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著者	TAKASHIMA Yoshihiro, SHINJO Akihisa, MIZUMA Yutaka
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Mitotic Chromosomes of Chinese Button-Quail (*Turnix susciator*)

Yoshihiro TAKASHIMA, Akihisa SHINJO*
and Yutaka MIZUMA

Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai 980, Japan
**Department of Animal Science, Faculty of Agriculture, Ryukyu University, Okinawa 903-1, Japan*

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Summary

Bone marrow chromosomes from male and female Chinese button-quail were studied in flame-dried preparations. The diploid number of chromosomes of male and female cells is 84. Except for the largest pair of submetacentric, all remaining chromosomes were acrocentric and of gradually decreasing size. It was impossible to identify the Z chromosome without G-banding technique. In G-bands in male cell, the 5th or 6th largest chromosome pair was recognized as Z chromosome. In C-bands, the W chromosome which is a medium-sized acrocentric (8-10th) was stained deeply as a heterochromatic element.

Many studies on avian chromosomes have been carried out. However, banding techniques have been applied to only a limited number of species. The chromosomes of Gruiformes have been studied for only a limited number of forms (1). This study describes the somatic chromosomes of Chinese button-quail (Order: *Gruiformes* Family: *Turnices*) prepared from bone marrow.

Materials and Methods

Five Chinese button-quail (4 males and one female) used in this study were collected from Okinawa in Japan. They were killed and sexed by anatomical inspection of the gonads. The femur was cut off and the bone marrow cells were removed to the centrifuge tube from the shaft of the bone in physiological buffer solution (PBS) by using a syringe. And 0.1 ml of 0.05% aqulous colcemid was added to 5 ml of bone marrow cell solution and incubated for 90 min at 37°C. Following 20 min treatment of 0.075 M KCl, five ml of cell solution was centrifuged and fixed in acetic methanol (1 : 3) with three changes. After the last fixation, slides were prepared by the flame-drying method.

The slides were stained with 5% Giemsa solution for 5 min and the chromosomes of well-spread metaphase cells were examined to determine the diploid number. For G-band patterns the trypsin-Giemsa technique of Wang and Fedoroff (2) with a slight modification was used. After harvesting, the slides were stored in an incubator for 3 days at 37°C and they were treated with 0.025% trypsin (Difco 1 : 250) in phosphate buffer solution (pH 7.4) for 30-45 sec at room temperature. The C-banding procedure used was based on the technique of Sumner (3). The batches of slides were incubated in a solution of saturated barium hydroxide for periods of time ranging from 1 min to 4 min at 40°C. The slides were aged at room temperature for 1-30 days before use.

The measurement of chromosomes was made with a microcomputer equipped with dual 5 inch mini-floppy disk drives and a graphic digitizer (4). Chromosomes were assigned to centromeric classes by the standard arm ratio criteria of Levan *et al.* (5).

Results

The karyotype of Chinese button-quail consisted of a relative small number of large chromosomes and a large number of minute chromosomes (Fig. 1). As the chromosomes formed a continuous graded series in length without distinct grouping, the recognition of individual pairs was not feasible. It was difficult to make definite counts of the number of minute chromosomes. However, in well-spread metaphase cell, it was found to be a maximum of 84 chromosomes (Fig. 2). The Chinese button-quail had a pair of the largest submetacentric chromosomes (Arm ratio 1.7-1.8) and all the remaining ones appeared to be acrocentrics. Six acrocentric chromosomes roughly similar in size, comprising the 4th, 5th and 6th pairs in length, were present in the homogametic male, while the female cell had only five acrocentrics of this size range. But Z chromosome could not be distin-

