Cytogenetical Analysis of the Somatic and Germ Cells in Ducks

1. A Cell Culture Method for the Analysis of Chromosome Karyotypes

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Summary

A modified cell culture method has been developed and used for preparation of chromosomes from Muscovy, Tsaiya duck and their intergeneric hybrid blood. Higher quality preparations than previous investigations can be made. It seems that conditions described as regards materials and methods are suitable for chromosome preparation of ducks.

The metaphase chromosomes in mitotic cells have also been analyzed. The diploid chromosome number with few exceptions in Tsaiya duck, Muscovy and their F_1 hybrid, mule duck, was 78. The female heterogamety of ZZ/ZW type in avian was found in these ducks. Sex chromosomes of these ducks were all pair 4, and W chromosome was smaller than Z chromosome. Pair 1 of Tsaiya duck and Muscovy chromosomes was submetacentric, and pair 2 of Muscovy chromosomes was submetacentric too. Pair 2 of Tsaiya duck chromosomes was metacentric. Pair 3 was telocentric in Tsaiya duck but acrocentric in Muscovy. All of the pairs 4 - 10 in both ducks other than the Z chromosome of

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Tsaiya duck was submetacentric, were telocentric. Pairs 1 - 5 in the hybrid were distinguished by which chromosome of each pair was derived from which parent, Tsaiya duck or Muscovy.

Introduction

In general, it is usually difficult to get many progeny from intergeneric hybridization, but a type of infertile hybrid, mule duck, can be produced by hybridization of Muscovy (*Cairina moschata*) with Tsaiya duck (*Anas platyrhynchos var. domestica*) and is raised for meat production in Taiwan. High fertility and hatchability can be obtained from this mating system[16,9,14] . Hoffmann[8] indicated that an efficient AI technique was developed in Taiwan, and in 1983, 30 million mule ducks were produced, many hybrid eggs were shipped by air from Taiwan for overseas trade after 28 days of incubation, which gives ample time for them to hatch after arrival at their destinations.

There has been interest in the chromosome investigation of the hybrid and its parents. Yamashina[17] conducted an experiment, based upon histological method, to investigate the chromosomes of these ducks and reported that there may sometimes exist a slight difference in morphology of chromosomes among these ducks, but sex chromosomes were considered indistinguishable. Chang *et al.*[2] compared the chromosome arm ratios in these ducks, since good quality chromosomes had not been obtained, some of their conclusions seem to need to be confirmed.

A modified cell culture method for duck chromosomes was developed in this experiment. Meanwhile, the metaphase chromosomes in mitotic cells have also been analyzed.

Materials and Methods

Twenty Tsaiya ducks (10♀ +10♂), 10 Muscovy ducks (5♀ +5♂) and 20

their F_1 hybrids, mule ducks, (10♀ + 10♂) were reared for this studies. The procedure subsequently described is a modification of that suggested by Vincent *et al.*[15] and Shoffner *et al.*[12] . Five ml of blood from duck vein were collected into an heparinized tube and mixed well. After sitting for 30 minutes at room temperature, the tube was spun at 300 g in a centrifuge for 10 minutes. The plasma, in which the leucocytes were suspended, was transfered in 1 ml portion to culture dish containing 5 ml of RPMI 1640 medium (Gibco) supplemented with 15% fetal bovine serum (Gibco), 1% antibiotic/antimycotic 100x mixture (Microbiological Associates), and 1.5% phytohemagglutinin M (Difco). The culture was incubated for 70 hours at 37°C in incubator (Quene). Mitotic cells were arrested for 2 hours with 10 μg/ml colchicine(Sigma). Cells were collected by centrifugation at 300 g for 10 minutes. The cell pellet was resuspended in 0.45% sodium citrate hypotonic solution for 10 minutes at room temperature. After which the cells were collected by centrifugation, fixed and resuspended in methnol/acetic acid (3:1) in an ice box. Slide preparations were made by 2 drops of cool cell suspension from a height of approximately 1 M on each slide set at a 30 to 45 degree angle. The slides thus prepared were rapidly dried to enhance the spreading and flattening of the cells. The slides were stained in Giemsa for 10 minutes. This stain consisted of 2 ml of Giemsa (Merck) and 98 ml 0.9% saline. After staining the slides were rinsed in distilled water and air dried, then mounted in balsam.

Suitable cells were photographed, and karyotypes for both sexes were constructed. The chromosome models in karyotypes were measured, and proportional lengths were calculated. The method of classification and terminology used to designate the different chromosome were adapted from the report of Chang *et al.*[2] . The arm ratios of chromosomes were also analyzed statistically[13] .

Results and Discussion

Different culture conditions for the chromosome analysis in various species had been proposed, such as fowl[12] ; goat and sheep[1, 6] ; pig[11] ; cattle[7] ; cat[4] and mouse[5] . Since the gravity and osmotic pressure of leucocyte, the temperature of culture, the havesting time and the hypotonic treatment condition in various species are sometime different, therefore, how to get the optimal conditions for chromosome preparation is important. In our experiment, high frequency and good spreading of metaphase chromosomes of ducks could be observed. It seems that conditions described as regards materials and methods are suitable for chromosome preparation of ducks.

