FREQUENCY OF GIEMSA C-BAND CHROMOSOMES IN THREE INBRED LINES OF CHICKENS

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Summary

Giemsa C-banded mitotic chromosome preparations from White Leghorn, New Hampshire and Rhode Island Red inbred lines were compared for frequency of C-band regions on individual chromosomes. Except for autosomes 3, 6, 8 and 9 and W sex chromosomes, C-banding was extremely variable in other macrochromosomes. No divergence for C-band difference between homologous chromosomes of these lines was detected. Approximately 75% of the mitotic metaphase microchromosomes have recognizable C-band regions with the current technique.
(Key Words: Chicken, C-banding and Heterochromatic Polymorphism)

Introduction

The cytochemical differentiation C-band technique defines specific heterochromatic regions, usually found at the centromeric or telomeric portions of the chromosomes. When the chromosomes are subjected to the acid-base-hot salt in situ treatment, the highly repetitive DNA in the C-band regions is not degraded to the same extent as the DNA in the remainder of the chromosome, hence the differential appearance of the constitutive heterochromatic portion.

The size and location of the C-bands in birds vary from species to species (Stock et al., 1982), and may be polymorphic within a species (Pollock and Fechheimer, 1981; Koop et al., 1983). The reports (Stephos and Arrigi, 1971; Stock, 1974; Stock et al., 1982; Pollock and Fechheimer, 1981; Wooster et al., 1977) have defined the C-band pattern for the larger chromosomes of the chicken. While numerous microchromosomes are largely or wholly heterochromatic following the C-banding treatment, individual chromosomes in this size category are extremely difficult to identify. The W sex chromosome of the heterogametic female is almost wholly heterochromatic and the q-arm telomere region of the Z chromosome has a large C-band which exhibits a size polymorphism (Pollock and Fechheimer, 1981). The centromeric regions of chromosomes 3 and 6 are consistently C-band while the remainder of the macrochromosomes are variable in staining density of the C-band regions.

If C-band polymorphisms exist in chickens other than the one found for the Z-chromosome, one approach to detecting such differences between homologous chromosomes would be to compare genetically divergent population of chickens for appearance of C-band regions. Any C-band polymorphism which might exist between or within breeds could by chance become fixed in one inbred line and not in another. Lines that differ in C-band appearance or morphol ogy, hypothetically would be easier to distinguish than segregating polymorphisms in a heterozygous population.

The objective of this study was to determine if there were C-band polymorphism between homologous chromosomes in three inbred lines of chickens and at the same time secure an estimate of the number of microchromosomes that have definite C-bands.

Materials and Methods

The three inbred lines compared were a highly

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inbred line of White Leghorns (Fx = 95%); a lesser inbred line of Rhode Island Reds (Fx=80%) and a New Hampshire line with Fx=60%. The White Leghorn and Rhode Island Red lines are homozygous at 8 blood group loci and the New Hampshire at 6 of 8 loci. The lines had different origins and differ in blood group loci and other phenotypic traits, such as plumage color, size, reproduction and behavior.

Embryos at twenty-four hours of incubation were the source of mitotic material (Wang and Shoffner, 1974; Bitgood et al., 1982). The C-banding procedure was slightly modified from Sumner (1972) as follows: 1) 0.2 N HCl for 1 hr at room temperature followed by two rinses in nanopure H_2O; 2) 5% $Ba(OH)_2$ for 2 minutes at 41 °C with a rinse in tap H_2O followed by nanopure H_2O; 3) 0.05 N HCl for 15 or 30 seconds at room temperatures; 4) 2X SSC for 1 hr at 60 °C and 5) 4% Giemsa stain in phosphate buffer for one and half hours.

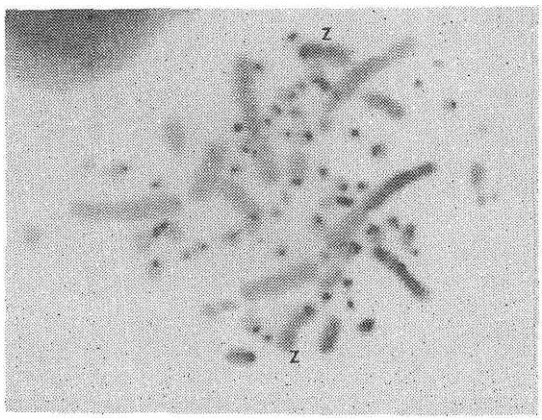

Figure 1. (a) Giemsa C banded chicken male mitotic chromosomes.

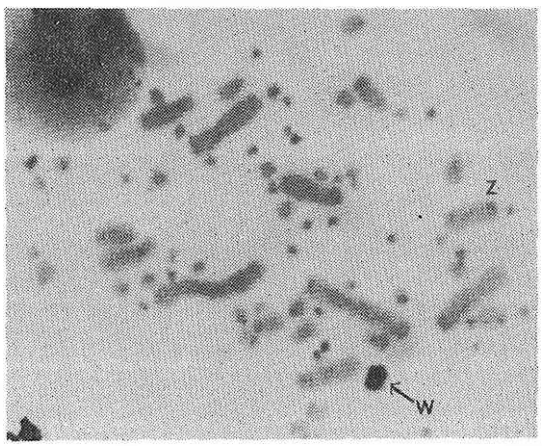

Figure 1. (b) Giemsa C-banded chicken female mitotic chromosomes.

