

METHODOLOGY

AN INEXPENSIVE METHOD FOR CHROMOSOME PREPARATIONS FROM FEATHER PULP IN BIRDS, USING SHORT TERM TREATMENT WITH COLCHICINE *in vitro*, DEMONSTRATED ON *Amazona amazonica* (PSITTACIDAE)

M.L. Giannoni¹, F. Foresti², C. Falcone¹ and P.A. Tosta¹

ABSTRACT

In order to develop an efficient and low-cost technique for obtaining bird chromosome preparations, and to adapt the cytogenetic process of bird sexing for general use at zoos and breeding farms with the technical support of cytogenetics laboratories, we tested variants of the technique described by Giannoni *et al.* (Genet. Sel. Evol. 23: 123-125, 1991), based on the utilization of cellular material from growing feather pulp cultured in complete medium for six hours. Hanks' saline solution gave satisfactory performance as a substitute for complete medium, with no need to use PHA, serum or collagenase, when utilized in material obtained from feather pulp of *Amazona amazonica* (Psittacidae).

INTRODUCTION

Growing feather pulp has been used to study bird cytogenetics for approximately four decades (Shoffner, 1985). However, cytogenetics laboratories continue to develop the techniques (Giannoni *et al.*, 1991) and to lower their cost.

Use of this material for the chromosome sexing of birds has been promoted by various researchers such as Mengden and Stock (1976), Sasaki *et al.* (1984) and Aquino and Ferrari (1986, 1990). According to Sasaki *et al.* (1984), the Chromosome Research

¹ Departamento de Melhoramento Genético Animal, FCAVJ/UNESP, 14870-000 Jaboticabal, SP, Brasil. Send correspondence to M.L.G.

² Departamento de Morfologia, Instituto de Biociências, UNESP, 18618-000 Botucatu, SP, Brasil.

Unit at the University of Hokkaido, Japan, has been studying the cytogenetics of birds for a long time and, since 1970, has performed chromosomal sex identification of birds for zoos and other institutions, with about 90% success in the preparations and diagnosis of the cases analyzed until May 1983, utilizing for this purpose blood culture, feather pulp and fibroblasts. They obtained better results with blood (85.77%) and skin (83.2%) culture than with feather pulp (42.9%), although certain differences in results have been associated with specific characteristics of some bird groups. In Falconiformes, for instance, skin culture gave the best results.

In the USA, the method of long-term culture of cell material obtained from feather pulp, is utilized with excellent results (Van Tuinen and Valentine, 1982). However, it requires well-equipped laboratories as well as experience in tissue culture, with a consequent limitation of its use in developing countries. A similar problem occurs with the leucocyte culture technique which, although yielding very satisfactory results, is somewhat traumatic for the bird, because of the blood collection procedure (De Boer and Belterman, 1980; Van Dongen and De Boer, 1984; Sasaki *et al.*, 1984).

The tissue culture method which utilizes complete culture medium, with the addition of PHA and serum, requires relatively high-cost products, which is the main limiting factor for widespread utilization of this method of bird sexing. The replacement of complete medium with Hanks' saline solution, which is easy to prepare and store and of low cost, as proposed by Foresti *et al.* (in press), who utilized this with success of fish cells and also on cells of some other vertebrate as well as invertebrate species, could therefore favor ample use of cytogenetics bird sexing.

MATERIAL AND METHODS

Growing feather pulp was collected from males and females of the species *Amazona amazonica* and processed in three different ways, as described below:

a) The cellular material of the pulp of eight feathers was cultured on Petri dishes containing 8 ml of complete McCoy's culture medium, to which 0.6 ml PHA and 1.0 ml of inactive equine serum were added. The material was incubated in an oven for four hours at 39°C, followed by the addition of three drops of 0.0016% colchicine and additional incubation for two hours. The material was then centrifuged and the cells were submitted to hypotonization with 0.075 KCl for 15 minutes, followed by fixing in 3:1 methanol-acetic acid. The fixative was changed three times to discard the cellular remains and to obtain the final suspension, which was dropped on the slides. The preparations were stained with Giemsa.

b) The same procedure as described in a), but replacing the complete medium with Hanks' saline solution.

c) The cellular material resulting from the feather pulp was suspended in 5 ml Hanks' salt solution, containing 1 drop of 0.05% colchicine solution, for 1 hour, followed by the same procedures of hypotonization, fixing, preparation of slides and staining as described in a).

