Theor Appl Genet (1985) 71:408–412

Fluorescent chromosome banding in inbred chicken: quinacrine bands, sequential chromomycin and Dapi bands

S. Fritschi and G. Stranzinger

Federal Institute of Technology, Institute of Animal Production, Section Animal Breeding, Zurich, Switzerland

Accepted May 21, 1985
Communicated by H. Abplanalp

Summary. Highly inbred White Leghorn chickens were used for an investigation of the banding pattern of the macrochromosomes. A standard was set for the Q-bands. GC-specific fluorochrome chromomycin and the AT-specific Dapi were used in a sequential stain. The comparison of these two stains disclosed quantitative differences in the base distribution of the DNA. Factors responsibe for the binding mechanism and the appearance of the bands are discussed.

Key words: Inbred chicken – Chromosomes – Banding pattern

Introduction

The domestic fowl (*Gallus domesticus*) is cytogenetically the most investigated bird. Its diploid chromosome number is 78 (Pollock and Fechheimer 1981), and its chromosomes vary in size from 8 μm to particles almost invisible under the light microscope. The largest eight pairs and the sex chromosomes are designated as macrochromosomes; the other 30 pairs as microchromosomes. The sex determining chromosomes are reversed in comparison to mammals with the cock being homozygous (*ZZ*) and the hen hemizygous (*ZW*).

The aim of this article is to characterize the banding pattern of the chicken macrochromosomes by using different fluorescent dyes, namely quinacrine stain, chromomycin and Dapi sequential staining, respectively. The use of highly inbred strains of chicken cell material offers a precise description of bands since the polymorphic appearance of chromosome structures should be limited. Up to now, only a few studies have been published describing bands of chicken chromosomes; namely Q-band (Stahl and Vagner-Capodano 1972), G-band (Stock et al. 1974; Wang and Shoffner 1974; Pollock and Fechheimer 1981; Carlenius et al. 1981) and R-band (Carlenius et al. 1981). The banding pattern can be subdivided into two categories: AT-rich regions staining with quinacrine mustard (Comings 1978), and 4,6-Diamidino-2-phenylindol · 2 HCl (Dapi) (Lin et al. 1977) and GC-rich DNA regions staining with Chromomycin A_3 (Schweizer 1976).

Chromosomes stained with Dapi show a qualitatively similar, though not identical, banding pattern as Q-bands. The dye chromomycin produces a type of R-band pattern. Sequential staining of Dapi and chromomycin of the same metaphase allows the distinction of differences between the distribution of base pairs (Schnedl 1981; Schweizer et al. 1978). The two fluorescence dyes can clearly be separated with the use of different excitation wavelengths of the filters.

Materials and methods

Metaphase preparations were made from embryonic liver cells taken from nine highly inbred (inbreeding coefficient = 94%) White Leghorn strains (Abplanalp et al. 1981) bred at the experimental farm, Chamau, of the ETH Zurich. For producing embryonic liver cells the hens were inseminated twice a week with undiluted ejaculates and eggs collected daily for ten days. Fertilized eggs were incubated 11–13 days in a standard incubator at 37.6 °C. Four hours before the preparation of the chromosomes, 0.4 ml Colcemid (10 μg/ml) was injected into the aircell of the fertile eggs and the eggs returned to the incubator. Thereafter, embryos were taken out of the egg and liver cells were directly prepared without the preceding incubation. The method was modified after Fechheimer (Fechheimer et al. 1970). As hypotonic fluid, 1% prewarmed (39 °C) sodium citrate with added Colcemid (0.3 ml

Colcemid – 10 µg/ml – per 10 ml sodium citrate) was chosen. A hypotonic treatment of 30 min at 39 °C was applied. For the first two fixations ice-cold methanol/acetic acid mixture in the proportion 3 : 1 was used. In the last fixation the proportion was 4 : 1. The samples were put into the freezer before the fixations and also before preparing the slides. Two to three drops of the cell suspension were put onto a slide and were air-dried overnight.

Staining procedures

Quinacrine mustard (Sigma, St. Louis, USA). Quinacrine mustard was dissolved in Sørensen phosphate buffer, pH 6.8, (f.c. 0.1 mg/ml) with the addition of some thymol to prevent bacterial development. The dry chromosome preparations were put into the dye solution and stained for 30 min. After staining, the slides were rinsed with tap water and put into the buffer solution (pH 6.8) for three min. Afterwards the slides were covered with a 3 : 1 glycerolphosphate buffer mixture (glycerol for fluorescence microscopy by Merck, Darmstadt, BRD) and the coverslip sealed with nail polish (modification after Caspersson et al. 1970).

