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GENE EXPRESSION OF TRIPLOIDY IN SIX ADULT INTERSEXUAL CHICKENS

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The peripheral blood of 6 adult intersexual chickens was examined chromosomally. The cases were chromosomal triploidy (3n) with the ZZW sex chromosomes. Erythrocyte size of triploid chickens was about 1.5 times that of normal diploid cell size. The difference between triploid cell size and diploid cell size was significant ($P<0.001$). The analyses of protein and estrase activity revealed that the triploidy had similar levels of gene expression to that of the diploid chickens. Macroscopically, three cases had a lateral streak gonad and two cases had bilateral gonads (a streak gonad on the left side and a small testis-like gonad on the right side). In the microscopical observations, all cases showed seminiferous tubeles without a spermatogenetic figure.

Key words: chicken intersexes, chromosomal triploidy, level of protein and isozyme, streak gonad

In general, intersexes among chickens appear at a low frequency of about 0.05% in normal commercial laying flocks (ABDEL-HAMEED, 1972). Surprisingly, however, in certain laying flocks, the frequency of intersex is 30–80 times higher than the general trend.[1)] Chromosomal studies have revealed that the characteristic of intersex in chickens is 3 sets of chromosomes (triploidy).[1–3, 12, 13, 18)] In order to elucidate the mechanisms of intersex, chromosomal analysis, measurements of cell size, protein and isozyme analysis and macro- and microscopical observations of gonads were carried out in 6 intersexual chickens. In this paper, the gene expression in triploid chickens is described.

MATERIALS AND METHODS

A large number of intersexual chickens were found in some laying flocks of chickens examined by us in Japan. The intersexes were initially sexed as female chickens at hatching. But when they were about 140 days old, they gradually showed

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a male-like appearance and did not produce any eggs. The incidence of occurrence was 61/3937 (1.55%) in a certain flock in which an actual occurrence was recorded. Total incidence of occurrence, including that of other flocks, was estimated as 4.03%, as far as the authors could examine.

Six adult intersexes were examined chromosomally by using blood culture methods (BIEDERMAN & LIN, 1982). As a control, 2 males and 2 females from the parent flock, and 5 females from the same flock as the intersexes were examined in the same manner. Interpretations were based on the 9 large types of autosomes plus the sex chromosomes. The lenght and width of both erythrocytes and nuclei were measured in blood smear preparations stained with Giemsa solution from 3 intersexes, and from 2 males and 1 female in the parent flock. The patterns of serum protein bands in 2 intersexes and in 4 normal adult chickens were analyzed by the controlled electrophoresis on polyacrylamide gel (SYUTO et al., 1981). The activity of serum estrase was examined by 8% slab-gel-electrophoresis (MATSUMOTO et al., 1979).

The gonads of intersexes were examined macroscopically at the time of slaughter, and were then fixed with formalin solution, embedded in paraffin and sectioned at 4 μ m. The sections were stained with hematoxylin and eosin and then observed microscopically.

OBSERVATIONS AND DISCUSSION

1) Chromosomal analysis

The results of chromosomal analysis of the intersexes are shown in table 1 and figure 1. In each metaphase plate examined, 10 large types of chromosomes including the sex chromosomes were analyzed. The blood samples of all intersexes showed 3 sets of chromosomes, that is, triploidy. The sex chromosomal consitution was ZZW only. No cases with mosaicism were detected. Two males and 2 females from the parent flock, and 5 females in the same flock as the intersexes had a normal diploid karyotype and no aberrations of chromosomes (tab. 1 & fig. 2a & b). The other

TABLE 1 *Chromosomal analysis in blood*

CASES		CHROMOSOME CONSTITUTION	
6 intersexs		3n,	ZZW*
2 males	from a parent flok	2n,	ZZ*
2 females	from a parent flok	2n,	ZW*
5 females in the same flock as intersexes		2n,	ZW*

Over 30 metaphase plates were counted for each case.

* ZZW, ZZ and ZW show the complement of sex chromosomes.

tissues failed to culture and were not chromosomally analyzed.