The chromosome preparations were submitted to banding procedures and marking of specific chromosome regions. The nucleolus organizing regions (NORs) were identified by the technique of Howell and Black (1980). C-bands were obtained according to Sumner (1972), with some adaptations.

RESULTS AND DISCUSSION

Although treatments *a* and *b* proved to be slightly superior in relation to treatment *c* in terms of the number of metaphases, all of them were efficient for bird sexing, as shown in Figures 1 and 2.

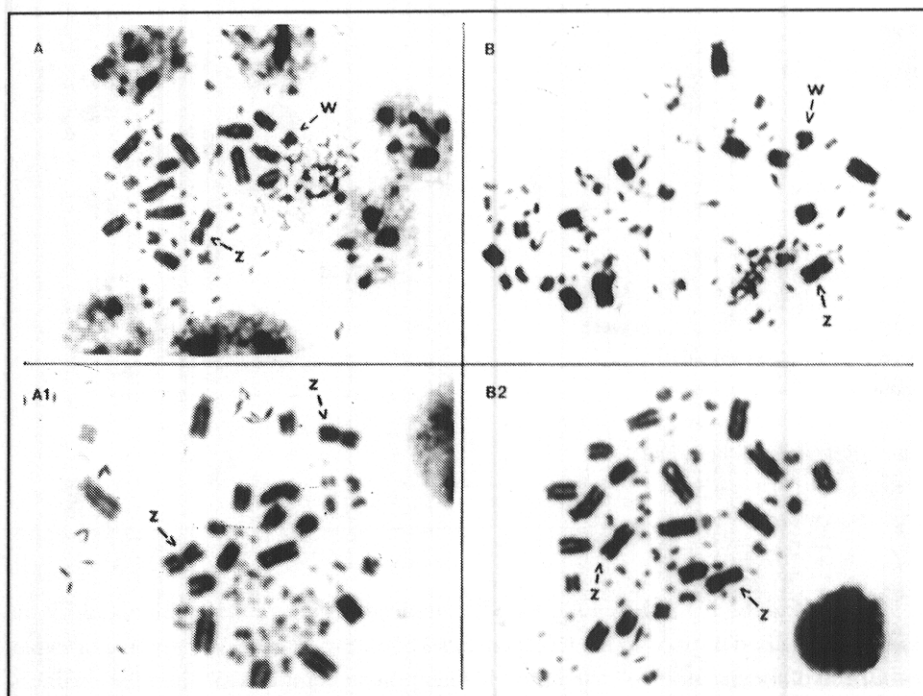


Figure 1 - Female (A and B) and male (A1 and B2) mitotic metaphases of the species *Amazona amazonica* obtained by culturing feather pulp for short period of time (6 hours) (A). Complete culture medium, (B), Hanks' salt solution with colchicine.