Dapi and chromomycin. The combination of Dapi (4,6-Diamidino-2-phenylindol · 2 HCl; Serva Heidelberg, BRD) and chromomycin A_3 (Serva Heidelberg, BRD) staining provides an opportunity to observe banding differences at the same metaphase. Air-dried slides were incubated in McIlvaine buffer (pH 7) for 15 min and subsequently stained with Dapi (0.3 µg Dapi/ml buffer) for 15 min. The slides were then rinsed in McIlvaine buffer (pH 7) to which was added 10 mM $MgCl_2$. The chromomycin solution (0.1 mg chromomycin/ml buffer with $MgCl_2$) was dropped onto the slides and those kept in the dark for 30 min. The slides were then rinsed in the buffer and sealed with glycerol buffer mixture (modification after Schweizer 1980).

Microscopic observation and photography

A Zeiss Microscope No. III with fluorescent equipment and a 63× oil immersion objective was used. To obtain clear banding patterns from the quinacrine stain, the preparations were immediately photographed with a Zeiss Filter No. 48.77.06 (wavelength 436 nm) in the blue field. The best banding effect from the Dapi staining resulted when the preparations were stored up to 14 days in the refrigerator, so that the stain could stabilize (Zeiss Filter No. 48.77.02, wavelength 365/366 nm). Chromomycin fades very quickly, and it was only possible to take one photograph per metaphase. The filters for chromomycin A_3 were in the blue field (wavelength 436 nm), Zeiss Filter No. 48.77.06. On the other hand, the fluorescence of Dapi is quite stable and several pictures could be taken. All the fluorescence photographs were taken on Kodak 2415 film. The analysis of the karyotypes was done by means of paper prints.

Results and discussion

Q-banding

The quinacrine bands of the nine identifiable chromosome pairs are distinct, as shown in Fig. 1.

The microchromosomes fluoresce weakly and the intensity of the fluorescence is quite variable.

The Q-banding of these chicken chromosomes is schematically shown in Fig. 2.

Based on our preparations, a standard banded karyogram is proposed. The diagram takes into consideration the distribution and the relative width of the bands. Until now only one description has been published (Stahl and Vagner-Capodano 1972) and it seems to be preliminary and incomplete. In Fig. 2 we tried to subdivide the chromosome arms into regions and landmarks (Paris Conference 1972). The Q-bands are characteristic for the individual chromosome and consist of reproducible patterns (Caspersson et al. 1970; Schweizer 1976). With the aid of the banding, the chromosomes may be clearly identified.

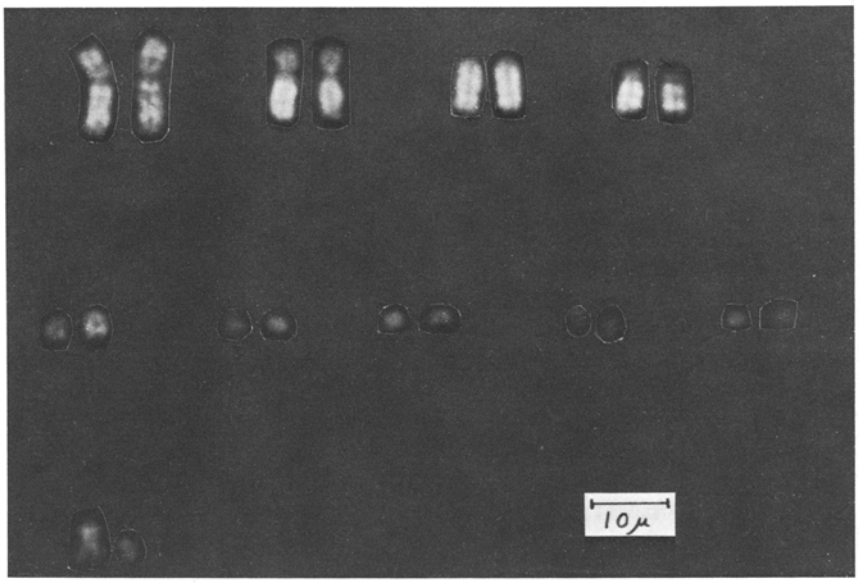

Fig. 1. Karyotype of a female animal with the sex chromosomes ZW, stained with the Q-band technique

