High Resolution G-banding Chromosomes of Japanese Quail (Coturnix coturnix japonica)

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High Resolution G-banding Chromosomes of Japanese Quail (*Coturnix coturnix japonica*)

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Summary

The high resolution G-banding patterns of Japanese quail (*Coturnix coturnix japonica*) were analyzed by using a technique combining Ethidium Bromide (EB) pretreatment and trypsin-Giemsa staining. The method yielded a high frequency of various early mitotic stages and the prometaphase G-banding patterns showed distinctive and consistent bands on the six pairs of macrochromosomes including the Z chromosomes and the larger microchromosomes. The EB-treated lymphocyte culture may be not only a simple but also a reliable technique for detailed analysis of chromosomes in Japanese quail.

Recently, the high resolution G-banding technique has been developed to obtain a large number of late prophase or prometaphase cells with high elongated chromosomes. The characterization of these G-banding patterns of human chromosomes has proved to be highly useful for cytogenetic analyses (1-2). Relatively little progress has been made in applying prometaphase G-banding to other mammalian and avian species (3). In this paper, the high resolution G-banding technique was applied to the study of chromosomes in Japanese quail (*Coturnix coturnix japonica*).

Materials and Methods

The peripheral blood samples of Japanese quail were drawn by sterile, heparinized syringes. Chromosome preparations for this study were obtained from two methods, leukocyte culture and whole blood culture. For leukocyte culture, one ml of whole blood was layered over one ml of Ficoll-Conray density gradients in a siliconized sterile tube and centrifuged at 1,550 rpm for 20 min at

room temperature. The leukocytes at the interface of erythrocytes and Ficoll-Conray mixture were collected by a siliconized pasteur pipette and rinsed 3 times in Hank's balanced salt solution. The leukocytes were cultured in 5 ml RPMI 1640 medium supplemented with 10% fetal calf serum, 0.1 ml phytohemagglutinin-P (Difco), penicillin and streptomycin at pH 7.2 and 39°C for 72 hr. Whole blood culture was made adding 0.1-0.2 ml peripheral blood to the same medium and further processings were done in a similar way. The ethidum bromide (EB, 5-10 μg/ml) and colcemid (0.04 μg/ml) were added for 2 hr prior to harvest. The cells were submitted to a hypotonic shock with 0.075 M KCl at 37°C for 20 min and fixed in several changes of methanol-acetic acid (3 : 1) mixture. Chromosome spreads were prepared by the standard procedures and flame dried. For G bands, the slides were stored at 37°C for three days before treatment and stained according to trypsin-Giemsa technique of Wang and Fedoroff (4) with a slight modification. The treatment time in a solution of 0.05% trypsin (Difco 1 : 250) in phosphate-buffered saline (without Ca^{++} or Mg^{++}) was 30-45 sec at room temperature. After this treatment the slides were immediately rinsed in tap water and stained for 5 min with 5% Giemsa in 0.01 M phosphate buffer (pH 7.2).

Results and Discussion

By the whole blood culture of Japanese quail, it was difficult to obtain satisfactory numbers of mitotic cells because of mitotic inhibitor of agglutination of erythrocytes. In addition, on the observation under a light microscope, the chromosome spreads were sometimes disturbed by the nuclei from erythrocytes of Japanese quail. On the other hand, from the leukocyte culture many chromosome spreads of high quality were obtained. For these reasons, it appears that the culture technique, using lymphocytes separated by the Ficoll-Conray method before culture is superior to whole blood culture technique.

This EB-pretreated technique resulted in a higher yield frequency (15-30%) of various early mitotic stages in the total number of mitotic cells than in the colchicine only technique (below 5%) because of the inhibitory effect of EB on mitotic chromosome condensation. The EB-pretreated chromosomes of Japanese quail were arranged in karyotypes for comparisons (Fig. 1). The chromosome number of Japanese quail was $2n=78$. The karyotype consisted of 6 pairs of macrochromosomes including the Z chromosomes and 33 pairs of microchromosomes. The Z chromosome ranked fourth in size with metacentric shape. The six larger pairs of Japanese quail chromosomes and nearly the first 10-20 pairs of the larger microchromosomes could be identified on the basis of EB treated chromosomes. But the smaller chromosomes were not able to be classified morphologically because they seemed only dots. More detailed studies are necessary to exactly assess the shape of smaller microchromosomes by using prophase or late prophase preparations.

