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日本産메추리(*Coturnix coturnix japonica*)의 染色體 多型現象

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Chromosomal Polymorphism of Japanese Quail(*Coturnix coturnix japonica*)

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SUMMARY

Chromosomal polymorphism involving constitutive heterochromatin has been reported in man, pigs, mouse, horse, chicken and so on. The chromosomal polymorphism of Japanese quail which includes constitutive heterochromatin as well the chromosomes without banding treatment has now been found. Through the use of a general technique that permits visualization of chromosome morphology and heterochromatin, three chromosomal variants have been found among birds ; +/+ homozygous from, +/− heterozygous form and −/− homozygous form in chromosome 4. This variants appear to be common in the randombred population and stably inherited in a Mendelian fashion. These results suggest that the variants would be useful as chromosomal markers for various types of cytogenetic studies.

I. INTRODUCTION

Compared with the number and diversity of types of chromosomal polymorphisms that have been noted in the primates, particularly in man(Craig-Holmes and Shaw, 1971 ; Craig-Holmes et al., 1973 ; 1975), relatively few have thus far been reported in animals. Variations in length and other features of the Y chromosome constitute one exception. The occurrence of centric fusions in a number of breeds of cattle and a few breeds of sheep is another.

Many studies have been carried out on avian chromosomes. However banding techniques have been applied to only a limited number of species. The studies of C-banding patterns of *Coturnix coturnix* in particular were very few. C-banding shows the location of constitutive heterochromatin which consists of highly repetitive DNA (Hsu, 1975). Polymorphisms in size of C-bands have been found in pigs(Christensen and Smedegard, 1978 ; 1979), mouse(Forejt, 1973 ; Baverstock et al., 1977), horse(Buckland et al., 1976), chicken(Pollock and Fechheimer, 1981 ; Ohh et al., 1990) and quail (Sasaki and Nishida, 1980). But compared to the large number of polymorphisms that have been found in man, the number and variety of types in any one class of livestock remains very small. The reason of this is because concerted efforts to find more have not been made.

To the study of chromosomal polymorphism, this study focuses on the finding of morphological variants and the indentification of C-band variants. According to identify the variant patterns, the chromosomal markers

also will be determined.

II. MATERIALS AND METHODS

All animals were randomly selected, housed and cared for in the facilities maintained by Poultry Science Department at OARDC(Ohio Agricultural Research Development Center) during experimental periods. To analysis, 100 samples of whole blood(1㎖/bird) were transferred.

The methodology of chromosome analysis is as follows,

1. Chromosome Analysis from Leukocyte Culture

The procedure used to prepare chromosome spreads from leukocyte culture is that of Sohn et al.(1990)

1) Culturing

A blood sample of about 1㎖ is drawn from the jugular vein. This blood transfer to 3㎖ centrifuge tube and then spin at 1550 rpm for 20 minutes. Buffy coat is only picked up and injected into a culture tube which contains 5㎖ RPMI 1640, 0.5㎖ ml Fetal calf serum, 0.1㎖ Pokeweed mitogen, 0.05㎖ heparin and 100 IU of penicillin and 100 μg of streptomycin per ml.

Incubate the culture for 72 hours, undisturbed at 41 ℃. Two hours prior to harvesting inject 0.05㎖ of 0.2% colchicine into culture.

2) Harvesting

The culture contents spin and then remove supernatant fluid and add about 1㎖ of hypotonic solution(0.3 % sodium citrate+calf serum, 6 : 1). After centrifugation, add freshly made fixative(3 : 1, methanol and acetic acid) to each tube. This fixative treatment is repeated 3 times.

3) Slide preparation and staining

A clean slide is prepared in distilled cold water. Five drops of the suspension are dropped onto the slide. The slide is placed in room temperature and air dried. When the slide is completely dried, stain with a solution of Giemsa stain diluted in Wright's buffer(1 : 20) for 10 minutes.