Quinacrine seems to bind specifically to AT-rich DNA (Schnedl 1974; Comings 1978). The appearance of the quinacrine bands is a very sensitive measure for alterations in the base composition. Results from autoradiographic experiments show that the Q-band corresponds to late replicating regions (Bianchi and Molina 1967; Schnedl 1974; Grzeschik et al. 1975; Latt 1977). The microchromosomes fluoresce only faintly. The hypothesis by Schmid (1962) and Bianchi and Molina (1967) is therefore supported; these authors reported that microchromosomes do not show a late replication and that they have very few heterochromatic regions. The predispositions which are responsible for the appearance of the Q-band pattern seem to be very conservative and show the genetic significance of these bands. The number and arrangement of the main bands are in the same order (Schnedl 1974) at closely related species.

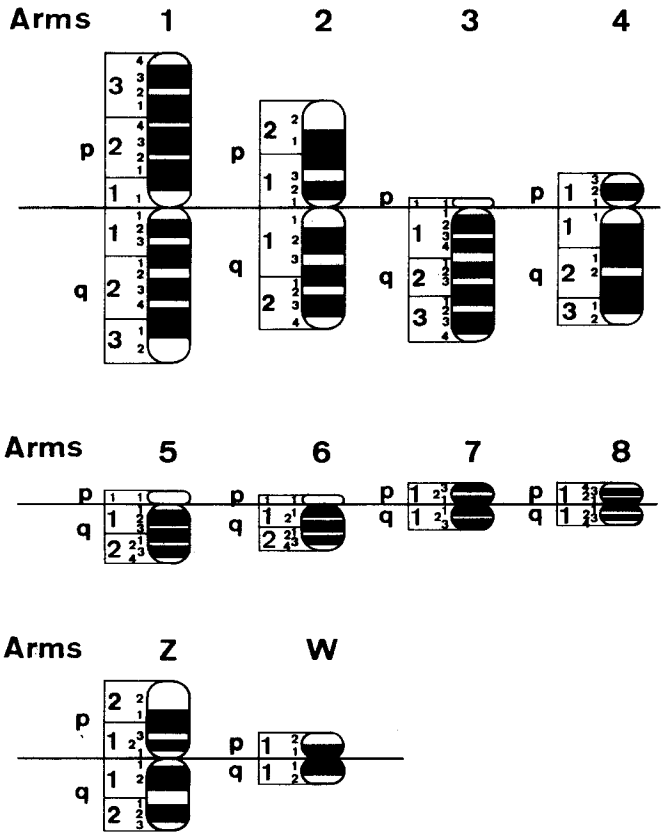

Fig. 2. Scheme of the Q-banding of a female karyotype, derived from different preparations (after the Paris Conference 1971)

Dapi/chromomycin banding

In Fig. 3 the observed fluorescence patterns are shown for the first four chromosome pairs and the sex chromosomes. Both stains were carried out on the same metaphase. The microchromosomes fluoresce very strongly with the chromomycin stain, however with the Dapi stain they do not fluoresce. The fluorescing bands are not as distinct as the bands of the quinacrine stain. Dapi is comparable to quinacrine for the position of the bands (see Fig. 4). Dapi and chromomycin appear to be complementary in only a few positions.

The microchromosomes fluoresce more intensively with the chromomycin stain than with the Dapi stain. The telomeric end of the Z-chromosome fluoresce brilliantly with chromomycin (Fig. 4), while with the Dapi stain the telomeric end of the Z-chromosome appears negative. In addition, the telomeric end of the first chromosome fluoresces with chromomycin but not with Dapi. The W-chromosome is highly fluorescent with the chromomycin stain, but pale with the Dapi stain. These results indicate that the proportion of bases AT and GC is different. With the chromomycin stain a very strong fluorescence has been found for the microchromosomes, indicating that they are rich in GC-sequences.