The karyotypes of metaphase spreading from the cultured cells of Tsaiya ducks, Muscovy ducks and mule ducks printed at the same

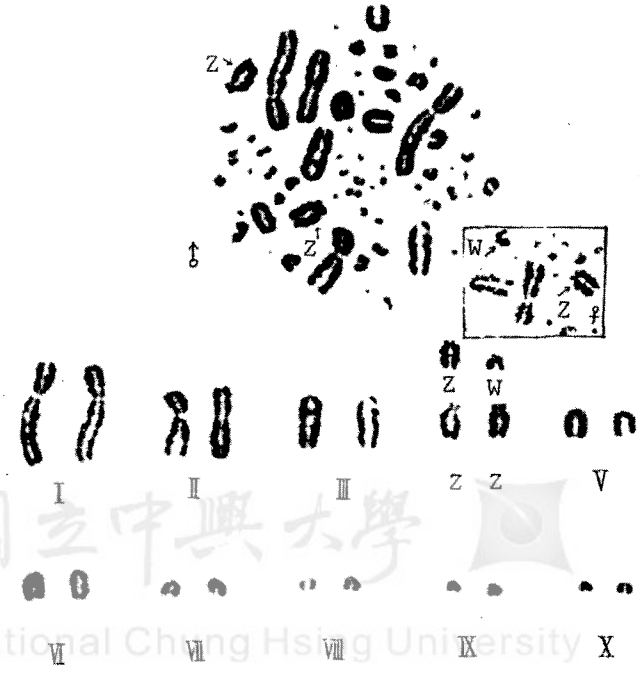

Fig. 1. Karyotype of the first 10 pairs of chromosomes in the Tsaiya ducks (x1000).

Z : Z chromosome W : W chromosome

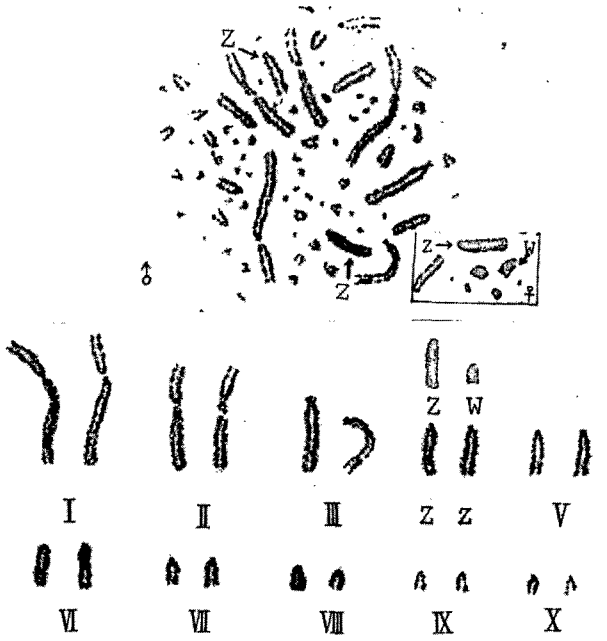

Fig. 2. Karyotype of the first 10 pairs of chromosomes in the Muscovy ducks (x1000).

Z : Z chromosome　　W : W chromosome

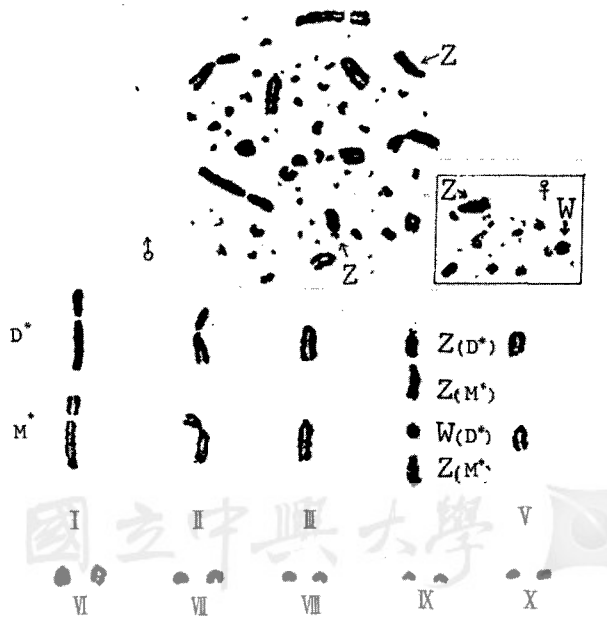

Fig. 3. Metaphase and serial alignments of the first 10 pairs of chromosomes in the mule ducks (x1000).

Z : Z chromosome　　W : W chromosome

D : Tsaiya duck　　M : Muscovy

magnification (x1000) are shown in Figs. 1, 2 and 3. The karyotype of birds consists of a number of macrochromosomes and in addition a large number of microchromosomes[10]. Both macro- and microchromosomes are also present in these ducks (Figs. 1, 2 & 3).