2. C-banding Analysis

A good C-band is obtainable after the slides with fixed cells are kept at room temperature for two weeks to month. The C-banding technique in used is that of Sumner(1972) with slightly modifications.

Slides are immersed in 0.2N HCl for 1 hour at room temperature, rinsed with distilled water and placed in a freshly prepared 5% aqueous solution of barium hydroxide octahydrate at 40~42℃ for 2 minutes. The slides are then dipped quickly into a 0.05N HCl solution 10 times, and then thoroughly rinsed with several changes of distilled water. After rinsing, the slides are incubated for 1 hour in 2XSSC(0.3M sodium chloride+0.03M trisodium citrate) at 60℃, rinsed with several changes of distilled water and allowed to air-dry. Slides are then stained in Giemsa solution(1 : 50 Wright's buffer diluted) for 1.5 hours. After thorough rinsing with distilled water, slides are air dried and cover slips are affixed to them using Permount.

III. RESULTS AND DISCUSSION

To identify the chromosomal variants of Japanese quail, the 8 largest autosomes and the sex chromosomes are observed and analyzed. The analysis indicated that chromosome 2 and the Z chromosome are metacentric, chromosome 1 and chromosome 8 are submetacentric whereas chromosomes 3, 4, 5, 6, 7 and the W chromosome are acrocentric. The morphology between homologues in each chromosomes is almost same pattern and highly consistent on the cells. Due to the presence of p-arm, however, the homologues of chromosome 4 show considerable dissimilarity in it's type. According to the morphological variants of chromosome 4, the representative karyotypes of male and female cells are shown in Fig. 1-A, B, C.

A total 92 C-banded metaphase spread from leukocyte cultures which are used in normal karyotype anal-

ysis are analyzed. All of the macrochromosomes examined have at least one C−band that is detected at a frequency about 100%. However, some variability of C-banding is observed among individuals and among cells from the same individuals. The most consistently detectable C-band in the macrochromosomes is at the centromere region except the Z chromosome. The centromere region of the Z chromosome is C-band negative. But the longer q-arm of the Z chromosome consistently displays an interstitial C-band usually, the p-arm also shows a smaller interstitial C-band. The W chromosome is entirely C-band positive. Most of the chro-