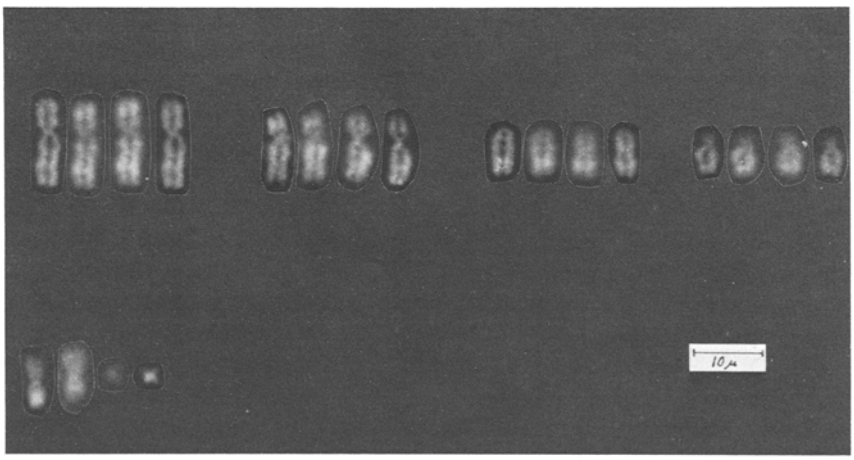

Fig. 3. Double karyotype of a female animal with the sex chromosomes ZW, stained following the chromomycin and Dapi techniques. The chromosome pairs are placed in such a way that the chromomycin stained chromosomes can be found to the outside and the Dapi fluorescing chromosomes to the inside

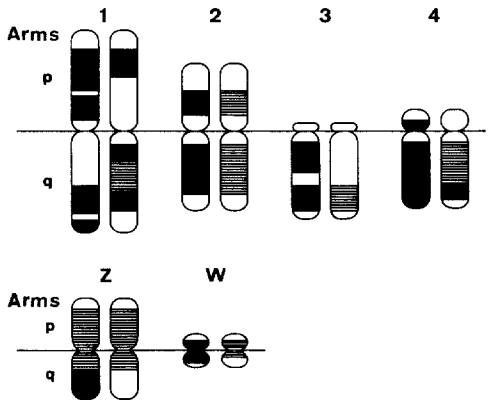

Fig. 4. Scheme of the chromomycin (*left*) and Dapi banding (*right*) of a female karyotype, derived from the photographic analysis of several preparations

The antibiotic, chromomycin A_3, gives a similar pattern as the R-banding-it binds specifically to GC-rich sequences (Goldberg and Friedman 1971; Schweizer 1976) and fluoresces yellow-green.

Dapi binds preferentially – as does quinacrine – to AT-rich positions; it fluoresces blue (Müller and Gautier 1975; Schweizer and Nagl 1976; Lin et al. 1977).

In the most recent papers fluorescent dyes are described which bind to DNA without pretreatment by hydrolysis (Caspersson et al. 1970; Dutrillaux et al. 1972; Schnedl 1974; Grzeschik et al. 1975; Lin et al. 1977; Comings 1978). To this group of dyes belong quinacrine mustard, chromomycin A_3 and 4,6-Diamidino-2-phenylindol (Dapi).

The mechanism of dye-DNA binding in all three cases is not as yet clearly understood. It seems that the base sequences and the chromosomal proteins play an important role, whereas the significance of the chromosomal proteins is still not well explained (Schweizer et al. 1978).

Schweizer (1979) indicates the sensitivity of the Dapi/chromomycin combined method as 10^{-2} picogram DNA. The cells of birds contain very little DNA. The following table indicates the DNA content after Feulgen absorption in relative units (Bachmann et al. 1972):

Species	Tissue	relative DNA-unities
mouse	liver	173
heron	liver	83
chicken	liver	78

The diploid chromosome set of the chicken corresponds to 3.5 pg DNA. It is supposed that 25% of the chicken genome is formed by repetitive sequences (Schultz and Church 1971). The chromomycin and Dapi bands are assumed to correspond to GC-respectively AT-richness of chromosome regions. As a consequence of the small DNA-content of the chicken genome, the result is not comparable to the distinct patterns found in plants.

Acknowledgements. The authors wish to thank Dr. C. Hagger and Mrs. D. Steiger-Stafl for supplying the egg material for this investigation and to Mrs. V. Madison for correcting, and Mrs. R. Jenny for typing, the manuscript. Please address reprints requests to G. Stranzinger.

