AMCoR
Asahikawa Medical College Repository http://amcor.asahikawa-med.ac.jp/

Hereditas (2003) 138(2):158-159.

Avian chromosomes can be examined by injection of erythrocyte nuclei into mouse oocytes

Tateno, Hiroyuki ; Kosuge, Masao ; Yanagimachi, Ryuzo

Hereditas 138: 158–159 (2003)

Brief report
Avian chromosomes can be examined by injection of erythrocyte nuclei into mouse oocytes

HIROYUKI TATENO[1], MASAO KOSUGE[2] and RYUZO YANAGIMACHI[3]

[1] *Department of Biological Sciences, Asahikawa Medical College, 2-1 Midorigaoka-higashi, Asahikawa 078-8510, Japan.* E-mail: htateno@asahikawa-med.ac.jp
[2] *Asahiyama Zoo, Asahikawa 078-8205, Japan*
[3] *The Institute for Biogenesis Research, Department of Anatomy and Reproductive Biology, University of Hawaii, Honolulu, Hawaii, USA*

Received April 22, 2003. Accepted May 13, 2003

Examination of the complete set of chromosomes is essential for the study of karyological evolution and gene mapping. Avian chromosomes have been examined by direct preparation of adult bone marrow cells (MITTAL and SAKHUJA 1980) or after in vitro culture of adult feather pulp (SASAKI et al. 1968), adult peripheral lymphocytes (TAKAGI and SASAKI 1974), adult skin cells (KOSUGE and TATENO 1982), or embryonic somatic cells (HAMMAR and HERLIN 1975). We report here that we can perhaps analyse the karyotype of any birds using peripheral erythrocytes.

MATERIAL AND METHODS

Mouse oocytes, collected from the oviducts of superovulated females (B6D2F1), were freed from cumulus cells and kept in Hepes-buffered CZB (Hepes-CZB) medium for less than 2 hr at 37°C (KIMURA and YANAGIMACHI 1995). The skin of the tip of brachium of the Bengalese finch (*Lonchura striata* var. *domestica*) was pricked with a sterile 26-gauge needle. The blood sample (about 5 μl) was mixed with 1 ml of Hepes-CZB and kept on ice until use. This amount of blood sample is far less than 1 % of bird's body weight, which is set as the threshold for blood draw from wild birds. The finch weights about 15 g. In the case of white-tailed eagles (*Haliaeetus albicilla*; listed as an endangered species in Japan), which are being preserved in Asahiyama Zoo, blood (1–2 ml) was withdrawn into a heparinized syringe from the vein of the brachium. Erythrocytes were separated by centrifugation (500g, 5 min) and mixed with 5 ml of Dulbecco's minimum essential medium (D-MEM) containing 10 % fetal bovine serum and 10 % dimethyl sulfoxide. The suspension was divided into 0.5 ml aliquots, kept at −80°C overnight, plunged into liquid nitrogen and stored until use.

Immediately before use, erythrocytes were treated with 0.5 % NP-40, a non-ionic detergent, in Hepes-CZB for 10 min at 0°C to disrupt both the plasma membrane and nuclear envelope. The nuclei were rinsed and individually injected into mouse oocytes according to the procedure of intracytoplasmic sperm injection described previously (KIMURA and YANAGIMACHI 1995). The time interval between demembranation of erythrocytes and their injection into oocytes was less than 1.5 hr. Within 30 min after injection, oocytes were activated by incubating them for 2–3 hr in calcium-free CZB medium containing 5 mM $SrCl_2$. Activated oocytes that emitted the second polar body were returned to normal CZB medium for further culture. All erythrocyte chromosomes were retained within oocytes. Approximately 6 hr later, eggs were transferred to CZB medium containing vinblastine sulfate (0.01 μg/ml). Between 18 and 20 hr after nuclear injection, the eggs arrested at the first cleavage metaphase were processed for chromosome examination using the gradual-fixation/air-drying method (MIKAMO and KAMIGUCHI 1983).

RESULTS AND DISCUSSION

Of 42 mouse eggs that were successfully injected with finch erythrocyte nuclei, 40 (95 %) each had one pronucleus of egg origin and one pseudo-pronucleus of erythrocyte origin. 39 eggs (93 %) at the metaphase of the first cleavage had both a mouse egg chromosome set and a finch erythrocyte chromosome set, which could be readily distinguished from each other (Fig. 1a). Eagle chromosomes were seen in 64 (94 %) of 68 injected eggs (Fig. 1b). Chromosome C-banding revealed that two of the three eagles used were female (ZW) as evidenced by the presence of a heterochromatic W chromosome (Fig. 1c).