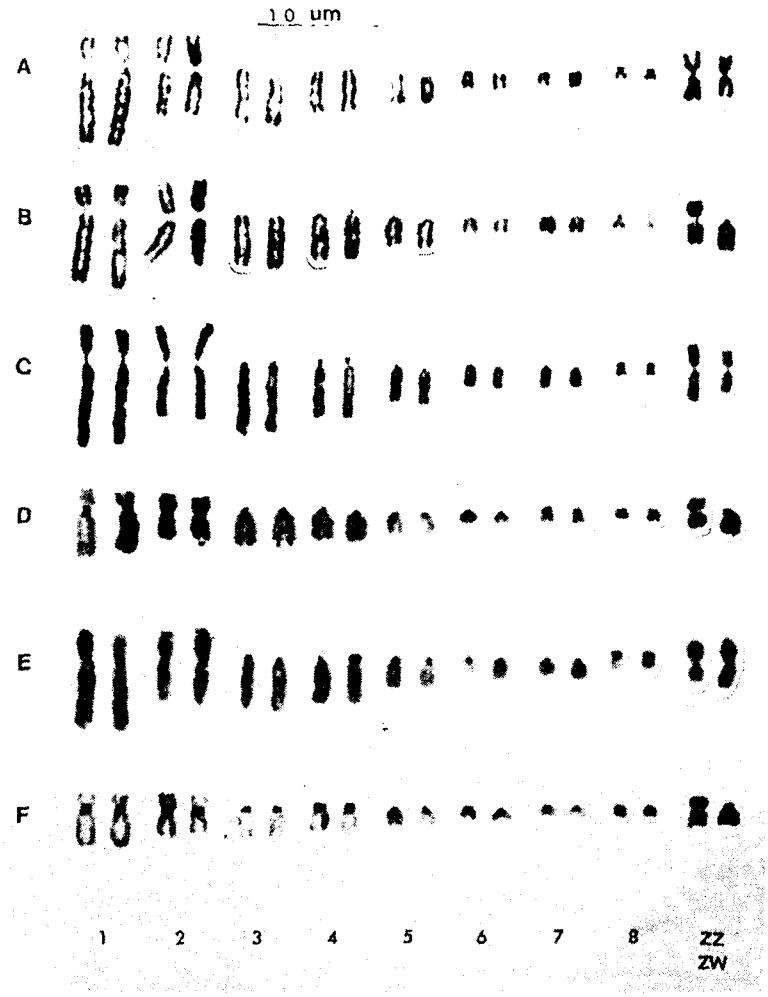

Fig. 1. The partial karyotypes of chromosomal variants in Japanese quail : A-C ; morphological variants, D-F ; C-banded variants,
A ; the karyotype with −/− homozygous form of chromosome 4, B ; the karyotype with +/− heterozygous form of chromosome 4, C ; the karyotype with +/+ homozygous form of chromosome 4, D ; C-banded karyotype from the A cell(−/− homozygous form), E ; C-banded karyotype from the B cell(+/− heterozygous form), F ; C-banded karyotype from the C cell(+/+ homozygous form).

mosome analyzed show a high correspondence of C-band patterns between homologues. However, the homologues of chromosome 4 show considerable dissimilarity in C-band patterns. The representative C-band variants karyotypes of male and female cells are shown in Fig. 1−D, E, F.

The different forms of chromosome 4 variants are arranged on Fig. 2. One form of chromosome 4, designated as the "−/−" homozygous form, shows no short arms(Fig. 2−A) and small C-bands at the centromere regions in the homologues(Fig. 2−D). Another form, designated as the "+/−" heterozygous form, is possessed of only a short arm(Fig. 2−B) and only a very prominent C-banded block which including centromere region and entirely short arm(Fig. 2−E). The other form, designated as the "+/+" homozygous form, displays both short arms(Fig. 2−C) and both prominent C-banded blocks at centromeres and p-arm regions in the homologues(Fig. 2−F). These forms are consistent patterns among individuals and among cells from same individuals.

The C-banding patterns that are observed generally agree with the relatively fewer observations made by Comings and Mattoccia(1972), Sasaki and Nishida(19