Intersexes with chromosomal triploidy were first reported in adult chickens by OHNO et al. (1963). Subsequent studies reported a high relationship between intersexuality and chromosomal triploidy (SARVELLE, 1970 ; ABDEL-HAMEED & SHOFFNER, 1917 ; ZARTMAN & SMITH, 1975). Based on a series of 10 experiments in chicken embryos (FECHHEIMER, 1981), it was observed that 17.7% of triploidy had a ZZW sex chromosomal constitution. FECHHEIMER (1981) described that two-thirds of the triploid embryos with ZZW were caused by diginy ; suppression of the first meiotic division of oogenesis (M I suppression) induced eggs with the ZW sex chromosomes. The remainder were caused by diandry. In our adult intersexes, the possible mechanism of occurrence of triploidy with ZZW may be fertilization of a ZW egg due to M I suppression by a haploid Z sperm. In mammalian species, spontaneous diploid oocytes as the cause of diginic triploidy were found by FUNAKI & MIKAMO (1980).

In all 26 cases of adult triploid chicken examined, only the ZZW sex chromosomal complement was found.[3, 12, 13, 18] There is another interesting point as to why cases with triploidy are born and develop into adults. In human triploidy, the condition appears to be completely lethal (SCHINDLER & MIKAMO, 1970). It seems that triploidy with ZZW sex chromosomes has a genetic mechanism which prevents triploid chickens from the lethal effects.

2) Erythrocytes measurements

Measurements of the length and width of whole cells and nuclei revealed that the erythrocytes of intersexes were significantly larger than those of normal diploid chickens ($P<0.001$, tab. 2, fig. 3a & b). Particularly, the length of erythrocytes in intersexes was about 1.5 times that in normal diploidy.

TABLE 2 *Comparison of dimensions of erythrocytes from 2 adult males and an adult female in a parent flock and from 3 intersexes (mean ± SD)*

CASES	WHOLE CELLS		NUCLEI	
	length	width	length	width
	(μm)	(μm)	(μm)	(μm)
Male & female	10.32 ± 0.778	6.62 ± 0.74	4.98 ± 0.42	3.20 ± 0.23
Intersex	$14.98 \pm 1.48^{***}$	$7.41 \pm 0.97^{***}$	$6.59 \pm 0.62^{***}$	$3.78 \pm 0.34^{***}$

Total of 50 cells were measured each case.
*** $p<0.001$

Comparisons between normal adult chickens and intersexes indicate that the erythrocyte measurements coincide closely with the ploidy level of somatic nuclei (ABDEL-HAMEED et al., 1971). ABDEL-HAMEED (1972) reported that cells in triploidy had a DNA content which was 1.4 times that of diploid chickens, and observed that the differences of cell size were reasonable for the increasing number of chromosomes. Other studies have clarified that cell size measurements are suitable as an index, such as sex chromatin and/or drumstick in mammalian species (HARE & SINGH, 1979), for use in a screening test of intersexes in chickens.

3) Analysis of serum protein and isozyme

The results of serum protein and estrase activity revealed that genetic expression in the triploid chickens was not different in comparison with those of diploidy. Thirty-six to 51 bands of proteins were detected by the controlled electrophoresis in the 2 triploid and 4 diploid chickens from the parent flock (fig. 4). Two groups showed identical electrophoretic patterns of serum protein, except for minor genetic differences in each case. Furthermore, similar levels of serum estrase were detected between triploidy and diploidy (fig. 5).

From the results obtained, it is clear that triploidy does not suppress certain genes that are normally active, nor does it activate other genes that are normally inactivated in the diploid cells. As mentioned earlier, triploid condition appears to be lethal in humans and mice ,[16, 17] although in chickens, it is not. It is suggested that gene expressions on the chromosomes are controlled either by genetic inactivation of one set of chromosomes in the triploid cells, or by other mechanisms which reduce the levels of gene expressions on the 3 sets of chromosomes to the levels of diploidy. ABDEL-HAMEED (1972) reported the possibility that in his cases all 3 sets of chromosomes in triploidy were equally active genetically, from the results of his examination of isoantigens. However, it has not been demonstrated in triploid chickens whether or not 1 set of chromosomes is inactivated. Therefore, further studies are necessary to clarify this point.

4) Macro- and microscopical observations of gonads

Macro- and microscopical findings of the gonads in 5 out of 6 intersex chickens are shown in table 3 and figures 6a, b and 7a, b. In normal female chickens, the right gonad degenerates before hatching, and only the left gonad develops into an ovary. Cases No. 1, 2 and 5 had a gonad in the left side only. However, the gonad was very small and showed underdeveloped streak gonads. Cases No. 3 and 4 had two gonads in both sides. The appearances of the left gonad were similar to those of the former cases, and the right gonads resembled small testes. The oviduct showed an underdeveloped appearance similar to that in prepubertal chicken.