References

Abplanalp H, Hagger C, Briles R (1981) Genetic variation of blood groups in inbred lines of leghorns, derived from a common base population. J Hered 72:224–226

Bachmann K, Harrington BA, Craig JP (1972) Genome size in birds. Chromosoma 37:405–416

Bianchi NO, Molina OJ (1967) Chronology and pattern of replication in the bone marrow chromosomes of Gallus domesticus. Chromosoma 21:387–397

Carlenius C, Ryttman H, Tegelström H, Jansson H (1981) R-, G- and C-banded chromosomes in the domestic fowl (*Gallus domesticus*). Hereditas 94:61–66

Caspersson T, Zech L, Johansson C (1970) Differential binding of alkylating fluorochromes in human chromosomes. Exp Cell Res 60:315–319

Comings DE (1978) Mechanisms of chromosome banding and implications for chromosome structure. Annu Rev Genet 12:25–46

Dutrillaux B, Finaz C, de Grouchy J, Lejeune J (1972) Comparison of banding patterns of human chromosomes obtained with heating fluorescence and proteolytic digestion. Cytogenetics 11:113–116

Fechheimer NS, Lodge JR, Miller RC (1970) Sex proportion of domestic chicken at 16 hours of incubation. J Reprod Fertil 23:365–367

Goldberg JH, Friedman PA (1971) Antibiotics and nucleic acids. Annu Rev Biochem 40:775–810

Grzeschik K-H, Kim MA, Johannsman R (1975) Late replicating bands of human chromosomes demonstrated by fluorochrome and Giemsa staining. Humangenetik 29:41–59

Latt SA (1977) Fluorescent probes of chromosome structure and replication. Can J Genet Cytol 19:603–623

Lin MS, Comings DE, Alfi OS (1977) Optical studies of the interaction of 4′-6-Diamidino-2-Phenylindole with DNA and metaphase chromosomes. Chromosoma 60:15–25

Müller W, Gautier F (1975) Interactions of heterochromatic compounds with nucleic acids. A-T-specific non-intercalating DNA ligands. Eur J Biochem 54:385–394

Paris Conference 1971 (1972) Standardization in human cytogenetics. Cytogenetics 11:313–362

Pollock DL, Fechheimer NS (1976) The chromosome number of *Gallus domesticus*. Br Poult Sci 17:39–42

Pollock DL, Fechheimer NS (1981) Variable C-banding patterns and a proposed C-band karyotype in *Gallus domesticus*. Genetica (the Hague) 54:273–279

Schmid W (1962) DNA replication patterns of the heterochromosomes in *Gallus domesticus*. Cytogenetics 1: 344–352

Schnedl W (1974) Banding patterns in chromosomes. Int Rev Cytol (Suppl) 4:237–272

Schnedl W, Abraham R, Förster M, Schweizer D (1981) Differential fluorescent staining of porcine heterochromatin by chromomycin A_3/distamycin A/Dapi and D 287/170. Cytogenet Cell Genet 31:249–253

Schultz GA, Church RB (1971) DNA base sequence heterogeneity in the order Galliformes. J Exp Zool 179:119–128

Schweizer D (1976) Reverse fluorescent chromosome banding with Chromomycin and Dapi. Chromosoma 58:307–324

Schweizer D (1979) Fluorescent chromosome banding in plants: applications, mechanisms and implications for chromosome structure. Proc 4th John Innes Symp, pp 61–72

Schweizer D (1980) Simultaneous fluorescent staining of R bands and specific heterochromatic regions (Da-Dapi bands) in human chromosomes. Cytogenet Cell Genet 27:190–193

Schweizer D, Nagl W (1976) Heterochromatin diversity in *Cymbidium* and its relationship to differential DNA replication. Exp Cell Res 98:411–423

Schweizer D, Ambros P, Andrle M (1978) Modification of Dapi banding on human chromosomes by prestaining with a DNA binding oligo-peptide antibiotic, Distamycin A. Exp Cell Res 111:327–332

Stahl A, Vagner-Capodano AM (1972) Cytogénétique – Etude des chromosomes du poulet (*Gallus domesticus*) par les techniques de fluorescence. C R Acad Sci Paris 275: 2367–2370

Stock AD, Arrighi FE, Stefos K (1974) Chromosome homology in birds: banding patterns of the chromosomes of the domestic chicken, ring-necked dove and domestic pigeon. Cytogenet Cell Genet 13:410–418

Wang N, Shoffner RN (1974) Trypsin G- and C-banding for interchange analysis. Chromosoma 47:61–69