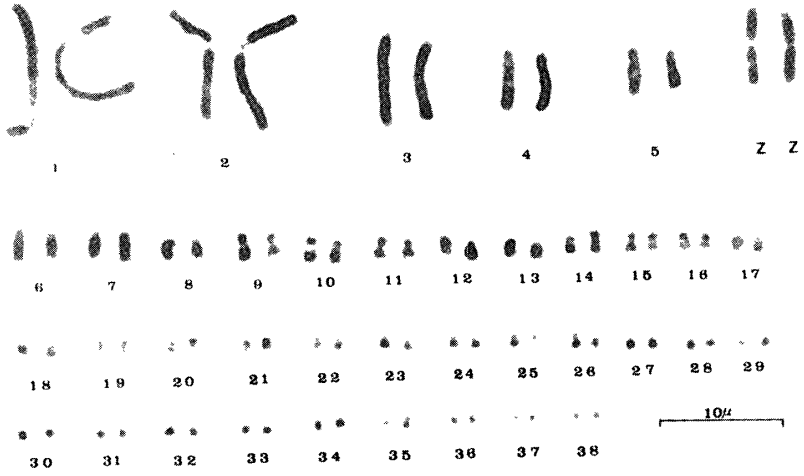

FIG. 1. The EB treated karyotype of Japanese quail (Coturnix coturnix japonica)

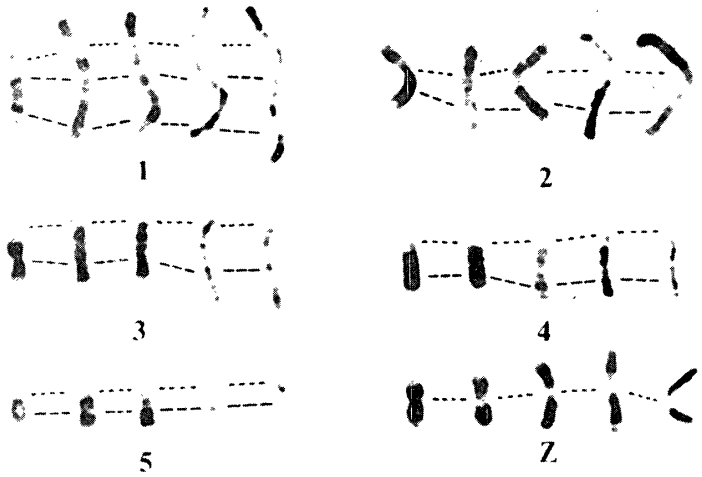

FIG. 2. High resolution banding of macrochromosomes including Z chromosomes

The high resolution G-banded macrochromosomes of Japanese quail were shown to compare the staining patterns from prophase to mid-metaphase (Fig. 2). In prometaphase the trypsin G-banding treatment produced distinctive and consistent bands on the macrochromosomes and larger microchromosomes, and it was possible to identify their chromosomes. As prometaphase G-banding patterns give more accurate, detailed information about the breakpoint or identification of structural changes such as deficiencies, inversions or translocations, the high resolution G-banding method by EB treatment and trypsin-Gimesa staining, which is simple and reliable, is highly useful for cytogenetic analysis of Japanese quail.

There were a greater number bands in the prophase chromosomes but poor

differentiation of bands as compared to the chromosomes of later mitotic stages. The trypsin G-banding technique produced a dark band at the distal end of the longer arm of the Z chromosome and a distinct light band at the distal end of the other arm. The W chromosome of the Japanese quail was the smaller submetacentric chromosome (7-9th).

The similarity of karyotypes of closely related avian species has been denonstrated (5-6). And this similarity has been confirmed by modern chromosome banding techniques (7-8). It may be of interest to determine the degree of chromosomal homology among Meleagris, Gallus and Phasianus by using the high resolution G-banding technique in order to study the evolutional relationship of the order Galliforms. And also further studies on evolution of these species will have to be done, utilizing contemporary methods of molecular genetics (10-11), because DNA changes such as gene mutations or DNA sequencing in animals are usually not detected by using a high resolution G-banding.

Acknowledgements

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