TABLE 3 *Macroscopical findings of gonads in intersexes*

CASE NO.	LEFT GONAD		RIGHT GONAD	
	(cm)	(g)	(cm)	(g)
1	streak gonad 2.2×1.2	0.5		
2	streak gonad 2.4×0.9	0.4		
3	streak gonad 0.7×0.6	0.2	testis-like 1.3×0.6	0.2
4	streak gonad 2.0×1.1	0.3	testis-like 0.9×0.6	0.2
5	streak gonad 2.9×1.1	0.7		

Microscopically, the streak gonads in the left side had different charateristics in comparison with those of other mammalian intersexes. In the ovotestes of intersexual pigs[10] and dogs,[11] two portions, the ovary and the testis, were apparently divided by connective tissues. However, in the left streak gonads of our intersex chickens, some testis-like structures were independently spread all over the gonad. No spermatogenetic figures were detected, and only the sustentacular cells which lined the basement membrane, were observed in the testis-like structure (fig. 8). The other portions of the left gonad had no oocytes. The right gonads of Cases No. 3 and 4 were filled with seminiferous tubeles without spermatogenetic figures. The presence of interstitial cells was not obvious in the gonads. Clear cell nests (tentatively non-pheochrome paraganglia) were obseved frequently in the intra- and extra-gonadal areas of the right and left gonads in all cases (fig. 8). Although the oviduct was distinctly observed, the ductus deferens was not detected.

Intersex in chickens can be induced by ovariectomy (MASUI, 1967). But it seems that the intersexuality of our cases was induced by different effects, perhaps by the disturbance of sex determinant gene(s) expression on the sex chromosomes, because our cases had 3 sets of sex chromosomes, ZZW. According to the H-Y theory of mammals (YAMADA & ISURUGI, 1981), in the female chicken with ZW sex chromosomes, the inducer is produced by a W-linked regulatory gene on the W sex chromosome. The structural gene on autosomes, which is stimulated by the inducer, produces the H-W antigen. Consequently, by the H-W antigen, the female gonad is directed to the ovary. In the male with ZZ sex chromosomes, the repressor is produced by the ZZ sex chromosomes, and it suppresses the structural gene. Therefore, the male gonad is directed to the testis. In the cases with ZZW sex chromosomes, it is conjectured that the repressor derived from 2 Z-linked regulatory genes is not completely suppressed by the inducer from 1 W sex chromosome, but the expression of the H-W antigen is suppressed. Subsequently, a large portion of the gonads is directed to testis-like structures.

During one particular period of our study, a large number of intersexual chickens were discovered in a certain line of laying flocks. Thereafter, no intersexes were detected in the same line by us. It is unknown why so many intersexes with triploidy occurred in that particular line of chicken during that period, and why no new cases have been detected in the same line. Further studies to ascertain the cause of occurrence of triploidy and intersexuality in chickens will be conducted using an experimentally produced model.

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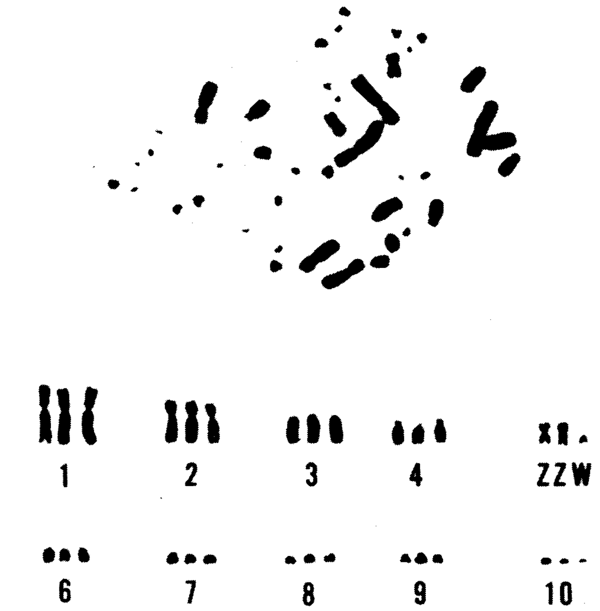
EXPLANATION OF PLATES