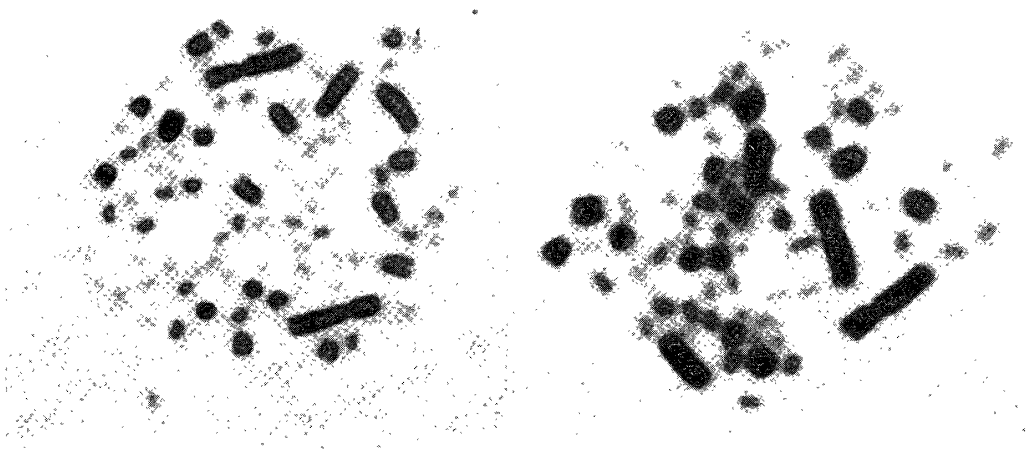

FIG. 1. Metaphase plates of specimen of Chinese button-quail (Left : male Right : female)

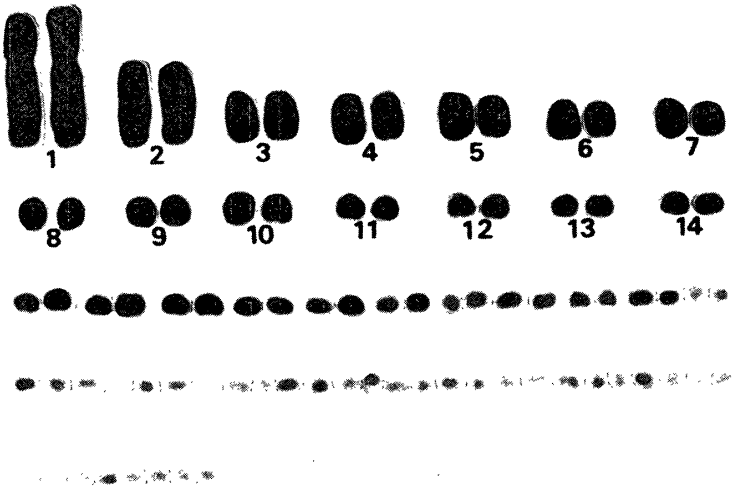

FIG. 2. Conventional, nonbanded Chinese button-quail (Male)

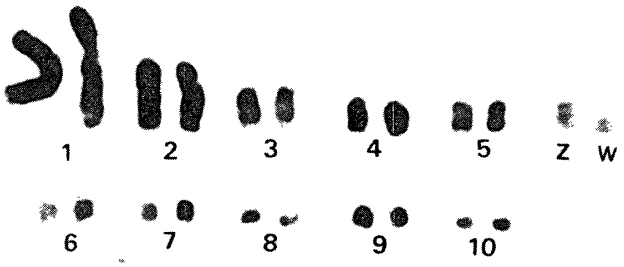

FIG. 3. G-band pattern of a karyotype of a female Chinese button-quail

guished from other elements in this group on the basis of conventional Giemsa staining.

The G-banded chromosomes of a female Chinese button-quail are shown in Fig. 3. As the trypsin G-banding technique produced characteristic band patterns, the individual pairs of larger chromosomes including Z chromosomes could be recognized. However, many small chromosomes were stained faintly and no clear-cut banding patterns were distinctive.