The chromosome arms of the pairs 1 - 4 of non-telocentric chromosomes in the Tsaiya ducks and Muscovy ducks were measured from the centromere to its end. These measurements were then averaged and an arm ratio for each pair obtained by dividing the length of its short arm by its longer arm. The following four groups according to the arm ratios had been defined by Chang *et al.*[2] : metacentric with an arm ratio of 1.0 - 0.6; submetacentric with an arm ratio greater than 0.2 but less than 0.6; acrocentric with measurable short arm and an arm ratio less than 0.2; and telocentric with a terminal centromere or immeasurable short arm. The classification of duck chromosomes according to the arm ratios is shown in Table 1.

Pair 1 of Muscovy chromosomes was submetacentric (Table 1). The result confirmed previous work of Chang *et al.*[2] . Pair 2 of Tsaiya duck chromosomes was metacentric (Table 1). This was also in agreement with previous finding by them. However, they indicated that pair 1 of Tsaiya duck chromosomes and pair 2 in Muscovy were metacentric. Our observations conflicted with those of their report (Table 1). The arm ratio of the pair 1 between the Tsaiya duck and Muscovy existed a significant difference ($P < 0.05$), it was also found in the pair 2. Low coefficients of variance of the arm ratios of pair 1 and 2 in both these ducks were obtained (Table 1). These results could be helpful in distinguishing in their hybrid, which chromosome of each pair is derived from which parent, the Tsaiya (♀) or Muscovy (♂) (Fig. 3). Pair 3 was telocentric in Tsaiya duck but acrocentric in Muscovy. All of the pair 4-10 in both ducks other than Z chromosome of Tsaiya duck was submetacentric, were telocentric. But the Z chromosome and the pair 5 in Muscovy were nearly acrocentric (Figs. 1, 2 & 3; Table 1).

The female heterogamety of the ZZ/ZW type was found in these

Table 1. Arm ratios and classification of chromosomes in the Tsaiya duck and the Muscovy.

Material	Exam. cell no.	Pair of chromosome 1 Mean ± SD	1 C.V.* %	2 Mean ± SD	2 C.V. %	3 Mean ± SD	3 C.V. %	4 Mean ± SD	4 C.V. %
Tsaiya duck	20	0.53± 0.05[a]** (SM)***	9.4	0.66± 0.06[a] (M)	9.1	- (T)	-	0.24± 0.02 (SM)	8.3
Muscovy	10	0.43± 0.03[b] (SM)	7.0	0.52± 0.04[b] (SM)	7.7	0.12± 0.02 (A)	16.7	- - (T)	- -

* C.V. : coefficient of variance.

** The values with different superscripts within the same column differ significantly ($p < 0.05$) from each other.

*** SM : submetacentric ; M : metacentric ; T : telocentric ; A : acrocentric.

Table 2. The distribution of the duck cells with various chromosome numbers.

Material	Chromosome number < 77	77	78	79	> 79	Total of cells	% of cells in modal class
Tsaiya duck	8	2	56	2	7	75	75
Muscovy	7	1	42	2	1	53	79
Mule duck	8	3	52	4	4	71	73

ducks, i.e., a ZZ complex was present in the male and a ZW complex in the female. Sex chromosomes of these ducks were all pair 4 (Figs. 1, 2 & 3). Z and W chromosome could be identified. W chromosome was smaller than Z chromosome. Furthermore, the Z chromosome of the Tsaiya duck was submetacentric but telocentric in Muscovy (Figs. 1 & 2). The female (ZW) and the male (ZZ) mule ducks were sterile.

Cheng & Wu[3] reported the diploid chromosome numbers of Muscovy and Liancheng duck were 78. In our experiment, the distribution of cells with various chromosome numbers was summaried in Table 2. Although the diploid chromosome numbers ranged from < 77 to > 79, there were 78 in 75%, 79% and 73% of the Tsaiya duck, the Muscovy and the mule duck cells, respectively. In other words, the diploid number with few exceptions in these ducks was 78.

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鴨體細胞及生殖細胞之細胞遺傳學分析

1. 細胞培養法分析染色體核型

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中文摘要

本研究以改良之細胞培養法自番鴨、菜鴨及其屬間雜交子代之血液來製備染色體，製得之染色體品質較之前人所得者爲佳。顯示如材料與方法所述之條件適合於鴨染色體之製備。

分析有絲分裂期間細胞之中期染色體核型，顯示菜鴨、番鴨及其子一代雜種—土番鴨之二倍體染色體數目，除少數外，均爲 78。在家禽中，ZZ/ZW 型母性異配之現象，各鴨亦復如此。這三種鴨之性染色體均爲第 4 對，W 染色體比Z 染色體爲小。菜鴨及番鴨之第一對染色體爲次中位中節染色體，番鴨之第 2 對染色體亦同。菜鴨之第 2 對爲中位中節染色體，其第 3 對則爲末端中節染色體，但番鴨者爲近端中節染色體。此兩種鴨之第 4～10 對之所有染色體中，除了菜鴨之Z染色體爲次中位中節染色體外，餘均爲末端中節染色體。本研究並將雜種之第 1～5 對之各對中之染色體加以區別其來自菜鴨或番鴨親代。

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