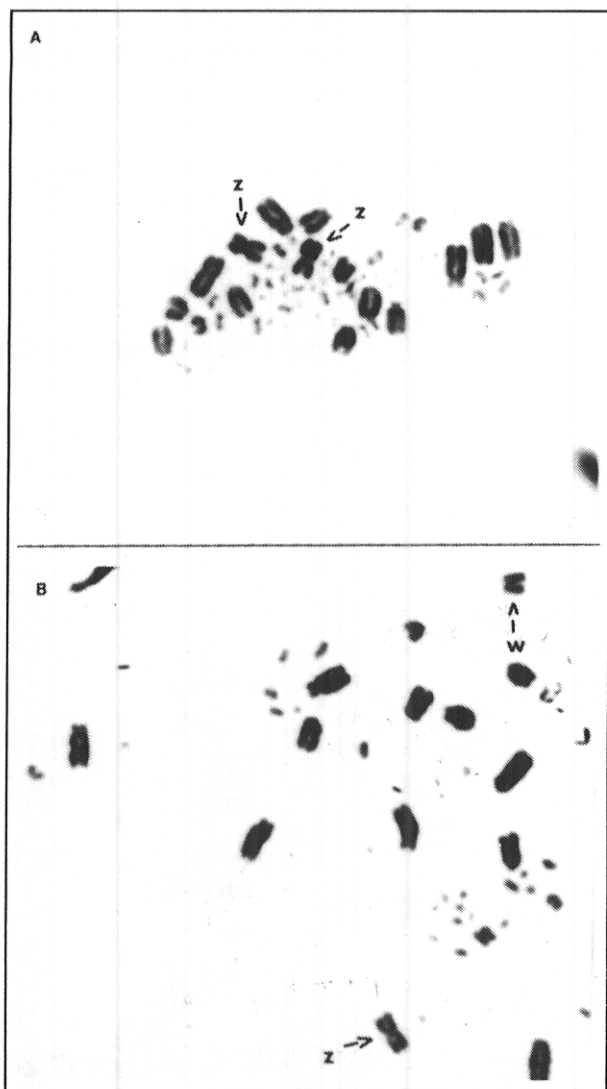


Figure 2 - Male (A) and female (B) mitotic metaphases of the species *Amazona amazonica* obtained by culturing the material for a short period of time (1 hour) in Hanks' salt solution with colchicine.

In Figure 1, the letter A is used to indicate the metaphases obtained by the traditional method utilized in the Cytogenetics Laboratory of FCAVJ, which consists of incubation of the material collected from feather pulp in complete medium for six hours. The metaphases indicated by the letter B were obtained by incubation of the material in Hanks' saline solution during the same period of time and with the same number of treatments.

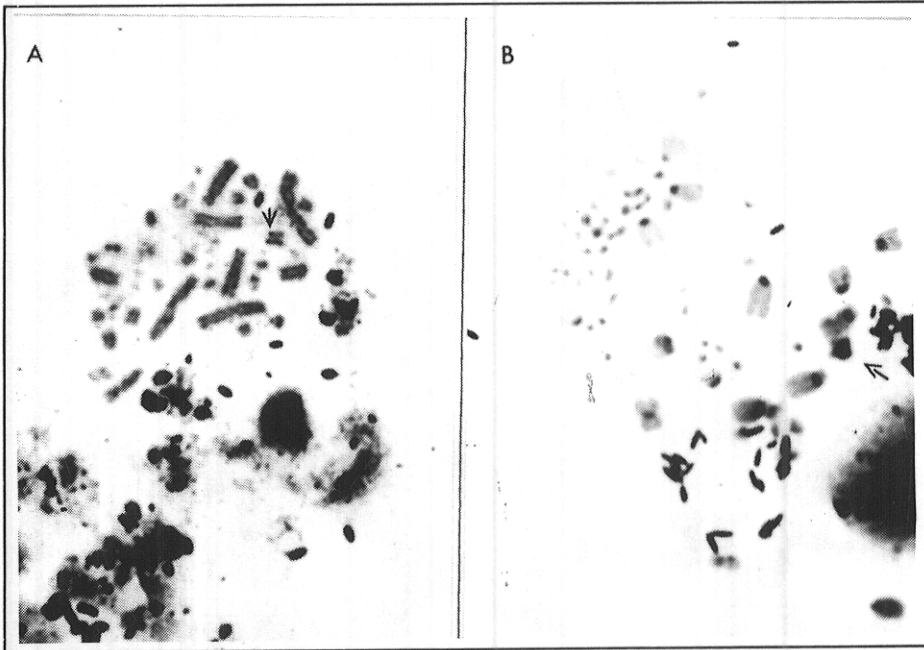


Figure 3 - Mitotic metaphases of the species *Forpus crassirostris* (A) and *Amazona amazonica* (B) submitted to different treatments to visualize bands or to identify specific chromosomes in the complement. (A) Metaphase stained with silver for the identification of the nucleolus organizing regions (NORs) (arrows). (B) Metaphase submitted to C-banding. Notice the heterochromatic character of the W chromosome (arrow).

In Figure 2, the letters A and B indicate male and female metaphases, respectively, obtained from material incubated in Hanks' salt solution containing colchicine for a short period of time (1 hour).

The quality of the metaphases obtained with the three treatments permitted easy identification of sex chromosomes Z and W, i.e., the birds could be sexed by standard Giemsa staining of the material.

To test the possibility of applying banding techniques and techniques for the identification of specific chromosome regions, the preparations obtained by the method of short-term incubation, with colchicine in Hanks' salt solution were submitted to C-banding and to silver staining for the identification of nucleolus organizers regions (NORs) (Figure 3). The results permit us to state that the technique of short-term incubation of feather pulp in Hank's saline solution with colchicine, used to obtain bird chromosome preparations as an alternative to six-hour cell culture in complete medium, is an efficient and low-cost variation of the method. On this basis, the method may prove to be a viable alternative for routine bird sexing in zoos and in bird breeding farms.

