A Feather Pulp Culture Technique for Avian Chromosomes, with Notes on the Chromosomes of the Peafowl and the Ostrich

Older concepts of the chromosomes of animals in general have been greatly revised in recent years by the increase of cytogenetic knowledge, due to rapid technical advances. The chromosomes of birds have been studied by many earlier investigators with classic sectioning methods (for reference, see MAKINO[1]). Recently, many avian species have been re-investigated with modern tissue culture techniques with revised results (for details, refer to TAKAGI and MAKINO[2]).

SANDNES[3] developed feather pulp techniques involving the hypotonic pretreatment and squash procedures for the demonstration of avian chromosomes. This method was slightly modified by KRISHAN[4] and his associates[5]. The present authors modified further the feather pulp techniques in combination with tissue cultures. This technical method produces more reliable results, based on the excellent metaphases, than those obtained with the squash method. Without sacrificing the birds, this feather pulp culture method is advantageous in facilitating very precise analyses of the chromosomes in various species, particularly in taxonomically interesting and rare birds.

Technical procedures. Growing tail or wing feathers were removed from living birds in the field or at the zoo. After sterilizing the surface of the feathers with 70% alcohol, the bases were cut off and placed in a tube containing 3–5 ml of culture medium 109. The feather bases thus kept in the medium were slit with fine scissors in the culture room, and the proximal inside parts from each feather base were scraped with tweezers in a Petri dish. Small pieces of the feather pulp tissue thus obtained were washed with 3 changes of Ringer's solution containing antibiotics, and cut into shreds with fine scissors. Then, they were planted directly on the glass surface of the culture bottle with plasma-clots, fed culture medium 109 supplemented with 15% calf serum, and incubated at 37 °C. After 2–3 days incubation, an outgrowth of fibroblast-like cells from the pieces of tissues became recognizable. After 7–10 days incubation, sufficiently grown cells on the glass surface were treated for 8–10 h with colcemid at a concentration of 0.1 µg/ml. The culture medium containing colcemid was decanted. By applying trypsin solution (0.15%), the growing cells were dislodged from the glass wall. The cell suspension was centrifuged for 5 min at 1000 rpm. After removal of the supernatant, the cells were treated with a phosphate-buffer hypotonic solution for 20 min at 37 °C and fixed with methanol-acetic acid (3:1). Fixed cells were air-dried on clear slides and stained with Giemsa.

Results. Accounts of the chromosomes of the peafowl and the ostrich studied by the method described above are given in the following:

The results of chromosome counts are summarized in the Table. A wide distribution of chromosome numbers is seen in the Table. Most probably the variations in number of the chromosomes may be due to artifacts caused by certain difficulty in counting the exact number of microchromosomes.

Peafowl (Figure 1): It should be mentioned that the birds here dealt with are of impure breed derived from a

[1] S. MAKINO, *A Review of the Chromosome Numbers in Animals*, 2nd edn (Hokuryukan, Tokyo 1956).
[2] N. TAKAGI and S. MAKINO, Caryologia *19*, 443 (1966).
[3] G. C. SANDNES, Science *119*, 508 (1954).
[4] A. KRISHAN, Experientia *18*, 100 (1962).
[5] A. KRISHAN, J. D. HAIDEN and R. N. SHOFFNER, Chromosoma *17*, 258 (1965).

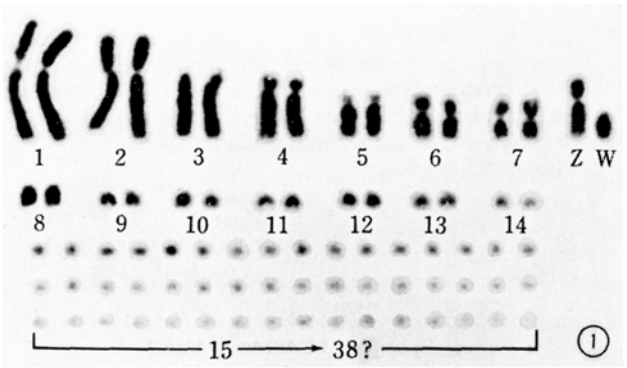

Fig. 1. Karyotype of a female peafowl, with 78 chromosomes.

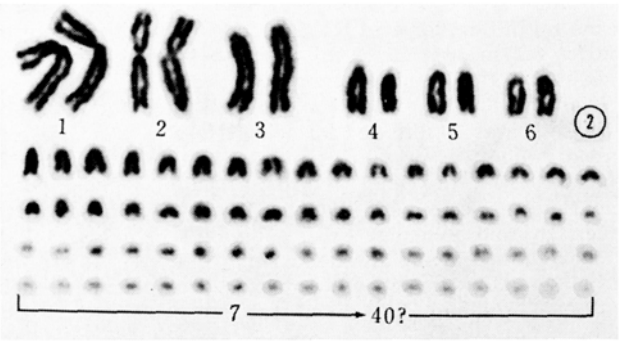

Fig. 2. Karyotype of an ostrich, with 80 chromosomes.

Chromosome counts in 2 species of birds under study

	Chromosome No. distribution											Total cells counted
	70	71	72	73	74	75	76	77	78	79	80	
Peafowl ♀	1	1		1	1	1	2	2	2			11
♂	5	2	5	1	5	1	3	2	1	1		26
Ostrich			1	1	2	1	4	5	9	3	3	29

hybrid between *Pavo cristatus* and *P. muticus muticus*. Growing tail-feathers from both sexes provided material for study.