PLATE I

Fig. 1	A metaphase (upper) and a karyotype (low) in blood samples from an intersexual chicken show the chromosomal triploidy (3n). In the karyotype, the micro chromosomes were not analyzed.
Figs. 2a & b	A karyotype of a normal diploid female (fig. 2a) and a normal diploid male (fig. 2b).
Figs. 3a & b	Erythrocytes of a diploid chicken (fig. 3a) and a triploid chicken (fig. 3b). Note the differences of cell size. × 570
Fig. 4	Patterns of serum protein by controlled electrophoresis. Nos. 1 & 2 were derived from 2 cases of triploidy ; Nos. 3–6 were derived from 4 cases of diploidy.
Fig. 5	Serum estrase activity by 8% slab-gel-electrophoresis. Analysis was conducted on the same samples as those used for protein analysis.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3
4
ZZW
6
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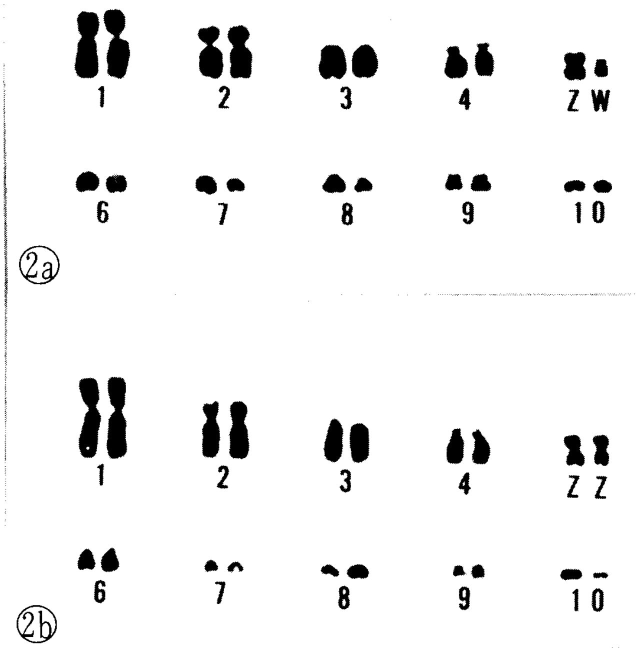
9
10