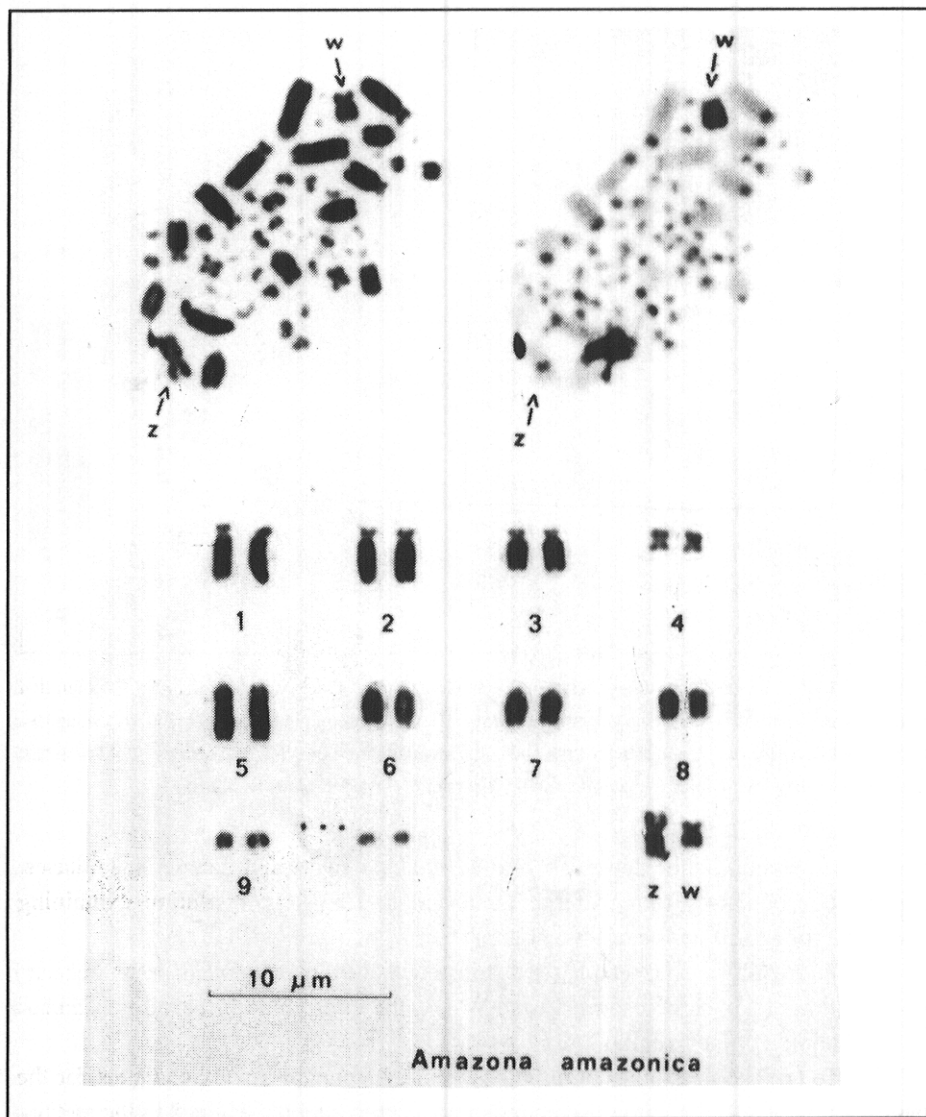


Figure 4 - Karyotype and sequential banding analyses of material from *A. amazonica*, with characteristic C-banding patterns in the W chromosome (arrows).

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RESUMO

Com o objetivo de desenvolver técnica eficiente e de baixo custo para obtenção de preparações cromossômicas em aves, visando popularizar o processo citogenético de sexagem de aves e permitir, com o apoio técnico de laboratórios de citogenética, sua ampla utilização em zoológicos e criatórios, foram testadas variantes da técnica descrita por Giannoni *et al.* (Genet. Sel. Evol. 23: 123-125, 1991), baseadas na utilização de material celular de bulbos de pena em crescimento, cultivado em meio de cultura completo durante período de incubação de 6 horas. A solução salina de Hanks mostrou desempenho satisfatório como substituto do meio completo, dispensando o uso de fitohemaglutinina, soro e colagenase, quando utilizada em material obtido de penas de *Amazona amazonica* (Psittacidae).

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