The Chinese button-quail metaphase showing the distribution of constitutive heterochromatin as revealed by C-banding was presented in Fig. 4. Since most chromosomes appeared to contain a block of constitutive heterochromatin localized to the centromeric region, C-banding pattern could not aid in distinguishing the chromosome pairs. In C-banding preparations, especially the W chromosome was usually heavily stained and easily identified. The W chromosome was an acrocentric, ranking 8-10th in the order of decreasing size. The C-banding

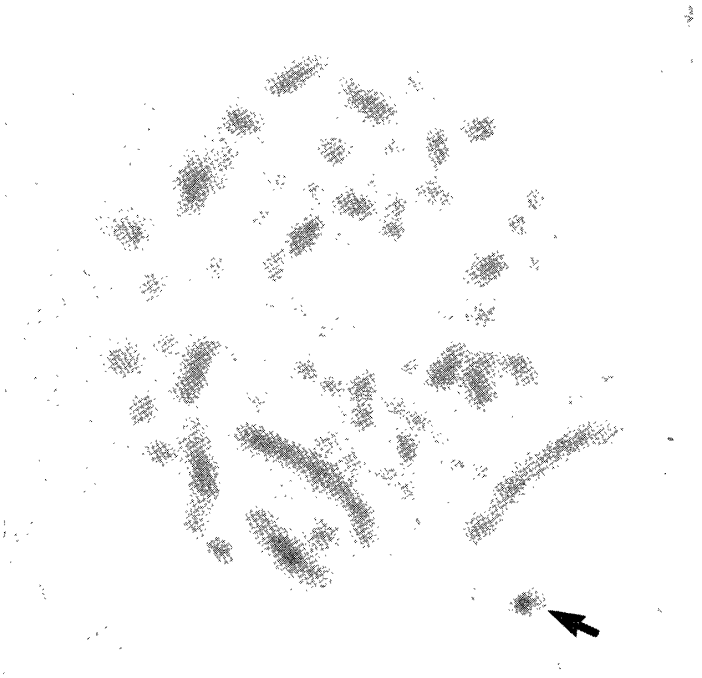

FIG. 4. Metaphase cell of a female Chinese button-quail stained for C-band. (Arrow indicates W chromosome)

patterns of chromosomes on preparations varied depending upon the degree of denaturation and extraction of the DNA. Owing to the complexity of technical aspects such as acid, alkali, or hot salt treatment time, and age of slides, it was not easy to yield identical results in all cells. Best quality was obtained when one- or two-day old slides in saturated 5% barium hydroxide solution were treated at 40°C for 90-120 sec.

Discussion

To date, around 310 karyotypes of birds have been published but only around 20 karyotypes have been characterized with modern chromosome banding techniques (6). But the karyotype and banding patterns of Chinese button-quail have not been previously reported.

In the cell of Chinese button-quail, the chromosomes were estimated to be $2n=84$. This number is slightly greater than the mean number of chromosomes of 234 avian species ($2n=80$) studied by Tegelström and Ryttman (7). And the largest chromosome of this species is submetacentric. This situation is found in about 30% of avian species karyotypes investigated (7). The karyotypes of avian species often display the subdivision into "macro-" and "microchromosomes" (1). However, in this species, the separation between the two categories was not clear-cut.

It is difficult to distinguish, by the means of conventional Giemsa staining,

5th and Z chromosome pairs. The G-banding technique has improved the possibility of chromosome identification. The G-banding technique of bird chromosomes, particularly when they consist of morphological similar shape and size, is very convenient for identifying the homologous larger chromosomes including Z chromosomes.

Identification of an avian W chromosome has always been more difficult than that of a mammalian Y chromosome because often it is probably one of the microchromosomes. Since the W chromosome of the Chinese button-quail is strongly C-band positive, the C-banding technique in this species makes it easy to identify the W chromosome and determine sex. This characteristic feature of the W chromosome is in accordance with what has been found by several investigators (8–11). In this monotypic avian species at an early stage of life, the identification of bird sexes by the presence of the heterochromatic W chromosomes has a practical use.

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