①


1
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Z W
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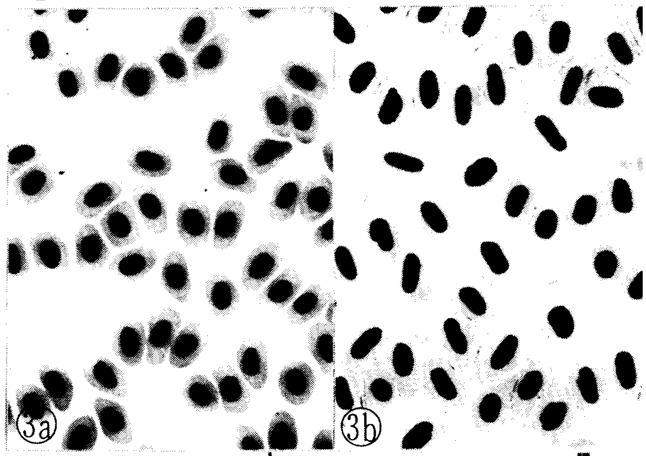
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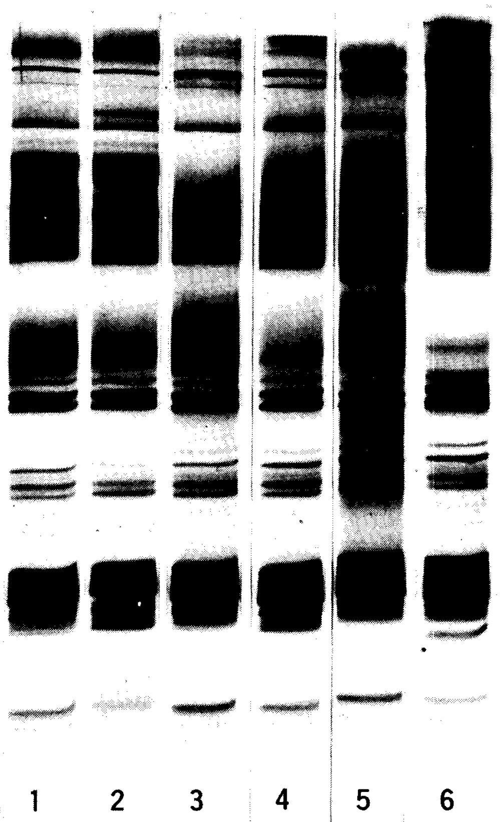
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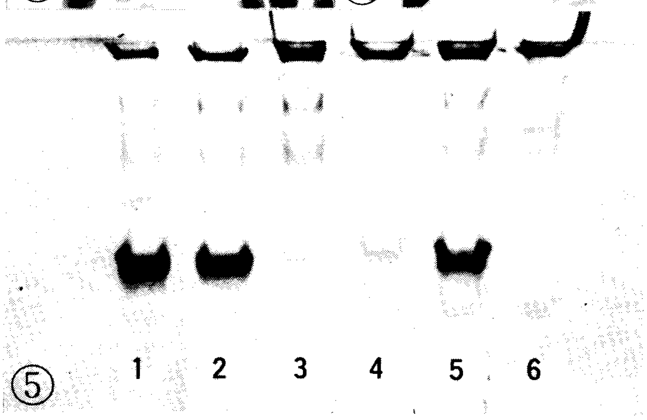
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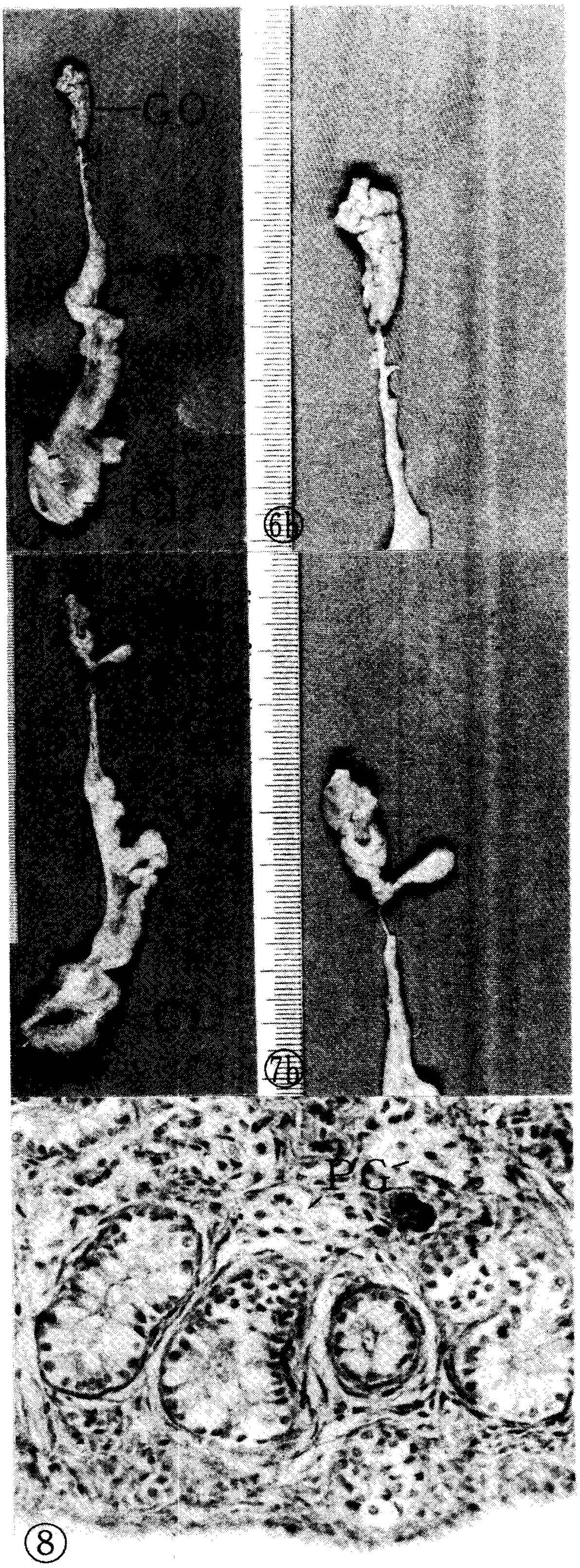
⑤

PLATE Ⅱ

Figs. 6a & b Genital organs of intersex No. 5. Note small streak gonad (shown in fig. 6b). GO : gonad, OV : oviduct, CL : cloaca

Figs. 7a & b Genital organs of intersex No. 4. Note 2 gonads, which are streak gonads, in the left side, and the testis-like gonad in the right side (see fig. 7b).

Fig. 8 Cross section of the streak gonad in case No. 5. In the seminiferous tubeles, only sustentacular cells are observed (H. & E). × 210
PG : non-pheochrome paraganglia-like clear cell nests


GO
6b
7b
PG
8