Figure 1 shows the karyotype of a female peafowl. The first and second largest pairs consist of submetacentric chromosomes clearly distinguishable from each other by size difference. No. 3 is represented by an acrocentric pair and No. 4 by a subtelocentric pair. They are the same length as the long arm of No. 1. A *Z*-chromosome was identified as a submetacentrics similar in size to Nos. 3 and 4, remaining unpaired in female cells. The chromosomes Nos. 5, 6 and 7 are of the same length, being about $^3/_4$ the length of Nos. 3 and 4. No. 5 is an acrocentric pair with a definite short arm. Nos. 6 and 7 are submetacentric and metacentric chromosomes, respectively. The chromosome identified as a *W*-chromosome in female cells is one of the small acrocentrics approximately $^2/_3$ the size of chromosomes Nos. 5–7. The eighth pair consists of small acrocentrics slightly shorter than the *W*-chromosome. There are at least 30 pairs of microchromosomes giving a possible chromosome number of 78 in total. They form a continuous series in size without any visible morphological difference.

Ostrich (*Struthio camelus camelus*) (Figure 2): Developing wing-feathers of a young ostrich of unknown sex were used for study.

Karyotype analysis revealed that the largest 6 pairs were recognizable individually on account of their characteristic configurations. The first 2 pairs of chromosomes consist of submetacentrics, the No. 2 chromosomes being slightly shorter than No. 1. The chromosomes of No. 3 are acrocentric, being similar in length to the long arm of No. 1 chromosomes. Chromosomes 4, 5 and 6 are acrocentric, having slightly decreasing size in order, but they are not always distinguishable from one another. In addition, there are a number of small acrocentrics. The diploid number of the chromosomes available for counting in this species seems to be 80. It seems probable that the bird under study may be a male, because of the fact that there was no heteromorphic pair amongst the larger chromosomes. More detailed studies are in progress[6].

Résumé. Description d'une technique de culture à partir de la moëlle des plumes, cette technique ayant permis l'analyse du caryotype du Paon et de l'Autruche.

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A Refined Test for X-Ray Induced Dominant Lethals in *Drosophila*

Samples of newly inseminated *Drosophila* eggs can be obtained by the egg collection method of Würgler et al.[1]. The eggs deposited by the females on a moist black paper are transferred to a small plexiglass plate (1 mm thick) with the aid of a small brush. Under a dissecting microscope they are arranged side by side in the centre of the plate and kept wet by adding a small drop of water. The sample can thus be irradiated the moment it has reached a desired age. Egg collection and irradiation are carried out in a climatized room with a temperature of 25 °C and 96% relative humidity.

After irradiation the eggs are transferred to a strip of agar (2% agar, 5 mm thick) lying on a glass plate (7.5 × 1.5 cm, one end cut to a point). The number of irradiated eggs (EI) is counted under a dissecting microscope by placing them in groups of 10 beginning about 1.5 cm from the tip of the agar strip. The glass plate with the agar strip and the eggs is then brought into a special culture tube. The latter consists of a glass tube (2 cm in diameter) of about 9 cm length whose upper end can be closed by a foam rubber stopper while the lower end fits onto a hollow plastic bowl (2 cm high). The bowl contains 2.5 ml of dry yeast medium (200 ml water, 5 g dry yeast and 30 g sugar are boiled; upon cooling, 4 ml of a Nipasol solution (4.28 g Nipasol-M-sodium in 200 ml 70% alcohol) and 4 ml of a Terramycin solution (0.0022 g in 200 ml 70% alcohol) are added). 1 drop of a concentrated aqueous suspension of living yeast is added to the surface of the medium and partially covered with a small piece of blotting paper.

The culture tubes containing the eggs are kept in a room with a temperature of 25 °C and 60% relative humidity. After about 21 h the surviving larvae hatch. Attracted by the yeast[2] and following a humidity gradient[3] they crawl into the medium. 48 h after egg collection practically all surviving larvae have reached the medium. Now the glass plate with the agar strip is removed from the culture tube, and surviving larvae found on the agar are brought back into the tube. The dead embryos and the empty egg shells lying on the agar strip are gently dried in a stream of heated air and covered with paraffin oil. Since the egg shells are now transparent the dead embryos can be classified under a dissecting microscope and counted according to the following syndromes: WE, white mottled eggs; KU, eggs with a typical spherical concentration of opaque material in the central part of the egg; BR, embryos with a brownish colour; LL, white embryos with larval structures visible; HL, larvae which opened the egg shell but did not hatch; TL, larvae which died immediately after hatching. In addition living hatched larvae (NL) which occasionally have not been transferred back from the agar strip into the tubes are noted.

The tubes with the surviving larvae are brought into a room with a temperature of 25 °C and 96% relative humidity. The high humidity in the vials causes most of the individuals to pupate outside the medium (about 4 cm above the surface). On the eighth day after irradiation the pupae are carefully removed from the culture tube with a wet brush and brought into small empyt tubes, individually for the collection of virgin females or

[1] F. E. Würgler, H. Ulrich and H. W. Spring, Experientia *24*, 1082 (1968).
[2] G. Benz, Revue suisse Zool. *62*, 305 (1955).
[3] G. Benz, Experientia *12*, 297 (1956).