This procedure creates minimum discomfort for the birds used, and to our knowledge is the first observation of avian chromosomes using their ery-

throcytes, which do not normally divide. The appearance of normal-looking avian chromosomes within mammalian egg cytoplasm suggests that the nucleus-cytoplasm interaction is not strictly species-specific, at least before the first cleavage. Karyoanalysis of human spermatozoa using hamster or mouse oocytes has been used (RUDAK et al. 1978; MARTIN 1983; KAMIGUCHI and MIKAMO 1986; LEE et al. 1996). It seems likely that the oocytes of mice, and probably other mammals, can be used as universal recipients for somatic nuclei from any avian and mammalian species. If oocyte's metaphase II chromosomes are removed prior to injection of a donor nucleus, chromosomes that appear at the first cleavage metaphase are all of donor origin. Unless the chromosomes of the nucleus donor are similar in structure and number to those of the egg, prior removal of egg chromosomes would be unnecessary for recognition/identification purposes. In fact, oocyte chromosomes can serve as an internal control of experiments. In contrast to our experience here with birds, our pilot experiments with cold blooded animals (lizard, frog and fish) revealed that their erythrocyte nuclei did not transform into normal chromosome within mouse eggs that must be kept around 37°C for their survival. The method reported here should be useful for basic studies on karyological evolution and gene mapping in avian species, as well as breeding of endangered avian species without distinct sexual dimorphisms.

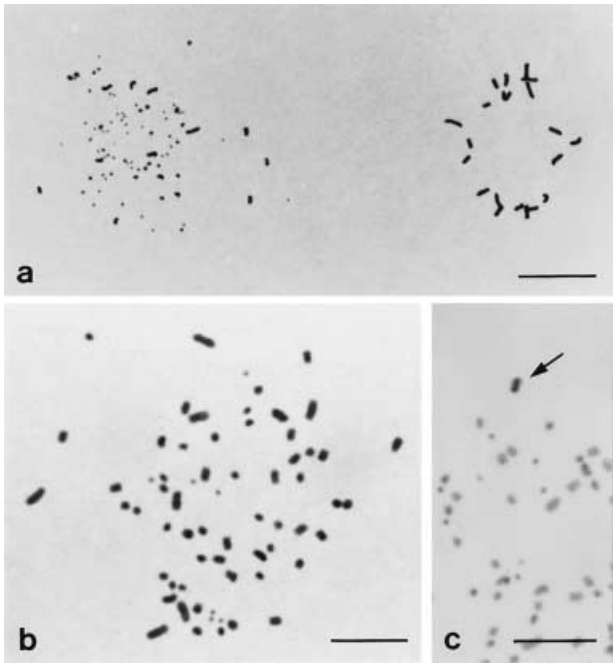

Fig. 1. Avian erythrocyte chromosomes in mouse egg at the first cleavage metaphase. **a**, This shows both finch diploid (2n = 80) chromosome set (left) and mouse haploid (n = 20) chromosome set (right). **b**, An eagle chromosome set (2n = 66). **c**, A heterochromatic W chromosome positively stained by C-banding (arrow). Scale bars: a, 20 μm; b and c, 10 μm.

ACKNOWLEDGEMENTS

We are indebted to Ms. Soccoro Tauyan and Dr. Ben Okimoto (Honolulu Zoo) for providing chicken, duck and frog blood samples in the pilot experiments. We are grateful to Drs. J. Michael Bedford and Rebecca L. Cann for reviewing the manuscript and giving us invaluable comments. This work was supported by the Ministry of Education, Science, Sports and Culture of Japan (H.T.).

REFERENCES

Hammar, B. and Herlin, M. 1975. Karyotypes of four bird species of the order Passeriformes. – Hereditas 80: 177–184.

Kamiguchi, Y. and Mikamo, K. 1986. An improved, efficient method for analyzing human sperm chromosomes using zona-free hamster ova. – Am. J. Hum. Genet. 38: 724–740.

Kimura, Y. and Yanagimachi, R. 1995. Intracytoplasmic sperm injection in the mouse. – Biol. Reprod. 52: 709–720.

Kosuge, M. and Tateno, H. 1982. Chromosomal sex diagnosis in four species of Strigiformes. – JJAZA 24: 6–9.

Lee, J. D., Kamiguchi, Y. and Yanagimachi, R. 1996. Analysis of chromosome constitution of human spermatozoa with normal and aberrant head morphologies after injection into mouse oocytes. – Human Reprod. 11: 1942–1946.

Martin, R. H. 1983. A detailed methods for obtaining preparations of human sperm chromosomes. – Cytogenet. Cell Genet. 35: 252–256.

Mikamo, K. and Kamiguchi, Y. 1983. A new assessment system for chromosomal mutagenicity using oocytes and early zygotes of the Chinese hamster. – In: Ishihara, T. and Sasaki, M. S. (eds), Radiation-induced chromosome damage in man. Alan R Liss, New York, pp. 411–432.

Mittal, O. P. and Sakhuja, S. 1980. Bone marrow chromosomes in *Corvus* species (Corvidae: Passeriformes: Aves). – Cytobios 29: 81–89.

Rudak, E., Jacobs, P. A. and Yanagimachi, R. 1978. Direct analysis of the chromosome constitution of human spermatozoa. – Nature 274: 911–913.

Sasaki, M., Ikeuchi, T. and Makino, S. 1968. A feather pulp culture for avian chromosomes, with notes on the chromosomes of the peafowl and the ostrich. – Experimentia 24: 1923–1929.

Takagi, N. and Sasaki, M. 1974. A phylogenic study of bird karyotypes. – Chromosoma 46: 91–120.