, to show that allopreening does reduce the parasite load.

I studied two colonial penguin species, the Macaroni (*Eudyptes chrysolophus*) and the Rockhopper (*E. chrysocome*), that breed on Marion Island (46°53'S, 37°52'E). The Macaroni Penguin colony studied was at Kildalkey, a large expanse of stony ground holding about 195,000 pairs (FitzPatrick Inst. unpubl. data). Rockhopper Penguins were studied in the vicinity of the island's meteorological station, where the birds nest in small colonies (ca. 100 pairs) among boulders a few meters above high tide. Both areas have numerous moist cracks and rocky crevices to which the ticks (*Ixodes uriae* White) can return after feeding on their penguin hosts.

Penguins were first caught in mid-February 1984 when the colonies contained adults in seagoing plumage, well-grown chicks, and molting birds. Some molting birds were immature, as judged by their short, drab head plumes. All birds were caught by hand and examined thoroughly, and any ticks discovered were counted. The ticks were mostly adult females and nymphs.

Further counting was done at the end of March (Macaroni Penguin) or in mid-April (Rockhopper Penguin) at a time when all birds were molting and when it was easy to see which birds were single and which were paired. Paired birds stood very close, if not in actual contact, allopreened each other, and acted in concert to display aggressively to intruding penguins. It was possible, therefore, to sample both single and paired birds. The selection ensured that the birds in the two groups were at the same stage of molt, because this influences the tick load (Murray and Vestjens 1967, pers. obs.). Control for location was achieved by catching paired and single birds within a few meters of each other. The ticks on the head and neck of the penguins caught then were

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