Slides were scanned and 15 well-defined spreads from 6 to 8 embryos for each line were photographed (Leitz Orthomat, 100X, 35 mm camera and Kodak film). Dark room procedure was based on Kodak film recommendation with F-5 paper with prints enlarged to about 200X. Several photographs at different focal depth were taken to capture all C staining regions. Enlargement, exposure time and print processing were varied to maximize recognition of heterochromatic regions. Presence of heterochromatic regions were recorded for each chromosome at the telomere and centromere regions. Staining intensity varied considerably but was recorded as positively heterochromatic when the region was recognizably darker than the surrounding area. A typical photograph of female and male C-banded chromosome spreads is shown in figure 1.

Results and Discussion

The frequency of C-band patterns for the large chromosomes 1-9 and the Z and W sex chromosomes for each inbred line are shown in table 1, 2 and 3. The counts include both homologues of each pair, but there may have been one for each pair of chromosomes in a cell because of entangled chromosomes. The telomere of the q-arm of the Z and the whole of the W were consistently heterochromatic. The centromeric regions of autosomes 3, 4, 6, 8 and 9, stained with a high frequency in all three lines. Chromosome 3, 4 and 8 varied some what as the darker staining regions were not discernable in all preparations. Chromosome 4 centromere regions although recognizable was usually fainter than 3, 6 or 9. Chromosomes 1 and 2 in all lines exhibit somewhat more than 50% C-banding at the centromere and a lower, more variable frequency at the p and q arm telomeres. On the average about 75% of the mitotic metaphase microchromosomes have recognizable C-bands with the current methodology (table 4). This is in contrast to the 100% C-banding in mitotic MI microchromosomes described by Pollock and Fechheimer (1981).

TABLE 1. LOCATION AND FREQUENCY OF C-BANDS IN THE METAPHASE CHROMOSOMES OF THE WHITE LEGHORN INBRED LINE

Chromosome	Number observed	P arm		Centromere		Q arm	
		No.	%	No.	%	No.	%
1	30	5	(17)	13	(43)	11	(37)
2	30	4	(13)	22	(73)	9	(30)
3	29			28	(97)	7	(24)
4	30	1	(3)	27	(90)	2	(7)
5	30			3	(10)	4	(13)
6	26			26	(100)	2	(8)
7	29			12	(41)	3	(10)
8	28	2	(7)	21	(75)	3	(10)
9	28			28	(100)		
Z	24	2	(8)	3	(13)	24	(100)
W	4	4	(100)	4	(100)	4	(100)

TABLE 2. LOCATION AND FREQUENCY OF C-BANDS IN THE METAPHASE CHROMOSOMES OF THE RHODE ISLAND RED INBRED LINE

Chromosome	Number observed	P-arm		Centromere		Q arm	
		No.	%	No.	%	No.	%
1	30	8	(27)	20	(67)	6	(20)
2	30	1	(3)	16	(53)	8	(27)
3	29			24	(83)	4	(14)
4	27	1	(4)	23	(85)	5	(19)
5	23			7	(30)	1	(4)
6	24			21	(88)		
7	23			9	(39)	1	(4)
8	22			18	(82)		
9	24			24	(100)		
Z	19	1	(5)			18	(95)
W	11	11	(100)	11	(100)	11	(100)

Variability could result from intrinsic differences, such as small amounts of constitutive heterochromatin difficult to visualize with the staining process method or to packaging so that DNA was digested by the procedure in differing degrees. Since the lines examined here were highly inbred, one would expect little variability for constitutive heterochromatin between homologues within lines. Detection of C-band polymorphism depends upon presence or absence, size difference or staining intensity and because of the general impreciseness of the current technique, C-band differences between homologous chromosomes of these three lines were not detected. Special attention was given to the Z-sex chromosome to determine if there was C-band polymorphism as described by Pollock and Fechheimer (1981) and none was detectable either within or between lines.

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TABLE 3. LOCATION AND FREQUENCY OF C BANDS IN THE METAPHASE CHROMOSOMES OF THE NEW HAMPSHIRE INBRED LINE

Chromosome	Number observed	P-arm		Centromere		Q arm	
		No.	%	No.	%	No.	%
1	30	12	(40)	16	(53)	11	(37)
2	29	11	(38)	16	(55)	16	(55)
3	30			26	(87)	8	(27)
4	30	1	(3)	27	(90)	11	(37)
5	30			4	(13)	8	(27)
6	29			29	(100)		
7	28			19	(68)	2	(7)
8	25	1	(4)	22	(88)	1	(4)
9	30			30	(100)		
Z	29	6	(20)	3	(10)	29	(100)
W	1	1	(100)	1	(100)	1	(100)

TABLE 4. COMBINED C-BAND FREQUENCY FOR MICROCHROMOSOMES (10-38) IN THE THREE INBRED LINES

	420 WL		New Hamp		RIR		Average	
	Obs. No.	%	Obs. No.	%	Obs. No.	%	Obs. No.	%
Partial band	241	40.1	173	32.5	199	31.2	613	34.6
Whole band	266	44.3	184	34.6	236	37.0	686	38.7
Non band	94	15.6	175	32.9	203	31.8	472	26.7
Total	601		532		638		1771	

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