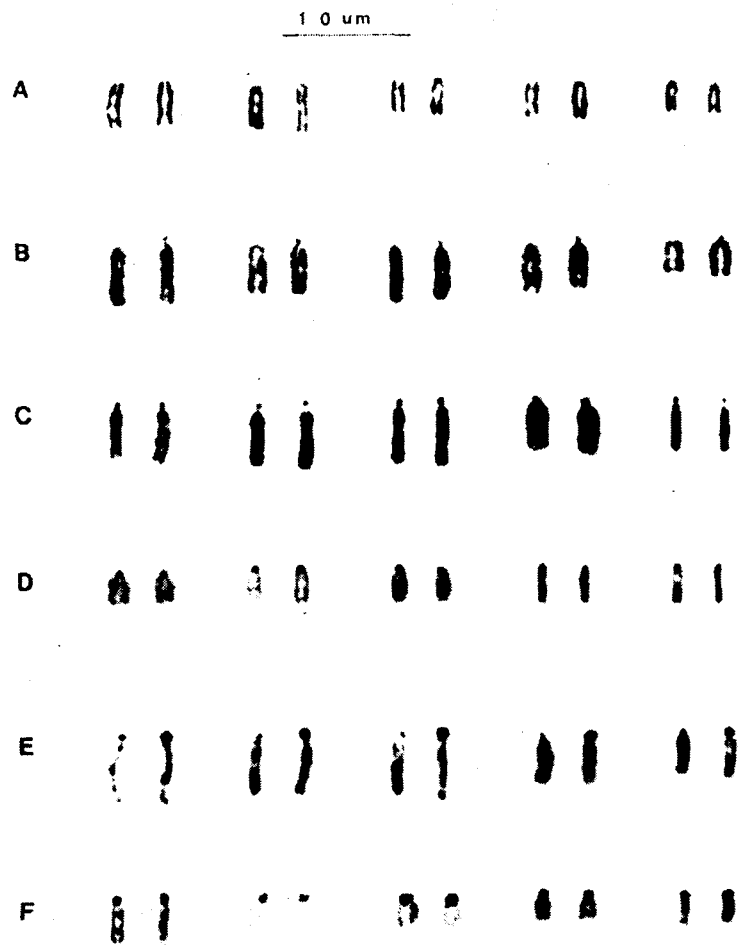

Fig. 2. The arrangement of number 4 Chromosomal variants ;
A ; −/− homozygous form, B ; +/− heterozygous form, C ; +/+ homozygous form, D ; C-banded −/− homozygous form, E ; C-banded +/− heterozygous form, F ; C-banded −/− homozygous form.

80), Stock and Bunch(1982), and Cesar(1990). However, the present study has clearly demonstrated the existence of morphological variants and the occurrence of C-band variants of Chromosome 4. The results of this study as well as those of previous studies indicate that the 8 largest autosomes in the quail exhibit more consistent C-band than those in the chicken(Pollock and Fechheimer, 1981 ; Ohh et al., 1990). C-banding studies of both the chicken and the quail have shown that microchromosomes are not entirely made up of constitutive heterochromatin. Most of the microchromosomes display prominent C-bands only at the pericentromeric region, indicating that the other portions may be genetically active.

The observed variability of C-banding among birds can be related to the factors which are believed to be important in inducing C-bands. Comings(1978) insists that the mechanism of C-banding is the extraction of non-C-band DNA and the retention of C-band DNA on the chromosome. Therefore, the Giemsa stain simply forms a complex with the remaining DNA. In general, the constitutive heterochromatin consists usually highly repetitive DNA which remains condensed during interphase and replicates during late S, may be GC-rich, neutral or AT-rich and frequently may be highly methylated. Accordingly this fact, this results show the p-arm of chromosome 4 consists of almost constitutive heterochromatin. The relative distribution of the chromosomal variants among birds indicate that they are stably inherited in a Mendelian fashion. In man, the mode of inheritance of chromosomal heteromorphism is Mendelian, but a few exceptions have been recorded (Verma, 1988).

There is a recent report from Cesar(1990) that the C-band variants of chromosome 4 and the Z chromosome are common in the randombred population of Japanese quail. Takashima et al. (1989) also reported the polymorphic C-banding was seen on the third, fourth and Z chromosomes of Japanese quail. However, these reports are limited in the heterochromatic variants and the specific descriptions of those polymorphisms are not established yet.

In this results, the observed chromosomal variants which include C-band variants and morphological variants should be useful as chromosomal markers. In order to maximize the use of the chromosomal variants in future research, work should be undertaken to develop stocks of birds which are homozygous for each variant.

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Ⅳ. 摘　要

Constitutive heterochromatin의 染色體 多型現象에 대해 사람을 비롯하여 돼지, 생쥐, 말, 닭 등에서 보고된 바 있다. 本 硏究에서는 日本産메추리의 C-band 多型體 뿐만 아니라 염색체의 形態的 多型體를 발견하여 이의 多型現象을 밝혔다. 일반적인 염색체 분석방법 및 C-banding 방법으로서 밝힌 3가지 염색체 多型體는 4번 염색체 +/− 同型體, +/− 異型體 및 −/− 同型體이다. 이와 같은 多型體들은 무작위 집단 내에서 일반적이고 지속적으로 나타나며 Mendel 법칙에 따른 유전양상을 보인다. 따라서 본 연구에서 밝힌 染色體 多型體들은 여러 細胞遺傳學的 연구에 表識因子(chromosome marker)로서 유용하게 이용되어질 수 있을 것으로 생각된다.

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